STUDIES OF AURORA AND POLO KINASES DURING CELL DIVISION IN *C. ELEGANS*

APPROVED BY SUPERVISORY COMMITTEE

_____
Rueyling Lin, PhD
Associate Professor of Molecular Biology

_____
Leon Avery, PhD
Professor of Molecular Biology

_____
Melanie Cobb, PhD
Professor of Pharmacology

_____
Andrew Zinn, MD, PhD
Associate Professor of Internal Medicine

DEDICATION

This dissertation is dedicated to my father, Walter R. Rogers,

who always will be my greatest mentor.

STUDIES OF AURORA AND POLO KINASES DURING CELL DIVISION IN *C. ELEGANS*

by

Eric Jason Rogers

DISSERTATION

Presented to the Faculty of the Graduate School of Biomedical Sciences

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

The University of Texas Southwestern Medical Center at Dallas

Dallas, Texas

January, 2005

Copyright

by

Eric Jason Rogers, 2005.

All Rights Reserved

ACKNOWLEDGEMENTS

I would like to first thank my mentor Dr. Rueyling Lin. Without her patience and support, I would not have been able to do this research. I would like to also thank my committee members, Dr. Leon Avery, Dr. Melanie Cobb, and Dr. Andrew Zinn, for making my graduate experience both fruitful and enjoyable. I thank Dr. Eric Olson and the Department of Molecular Biology for providing excellent facilities and assistance.

Much of the data presented in this dissertation was done in collaboration with others. In particular, I would like to thank Dr. James Waddle for the microscopy work. Also, I would like to thank Dr. Jill Schumacher and her postdoctoral fellows, Dr. John Bishop and Dr. Phedra Amayed, who performed the in vitro kinase experiments shown in the results.

The generosity of the *C. elegans* research community provided me with access to many reagents. In particular, I would like to thank the *C. elegans* Genome Consortium, Dr. J. Austin (University of Chicago, Chicago, IL), Dr. B. Horvitz (Massachusetts Institute of Technology, Boston, MA), Dr. J. Loidl (University of Vienna, Vienna, Austria), Dr. Y. Kohara (National Institute of Genetics, Shizuoka, Japan), Dr. A. Golden (National Institutes of Health, Bethesda, MD), Dr. Anthony Hyman (Max Planck Institute, Dresden, Germany), Dr. G. Seydoux (Johns Hopkins, Baltimore, MD), Dr. J. Priess (Fred Hutchinson Cancer Center, Seattle, WA), and Dr. S. Ohno (NEC Corporation, Japan) for sharing reagents such as worm strains, antibodies, and cDNAs.

STUDIES OF AURORA AND POLO KINASES DURING CELL DIVISION IN

C. ELEGANS

Publication No._____

Eric Jason Rogers, Ph.D.

The University of Texas Southwestern Medical Center at Dallas, 2005

Supervising Professor: Rueyling Lin, Ph.D.

Accurate chromosome segregation during cell division requires the precisely regulated release of chromosome cohesion. In mitosis, sister chromatids are linked by chromosome cohesion until the proteolysis of the cohesion Scc1 by separase triggers the separation of sister chromatids at anaphase. Chromosome dynamics during meiosis are more complex, as homologous chromosomes separate in anaphase I, whereas sister chromatids remain attached until anaphase II. In meiosis, separase must cleave the cohesin REC-8 in a stepwise manner to separate homologs in meiosis I and then sister chromatids in meiosis II. However, the mechanisms regulating the selective and sequential release of meiotic chromosome cohesion are unclear. Using *C. elegans,* we investigated the roles of Aurora and Polo kinases during the release of meiotic chromosome cohesion.

We found that the Aurora B kinase AIR-2 is localized to sub-chromosomal regions representing the last points of contact between homologous chromosomes in meiosis I and between sister chromatids in meiosis II. Depletion of AIR-2 by RNA interference (RNAi) prevented both chromosome separation and REC-8 removal during meiosis. We showed AIR-2 phosphorylated REC-8 at a major amino acid *in vitro* (T625). The depletion of two phosphatases, GSP-1 and GSP-2, altered the localization pattern of AIR-2, such that AIR-2 is detected throughout the chromosome. Concurrently, there was a chromosome-wide reduction in REC-8 and sister chromatids precociously separated at anaphase I. We propose that AIR-2 promotes the selective release of meiotic chromosome cohesion via the phosphorylation of REC-8 at specific chromosomal locations and that GSP-1/2 antagonize AIR-2 activity.

We also described that the Polo-like kinase PLK-1 is required for the release of meiotic chromosome cohesion during meiosis II. Depletion of PLK-1 by RNAi did not block the separation of homologous chromosomes, but the resulting dyads fail to separate during meiosis II. Furthermore, in *plk-1(RNAi)* embryos, REC-8 was not removed from these dyads. PLK-1 was capable of phosphorylating REC-8 in vitro. The *gsp-1/2(RNAi)* phenotype of precocious loss of REC-8 at anaphase I was suppressed by the simultaneous inhibition of PLK-1. We propose PLK-1 regulates the second phase of meiotic chromosome cohesion release. In summary, we propose that both Aurora B and Polo kinases phosphorylate REC-8 in order to regulate the selective and sequential release of chromosome cohesion during meiosis in *C. elegans*.

TABLE OF CONTENTS

PRIOR PUBLICATIONS

Detwiler, M. R, M. Reuben, X. Li, **E. Rogers**, and R. Lin. Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in *C. elegans*. *Developmental Cell*. **1**(2): 187-99 (2001).

Rogers, E., J. D. Bishop, J. M. Schumacher, J. A. Waddle, and R. Lin (2002). "The aurora kinase AIR-2 functions in the release of chromosome cohesion in *Caenorhabditis elegans* meiosis." *Journal of Cell Biology* **157**(2): 219-29.

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

4-D – four-dimensional

ABI – protein complex composed of Aurora B kinase, Bir1/Survivin, and INCENP

APC – anaphase promoting complex, also called the cyclosome

ATP – adenosine triphosphate

Cdk – cyclin-dependent kinase

DAPI – 4',6'-diamidino-2-phenylindole hydrochloride

DIC – differential interference contrast

DNA – deoxyribonucleic acid

dsRNA – double-stranded RNA

EMS – ethylmethanesulfonate

GFP – green fluorescent protein

H3P – phosphorylated-Ser10 of histone H3

IAP – inhibitor of apoptosis

MAP – microtubule associated protein

MAPK – mitogen-activated protein kinase

PBD – Polo box domain

RNA – ribonucleic acid

RNAi –RNA interference

CHAPTER 1: Introduction and literature review

A. Cell division: Mitosis

Cell division, one of the most central processes in all of biology, is a complex process by which a 'mother' cell divides into two 'daughter' cells. The purpose of cell division is to generate new cells, thus forming an immortal lineage of life. Cell division was first observed in the 19th century by biologists, such as Rudolf Virchow, using available microscopes. Virchow's insight from these observations lives with us today in the phrase "*omnis cellula a cellula*," meaning that all cells are derived from other cells.

Each cell division requires the proper partitioning of all vital components into the daughter cells to ensure their viability. For example, prior to every division, the mother cell replicates its genetic information in the form of chromosomes. The chromosomes must be segregated during cell division, such that each daughter normally receives precisely one set of chromosomes in order to ensure their viability.

1. Traditional stages of mitosis

Contemporaries of Virchow, such as Walther Flemming, separated cell division into different phases, based on events observable using light microscopy. Prophase (Fig. I.1 B), the opening stage of cell division, was defined by the first appearance of condensed chromosomes (Flemming, 1879; Wilson, 1928). The condensed chromosomes resemble threads; thus the name

mitosis, from the Greek μιτο, meaning "thread" (Flemming, 1882). Mitosis is the process used for most cell divisions that occur in eukaryotes.

During prophase, another key event is the degradation of the nuclear envelope, a membrane-bound compartment separating the nuclear material, mainly the chromosomes, from the rest of the cytoplasm. The complete disappearance of the nuclear envelope marks the end of prophase and entry into prometaphase (Fig. I.1 C). Prometaphase involves the formation of the bipolar spindle, a mitotic structure that exerts on the chromosomes the forces required for their proper segregation. The spindle is a large structure composed of tiny 'cables', which are microtubules that are organized by centrosomes (Flemming, 1875; Boveri, 1888). In general, the centrosomes nucleate microtubules, which radiate in all directions forming the astral fibers (Flemming, 1875; Boveri, 1888).

During prometaphase, the two centrosomes migrate apart, the astral microtubules shorten, and a growing concentration of microtubules asymmetrically extends toward the chromosomes. This results in the formation of the bipolar spindle, which is composed of the separated centrosomes connected by a concentration of microtubules meeting at the chromosomes (Fig. I.1 D). The bipolar spindle is composed of three main groups of microtubules: (1) astral microtubules, which radiate in all directions; (2) spindle microtubules, which connect the centrosomes to the chromosomes; and (3) a variable number of microtubules, which meet from opposing centrosomes at the center of the cell.

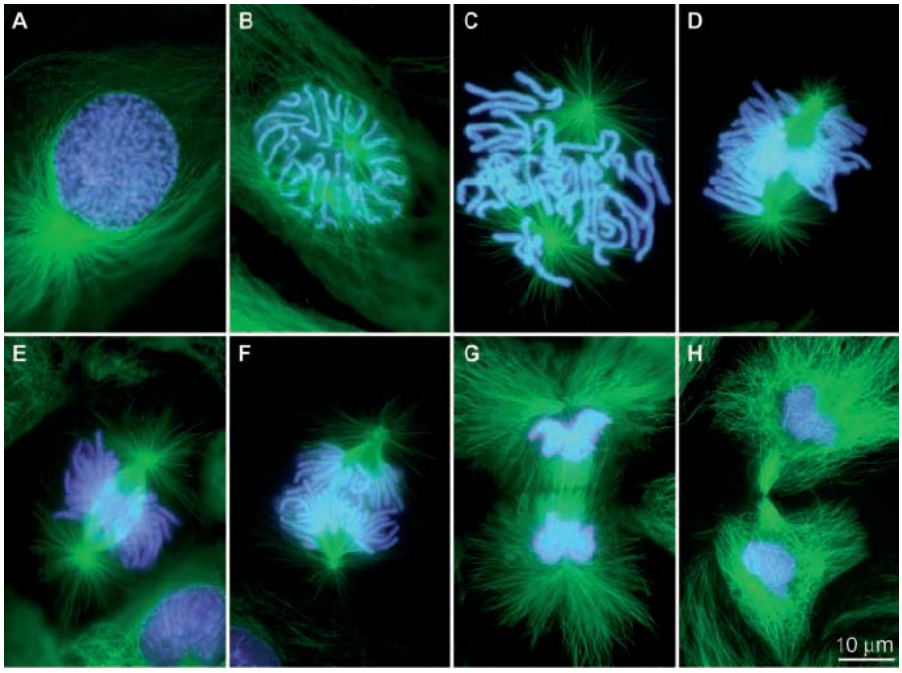

Figure I.1. The stages of mitosis. (Reproduced from Rieder and Khodjakov, 2003.) Panels show fixed newt lung cells stained for DNA in blue and microtubules in green. A, interphase; B, prophase; C-D, prometaphase; E, metaphase; F-G, anaphase; and H, telophase (with cytokinesis).

The microtubules of the bipolar spindle exert forces on the chromosomes to precisely move them around the cell during division (Inoue and Sato, 1967). The first chromosomal movement is the congression of the chromosomes to the center of the cell (Fig. I.1 D). The process of congression culminates with the alignment of all the chromosomes at the equator of the cell; this arrangement is termed the metaphase plate. Visually, metaphase is the most impressive stage of cell division. Metaphase is defined by the appearance of all the chromosomes aligned exactly at the equator (Fig. I.1 E).

The subsequent entry into anaphase is defined by the separation of the chromosomes towards the opposite poles of the spindle. During early anaphase, chromosomes are pulled apart by the shortening of the spindle microtubules while the centrosomes remain stationary. This phase of spindle behavior is referred to as anaphase A. Then, in anaphase B, the distance

between centrosomes increases, resulting in a pulling of the chromosomes even further apart. Lastly, the end of anaphase involves the formation of the central spindle, a microtubule structure between the separating chromosomes that pushes the chromosomes apart and prepares the cell for cytokinesis (Fig. I.1 G).

The final stages of cell division include the events of telophase and the process of cytokinesis (Fig. I.1 H). Telophase is defined by the appearance of new nuclear envelopes, thereby completing karyokinesis, which is the process of nuclear division (Schleicher, 1879). Telophase also involves the disassembly of the mitotic spindle and decondensation of the chromosomes. In many cells, including all animal cells, the process of cytokinesis concludes cell division. Cytokinesis is the irreversible cleavage of the mother cell into two daughter cells.

2. Modern molecular landmarks of mitosis

Over the last 25 years, significant progress has been made in understanding how the life cycle of the eukaryotic cell is regulated at the molecular level. In particular, studies of cyclin-dependent kinases (Cdks) have provided a conceptual framework for the description of the temporal control of the cell cycle.

Seminal studies of Cdks and cyclins led to the awarding of the 2001 Nobel Prize in Physiology or Medicine to Dr. Lee Hartwell, Dr. Tim Hunt and Dr. Paul Nurse (Nasmyth, 2001). Through different methods, these researchers demonstrated that the cell cycle is regulated by the rise and fall of multiple Cdk activities that depend on the abundance of their cognate cyclins (Swenson et al., 1986; Alfa et al., 1989; Draetta et al., 1989; Maller et al., 1989). The abundance of different cyclins oscillates in strict temporal patterns, correlating with progression through the

cell cycle (Evans et al., 1983; Draetta et al., 1989; Murray et al., 1989). This molecular system is remarkable conserved throughout the eukaryotic kingdom, providing a universal model of regulation of the cell cycle in all eukaryotes (Nurse, 1990).

a. The cyclin-dependent kinases

Kinases are enzymes that transfer phosphates from ATP molecules and covalently attach the phosphates to their targets. Although kinases play significant roles throughout biology, this is especially true with regards to the cell cycle (Maller, 1993). The cell cycle is ruled by protein kinases that use various phosphorylation-based mechanisms to orchestrate numerous molecular events (Nigg, 2001).

Among the cell cycle kinases, the most prominent and well studied are the Cdks. Most kinases initially are inactive, because a domain called the activation loop physically blocks access to the catalytic site (Hubbard, 1997). Therefore, most kinases are activated by phosphorylation within the activation loop; this opens access to the catalytic site (Johnson et al., 1996). With Cdks, kinase activation requires conformational changes induced by the binding of cyclins. The binding of cyclins promotes the phosphorylation of the Cdk in the activation loop by Cdk-activating kinases and promotes the binding of Cdk substrates (Fisher et al., 1994; Tassan et al., 1994; Desai et al., 1995; Fesquet et al., 1998).

The activation of Cdk1 is sufficient to drive cells into mitosis (Newport and Kirschner, 1984). Cdk1 is activated specifically during mitosis by various regulatory mechanisms. Primarily, Cdk1 is activated by a rise in the concentrations of the mitotic cyclins, cyclin-A and cyclin-B, which peak at metaphase (Fig. I.2 A; Evans et al., 1983). However, Cdk1 is kept

inactive, even in the presence of mitotic cyclins, by inhibitory phosphorylations added by the Wee1/Myt1 kinases (Gould and Nurse, 1989; Lundgren et al., 1991; Parker et al., 1992; McGowan and Russell, 1993; Mueller et al., 1995). The kinase activities of Wee1/Myt1 towards Cdk1 are counter-acted by the phosphatase Cdc25 (Russell and Nurse, 1987; Dunphy and Kumagai, 1991; Gautier et al., 1991; Kumagai and Dunphy, 1991; Lundgren et al., 1991; Strausfeld et al., 1991). Phosphatases function to remove phosphates from their substrates, operating in direct opposition to kinases.

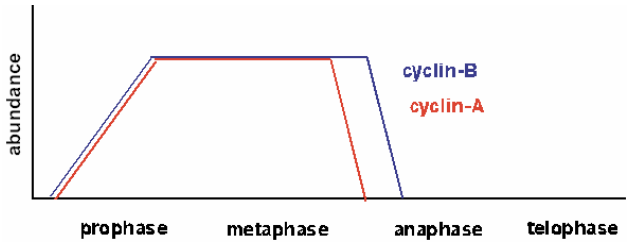

Figure I.2 A. Mitotic cyclin levels peak at metaphase. The abundance of the mitotic cyclins begins to rise prior to prophase and peaks at metaphase. During anaphase, the mitotic cyclins are degraded rapidly.

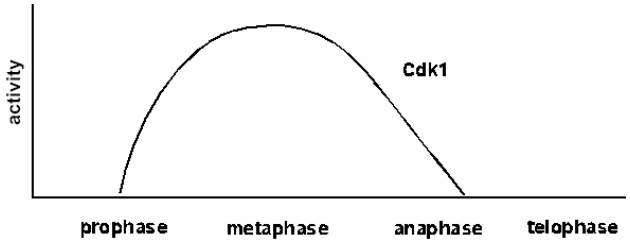

Figure I.2 B. Cdk1 activity peaks at metaphase. As a direct result of the abundance of mitotic cylcins, Cdk1 kinase activity rises during prophase, peaks at metaphase, and declines during anaphase.

In general, the ease and reversibility of phosphorylation allows the cell to use kinase/phosphatase systems to precisely regulate molecular events requiring great temporal

accuracy (Maller, 1993). One example is the regulation of Cdk1 by the Wee1/Cdc25 kinase/phosphatase pair. Entry into mitosis involves the activation of a small pool of Cdk1 complexed with the mitotic cyclins, which then invokes two feedback loops. There is negative feedback inhibiting Wee1/Myt1 kinase activities and positive feedback promoting Cdc25 phosphatase activity (Hoffmann et al., 1993; Izumi and Maller, 1993; Strausfeld et al., 1994; Mueller et al., 1995). Initially, the rate of Cdk1 activation is slow (Fig. I.2 B); however, above some threshold, the two complementary feedback mechanisms result in a precipitous feed-forward mechanism that greatly accelerates the activation of the remaining Cdk1/cyclin complexes (Novak and Tyson, 1993). Once achieved, the high activity of Cdk1/cyclin drives the cell into prophase by promoting such processes as chromosome condensation, nuclear envelope breakdown, and bipolar spindle formation (Peter et al., 1990; Ward and Kirschner, 1990; Desai et al., 1995).

b. The anaphase promoting complex

Over the last 25 years, significant progress has been made in understanding how proteins are destroyed by the cell in a molecularly regulated manner. Pioneering studies of the process of ubiquitin-mediated proteolysis led to the awarding of the 2004 Nobel Prize in Chemistry to Dr. Aaron Ciechanover, Dr. Avram Hershko, and Dr. Irwin Rose (Finley et al., 2004). Their research focused on the biochemistry of numerous ubiquitin ligases and how modification of their substrates leads to their proteolysis by the proteasome (Finley et al., 2004). One such ubiquitin ligase is the anaphase promoting complex (APC), an important regulator of the cell cycle.

The APC, also known as the cyclosome, is required for entry into anaphase. The function of the APC was discovered by studies into the regulation of the abundance of mitotic cyclins (Irniger et al., 1995; King et al., 1995; Sudakin et al., 1995; Yu et al., 1996). The APC is a large, multi-protein complex with ubiquitin ligase activity that specifically targets the degradation of cell cycle proteins. Functionally, the APC covalent fastens ubiquitin chains to target proteins, thereby allowing their recognition and degradation by the proteasome. The proteasome is a large protein complex with proteolytic activity that is constitutively active throughout the cell cycle (Glotzer et al., 1991; Hershko et al., 1991; Mahaffey et al., 1993).

(1) APC/Cdc20 activity regulates anaphase

The temporal activity of the APC is regulated within the cell cycle. The APC is first activated during metaphase (Fig. I.3), in a Cdk1/cyclin-dependent manner, to target the degradation of multiple anaphase inhibitors (Irniger et al., 1995; Zachariae and Nasmyth, 1996; Zachariae et al., 1996). The APC associates with an activating subunit, Cdc20, whose abundance peaks at the metaphase-anaphase transition (Dawson et al., 1993; Sigrist et al., 1995; Visintin et al., 1997; Weinstein, 1997; Fang et al., 1998; Kramer et al., 1998; Lim et al., 1998; Prinz et al., 1998). Both Cdc20 and APC mutants arrest in a metaphase state, without separating their chromosomes (Palmer et al., 1989; Holloway et al., 1993; Dawson et al., 1995; Irniger et al., 1995; Sigrist et al., 1995; Ciosk et al., 1998; Lorca et al., 1998; Furata et al., 2000; Golden et al., 2000). Biochemically, Cdc20 has been shown to both activate APC activity in vitro and to bind to specific APC substrates in vivo (Fang et al., 1998; Kramer et al., 1998; Jaspersen et al., 1999; Pfleger et al., 2001; Schwab et al., 2001).

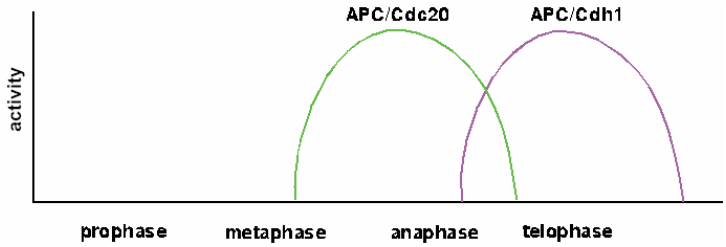

Figure I.3. The activity of the anaphase promoting complex APC. APC/Cdc20 activity peaks at anaphase, whereas APC/Cdh1 activity peaks after telophase.

The most significant substrates of the APC/Cdc20 complex are the mitotic cyclins (Irniger et al., 1995; King et al., 1995; Sudakin et al., 1995). The destruction of mitotic cyclins during anaphase serves to inhibit, via a negative feedback loop, Cdk1/cyclin-activity (Fig. I.2; Novak and Tyson, 1993). The decline in Cdk1/cyclin activity is essential for the progression of the cell cycle in all eukaryotes that have been examined (Zachariae and Nasmyth, 1999). This is attributed to the fact that high Cdk1/cyclin activity inhibits the later cell division events: spindle disassembly, nuclear envelope reformation, and cytokinesis (Holloway et al., 1993; Surana et al., 1993).

(2) APC/Cdh1 activity regulates the end of cell division

The exit from telophase requires the activity of the APC, now complexed with Cdh1 in lieu of Cdc20 (Fig. I.3; Sigrist and Lehner, 1997; Visintin et al., 1997). However, unlike Cdc20, the abundance of Cdh1 is constantly maintained. Prior to telophase, Cdk1 phosphorylation of Cdh1 inhibits Cdhl-dependent APC activities. The decline of Cdk1 activity during anaphase allows for a rise in Cdh1-dependent APC activity (Fig. I.3; Zachariae et al., 1998; Jaspersen et al., 1999; Sorensen et al., 2000). The activity of APC/Cdh1 drives the cell through telophase and the end of mitosis (Zachariae et al., 1998). Cdh1 mutants re-enter the next cell cycle prematurely; therefore, APC/Cdh1 activity probably functions in the timing of the following cell division by

maintaining low Cdk activity and by degrading various factors that promote cell division (Schwab et al., 1997; Sigrist and Lehner, 1997; Kitamura et al., 1998; Kominami et al., 1998).

The activity of the APC/Cdc20 complex is required for such anaphase events as chromosome segregation and spindle elongation, whereas APC/Cdh1 activity is required for such telophase events as spindle disassembly and nuclear envelope reformation (Murray et al., 1989; Gallant and Nigg, 1992; Holloway et al., 1993; Surana et al., 1993; Rimington et al., 1994; Sigrist et al., 1995). Like the Cdks, the function and components compromising the APC are conserved widely throughout eukaryotes, providing another general regulator of the cell cycle (Peters et al., 1996; Yu et al., 1998; Zachariae et al., 1998; Grossberger et al., 1999). Together the Cdks and APC, through their protein kinase activity and protein degradation capability, respectively, control the major temporal transitions of mitosis and form the basis for contemporary understanding of the molecular control of cell division.

3. Molecular mechanisms regulating the events of mitotic cell division

Although biologists have visually described the events of cell division for over a century, it was not until recently that this process could be described at the molecular level. In the last few years, great strides have been made in understanding the molecular mechanisms behind the complex process of cell division. The insights include the discovery of two related protein complexes, cohesins and condensins, both of which regulate chromosome structure, albeit in different ways. Also, discoveries of some protein components of the spindle and kinetochore are beginning to give insights into various spindle behaviors.

However, the extent of progress is highlighted most by studies of the regulation of the metaphase-to-anaphase transition. Descriptions of the molecular functions of such important regulators as separase and securin have accelerated the pace of research on cell division. Finally, tying these discoveries to both the Cdks and the APC provides an integrated understanding of the molecular control of cell division.

a. Chromosome cohesion: Cohesins

Prior to mitosis, the mother cell must replicate its chromosomes. During the process of DNA replication, chromosomal cohesion is established between sister chromatids. After this stage, each chromosome is composed of two sister chromatids that remain linked together via chromosome cohesion. Chromosome cohesion is mediated by both topological DNA connections resulting from the replication process and by a proteinaceous structure composed of cohesin complexes (Holm et al., 1985; Uemura et al., 1987; Ciosk et al, 1997; Guacci et al., 1997; Michaelis et al., 1997; Losada et al., 1998).

The cohesin complex is composed of at least four protein subunits: Scc1, Smc1, Scc3, and Smc3 (Strunnikov et al., 1995; Guacci et al., 1997; Michaelis et al., 1997; Losada et al., 1998; Toth et al., 1999). Although the Scc subunits, named for sister chromatid cohesion, probably perform structural roles, both Smc subunits, named for structural maintenance of chromosomes, contain ATPase activity that is important for their function (Losada and Hirano, 2001). The cohesion holocomplex is believed to form a 'scaffold' between each pair of sister chromatids that has been detected throughout the length of chromosomes (Blat and Kleckner, 1999; Tanaka, et al. 1999). This proteinaceous scaffold is required to hold each pair of sister chromatids together until anaphase and is essential to resist the spindle forces that align the

chromosomes at metaphase (Tanaka et al., 2000; Sonoda et al., 2001; Vagnarelli and Earnshaw, 2001).

Cohesins are essential for life; cohesin mutants exhibit premature sister chromatid separation and chromosome segregation errors during cell division (Guacci et al., 1997; Koshland, 1997; Michaelis et al., 1997; Toth et al., 1999; Losada et al., 2000; Tomonaga et al., 2000). These results suggest the DNA linkages are not sufficient to link sister chromatids. The fate of the DNA linkages is unclear, but they probably are resolved by DNA enzymes, called topoisomerases, either prior to cell division or, perhaps, during the process of chromosome condensation (Swedlow et al., 1993; Saka et al., 1994; Gimenez-Abian et al., 1995; Andreassen et al., 1997).

b. Chromosome condensation: Condesins

Chromosome condensation serves to reduce the volume occupied by the chromosomes. Chromosome condensation also results in the individualization of single chromatids and the differentiation of chromosome arms (Steffensen et al., 2001; Hagstrom et al., 2002; Losada et al., 2002). This process requires the condensin complex composed of CAP-D2, CAP-G, CAP-H, Smc2, and Smc4 (Strunnikov et al., 1995; Hirano et al., 1997; Sutani et al., 1999; Kimura and Hirano, 2000). These subunits come together to form a complex with a very similar structure to the cohesin complex (Sutani et al., 1999). As with cohesins, the Smc subunits of condensin use ATP hydrolysis to produce mechanical energy to alter their substrates (Kimura and Hirano, 1997). Most notably, in the presence of topoisomerases, condensin holocomplexes can induce the condensation of DNA in vitro (Kimura and Hirano, 1997; Kimura et al., 1999a; Hagstrom et al., 2002).

Condensin mutants sometimes display chromosome condensation, but they invariably exhibit chromosome segregation defects resulting from the inability to completely separate sister chromatids (Saka et al., 1994; Strunnikov et al. 1995; Bhat et al. 1996; Lieb et al. 1998; Sutani et al. 1999; Freeman et al. 2000; Lavoie et al. 2000; Ouspenski et al., 2000). This might be explained by the different degrees of chromosome condensation observable in different organisms. The condensin machinery is regulated by both localization and activity. Condensin complexes first appear on the chromosomes in prophase, and the phosphorylation of condensin subunits is capable of stimulating condensin complex activity (Hirano and Mitchinson, 1994; Saitoh et al., 1994; Hirano et al., 1997; Kimura et al., 1998; Losada et al., 1998; Kimura et al., 1999a).

c. Cdk1 directly regulates prophase and prometaphase

The activation of Cdk1 drives cells into mitosis (Newport and Kirschner, 1984). Recently, new Cdk1 substrates have been described that might explain more precisely how Cdk1 functions to promote the events of prophase and prometaphase. For instance, both cohesins and condensins are phosphorylated during mitosis (Kimura et al., 1998; Losada et al., 2000, Tomonaga et al., 2000; Hoque and Ishikawa, 2001). Cdk1 is required for chromosome condensation, and Cdk1 is capable of phosphorylating condensins, which might be required for their activity (Kimura et al., 1998; Collas, 1999; Sutani et al., 1999; Steen et al., 2000; Kimura et al., 2001; Losada et al., 2000). Similarly, Cdk1 has been shown to phosphorylate cohesions, which might facilitate their re-organization or removal from the chromosome (Losada et al., 2000).

Another event during prophase that requires Cdk1 activity is nuclear envelope breakdown. Cdk1 activity has been proposed to directly disassemble nuclear envelopes via the

phosphorylation of laminins, which are structural components of the nuclear membrane (Suprynowicz and Gerace, 1986; Peter et al., 1990; Ward and Kirschner, 1990; Nigg, 1992; Schneider et al., 1999). Also Cdk1 activity has been proposed to directly disassemble another membrane-bound organelle during mitosis, the Golgi (Lowe et al., 1998).

There is evidence suggesting that Cdk1 might directly influence spindle behaviors via the direct phosphorylation of microtubule associated proteins (MAPs) and microtubule motors, such as CENP-E (Heald et al., 1990; Peter et al., 1990; Verde et al., 1990; Buendia et al., 1992; Liao et al., 1994; Tournebize et al., 2000). Finally, it has been suggested that Cdk1 might directly trigger anaphase by activating the APC. The activity of the APC is temporally correlated with the phosphorylation of the APC, and Cdk1 is capable of phosphorylating several APC subunits in vitro (Kramer et al., 2000; Rudner and Murray, 2000; Tang et al., 2001). The phosphorylation of the APC is proposed to increase the APC's affinity for its activator Cdc20; however, this mechanism is not yet proven (Rudner and Murray, 2000).

d. The kinetochore

Prometaphase involves the formation of the kinetochore, a specialized structure that connects the chromosomes to the microtubules of the spindle. The kinetochore performs three functions: (1) kinetochores are the sites where chromosomes capture spindle microtubules, (2) kinetochores monitor chromosome attachments to microtubules, and (3) kinetochores are responsible for chromosome motility on the spindle (for review see Mitchinson and Salmon, 2001.) Although many components of the kinetochore have been described, their exact functions remain a mystery. Important functional components of the kinetochore include the microtubule-based

molecular motors: dynein and two kinesins, CENP-E and MCAK (Schaar et al., 1997; Sharp et al., 2000; McEwen et al., 2001).

During prometaphase, spindle microtubules extend out from the centrosomes 'searching' for chromosomes; then kinetochores 'capture' the microtubules that they encounter (Kirshner and Mitchinson, 1986). Both dynein and CENP-E have been implicated in promoting microtubule capture at kinetochores (Rieder and Alexander, 1990; Schaar et al., 1997; Yucel et al., 2000). The spindle then is able to exert forces on the chromosomes that cause their congression and alignment at metaphase.

Although the spindle appears visually as a steady-state structure, it is a very dynamic structure composed of both polymerizing and depolymerizing microtubules (Mitchinson and Kirschner, 1984; Cassimeris et al., 1988; Sammak and Borisy, 1988; Belmont et al., 1990; Inoue and Salmon, 1995). Chromosome movements on the spindle are coupled to changes in the length of microtubules and require various motor proteins (Inoue and Salmon, 1995). For instance, the motor proteins dynein, CENP-E, and MCAK have been implicated in the congression of the chromosomes to the metaphase plate (Walczak et al., 1996; Wood et al., 1997; Maney et al., 1998; Starr et al., 1998; Bowman et al., 1999; Lee et al., 1999).

The final alignment of all the chromosomes at metaphase requires each chromosome to be oriented such that each sister chromatid kinetochore interacts with microtubules from only one pole: this arrangement is known as bi-orientation. The ability to achieve bi-orientation requires cohesins, condensins, and a fully functional kinetochore, although the mechanisms behind bi-orientation are not clear (Tanaka et al., 2000; Howe et al., 2001; Sonoda et al, 2001; Vagnarelli and Earnshaw, 2001; Hoque and Ishikawa, 2002; Toyoda et al, 2002).

e. The spindle checkpoint

There is evidence that kinetochores perform multiple rounds of stabilization and destabilization until the proper microtubule connections are established (Nicklas and Ward, 1994). This is part of a more robust mechanism called the spindle checkpoint (Musacchio and Hardwick, 2002). The spindle checkpoint functions by inhibiting entry into anaphase until all of the chromosomes are bi-oriented and aligned at the metaphase plate (Taylor and McKeon, 1997; Martinez-Exposito et al., 1999; Shonn et al., 2000; Hoffman et al., 2001; Skoufias et al., 2001; Stern and Murray, 2001; Taylor et al., 2001; Waters et al., 2001; Zhou et al., 2002).

In general, the spindle checkpoint is composed of multiple Mad and Bub proteins, which were first described in *S. cerivisae* (Hoyt et al., 1991; Li and Murray, 1991). The spindle checkpoint functions at the kinetochore to monitor chromosome attachments to the spindle, such that the presence of unattached or improperly attached kinetochores results in activation of the checkpoint and cell cycle arrest. Once proper attachments are generated, the cell cycle resumes.

During prometaphase, the Mad and Bub checkpoint proteins become enriched at unattached kinetochores, resulting in a diffusible signal dependent on Mad2 (Taylor and McKeon, 1997; Canman et al., 2002; Shannon et al., 2002). This signal globally delays entry into anaphase via the inhibition of Cdc20 and APC activation (Taylor and McKeon, 1997; Martinez-Exposito et al., 1999; Shonn et al., 2000; Hoffman et al., 2001; Skoufias et al., 2001; Stern and Murray, 2001; Taylor et al., 2001; Waters et al., 2001; Zhou et al., 2002). In fact, Cdc20 mutants that fail to bind Mad2 do not respond to the spindle checkpoint artificially induced by spindle poisons (Hwang et al., 1998; Kim et al., 1998). Exactly what the spindle

checkpoint detects remains unclear, but it might be a combination of sensing attachment and/or tension, i.e., bi-orientation (Yu et al., 1999).

In *S. cerivisae*, the Mad and Bub genes are absolutely essential for growth only in the presence of spindle poisons. However in vertebrates, an intact spindle checkpoint is required for each cell division (Li and Murray, 1991; Dobles et al., 2000). Recently, dynein, CENP-E, and MCAK all have been implicated to function in the spindle checkpoint (Chen et al., 1998; Chen et al., 1999; Abrieu et al., 2000; Basto et al., 2000; Chan et al., 2000; Yao et al., 2000; McEwen et al., 2001).

Once the checkpoint is satisfied, the requirements for entry into metaphase have been met. Metaphase requires the alignment of all chromosomes at the equator such that (1) all chromosomes are bi-oriented, (2) all chromosomes exhibit constant tension produced by opposing spindle forces, and (3) sisters remain linked together by the integrity of their chromosome cohesion.

The spindle forces pulling on the chromosomes are believed to be established fully by metaphase, such that opposing poleward forces are counter-balanced in equilibrium (Shelby et al., 1996; He et al., 2001). This has been demonstrated by experiments in which spindle microtubules from one centrosome are destroyed using a laser. This results in the movement of the chromosome toward the opposite centrosome, without the activation of the APC and entry into anaphase (McNeill and Burns, 1981).

f. Separase and securin regulate the metaphase-anaphase transition

Amazing insights into the metaphase-anaphase transition have been made in the last few years because of the discovery of separase, a protease required for anaphase. The separation of sister chromatids to opposite poles during anaphase is a synchronous process. Studies of *S. cerivisae* revealed that the cohesin Scc1 is proteolytically cleaved by separase, which probably accounts for this synchrony (Uhlmann et al., 1999). Separase is essential for chromosome segregation (Funabiki et al., 1996; Ciosk et al., 1998). The cleavage of Scc1 by separase irreversibly triggers anaphase by breaking the cohesin complexes linking sister chromatids and allowing the opposing spindle forces to separate the chromosomes (Uhlmann et al., 1999). Later experiments using other animals, including vertebrates, supported their results (Waizenegger et al., 2000; Hauf et al., 2001; Toyoda et al., 2002).

Elegant experiments completed using *S. cerivisae* demonstrated the cleavage of Scc1 is both necessary and sufficient for chromosome separation (Uhlmann et al., 2000). This also allowed further proof of the tension generated by spindle forces at metaphase, because the artificial induction of Scc1 cleavage in the absence of APC activation and entry into anaphase triggered chromosome separation (Uhlmann et al., 2000).

Separase must be regulated carefully because, once activated, the cleavage of Scc1 is irreversible. Securin is an important regulator of separase (Ciosk et al., 1998). Securin binds separase thereby keeping it inactive by physically blocking access to the catalytic domain of separase. Securin is a target of the APC/Cdc20 complex, which targets securin for degradation in anaphase and liberates separase to cleave Scc1 (Fig. I.4 A; Cohen-Fix et al., 1996; Funabiki et al., 1996; Yamamoto et al., 1996; Ciosk et al., 1998; Zou et al., 1999). Non-degradable securin

mutants block chromosome cohesion release and chromosome separation in vertebrate cells, an effect similar to blocking separase function or activating the spindle checkpoint (Zou et al., 1999; Zur and Brandeis, 2001). For a list of mutants that failed to separate chromosomes, see Appendix A.

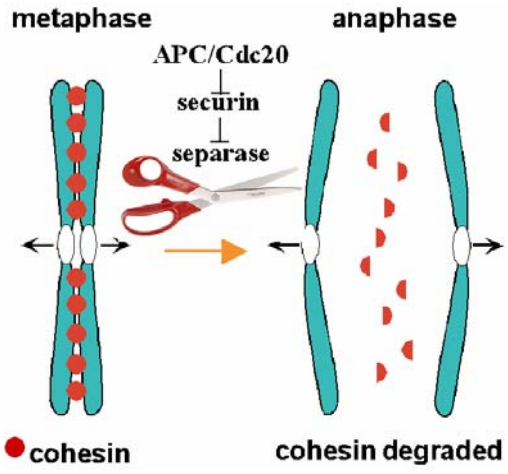

Figure I.4 A. The separase-securin-APC regulatory axis. The activation of APC/Cdc20 activity results in the degradation of securin, thereby releasing separase from inhibition by securin. Once active, separase destabilizes cohesin complexes by proteolytically cleaving Scc1, allowing sister chromatid separation.

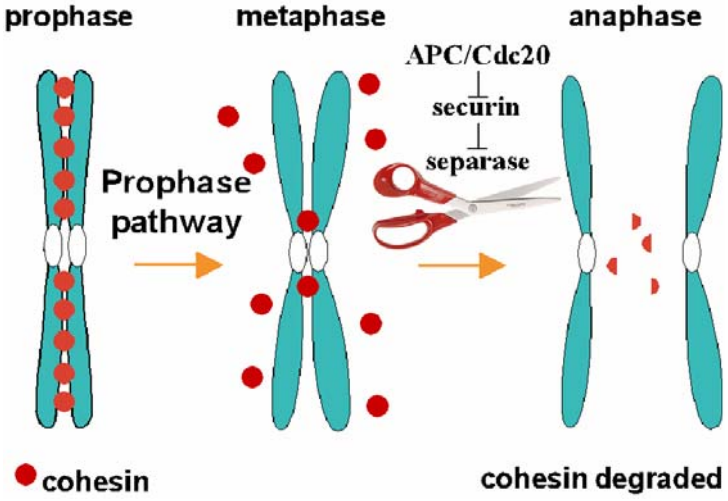

Figure I.4 B. Vertebrates display a prophase pathway of cohesin dissociation. In vertebrates, the majority of cohesions are removed from the chromosome arms prior to APC activation and independent of separase.

Separase is a cysteine protease that is distantly related to the caspases and cleaves itself once activated (Waizenegger et al., 2000). Separase auto-cleavage results in three subunits, which form a complex able to cleave Scc1 in vitro (Waizenegger 2000; Zou et al., 2002). However, the purpose of this auto-cleavage is unknown, because non-cleavable separase mutants appear fully functional (Waizenegger et al., 2000; Zou et al., 2002). It is clear that securin performs other functions as well, because in some species, securin mutants fail to separate their chromosomes (Funabiki et al., 1996; Stratmann and Lehner, 1996; Jallepalli et al., 2001; Mei et al., 2001). This probably is due to securin's role in positively regulating separases in other manners, such as sub-cellular localization or pre-activation priming (Kumada et al., 1996; Leismann et al., 2000; Jallepalli, et al., 2001; Jensen et al., 2001; Hornig et al., 2002; Waizenegger et al., 2002).

g. Vertebrates remove the majority of cohesins via a prophase pathway

Although many of these models are based on the results of studies using yeast, vertebrates appear to have important differences. In vertebrates, mitotic cohesins in the chromosomal arms are removed from the chromosome during prophase in a process that is independent of separase, securin, and the APC (Fig. I.4 B; Losada et al., 2000; Sumara et al., 2000). This prophase pathway results in the release of the majority (95%) of cohesin prior to metaphase, without the cleavage of Scc1 by separase (Losada et al., 1998; Sumara et al., 2000). The remaining cohesin is located near the kinetochore and is sufficient to hold sister chromatids together (Losada et al., 2000; Waizenegger et al., 2000; Hauf et al., 2001; Hoque and Ishikawa, 2001). Then, at the metaphase-to-anaphase transition, the residual cohesin is released by the cleavage of Scc1 by

separase in a process dependent on securin and the APC (Fig. I.4 B; Waizenegger et al., 2000; Hauf et al., 2001; Jallepalli, et al., 2001).

During telophase, the majority of cohesin removed during prophase returns to the chromosomes (Losada et al., 2000; Sumara et al., 2000). The reasons for the existence of the prophase pathway and the return of cohesion so quickly are unknown. During prophase, the process of chromosome condensation is simultaneous with the prophase removal of cohesion. This has led to the suggestion that cohesin removal from the chromosome arms during prophase might be required to facilitate the condensation of the chromosome arms in organisms, such as vertebrates, with longer chromosomes (Losada et al., 2000; Sumara et al., 2000; Losada et al., 2002). A similar phenomenon has been described in *Drosophila* (Warren et al., 2000).

Recent work has revealed an increased level of complexity in the regulation of separase. This is based on the discovery of an inhibitory phosphorylation of separase by Cdk1 in vertebrate cells (Stemmann et al., 2001). The phosphorylation of separase by Cdk1 suggests a model in which high levels of Cdk1 activity might inhibit separase activity. This conclusion is supported by the observation that expression of non-degradable cyclin-A, which presumably keeps Cdk1 active, blocks chromosomes separation despite APC activation and the destruction of securin (Geley et al., 2001). Both of these results suggest securin is not the sole inhibitor of separase.

The inhibitory phosphorylation of separase by Cdk1 is consistent with the observation that *securin* can be deleted in mouse embryos and in human tissue culture cells without causing defects in the timing of cohesion release (Jallepalli, et al., 2001; Mei et al., 2001). This idea is further supported by the fact that mammalian cells lacking *securin* are capable of blocking the

loss of sister chromatid cohesion when the spindle checkpoint is artificially induced by spindle poisons (Mei et al., 2001).

Recent work also has shown an increased level of complexity in the functions of separase. Separase might be involved in regulating the spindle during anaphase, based on recent evidence from *S. cerivisae*. Removing separase's main target Scc1 does not obviate the essential functions of separase during mitosis. Interestingly, these mutants display anaphase spindle defects instead of simply chromosome separation defects (Jensen et al., 2001; Severin et al., 2001).

The discovery of the first non-cohesin target of separase, Slk19, might explain this function of separase. Slk19 is a kinetochore component that is cleaved into two fragments by separase. The stable, N-terminal fragment of Slk19 transits to the central spindle during anaphase, and over-expression of a non-cleavable Slk19 disrupts microtubule stability of the spindle in anaphase (Sullivan et al., 2001).

h. The APC directly regulates anaphase and telophase

Recently, novel targets of the APC have been described that help explain how the APC regulates the numerous events of cell division. Although previously cell division was thought to be regulated indirectly by the APC's role in the degradation of mitotic cyclins, now there is evidence that APC-mediated degradation plays more direct regulatory roles.

The description of specific degradation domains in cyclins targeted by the APC led to the discovery of these domains in other proteins. For instance, the important regulator of anaphase entry, securin, contains a D-box motif that is similar in sequence to the D-box motif in cyclin-B

(Glotzer et al., 1991; Hilioti et al., 2001). The presence of a D-box in securin readily explains both (1) the coincident destruction of securin and cyclin-B (Cohen-Fix et al., 1996; Funabiki et al., 1996; Yamamoto et al., 1996; Ciosk et al., 1998; Zou et al., 1999) and (2) the observation that expression of non-degradable cyclin-B does not block chromosome separation whereas loss of APC function does (Glotzer et al., 1991; Davis et al., 2002). The presence of a D-box in securin is also suggestive of why over-expression of the D-box region from cyclin-B inhibits chromosome separation, whereas over-expression of non-degradable cyclin-B does not inhibit chromosome separation (Glotzer et al., 1991; Holloway et al., 1993; Zou et al., 1999). These studies show the importance of securin as a non-cyclin target of the APC.

Similar approaches led to the discovery of other non-cyclin substrates of the APC. The identities of these factors suggest direct roles for the APC in the regulation of the later events of anaphase and telophase. Several microtubule motors, for example the kinesin Xkid, are targeted for degradation by the APC (Luca and Ruderman 1989; Murray et al., 1989; Murray et al. 1989; Pellman et al., 1995; Juang et al., 1997; Hildebrandt and Hoyt, 2001). When a non-degradable form of Xkid is introduced into cells, it blocks spindle behaviors during anaphase A (Funabiki and Murray, 2000). Also, CENP-E is degraded during anaphase, and although not proven, this is suggestive that CENP-E is a target of the APC (Brown et al., 1994).

A degradation motif first described in Cdc20 and named the KEN-box is required for APC/Cdh1mediated destruction of Cdc20 (Pfleger and Kirschner, 2000). The KEN-box has subsequently been described to regulate numerous cell division regulators such as securin, aurora kinases, NIMA/Nek kinases, Polo kinases, Cdc25 phosphatases, and Xkid (Pfleger and Kirschner, 2000; Hames et al., 2001; Hildebrandt and Hoyt, 2001, Zur and Brandeis, 2001;

Donzelli et al., 2002; Hagting et al., 2002; Leismann and Lehner, 2003). These findings further support the hypothesis that the APC directly regulates cell division events.

4. Summary: Cdk1 and the APC regulate cell division

Now scientists can describe more fully the progression through the different stages of mitosis by unifying microscopic observations with molecular events. Different cell division events have been shown to require specific proteins and enzymatic activities. Furthermore, biochemical changes in these proteins, such as abundance or enzymatic activities, correlate with specific events in cell division. The fundamental models of Cdk and APC function now are linked directly to cell division events, such as the regulated separation of chromosomes at anaphase by separase and securin.

However, many of the molecular events still remain a mystery. More importantly, the spatial regulation of the cell cycle has just begun to be integrated with the better-understood process of temporal regulation of the cell cycle (Pines, 1999). For instance, the sub-cellular locations of important regulatory molecules throughout the cell are just beginning to be appreciated.

For instance, Cdk1/cyclin-B is localized to both the chromosomes and the spindle during cell division (Bailley et al., 1989; Riabowol et al., 1989; Alfa et al., 1990; Leiss et al., 1992). Even more striking is the observation in human cells that a subset of cyclin-B localized to the chromosomes and centrosomes is degraded during metaphase, whereas the majority of cyclin-B remains stable until anaphase (Huang and Raff, 1999). The APC is first observed on centrosomes and later in anaphase on the spindle (Huang and Raff, 2002; Raff et al., 2002).

Similarly, separase, securin, and cohesins have been observed initially on the chromosomes and then, during anaphase, on the spindle (Ciosk et al., 1998; Hoque and Ishikawa, 2001; Jensen et al., 2001; Severin et al., 2001). With the application of live imaging techniques, the movements of molecules can be tracked in real-time during cell division (Rieder and Khodjakov, 2003).

B. Cell division: Meiosis

Meiosis is a specialized type of cell division used by in species capable of sexual reproduction. The functions of mitosis and meiosis are quite different. The purpose of mitosis is to produce two identical copies of the mother cell. However, the purpose of meiosis is to reduce the chromosomal content in half. In fact, the word meiosis means "diminution" or "reduce by half" (Farmer and Moore, 1905; Janssens, 1909). In most species, the chromosome content is reduced by meiosis from diploid (two sets of each chromosome) to haploid (one set of chromosomes).

In general, sexually reproducing species use the process of meiosis to produce haploid gametes. Then, when female and male gametes are borught together by fertilization, the haploid gametes join to form diploid progeny with genetic contributions from both parents. Meiosis is observed in the majority of eukaryotic species, presumably because the generation of genetic diversity provides an evolutionary advantage.

Also of note is that, in sexual species, the meiotic cells are part of the germline, where germ cells and gametes form an immortal lineage. This is in contrast to the soma, which is important as a protector and caregiver to the germline but makes no genetic contribution. Therefore, the germline has many features that are not found in the soma.

For example, the germline is guarded carefully, because any genetic damage here has the potential of being inherited in the next generation. Also, the gametes, especially oocytes, must be able to generate a new organism, which requires specialized attributes and supporting cells. This fact often makes the experimental analysis of meiosis difficult, because gametogenesis usually occurs in well-protected, specialized organs.

Unlike mitotic cells, meiotic cells are not easily removed from an organism and cultured for use in ex vivo experiments. Unfertilized, vertebrate oocytes can be obtained readily and in large numbers, but they have already reached to prometaphase II and then arrest until fertilization. Therefore, the earlier processes of meiosis I cannot be studied in these oocytes. As in mitosis, the premiere model organisms for meiotic studies are genetically tractable species of yeast, and to a lesser extent, *C. elegans* and *Drosophila*.

1. The traditional stages of meiosis

Early cytological studies of meiosis described events that are dramatically different from those of mitosis. Just as in mitosis, meiotic cells duplicate their chromosomes prior to the meiotic divisions. However, meiosis results in halving the number of chromosomes, because meiosis consists of two consecutive cell divisions: meiosis I and meiosis II (Fig. I.5 B). Each of these divisions is sub-divided into the traditional stages of mitosis: prophase I, metaphase I, anaphase I, prophase II, metaphase II, anaphase II, etc.

The classic cytological studies also revealed unique chromosomal behaviors not seen in mitosis: pairing, synapsis, and chiasmata formation (van Benenden, 1883; Weismann, 1887). The length of meiosis I is increased, because prophase I contains many unique chromosome behaviors. The specialized meiotic prophase I has been further divided into five sub-stages: leptotene, zygotene, pachytene, diplotene, and diakinesis (Fig. I.5 A).

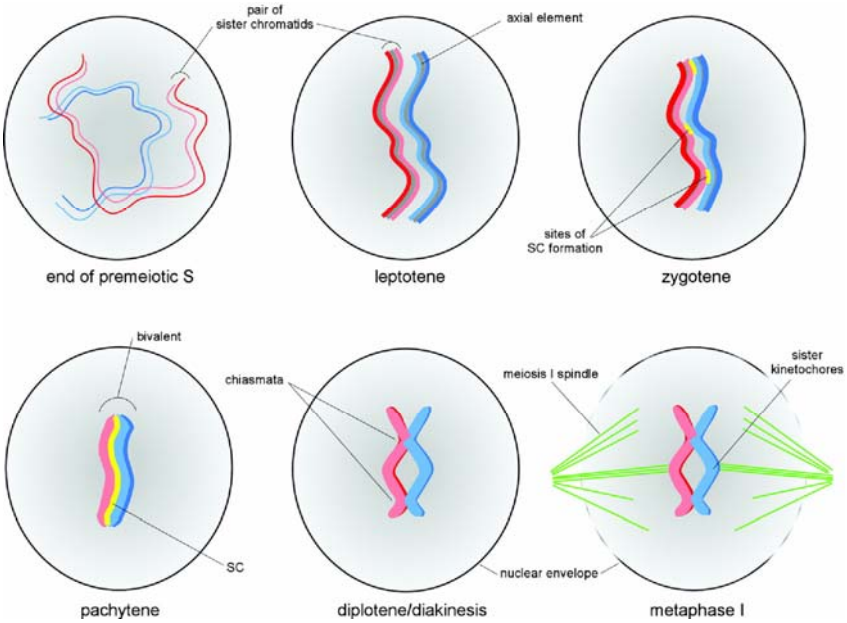

Figure I.5 A. Prophase I is divided into five stages. (Reproduced from Page and Hawley, 2003.) The sub-stages of prophase I are leptotene, zygotene, pachytene, diplotene, and diakinesis.

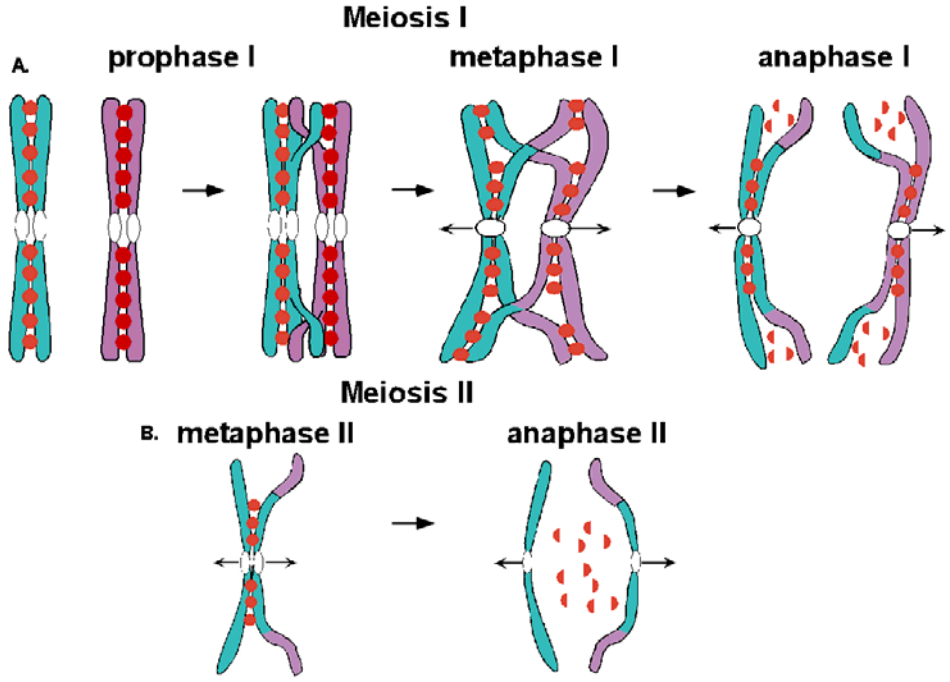

Figure I.5 B. Chromosome segregation during meiosis. During meiosis, homologous chromosomes, in this case one blue and one purple, become connected by chiasmata. Meiosis I results in the segregation of homologs. Meiosis II results in the segregation of sister chromatids; in this case, the blue dyad separates into individual sister chromatids.

Leptotene is when the chromosomes begin assembling specialized proteinaceous structures to prepare for synapsis (Fig. I.5 A). Zygotene involves the first pairings of homologous chromosomes and later their intimate association via synapsis. The pairing of homologous chromosomes, or homologs, is a process by which paternally-derived and maternally-derived chromosomes associate with each other based on DNA sequence homology. Pairing leads the more elaborate process of synapsis. Synapsis involves the formation of a large, complex, proteinaceous structure between the homologs, called the synaptonemal complex (Fig. I.5 A).

Entry into pachytene is defined when all the homologs have completed the process of synaptonemal complex formation. Then, also during pachytene, recombination between homologs occurs. Recombination is the physical exchange of DNA between homologs, which serves to further increase genetic diversity and is required for the formation of stable connection linkages between homologs.

Entry into diplotene is marked by the disassembly of the synaptonemal complex. This is the first stage when chiasmata can be observed. Chiasmata are the result of recombination and mark the physical linkages between homologs (Fig. I.5 A). Diakinesis involves chromosome condensation, nuclear envelope breakdown, and entry into prometaphase I.

2. Many molecular landmarks of meiosis are conserved with mitosis

Despite their fundamental differences, mitosis and meiosis share much in common, because the process of cell division is functionally the same. This idea is best demonstrated by molecular studies revealing that many of the factors regulating mitosis also perform similar functions in

meiosis. In fact, the discovery of Cdk1/cyclin-B activity was partly due to studies of meiotic cells (Masui and Markert, 1971; Smith and Ecker, 1971). Therefore, it has long been believed that meiosis is governed by the same paradigms that control mitosis: (1) Cdk-dependent phosphorylation mechanisms (Draetta et al., 1989; Labbe et al., 1989; Grallert and Sipiczki, 1990; Choi et al., 1991; Huchon et al., 1993; Liu et al., 1998; Boxem et al., 1999), and (2) APC-regulated protein degradation mechanisms (Sigrist et al., 1995; Lorca et al., 1998; Furata et al., 2000; Golden et al., 2000).

Also, meiosis requires both cohesin and condensin complexes (Klein et al., 1999; Watanabe and Nurse, 1999; Eijpe et al., 2000; Losada et al., 2000; Pasierbek et al., 2001; Pelttari et al., 2001; Hagstrom et al., 2002), separase and securin (Buonomo et al., 2000; Salah and Nasmyth, 2000; Siomos et al, 2001; Kitagawa et al., 2002), and many other factors, such as spindle checkpoint proteins that have been studied well in mitotic cells, (Kitagawa and Rose, 1999; King et al., 2000; Abrieu et al., 2001; Eaker et al., 2001).

3. Molecular mechanisms unique to meiosis

Just as cytological observations over a hundred years ago revealed major differences between mitosis and meiosis, modern molecular studies have revealed important molecular differences between mitosis and meiosis. Meiotic cells perform two consecutive rounds of chromosome segregation in order to reduce chromosome numbers by half. This special ability depends on the unique events of the extended first prophase and, presumably, requires a multitude of meiosis-specific factors. The most notable of these meiosis-specific factors are the cohesin Rec8 and the kinetochore component Monopolin (Mam1).

a. Chromosome cohesion during meiosis

During meiosis, the key cohesin subunit Scc1 is replaced by a homologous protein called Rec8 (Klein et al., 1999; Parisi et al., 1999; Watanabe and Nurse, 1999; Pasierbek et al., 2001). In both yeast and worms, Rec8 is required for meiosis and is expressed only during meiosis (Parisi et al., 1999; Watanbe and Nurse, 1999; Pasierbek et al., 2001). As for the other cohesin subunits, evidence from different organisms suggests there are various differences between mitosis and meiosis, depending on the species.

There also is evidence in some organisms that other cohesin subunits are replaced or that meiosis-specific versions are used in addition to mitotic versions. For example, in mammalian meiosis there are three additions: (1) the meiosis-specific Rec8 coexists with its mitotic homolog Scc1, (2) the meiosis-specific Smc1β coexists with the mitotic Smc1, and (3) the meiosis-specific STAG3 coexists with the mitotic Scc3 (Prieto et al., 2001; Revenkova et al., 2001; Prieto et al., 2002). The use of meiosis-specific subunits presumably enables cohesin to fulfill functions that are specific to meiosis, such as recombination, mono-orientation, and the step-wise release of chromosome cohesion.

Although normally Rec8 is not expressed in mitotic cells, the mis-expression of Rec8 during mitosis is capable of rescuing yeast lacking Scc1 (Toth et al., 2000). However, the converse is not true. This demonstrates Rec8 is functionally equivalent to Scc1 during mitosis, whereas Scc1 is not functionally equivalent to Rec8 during meiosis.

b. Prophase I: Recombination and chiasmata formation

Recombination is the crucial process of meiosis by which homologous chromosomes exchange genetic information and form chiasmata. Recombination is initiated by the intentional creation of double-stranded breaks by the endonuclease Spo11 (Keeney et al., 1997; Dernburg et al., 1998). The presence of double-stranded breaks induces DNA repair. However, in meiosis, the usual preference of mitotic cells to use a sister chromatid for repair is reversed in favor of using a homologous chromatid (Collins and Newlon, 1994; Schwacha and Kleckner, 1994). This results in recombination between non-sister chromatids, potentially generating genetic diversity on the recombinant chromosome. During pachytene recombination events are often resolved to form DNA crossovers between the non-sister chromatids. Crossovers are topological linkages of DNA. During most of the process of recombination, the homologous maternal and paternal chromatids are bound (or synapsed) together along their entire lengths to form a specialized meiotic structure, called the synaptonemal complex.

(1) Synapsis

During pachytene, the synamptonemal complex holds all four chromatids of the bivalent in a single bundle. Electron microscopic analysis of the synaptonemal complex in various species suggests that it is composed of two axial cores, one from each homologue, that are connected by a central element (Zickler and Kleckner, 1998). Within the context of the synaptonemal complex, the axial cores are referred to as the lateral elements, and they are composed primarily of cohesin complexes. The central element, which is composed of cohesion complexes and Red1, lies between the lateral elements (Smith and Roeder, 1997; Klein et al., 1999). In *S.*

cerivisae, the ability to form the central element and, therefore, the synaptonemal complex depends on the replacement of Scc1 with Rec8 (Klein et al., 1999).

(2) Crossing-over and chiasmata formation

At diplotene, the dissolution of the synaptonemal complex allows maternal and paternal sister chromatid pairs to separate, except in the regions of crossovers. The physical results of crossovers are observed as chiasmata; these are visible with conventional light microscopy. After the disappearance of the synaptonemal complex, chiasmata are visual manifestations of the physical connections between homologs. Chiasmata formation is required to link homologs together from this stage until anaphase I.

From diplotene until anaphase I, the meiotic chromosomes often are referred to as "bivalents" to denote the two linked homologs. The two linked homologs actually are composed of four individual chromatids. Stable chiasmata are required for both the proper attachment of bivalents to the spindle and to resist the bipolar spindle forces for alignment of the bivalents at metaphase I. As a consequence, in most eukaryotic organisms, recombination is obligatory for chromosome segregation at meiosis I. Chromosomes defective in recombination often display high rates of mis-segregation during meiosis I, which is clearly seen in many *C. elegans* Him mutants (Broverman and Meneely, 1994).

c. Meiosis I kinetochore: Mono-orientation

(1) Bivalents

In meiotic prometaphase, bivalents begin the process of aligning themselves on the spindle. In males, spermatogenesis involves the formation of a centrosomal-based spindle, similar to mitosis. However, in most female meiotic systems, the constructing of the meiotic spindle is initiated by the bivalents, and meiosis occurs in the absence of centrosomes. In both male and female systems, the structure of the bivalent chromosome is very different from chromosomes in both mitosis or meiosis II. The proper geometry of bivalent chromosomes is dependent on the presence of chiasmata and on the mono-orientation of sister chromatid kinetochores (Fig. I.6).

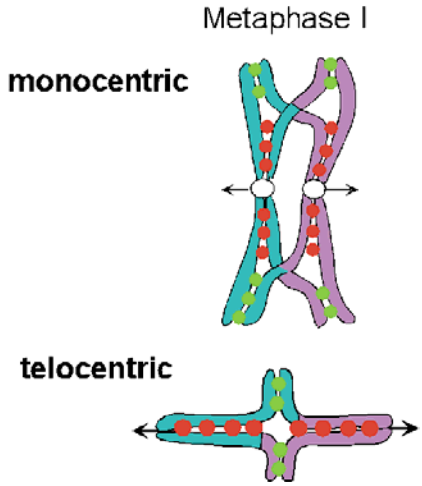

Figure I.6. The geometry of bivalent chromosomes. This drawing shows a conceptual representation of two types of bivalent chromosomes at metaphase I. The location of the kinetochore determines the shape of the bivalent. A typical monocentric bivalent is shown (above) with one chiasma per arm. The sister chromatids mono-orient their kinetochores such that the two homologs are pulled in opposite directions, denoted by the arrows. Telocentric chromosomes have their kinetochore located near the end of the chromosome. Telocentric bivalents form a single chiasma and appear as shown (above). In *C. elegans*, all bivalents have a telocentric geometry.

During prometaphase, the bivalents are structured with the two homologous kinetochores oriented in opposite directions. This geometry allows for congression and alignment at metaphase, because the two kinetochores of each bivalent are attached to opposite poles of the spindle. To orient homologs to opposite poles, each pair of sister kinetochores must function as a single unit: this geometry is called mono-orientation. Mono-orientation allows the two kinetochores from a sister chromatid pair to attach to the same pole. Thus, most bivalents immediately obtain a bipolar orientation that balances the bivalent on the metaphase plate, because maternal and paternal kinetochores are being pulled toward opposite poles with equal forces (Fig. I.6).

However, some bivalents fail to orient properly, having either both kinetochores attached to the same pole or only one kinetochore attached to a pole. In this case, the kinetochores are able to go through successive cycles of microtubule release and reattachment until stable bipolar orientation of the bivalent is established. This process is believed to be similar to the spindle checkpoint characterized in mitotic cells (Shonn et al., 2000).

(2) Monopolin is required for mono-orientation

Although mono-orientation is key to meiosis I, very little is known about this process. In *S. cerivisae*, mono-orientation requires the meiosis-specific factor Mam1 (Toth et al., 2000). However, the molecular function of Mam1 is not clear. Mam1 is first localized to kinetochores in pachytene, probably during the process of recombination, and it persists there until anaphase I. Also, factors homologous to Mam1 have yet to be found in other species. Therefore, nothing is known about the factors or mechanisms that confer mono-orientation in other organisms (Toth et al., 2000).

There are suggestions that mono-orientation is conferred intrinsically by the chromosome structures themselves and not by the cytoplasm or spindles of these cells. Cell fusion experiments using grasshopper sperm cells demonstrated that if bivalents are transferred to the meiosis II spindle, they align normally and disjoin to opposite poles at anaphase at the same time as do native meiosis II sister chromatids (Paliulis and Niklas, 2000). This result demonstrated that bivalents are structured such that sister chromatid kinetochores are mono-oriented on the spindle, regardless of the cellular environment. This experiment also suggested that the signal triggering the resolution of chiasmata – and the separation of homologs – is the same as the signal triggering the separation of sister chromatids at meiosis II.

d. Anaphase I: Resolving the chiasmata

Just as in mitosis, entry into anaphase I during meiosis requires activation of the APC/Cdc20 to destroy both M-cyclins and securing (Fig. I.7). This is demonstrated clearly by the metaphase I arrest observed in APC mutants in both yeast and worms (Salah and Nasmyth 2000; Chu et al., 2001). Similarly, both separase and securin mutants fail to resolve chiasmata and separate homologs during meiosis in yeast and worms (Buonomo et al., 2000; Salah and Nasmyth, 2000; Chu et al., 2001; Siomos et al., 2001; Kitagawa et al., 2002).

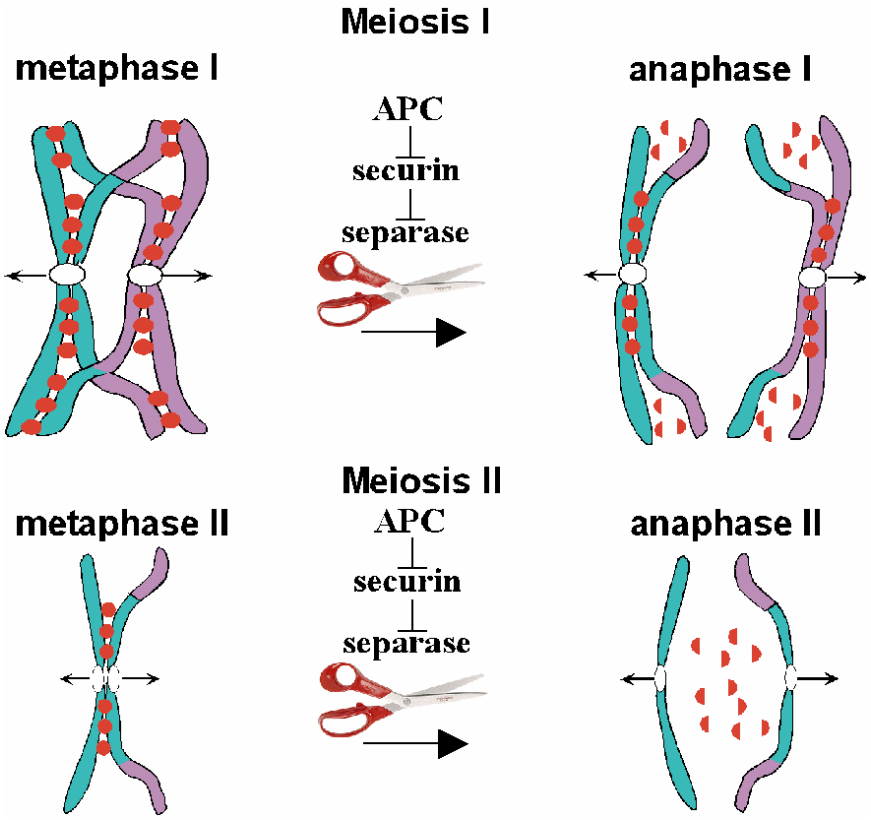

Figure I.7. Separase cleaves Rec8 to trigger anaphase I and anaphase II. Similar to mitosis, both meiotic divisions involve the separase-securin-APC regulatory axis; however, only a subset of meiotic cohesions is removed during meiosis I.

Just as Scc1 is cleaved by separase at the entry to anaphase of mitosis, Rec8 is cleaved by separase at the entry to anaphase I of meiosis (Fig. I.7). Separase is required for chiasma resolution and homologue separation in both yeast and worms. In *S. cerivisae* at the onset of anaphase I, separase cleaves the majority of Rec8 at two sites, both of which resemble the separase cleavage sites in Scc1 (Buonomo et al., 2000). Mutation of these cleavage sites blocks chiasmata resolution and homologue separation. In summary, the cleavage of Rec8 is both necessary and sufficient for homologue separation in anaphase I (Buonomo et al., 2000).

The block to meiosis I chromosome segregation imposed by separase inactivation or by non-degradable Rec8 is bypassed by Spo11 mutants. This observation indicates that cleavage of Rec8 by separase is required for chromosome segregation during meiosis I only if homologs are linked by chiasmata (Buonomo et al., 2000). Whether cleavage of Rec8 by separase also triggers anaphase I in vertebrate cells is unclear.

During congression and alignment of the bivalents, the integrity of the chiasmata is crucial. It long has been believed that sister chromatid cohesion distal to the chiasma maintains the connection between maternal and paternal homologs and resists the opposing forces of the spindle (Maguire, 1974; Carpenter, 1994). This was demonstrated for the first time using *S. cerivisae* (Buonomo et al., 2000). This report strongly supports the idea that, at metaphase I, homologs are held together by the chiasmata due entirely to chromosome cohesin complexes between sister chromatids located distal to the chiasmata.

In *S. cerivisae*, biochemical experiments show the majority of Rec8 is destroyed in meiosis I but some does persist until meiosis II (Klein et al., 1999; Buonomo et al., 2000). However, the localization of these subsets of Rec8 is not clear. Studies of the large chromosomes in mammals revealed the localization of Rec8 and STAG3 was along the axes that lie between sister chromatid arms from prior to meiosis until metaphase I (Prieto et al., 2001; Prieto et al., 2002). Only a minor proportion of Rec8 is retained around the kinetochore until the second meiotic division (Prieto et al., 2002). Similar observations were made for the localization of Rec8 in *C. elegans* and *Arabidopsis* (Pasierbek et al., 2001; Cai et al., 2003).

In vertebrates, the persistence of Rec8 along chromosome arms until the onset of anaphase I clearly contrasts with the behavior of Scc1 in mitosis, which largely disappears from chromosome arms via the prophase pathway. The persistence of meiotic cohesins along chromosome arms is particularly remarkable during diakinesis, because chromosome cohesins are believed to be removed during mitosis to allow the process of chromosome condensation to shorten and compact the chromosomes (Prieto et al., 2001; Prieto et al., 2002).

e. Meiosis II is similar to mitosis but starts with half the chromosomes

The second meiotic division is similar to mitosis in terms of sister chromatid orientation. The main difference is that meiosis II begins with half the number of chromosomes. During meiosis II, there is one set of dyads, composed of two sister chromatids each (Fig. I.7). At metaphase II, the kinetochores of these sister chromatids exhibit bi-orientation, analogous to mitotic kinetochores. Just like mitotic cells, meiotic cells attempt to pull sister kinetochores toward opposite poles at metaphase II but are prevented from doing so by the residual sister chromatid cohesion (Fig. I.7).

The cleavage of the remaining Rec8 by separase triggers the poleward migration of the individual chromatids (Fig. I.7). This is demonstrated best by experiments in *S. cerivisae* using the Spo11 mutant to bypass meiosis I. The function of Separase in meiosis II was shown by the inability of Separase mutants to separate sister chromatids during meiosis II, when meiosis I is bypassed by a Spo11 mutant background (Buonomo et al., 2000). Similarly, the expression of a non-degradable Rec8 prevents sister chromatid separation during meiosis II in a Spo11 mutant background (Buonomo et al., 2000). In both cases, Spo11 is used to alleviate the block of homolog separation during meiosis I in order to analyze meiosis II.

f. Counting chromosomes during meiosis and mitosis

Each diploid cell contains two copies of each chromosome and can be defined as 2C:2N. (The C number refers to the DNA content in multiples of a single genome content, and the N number refers to the actual number of chromosomes). Prior to mitosis, the entire chromosome content is replicated during a synthesis phase, doubling the chromosome content from 2C to 4C. However, because the newly replicated chromosomes remain linked to their sisters the number of individual chromosomes remains unchanged, staying at 2N. In other words, DNA replication results in the following: 2C:2N changes to 4C:2N (Fig. I.8 A). Then during anaphase, the disjunction of sister chromatids results in the doubling of the number of chromosomes, whereas the chromosome content remains unchanged. In other words, anaphase results in the following: 4C:2N changes to 4C:4N (Fig. I.8 A). After mitosis, each daughter cell receives half of the chromosomes and half of the total DNA content to become 2C:2N, or diploid like their mother (Fig. I.8 A).

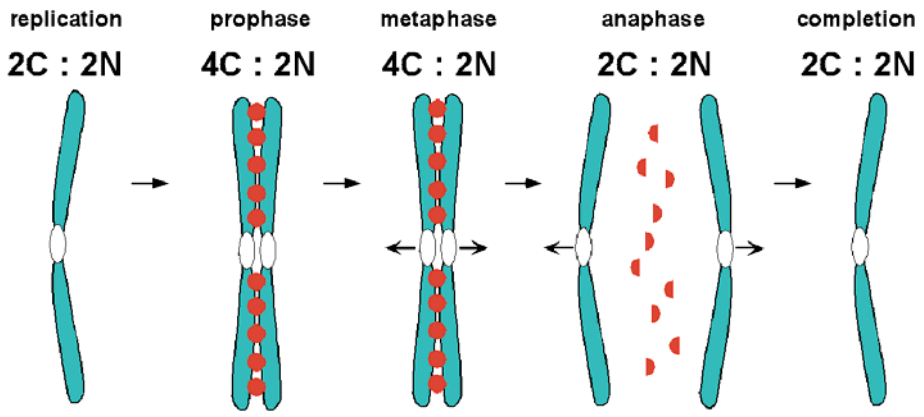

Figure 1.8 A. Counting chromosomes during mitosis. Each sister chromatid is shown in blue, with a white circle denote the kinetochore region. The red circles represent cohesin complexes that link the sister chromatids. The arrows indicate the direction of the pulling forces generated by the spindle.

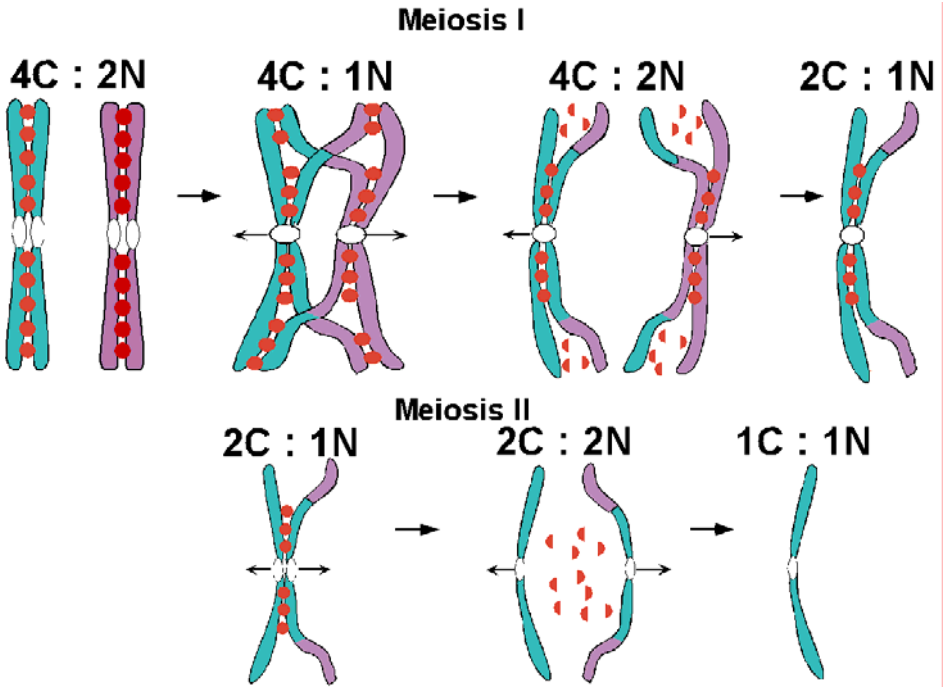

Figure 1.8 B. Counting chromosomes during meiosis. One maternal chromosome is shown in blue, and its paternal homolog is shown in purple. The red circles represent cohesin complexes that link the sister chromatids together. The arrows indicate the direction of the pulling forces generated by the spindle. After recombination, the homologs are held together by sister chromatid cohesin complexes located distal, with respect to the kinetochore, to the chiasmata. The diagram depicts segregation of the sister chromatids composing the maternal dyad, which displays evidence of recombination as shown by the small purple regions.

Meiosis consists of two consecutive rounds of chromosome segregation following a single round of DNA replication. During a pre-meiotic synthesis phase, chromosome replication results in the following: 2C:2N changes to 4C:2N, just as in mitosis (Fig. I.8 B). Then during the prophase I, the homologs become linked by chiasmata, reducing the number of chromosomes from 4C:2N to 4C:1N. During anaphase I, the disjunction of homologs results in the doubling of the number of chromosomes from 4C:1N to 4C:2N. After the first meiotic division, each daughter cell receives half of the chromosomes and half of the total DNA content to become 2C:1N.

In these 2C:1N cells, the meiotic cell has received the same amount of DNA content as in mitosis but the chromosomes are in the form of 1N paired sister chromatids or dyads. This is very different from mitosis, in which the daughter cell receives twice as many chromosomes in the form of 2N single chromatids. Then during anaphase II, the number of chromosomes doubles from 2C:1N to 2C:2N. After the second meiotic division, each daughter receives half of the chromosomes and half of the total DNA content to become 1C:1N (Fig. I.8 B). The result of meiosis is the formation of haploid cells (1C; 1N) with the chromosomes in the form of single chromatids.

g. Maintenance of proximal sister chromatid cohesion until meiosis II

The unique ability of meiotic cells to perform two successive rounds of division – without an intervening phase of chromosome duplication – is due to the persistence of sister chromatid cohesion located in the proximity of the kinetochores. The persistence of cohesion in the proximity of the kinetochore is crucial for aligning sister chromatids on the metaphase II spindle, and the destruction of this remaining cohesion triggers the separation of sister chromatids at the

onset of anaphase II. The mechanism underlying this differential treatment of cohesins located in the distal chromosome arms and cohesions located in the proximity of the kinetochore remains obscure.

(1) Scc1 cannot functionally substitute for Rec8 during meiosis

The differential regulation of cohesins might arise from differences between meiotic and mitotic cohesin complexes or from other molecular differences between the cytoplasmic environment of mitotic and meiotic cells. Some insight about Rec8 has been gained from analysis of meiosis in *S. cerivisae*, which has had the Rec8 coding sequence replaced by Scc1 coding sequence. In these *rec8* mutants, which mis-express Scc1 during meiosis, meiotic recombination is defective, thereby preventing homologs from becoming linked together by chiasmata (Toth et al., 2000). Despite this defect, sister kinetochores bi-orient at metaphase I. Then at the onset of anaphase I in this mutant, all the Scc1 is degraded by separase resulting in the complete loss of chromosome cohesion during meiosis I (Toth et al., 2000). This observation suggests it is differences in Rec8 versus Scc1 that account for the differential regulation of proximal sister chromatid cohesion.

However, there also is evidence that differences in other meiosis-specific factors besides the cohesion Rec8 account for the differential regulation of proximal cohesion during meiosis. For example, Spo13 is required in *S. cerivisae* to maintain sister chromatid cohesion during meiosis I. Spo13 is expressed only during meiosis.

(2) Mutants that fail to protect proximal cohesion during meiosis I

Finally, several other factors that are present during both mitosis and meiosis are involved in protecting proximal cohesion during meiosis I. Several mutants have been described that fail to

protect proximal Rec8 during meiosis I: Bub1, Mei-S322, Slk19, and Spo13 (see Appendix B). Currently, the molecular functions of these factors are not clear.

In *S. pombe*, *bub1* mutants separase degrades all Rec8 at anaphase I, resulting in the precocious separation of sister chromatids during meiosis I. Bub1 is a kinetochore factor that has a well-characterized function in the mitotic spindle checkpoint. The function of Bub1 in regulating Rec8 is believed to be independent of its function in the spindle checkpoint, because other spindle checkpoint mutants, such as Mad2, do not exhibit Rec8 defects (Bernard et al., 2001). However, Bub1's role in regulating Rec8 might be indirectly due to its function in orienting the kinetochore.

In *Drosophila mei-S322* mutants, all Rec8 is degraded by separase at anaphase I, resulting in the precocious separation of sister chromatids during meiosis I. Mei-S332 is also a kinetochore factor that persists in the kinetochore region throughout meiosis I and is not degraded until anaphase II (Kerrebrock et al., 1992; Moore et al., 1998; Tang et al., 1998). This localization mirrors the localization of proximal cohesion, thereby suggesting a role in protecting proximal Rec8 until anaphase II.

Finally in *S. cerivisae*, *slk19* and *spo12* mutanst display precocious separation of sister chromatids during meiosis I. Slk19 is a kinetochore factor that is cleaved during mitosis by separase and functions in spindle stability during anaphase. Spo12 is a novel factor with no homology to other proteins (Sharon and Simchen, 1990a; Sharon and Simchen, 1990b). Genetic analysis shows Slk19 functions downstream of Spo12. The observation that *spo11* does not suppress *slk19* suggests Slk19 has other roles besides regulating Rec8 (Kamieniecki et al., 2000; Zeng and Saunders, 2000).

Current evidence does not distinguish whether Bub1, Mei-S322, Slk19, Spo12, and Spo13 act primarily to protect proximal cohesion, to enable mono-oreintation of kinetochores, or serve as more general regulators of meiosis. Because of its chromosomal localization pattern, Mei-S322 is considered the best candidate for a direct protector of proximal Rec8 during meiosis I (Kerrebrock et al., 1992; Moore et al., 1998; Tang et al., 1998).

C. New cell division regulators: Aurora and Polo kinases

Although studies of Cdk1 and the mitotic cyclins have provided an important framework for the regulation of cell division, it is clear that other protein kinases also play important roles during cell division. Predominant among these other kinases are the Aurora and Polo kinases. Aurora and Polo kinases were first described in cell division mutants in both *S. cerivisae* and *Drosophila* (Sunkel and Glover, 1988; Llamazares et al., 1991; Chan et al., 1993; Kitada et al., 1993; Glover et al., 1995). Both Aurora and Polo kinases are similar to Cdk1, in that they are cell-cycle regulated with maximum kinase activity occurring during cell division and are highly conserved throughout the eukaryotic kingdom (Nigg, 2001).

Like Cdk1, both Aurora and Polo kinases are required for numerous processes during cell division (Nigg, 2001). However, unlike the Cdks, neither Aurora nor Polo kinases associate with cyclins. Instead, Aurora and Polo kinases are regulated directly by cyclical changes in their abundance, similar to the regulation of mitotic cyclins. Also, Aurora and Polo kinases show dynamic sub-cellular associations with various mitotic structures that probably play an important part of their regulation (see below). However, the precise roles of these relatively novel kinases remain unclear making them an active topic of research.

1. The Aurora kinase family

The first Aurora kinase was discovered in *S. cerivisae*, where there is a single Aurora kinase family member, named Ipl1 for increase-in-ploidy (Chan et al., 1993). In vertebrates, the Aurora kinases are divided into three sub-classes (A, B, and C) defined by their sub-cellular localizations (Adams et al., 2001a). Aurora-A kinases are localized to the centrosomes. Aurora-B kinases are chromosomal passengers, meaning they first localize to the chromosomes, then, in anaphase, they transit to the central spindle and cytokinetic midbody (Schumacher et al., 1998a; Terada et al., 1998; Adams et al., 2001b). Aurora-C kinases, which are restricted to the germline, are not well studied (Bernard et al., 1998; Tseng et al., 1998; Kimura et al., 1999b). In *Drosophila* and *C. elegans*, there are two Aurora kinases, which often are referred to by the vertebrate designations as Aurora-A and Aurora-B, although this is technically incorrect based on evolutionary relationships of these family members (Brown et al., 2004).

Studies of Aurora kinases have revealed their requirement in such diverse processes as chromosome structure, spindle assembly, and cytokinesis (Bischoff and Plowman, 1999; Adams et al., 2001b). Also, Aurora kinases are implicated strongly in oncogenesis; for review, see Giet and Prigent (1999). However, exactly how Aurora kinases function in these processes is not clear.

2. The Polo kinase family

In *S. cerivisae*, the single Polo kinase was first discovered in genetic screens by Dr. Lee Hartwell and named Cdc5 for cell division defective (Hartwell et al., 1974). Later, Cdc5 was found to be a member of the Polo kinase family, named after the *polo* mutant in *Drosophila* (Sunkel and

Glover, 1988; Golsteyn et al., 1994). In vertebrates, there are three distinct types of Polo kinase: Plk1, Plk2(Sak), and Plk3(Prk/Fnk). Polo kinase classification is determined solely by amino acid sequence conservation. Plk1 is the best studied of the three, whereas the functions of both Plk2 and Plk3 remain elusive. Plk1 has been implicated in many of the same processes as Cdk1: (1) nuclear envelope breakdown, (2) spindle assembly, (3) activation of the APC/Cdc20, (4) cell cycle checkpoints, and (5) cytokinesis (Nigg, 1998). Polo kinases have been implicated in oncogenesis (Knecht et al., 1999; Smith and Roeder, 1997; Wolf et al., 1997). Exploration of the molecular functions of Plk1 is a very active area of research.

3. The regulation of Aurora and Polo kinases

As cell cycle regulators, Aurora and Polo kinases are regulated by the two main paradigms of cell cycle control: phosphorylation and degradation. The transcription of Aurora and Polo kinases is constant during the cell cycle (Bischoff and Plowman, 1999; Nigg, 1998). However, there is evidence that their protein levels are regulated during the cell cycle by mRNA stability and translational mechanisms, which regulate their protein levels such that they peak during cell division (Lake and Jelinek, 1993; Lee et al., 1995; Cheng et al., 1998).

a. Activating kinase activity via phosphorylation

For both Aurora and Polo, kinase activation temporally mirrors Cdk1/cyclin activity but continues for much longer into the cell cycle, peaking during anaphase slightly after Cdk1 activity (Fenton and Glover, 1993; Lee et al., 1995; Bischoff et al., 1998). Both Aurora and Polo kinases contain conserved signature activation sequences in their kinase activation loops, similar to Cdk1 (Hamanaka et al., 1995; Abrieu et al., 1998; Giet and Prigent, 1999).

In general, both Aurora and Polo kinases have been shown to be phosphorylated in vivo when they are activated (Tavares et al., 1996; Kotani et al., 1998; Qian et al., 1998a). In Aurora-A, this can be mimicked by mutation such that T to D mutation is activated approximately 7-fold, whereas T to A mutation prevents activation of both human and *Xenopus* forms (Walter et al., 2000; Littlepage and Ruderman, 2002). Similarly, Aurora-B is activated by phosphorylation, although the phosphorylation sites are unknown (Bolton et al., 2002).

With regard to Polo kinases, several cell-cycle regulated phosphorylation sites on Plk1 have been mapped. One site in particular, T201 in human Plk1, results in activation of Plk1 kinase activity (Jang et al., 2002; Kelm et al., 2002; Wind et al., 2002). Unlike Aurora kinases, where the upstream kinase is unknown, the description of a Plk1 kinase kinase (xPlkk1) in *Xenopus* might represent the upstream regulator of xPlk1 activation (Qian et al., 1998a). However, it has also been suggested that both Aurora and Polo kinases are activated directly by Cdk1 phosphorylation (Hamanaka et al., 1995; Abrieu et al., 1998; Qian et al., 1998b; Giet and Prigent, 1999).

b. Degradation

During telophase, the decline in kinase activity of both Aurora and Polo kinases is due directly to degradation. Both Aurora and Polo kinases are degraded via their conserved KEN-box motifs, which are targeted for degradation by the APC/Cdh1 complex during telophase (Fang et al., 1998; Honda, et al., 2000; Pfleger and Kirschner, 2000; Walter et al., 2000; Castro et al., 2002). However, in *S. cerivisae*, Cdc5 was shown to be degraded via a D-box motif by the APC/Cdc20 complex during late anaphase (Charles et al., 1998; Shirayama et al., 1998). The reason for this

difference is unknown but might be related to the silencing of this conserved D-box domain in vertebrates (Castro et al., 2002; Littlepage and Ruderman, 2002).

4. The localization of Aurora and Polo kinases

Most kinases are capable of phosphorylating various targets; however, target specificity often is regulated by the sub-cellular localization of either the kinase or its substrates. This type of spatial regulation controls kinase activity towards its targets. Aurora and Polo kinases are regulated both spatially and temporally by their localization to different mitotic structures within the dividing cell. Both their localization and the timing of their localization require complex interactions with other proteins that have just begun to be appreciated. In summary, the spatial-temporal regulations of these novel, highly conserved kinases are critical for their many functions during cell division.

a. Sub-cellular localization of Aurora kinases

The sub-cellular localization of both Aurora and Polo kinases is quite intriguing and informative. Aurora-A kinases are stably localized to centrosomes during cell division; however, Aurora-B kinases are very dynamic during cell division. In many species, Aurora-B kinases are observed localized to the chromosomes from prophase until metaphase (Schumacher et al., 1998a; Terada et al., 1998; Adams et al., 2001b). Then at anaphase, Aurora-B transits to the central spindle; this is called chromosomal passenger behavior. Recently, it was shown that Aurora-B is part of a chromosomal passenger complex (see below). Less is known about Aurora-A, but its association with TPX2 is required for its localization to the spindle (Kufer et al., 2002).

Aurora-B is regulated by its association with a conserved protein complex consisting of Aurora-B, Bir1/survivin, and INCENP. This complex has been referred to as either the ABI complex or the chromosomal passenger complex (Kim et al., 1999; Adams et al., 2000; Adams et al., 2001a; Wheatley et al., 2001; Bolton et al., 2002). Bir1, also known as survivin, is a small protein containing a BIR (baculovirus inhibitor of apoptosis) motif (Clem et al., 1991). This inhibitor of apoptosis (IAP) domain is contained in many IAP proteins, which function to inhibit apoptosis (Deveraux and Reed, 1999). In fact, survivin was named for its ability to prevent apoptosis when over-expressed in human cells treated with apoptotic stimuli (Ambrosini et al., 1997). INCENP has long been studied, both as the first chromosomal passenger protein and for its role in cytokinesis (Cooke et al., 1987; MacKay et al., 1998). INCENP is a large protein with several domains. INCENP contains a domain that binds chromatin in the N terminus and a microtubule binding domain in the C terminus that is required for its passenger behavior (Ainsztein, et al., 1998; Wheatley et al. 2001).

Loss-of-function studies of the ABI complex revealed common phenotypes for each component (Cutts et al., 1999; Kim et al., 1999; Kaitna et al., 2000; Speliotes et al., 2000; Uren et al., 2000; Adams et al., 2001b; Oegema et al., 2001). Also, loss of function of any of the subunits disrupts the sub-cellular localization of the other components. In particular, loss of function of either Bir1/survivin or INCENP results in the disruption of Aurora-B localization (Adams et al., 2000; Kaitna et al., 2000; Speliotes et al., 2000; Adams et al., 2001c). In fact, Aurora-B directly binds to both Survivin/Bir1 and INCENP to form stable complexes in vivo (Adams et al., 2000; Kaitna et al., 2000; Bolton et al., 2002).

Survivin/Bir1 and INCENP direct the location of Aurora-B kinases in the cell. Both Survivin/Bir1 and INCENP are required for the movement of Aurora-B from the chromosome arms to concentrate at the inner kinetochore region at metaphase (Adams et al., 2000; Kaitna et al., 2000; Adams et al., 2001c; Bolton et al., 2002). Also at the onset of anaphase, Survivin/Bir1 and INCENP are required for Aurora-B to migrate to the central spindle (Adams et al., 2000; Adams et al., 2001c; Kaitna et al., 2000; Bolton et al., 2002). These observations suggest the ABI complex subunits function to target Aurora-B kinases in a manner regulated by the cell cycle. These results also suggest that the phenotypes of both Bir1/survivin and INCENP can be explained by the loss of function of the only known catalytic subunit of the chromosomal passenger complex, Aurora-B.

b. Sub-cellular localization of Polo kinases

With regard to Polo kinases, dynamic movement and localization to several mitotic structures have been observed. In prophase, Plk1 first appears on the nuclear envelope and centrosomes (Golsteyn et al., 1995; Lee et al., 1995; Arnaud et al., 1998; Moutinho-Santos et al., 1999; Hudson et al., 2001). In metaphase, Plk1 is found on kinetochores, centrosomes, and spindle microtubules. Then, in a manner similar to that of the passenger complex, Plk1 re-localizes to the central spindle at anaphase. However, a subset of Plk1 remains associated with centrosomes throughout mitosis (Golsteyn et al., 1995; Lee et al., 1995; Arnaud et al., 1998; Moutinho-Santos et al., 1999; Hudson et al., 2001).

There is evidence the sub-cellular localization of Polo kinases requires a highly conserved motif called the Polo box. Initially, the presence of the Polo box (S-K-W-V-D-Y-S) was used to define whether a kinase is a Polo kinase (Nigg et al., 1998). Both deletion of the

Polo box or mutations within the conserved sequence result in the mis-localization of Polo kinases (Lee et al., 1995; Lee et al., 1998; Lee et al., 1999; Song et al., 2000**)**.

5. Multiple events of cell division are regulated by Aurora and Polo kinases

a. Aurora-B and chromosome structure

A major area of research is to find how and through what targets these cell division kinases function. One of the first substrates of the Aurora kinases was discovered by Dr. David C. Allis's laboratory in collaboration with my mentor, Dr. Rueyling Lin. Dr. Allis and Dr. Lin showed – in *S. cerivisae* and *C. elegans*, respectively – that histone H3 is a conserved substrate of Aurora kinases (Hsu et al., 2000). Furthermore, they showed the loss of histone H3 phosphorylation was correlated with chromosome condensation defects and chromosome segregation failure (Hsu et al., 2000).

For a long time, it has been known that the phosphorylation of both histone H3 and histone H1 was correlated with chromosome condensation during mitosis in a variety of eukaryotic organisms (Bradbury et al., 1973; Gurley et al., 1974). However, researchers have shown more recently that the phosphorylation of histone H1, and even histone H1 itself, is dispensable for chromosome condensation (Ohsumi et al., 1993; Shen et al., 1995). Work done using *Tetrahymena* revealed the most direct connection between the phosphorylation of histone H3 and chromosome condensation (Hendzel et al., 1997; Wei et al., 1998). The mutation of the phosphoryation site, serine 10, in histone H3 in *Tetrahymena* was sufficient to reveal both mitotic and meiotic chromosome segregation defects that were attributed primarily to malfunctions in chromosome condensation (Wei et al., 1998).

Aurora kinases phosphorylate histone H3 on serine 10. The development of a phospho-serine 10 specific, histone H3 antibody by Dr. Allis has allowed Aurora kinase activity to be monitored in vivo. Although in vitro, both Aurora-A and Aurora-B are able to phosphorylate histone H3 at serine 10, the majority of in vivo phosphorylation appears to occur through Aurora-B. This agrees with the phenotypes of Aurora-A versus Aurora-B mutants. The loss-of-function of Aurora-B in worms or flies results in the abolishment of histone H3 phosphorylation, which correlates with failures in chromosome condensation and severe chromosome segregation defects during mitosis (Hsu et al., 2000; Adams et al., 2001b; Giet and Glover, 2001; Hagstrom et al., 2002). On the other hand, the loss of function of Aurora-A results in centrosomal defects and has little to no effect on histone H3 phosphorylation (Hsu et al., 2000; Hannak et al., 2001).

In both yeast and worms, the PP1 phosphatases Glc7p, CeGLC-7α, and CeGLC-7β have been shown to antagonize Aurora-B kinase activity towards histone H3 in vivo (Hsu et al., 2000). The mechanism of GLC7 function was suggested to be via direct activity towards histone H3, because the yeast Glc7p was shown to dephosphorylate histone H3 in vitro (Hsu et al., 2000). An *ipl1* mutation can be suppressed by some alleles of *glc7*, suggesting a balance of opposing activities (Hsu et al., 2000). Also, it was shown in *S. cerivisae* that Ipl1 and Glc7 regulate the balance of phosphorylation of at least one another common substrate, Ndc10 (Biggins et al. 1999; Sasson et al. 1999). The Glc7 phosphatases are required for cell division, and loss-of-function analysis in both yeast and worms revealed defects in chromosome condensation during mitosis (Hsu et al., 2000).

b. Plk1 and cell cycle regulation

(1) Cell cycle progression

There are several different lines of evidence suggesting Plk1 regulates entry into mitosis via the activation of Cdk1/cyclin complexes. Plk1 activates the Cdc25 phosphatase via phosphorylation, which antagonizes the effects of Wee1 inhibitory phosphates on Cdk1 (Kumagai and Dunphy, 1996; Ouyang et al., 1997; Qian et al., 1998b; Qian et al., 1999). Another target of Plk1 is cyclin-B, which might enhance its ability to activate Cdk1 (Toyoshima-Morimoto et al., 2001). These results have led to models where Cdk1 and Plk1 activation occur simultaneously and feed into each other to further promote Cdk1 activation.

Plk1 also is required for exit from mitosis. There are several observations suggesting Plk1 activates the APC directly via phosphorylation. The result of this would be the promotion of entry into anaphase via cyclin-B and securin degradation (Charles et al., 1998; Descombes and Nigg, 1998; Kotani et al., 1998). Specific Plk1 targets include APC1/Tsg24, APC6/Cdc16, APC3/Cdc27, and APC8/Cdc23 (Golan et al., 2002). Interestingly, experiments in *S. cerivisae* suggest Cdc5/Plk1 activates the APC to specifically target the degradation of cyclin-B but not securin (Jaspersen et al., 1998; Hudson et al., 2001).

Initially, Plk1 appears to activate Cdk1/cyclin-B activity and then later to inactivate Cdk1/cyclin-B activity via the promoting the activation of the APC. Both of these actions of Plk1 serve to drive the cell cycle forward, albeit at different stages.

(2) Plk1 and cell cycle checkpoints

In many systems, Plk1 is inhibited when either spindle poisons or DNA damage perturb the cell cycle. In *S. cerivisae*, *cdc5* mutants fail to arrest the cell cycle in response of DNA damage (Toczyski et al., 1997). In response to DNA damage, the checkpoint kinase Chk2/Rad53 has been shown to block Plk1 activation via direct inhibitory phosphorylation of Plk1 (Bernstein and Coughlin, 1998; Karaiskou et al., 1999; Smits et al., 2000; Kang et al., 2002). Also, in response to the spindle checkpoint, a proposed Cdc5/Plk1 substrate Bfa1 is not phosphorylated, and this requires an intact spindle checkpoint (Hu et al., 2001; Lee et al., 2001). The inhibition of Plk1 could contribute to cell cycle arrest conceivably through Plk1's role in activating Cdk1/cyclin-B or the APC.

c. Aurora-A and Plk1 both are required for spindle assembly

There are conflicting reports about the requirement of Aurora-A in the process of spindle assembly. Work done using *Drosophila* showed a high frequency of monopolar spindles in Aurora mutants (Glover et al., 1995; Giet et al., 2002). However, work in *C. elegans* showed bipolar spindle assembly occurs normally, but later the spindle collapses (Schumacher et al., 1998b; Hannak et al., 2001). Whatever the exact defect, Aurora-A kinases clearly regulate some aspect of spindle function during mitosis.

There are conflicting reports about Plk1's involvement in the process of spindle assembly. Some reports show a complete failure in spindle assembly, whereas others report functional spindle defects after rather normal spindle assembly. Plk1 is required for spindle assembly in human cells, *Xenopus,* and *Drosophila* (Lane and Nigg, 1996; Qian et al., 1998b; do

Carmo Avides et al., 2001; Donaldson et al., 2001). In *S. cerivisae* and *Drosophila*, a bipolar spindle is formed in the absence of Plk1, but numerous microtubule defects were present in these mutants (Schild and Byers, 1980; Kitada et al., 1993; Ohkura et al., 1995; Herrmann et al., 1998; Riparbelli et al., 1998). In *C. elegans*, Plk1-depleted embryos form bipolar spindles that are capable of organizing microtubules (Chase et al., 2000). Studies of human cells showed Plk1 inhibition does not prevent spindle assembly; however, spindle position defects and chromosome alignment anomalies were observed (Seong et al., 2002).

d. Aurora-B and Plk1 both are required for cytokinesis

Analyses of both Aurora-B and Plk1 mutants clearly demonstrated roles for each in cytokinesis. Both Aurora-B and Plk1 are required for cytokinesis in *S. cerivisae*, *C. elegans*, *Drosophila*, and vertebrate cells (Sunkel and Glover, 1988; Ohkura et al., 1995; Carmena et al., 1998; Herrmann et al., 1998; Riparbelli et al., 1998; Schumacher et al., 1998a; Terada et al., 1998; Chase et al., 2000; Severson et al., 2000; Adams et al., 2001b; Donaldson et al., 2001; Giet and Glover; 2001; Kawasaki et al., 2001; Song and Lee, 2001). Also the over-expression of Aurora-B or Plk1 causes cytokinesis defects in different organisms (Lee and Erikson, 1997; Terada et al., 1998; Lee et al., 1999; Song et al., 1999).

The Aurora-B/ABI complex has emerged as a critical coordinator of events in cytokinesis. Loss-of-function analyses of both INCENP and Survivin/Bir1 have shown their requirement for cytokinesis in various species (Cutts et al., 1999; Kim et al., 1999; Kaitna et al., 2000; Speliotes et al., 2000; Uren et al., 2000; Adams et al., 2001c; Oegema et al., 2001). The molecular function of either Aurora-B or Plk1 in cytokinesis is not clear. Both the Aurora-B/ABI complex and Plk1 are required for the stable recruitment of MKLP1/Eg5/ZEN-4, a

microtubule motor that functions to stabilize the central spindle in anaphase (Adams et al., 1998; Severson et al., 2000; Adams et al., 2001b). This observation is sufficient to explain the cytokinesis defects, but the relationship between MKLP1 and Plk1 is not clear.

e. Biological substrates of Aurora and Polo kinases

Both Aurora and Polo kinases have been proposed to regulate many different substrates, because of their involvement in diverse processes and their localization to diverse sub-cellular locations (Adams et al., 2001a; Nigg et al., 2001).

Aurora kinases have been proposed to phosphorylate both chromosome- and spindle-associated factors. In *S. cerivisae*, Ipl1 is implicated in phosphorylating several kinetochore proteins, Ndc10p, Sli15p, and Dam1p (Biggins et al., 1999; Kang et al., 2001). In *S. cerivisae*, *Drosophila*, and *C. elegans*, there is sufficient evidence to suggest Aurora-B phosphorylates histone H3 to regulate chromosome structure (Hsu et al., 2000; Giet and Glover, 2001). In *Xenopus*, Aurora kinases have been implicated in phosphorylating the kinesins MKLP-1/KLP3A and histone H3 to regulate cytokinesis (Giet et al., 1999; Murnion et al., 2000). For a comprehensive list of Aurora B substrates, see Appendix C.

Polo kinases appear to have more pleiotropy then Aurora kinases and, therefore, have been implicated to have even more diverse substrates. Proposed substrates of Plk1 include cyclin-B and Cdc25, during entry into mitosis, and multiple subunits of the APC to regulate entry into anaphase (Toyoshima-Morimoto et al., 2001; Golan et al., 2002; Toyoshima-Morimoto et al., 2002). Interestingly, Plk1 has been proposed to phosphorylate MKLP1, which is also a proposed substrate for Aurora-B kinases (Lee et al., 1995; Adams et al., 1998). In *S.*

cerivisae, Cdc5 phosphorylates Bfa1, which might be involved in regulating cytokinesis and exit from mitosis (Hu et al., 2001). For a comprehensive list of Plk1/Polo substrates, see Appendix D.

6. Summary

Both Aurora and Polo kinases constitute key regulators of cell division. To properly segregate chromosomes during cell division, the action of these kinases must be tightly controlled in both time and space. The mis-regulation of both Aurora and Polo kinases is frequently observed in human cancers. In order to understand their function, the substrates of these kinases must be identified, and how phosphorylation of these proteins contributes to cell division must be understood. This should promote understanding of how the mis-regulation of Aurora and/or Polo kinases contributes to the chromosomal instability that is typical of most human cancers.

D. Significance: Proper chromosome segregation is vital to life

1. Chromosome segregation errors during mitosis

The mis-segregation of chromosomes during meiosis or mitosis leads to cells with altered numbers of chromosomes, a situation known as aneuploidy. Most malignant solid tumor cells are highly aneuploid, especially those arising from the breast, colon, lung, oropharynx, and prostate. Karyotypic studies have shown that the majority of cancers have lost or gained chromosomes (Lengauer et al., 1998; Jallepalli and Lengauer, 2001). In particular, about 85% of colon cancer lines display aneuploid karyotypes. Most likely, this is due to chromosome segregation errors during mitosis. Though it has never been established whether aneuploidy promotes the genesis of tumors, intuitively it seems that losing a chromosome might be

tumorgenic, because of the loss of tumor suppressor genes. Also, it is important to note that not only are cancers aneuploid but the karyotype of a single tumor is commonly heterogeneous, reflecting a continuing defect in chromosome segregation (Lengauer et al., 1998; Jallepalli and Lengauer, 2001).

a. Defects resulting in aneuploidies might contribute to tumorgenesis

Defects in sister chromatid cohesion, resolution, and separation are capable of contributing to the genesis of aneuploid cells. In *S. cerivisae*, a large number of genes alterations can cause anueploidy. For example, non-lethal mutants in cohesins, condensins, kinetochore components, and checkpoint factors cause very high rates of aneuploidy (Michaelis et al., 1997; Skibbens et al., 1999; Toth et al., 1999; Mayer et al. 2001). Similarly, many examples of the same types of factors are implicated in human cancers. The human *securin* gene, originally named pituitary tumor transforming gene, was first described for its oncogenic ability (Pei and Melmed, 1997; Zhang et al., 1999; Zou et al., 1999). More striking, deletion of *securin* in a human colon carcinoma cell line with a stable karyotype is sufficient to cause extreme aneuplopidies (Jallepalli et al., 2001). Also mutations in APC subunits have been implicated in colon cancer progression (Wang et al., 2003). Mice heterozygous for the spindle checkpoint gene *mad2* are highly prone to lung cancer (Cahill et al, 1998; Jaffrey et al, 2000; Michel et al., 2001). Transient perturbations in any of these factors might enhance tumorgenesis, whereas continued perturbation is cell autonomous lethal (Shah and Cleveland, 2000).

b. Aurora and Polo kinases and human cancers

Aurora kinases are studied intensively by cancer researchers. The genes encoding the three human Aurora kinases map to regions that are affected by chromosomal abnormalities in different cancer types, and over-expression of each of the three human Auroras has been detected in tumor cell lines (Bischoff et al., 1998; Tatsuka et al., 1998; Kimura et al., 1999b). Immortalized human cell lines that over-express Aurora-B exhibit elevated levels of histone H3 phosphorylation and defects in chromosome segregation and cytokinesis (Ota et al., 2002). These cells are often aneuploid and can produce aggressive tumors in mice. Increased levels of phosphorylation of histone H3 also were shown to correlate with over-expression of Aurora-B in some human colorectal tumor cell lines (Ota et al., 2002).

Similarly, Polo kinases also are associated with human cancers. The expression of Plk1 is associated with tumorigenesis, but somewhat surprisingly, there is evidence for both up- and down-regulation of Plk1 in tumor cells (Knecht et al., 1999; Simizu and Osada, 2000). This suggests that Plk1 can act as both a tumor suppressor and an oncogene.

2. Chromosome segregation errors during meiosis

Errors in meiosis occur in as many as one in four human oocytes, resulting in the production of aneuploid zygotes, and the frequency of meiotic aneuploidy increases with maternal age (Hassold and Hunt, 2001). The prime consequence of meiotic aneuploidy usually is zygotic lethality, which probably accounts for the majority of spontaneous miscarriages (Griffin, 1996). It has been demonstrated that one third of all spontaneously aborted embryos are trisomic for at least one chromosome (Hassold and Hunt, 2001). In rare cases, such as trisomy 21, the zygotes

are viable, but the error leads to Down's syndrome, the single leading cause of mental retardation Hassold and Hunt, 2001).

There are several potential causes of chromosome mis-segregation during meiosis: lack of chiasmata formation, defects in maintaining sister chromatid cohesion or chiasmata, the precocious loss of chromosome cohesion between sister chromatids at anaphase I, and severe defects in spindle/kinetochore interactions. All of these potential causes might involve the same factors involved in generating aneuploidies during mitosis – such as cohesins, condensins, securin, kinetochore components, and checkpoint proteins. However, alterations in meiosis-specific factors also are expected to be involved in the mis-segregation of chromosomes during meiosis.

Unfortunately, despite extensive study of the etiology of trisomy, there is no predominant mechanism underlying meiotic aneuploidy. However, several significant conclusions have been made (Hassold and Hunt, 2001). First, the majority of trisomies arise due to mis-segregation in oocytes and not in sperm. Second, there is considerable variation in the incidence of trisomy between chromosomes, suggesting that unique structural differences between chromosomes, such as overall length or position of the centromere, might contribute to their mis-segregation. Third, the majority of segregation errors must have occurred during the first meiotic division, although a small percentage of errors occur during meiosis II as well.

The fidelity of chromosome segregation is vital to cell division, be it mitosis or meiosis. Mitotic chromosome instabilities might contribute to both tumor progression and tumor heterogeneity. Tumor heterogeneity probably aids the survival of cancer cells by allowing them to avoid otherwise normally effective therapeutic strategies. Also, chromosome segregation is

essential for meiosis. Meiotic anupleoidies probably underlie the majority of cases of infertility, spontaneous abortions, and in rare cases, congenital syndromes. Understanding Aurora and Polo kinases and their roles in chromosome segregation should aid our ability to detect and perhaps treat these problems in humans (Hassold and Hunt, 2001; Dove, 2003; Sausville, 2004).

CHAPTER TWO: The Aurora kinase AIR-2 regulates the selective release of chromosome cohesion during meiosis

A. Introduction

1. *C. elegans* offers many advantages to study meiosis and early embryogenesis

C. elegans is free-living soil nematode that has become a premiere model organism for biological research in the last 30 years (Ankeny, 2001). A community of researchers has developed a variety of genetic and molecular tools for studying *C. elegans*. Also the biology of *C. elegans* offers many advantages for studying meiosis and early embryogenesis, in particular, strong cell biology and the ease of RNA interference (RNAi) technology. The germline of *C. elegans* is well suited for studying the early stages of meiosis, because the nuclei are distributed throughout the gonad in a defined order that correlates with the sequential stages of meiosis. In addition, the *C. elegans* germline offers optimal visualization conditions for high-resolution imaging of meiotic chromosomes.

The *C. elegans* gonad is a tube-like syncytium consisting of thousands of germ nuclei at various developmental stages (Schedl, 1997). Nuclei at the most distal end, relative to the spermatheca, proliferate mitotically. Moving proximally, germ nuclei enter meiosis, progress through different stages of meiotic prophase I and cellularize to become oocytes (Fig. II.1). Full-grown oocytes are in diakinesis of prophase I and are referred to by their positions in the gonad relative to the spermatheca, with the most proximal oocyte being minus one (-1). In the presence of sperm, –1 oocytes sequentially undergo maturation, enter the spermatheca, and become

fertilized (McCarter et al., 1999). Following fertilization, the oocyte-derived nucleus completes two rounds of meiotic division, each marked by the extrusion of a polar body, to become a haploid pronucleus (Fig. II.1).

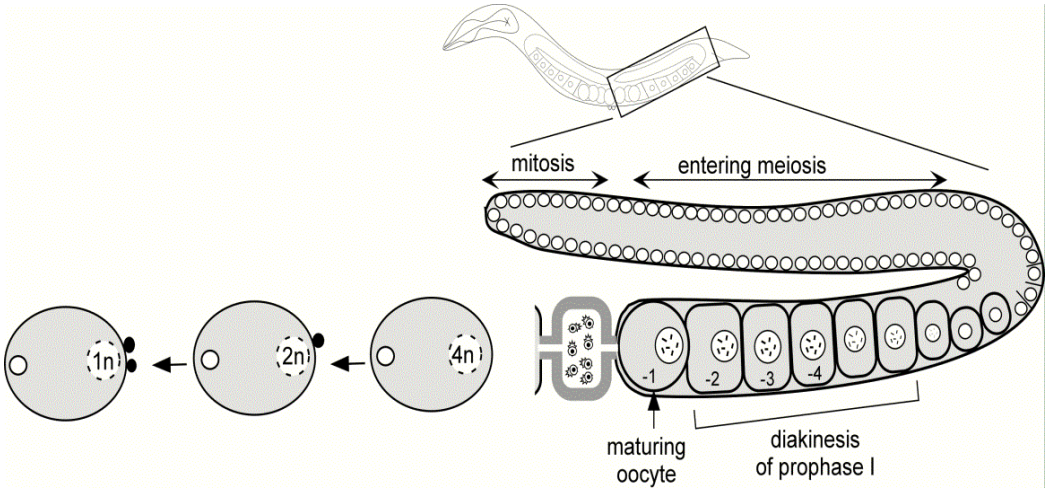

Figure II.1. The reproductive system of the *C. elegans* hermaphrodite. (Reproduced from Rogers et al., 2002.) Schematic representation of one arm of the *C. elegans* gonad, including newly fertilized embryos. Nuclei are represented by open circles. The DNA complement in oocyte-derived nuclei is indicated by the number inside each nucleus.

2. Both AIR-2 and PLK-1 are required for meiosis

To assay gene function, RNAi can be applied to the *C. elegans* germline relatively easily. The introduction of double-stranded RNAs (dsRNAs) into the germline results in a robust loss of gene function effect that is highly specific to the nucleotide sequence. In this thesis, several genes were analyzed by experiments using germline RNAi. In most cases, the technique of microinjection was utilized to introduce dsRNAs directly into the developing meiotic nuclei of the germline syncytium.

Wildtype zygotes develop into multicellular embryos, with each diploid cell containing one pair of centrosomes (Fig. II.2). Previously, Aurora and Polo kinases were studied in *C.*

elegans using RNAi (Schumacher et al., 1998a; Schumacher et al., 1998b; Chase et al., 2000). If either AIR-2 or PLK-1 is inhibited by RNAi, similar phenotypes were observed (Fig. II.2): the progeny of dsRNA-injected mothers arrest as single-cell embryos. These embryos are severely disorganized, highly polyploid, and contain multiple centrosomes (Fig. II.2). In particular, both *air-2(RNAi)* and *plk-1(RNAi)* embryos exhibit severe chromosome segregation and cytokinesis defects (Schumacher et al., 1998a; Chase et al., 2000). However, the failure of cytokinesis is not indirectly caused by defects in chromosome segregation, because other mutants are able to complete cytokinesis in the presence of un-segregated chromosomes (Severson et al., 2000).

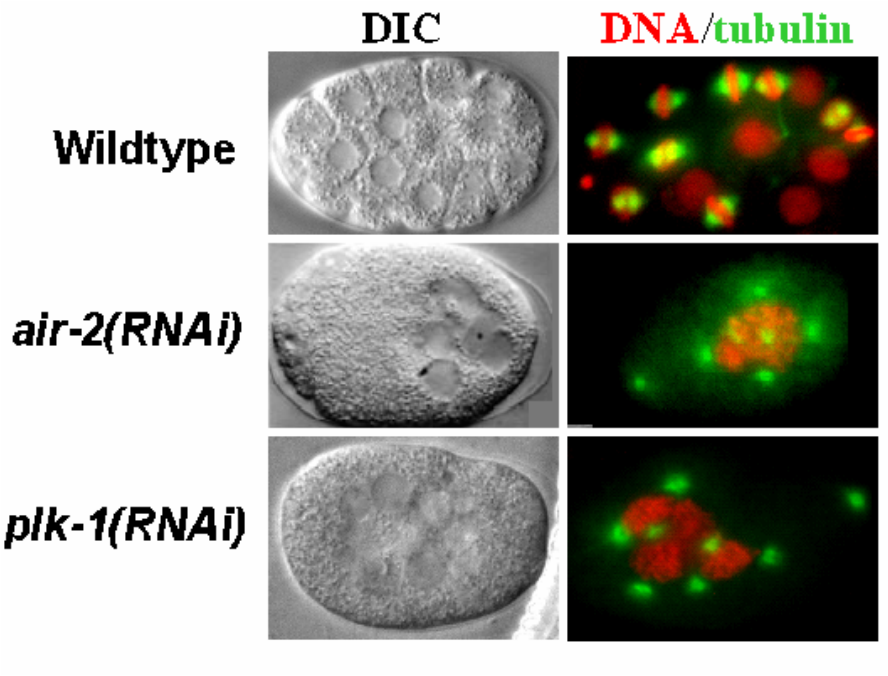

Figure II.2. Both *air-2(RNAi)* and *plk-1(RNAi)* result in 100% embryonic lethality. The left column shows differential interference contrast (DIC) pictures of live embryos from wildtype, *air-2(RNAi),* and *plk-1(RNAi),* respectively. The right column shows fixed embryos stained with a green marker for tubulin and a red marker for DNA.

Aside from the mitotic defects, it was observed that *air-2(RNAi)* and *plk-1(RNAi*) embryos lack polar bodies; this suggests defects in meiosis (Schumacher et al., 1998a; Hsu et al., 2000; Chase et al., 2000). RNAi studies of AIR-1, another Aurora kinase, revealed some similarities to AIR-2; however, meiosis appeared to be unaffected (Schumacher et al., 1998b). Also RNAi studies of PLK-2, another Polo kinase, revealed no RNAi phenotype and also no additive or synergistic effects when combined with *plk-1(RNAi)*.

Detailed analysis of meiosis discovered that both AIR-2 and PLK-1 are absolutely required for meiotic chromosome segregation and polar body extrusion (Chase et al., 2000; Hsu et al., 2000; Oegema et al., 2001). If RNAi embryos are examined prior to mitosis, the result of these meiotic defects is evident (Fig. II.3). Both *air-2(RNAi)* and *plk-1(RNAi)* zygotes retained a 4C DNA content in the oocyte-derived pronucleus. After pronuclei fusion in these RNAi embryos, the first cell is polyploid, containing 5C DNA content during the first cell cycle.

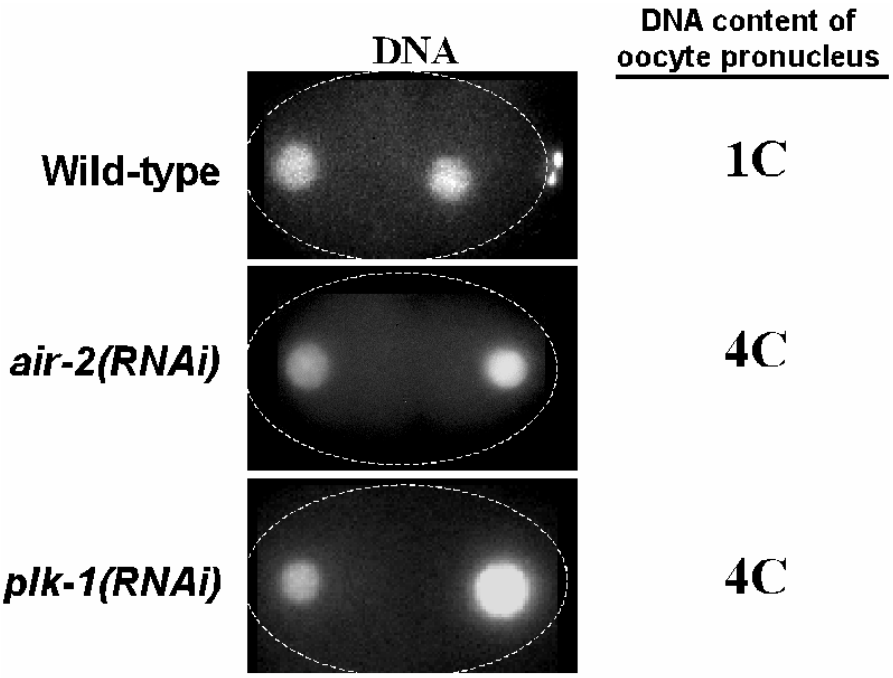

Figure II.3. Both *air-2(RNAi)* and *plk-1(RNAi)* result in meiotic failure. The DNA content of pronuclei stage zygotes from wildtype, *air-2(RNAi)*, and *plk-1(RNAi),* respectively. The sperm-derived pronucleus is oriented to the left, and the oocyte-derived pronucleus is to the right. In both *air-2(RNAi)* and *plk-1(RNAi)*, no polar bodies are observed and the oocyte-derived pronucleus is polyploid, compared to the sperm pronucleus.

This thesis explores why meiosis is defective in both *air-2(RNAi)* and *plk-1(RNAi)* zygotes. In particular, the role of AIR-2 and PLK-1 was examined in terms of chromosome segregation during the meiotic divisions of the fertilized oocyte. The results of this work are three-fold: (1) AIR-2 functions to promote the release of chromosome cohesion between homologs via phosphorylation of REC-8, specifically in the chromosome arms distal to the chiasmata during meiosis I; (2) PLK-1 might be involved in the release of the proximal/centromeric chromosome cohesion, specifically during meiosis II; (3) PLK-1 might play novel roles in establishing cell polarity and asymmetric cell division in the embryo immediately following meiosis.

B. Results

Accurate chromosome segregation during cell division requires the precisely regulated establishment and release of chromosome cohesion. Chromosome dynamics during meiosis are complex; as homologs separate at anaphase I, whereas sister chromatids remain attached until anaphase II. How the selective release of chromosome cohesion is regulated during meiosis had not been described previously. This research shows that the Aurora-B kinase AIR-2 regulates the selective release of chromosome cohesion during *C. elegans* meiosis.

AIR-2 localizes to sub-chromosomal regions corresponding to the last points of contact between homologs in metaphase I and between sister chromatids in metaphase II. Depletion of AIR-2 by RNAi prevents chromosome separation at both anaphases, with concomitant prevention of meiotic cohesin REC-8 release from meiotic chromosomes. We show that AIR-2 phosphorylates REC-8 at a major amino acid (T625) in vitro. Interestingly, depletion of two PP1 phosphatases, GSP-1 and GSP-2, abolishes the restricted localization pattern of AIR-2. In *gsp-1/2(RNAi)* zygotes, AIR-2 is detected on the entire bivalent. Concurrently, chromosomal REC-8 is reduced dramatically and sister chromatids are separated precociously at anaphase I in *gsp-1/2(RNAi)* zygotes. We propose that AIR-2 promotes the release of chromosome cohesion via phosphorylation of REC-8 at specific chromosomal locations and that GSP-1/2, directly or indirectly, antagonize AIR-2 activity.

1. Chromosome condensation appears normal in meiosis after AIR-2 inhibition

The meiotic failure of *air-2(RNAi)* zygotes most likely was not due to a defect in chromosome condensation. The six bivalent chromosomes in the oocytes of *air-2(RNAi)* animals condensed

during prophase to form short rods that were morphologically indistinguishable from those in wildtype oocytes (Fig. II.4; Speliotes et al., 2000). This observation suggests that AIR-2 functions in meiosis via a mechanism that is independent of chromosome condensation.

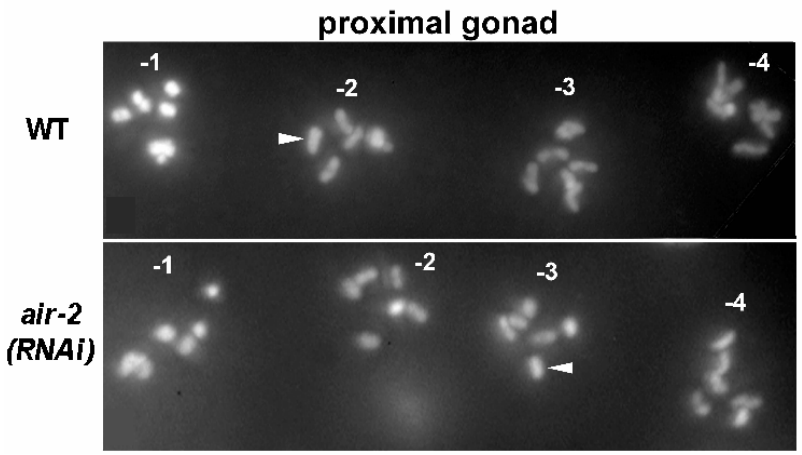

Figure II.4. Chromosome condensation appears normal in *air-2(RNAi)* oocytes. (Reproduced from Rogers et al., 2002.) DNA staining of fixed proximal gonads derived from wildtype or *air-2(RNAi)* animals.

It was shown previously that in *air-2(RNAi)* oocytes, histone H3 phosphorylation at Serine 10 is abolished (Hsu et al., 2000). In contrast, in *gsp-1/2(RNAi)* animals, histone H3 phosphorylation is increased beyond wildtype levels. We found that *gsp-1/2(RNAi)* results in severe meiotic defects (see below). However, in *gsp-1/2(RNAi)* animals, the increase in histone H3 phosphorylation was not correlated with chromosome condensation defects.

In summary, the severe meiotic defects in either GSP-1/2 or AIR-2 depletion can not be explained by defects in chromosome condensation. Similarly, changes in histone H3 phosphorylation in *air-2(RNAi)* and *gsp-1/2(RNAi)* animals do not correlate with chromosome condensation defects during meiosis. These observations do not indicate the purpose of histone

H3 phosphorylation during meiosis. Similarly, precisely what cell division processes are regulated by AIR-2 and GSP-1/2 during meiosis is not known.

2. Histone H3 phosphorylation occurs at sub-chromosomal foci

Histones are plentiful, composing about half of the mass of chromosomes (Kornberg and Lorch, 1999). Histone H3 is a core histone upon which DNA is wrapped. Aurora B kinases phosphorylate histone H3 at Ser10 (Hsu et al., 2000). A commercial antibody can be used to detect the highly conserved phosphorylated-Ser10 epitope (H3P) in various organisms. Previous studies have characterized H3P staining in *C. elegans*. In *C. elegans* meiosis, H3P is observed first on the chromosomes of oocytes in diakinesis (Hsu et al., 2000).

We performed a detailed examination of H3P staining during meiosis in *C. elegans*. H3P staining is observed in the last four to five oocytes (Fig. II.5 A). Under close examination, we observed two H3P foci associated with each bivalent chromosome (Fig. II.5 B). These foci were near the junction between homologous chromosomes and in the vicinity of the chiasma (Fig. II.5 D). Based on this observation, we hypothesized that the H3P sub-chromosomal foci might represent the distal arms of the bivalent.

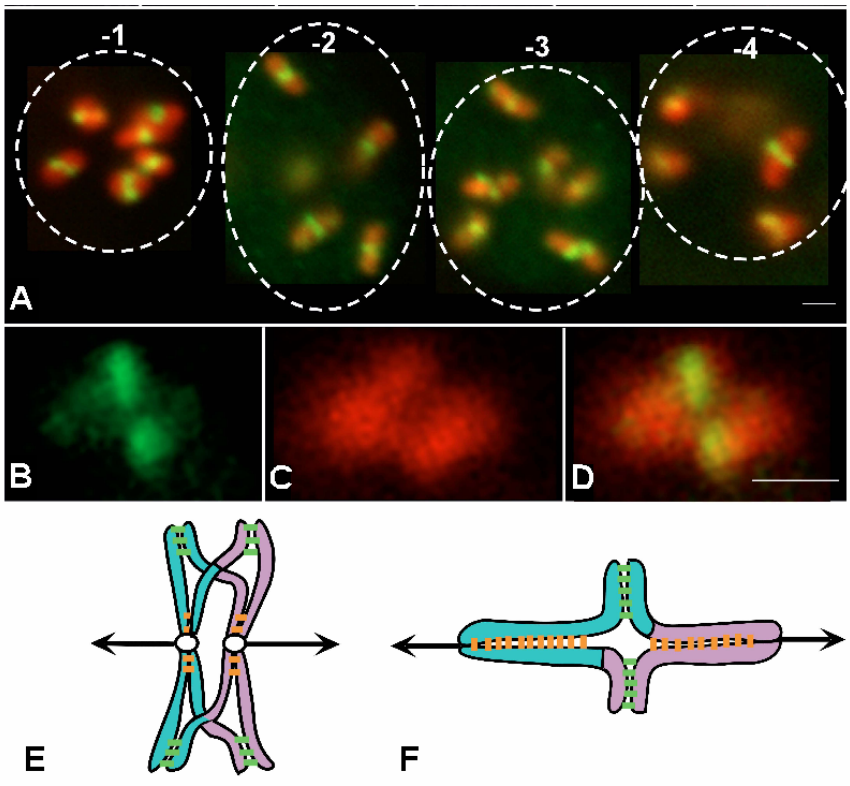

Figure II.5. Histone H3 phosphorylation occurs at sub-chromosomal foci. Staining of wildtype proximal gonad using an anti-phospho-Ser10 histone H3 antibody. (A) Merged image with H3P staining in green and DNA staining in red of the last four oocytes in the gonad. (B-D) Close-up of individual bivalent showing H3P (B), DNA (C) and merged image (D). Schematic drawing of a typical bivalent in vertebrates (E) and *C. elegans* (F). Sister chromatids are shown in the same color, whereas homologues are represented in different colors. The direction of pulling force is indicated by arrows. Green and orange bars represent cohesin molecules connecting chromosome arms distal and proximal, respectively, to the chiasmata.

3. In vivo 4-D analysis: AIR-2 is required for the separation of homologs

To better understand the defect in meiotic divisions observed in *air-2(RNAi)* zygotes, we performed four-dimensional (4-D) imaging of meiosis using a reporter strain carrying a transgene expressing a histone H2B–green fluorescent (GFP) fusion protein. This imaging approach allows the observation of chromosome dynamics in real-time during the meiotic divisions.

In the wildtype embryo immediately after fertilization, the six bivalents begin to align with the long axis of each bivalent parallel to the surface of the embryo (Fig. II.6, A and F; Herman et al., 1979; Albertson and Thomson, 1993). Each bivalent consists of two homologs arranged axially, i.e., end to end, with the inner ends being the point of contact between homologs and the outer ends leading toward the poles. Later, the chromosomes rotate 90 degrees, such that the long axis of each bivalent becomes perpendicular to the surface of the embryo (Fig. II.6, B and G). About 2 min later, anaphase begins and the homologous chromosomes separate into two groups (Fig. II.6, C and H). The group of chromosomes closer to the surface of the embryo is extruded as a polar body (Fig. II.6, D and I). In meiosis II, the sister chromatids arrange in a similar end-to-end configuration. After the completion of meiosis I, six univalent chromosomes appear. They remain unaligned for 5 min, before the whole process repeats to separate the end-to end–joined sister chromatids (Fig. II.6, E and J).

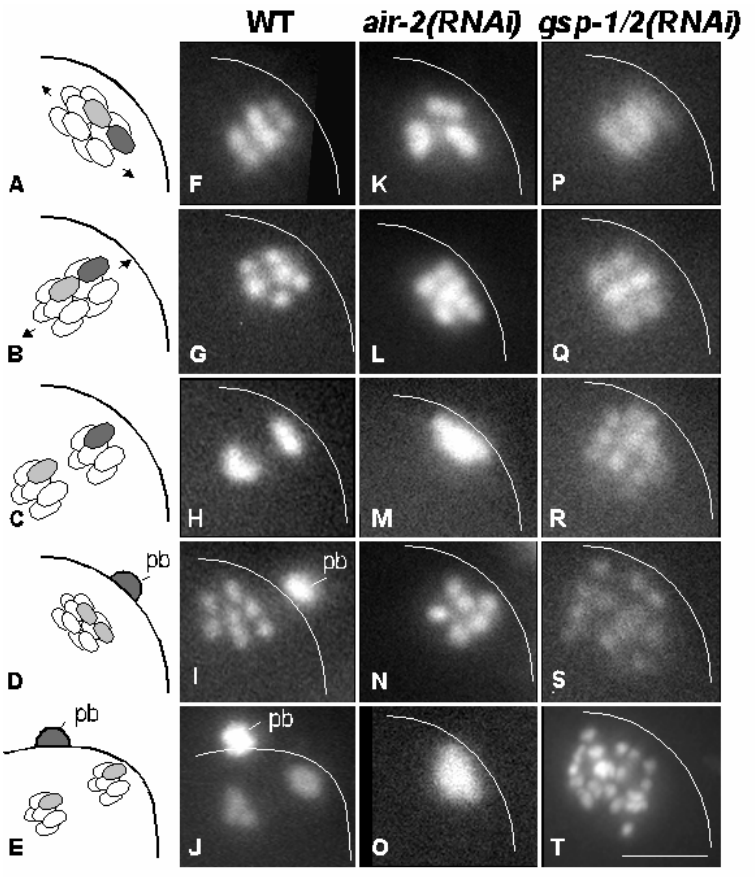

Figure II.6. AIR-2 is required for chromosome separation during meiosis. (Reproduced from Rogers et al., 2002). (A–E) Schematic drawing of meiotic chromosomes in the oocyte-derived nucleus in a wildtype embryo. Each bivalent is represented as two short rods joined end to end, with the outer ends leading toward poles. Homologues from one bivalent are indicated by different shades of gray in A–C. After the extrusion of a polar body (pb), sister chromatids are shown as small short rods in D and E. (F–J) Wildtype; (K–O) *air-2(RNAi)*; (P–T) *gsp-1/2(RNAi)* meiotic embryos. (A, F, and K) Initial alignment, parallel to the cell cortex. (B and G) After a 90° rotation, the chromosomes are perpendicular to the edge of the embryo. (C and H) Anaphase I; (D and I) metaphase II; (E and J) anaphase II. Embryos in K and P, L and Q, M and R, N and S, and O are at the same stage as those in F, G, H, I, and J, respectively. All images presented here are representative slices from each time point, except for that in T, which is a projection of the image stack of DAPI staining from a fixed embryo. The edge of the embryo is indicated by a black curve in A–E and a white curve in F–T. The bar indicates 2.5 μm.

In *air-2(RNAi)* zygotes, the bivalents appeared morphologically normal immediately after fertilization (Fig. II.6 K). However, the initial alignment on the metaphase plate was slightly disorganized, no rotation was observed, and the long axis of each bivalent remained parallel to the surface of the embryo (Fig. II.6, K and L). About 3 min after the initial alignment, a time

point equivalent to the onset of anaphase I in wildtype zygotes, all the chromosomes moved toward and were compressed against the edge of the embryo (Fig. II.6 M). No separation of chromosomes was observed (n=50). Then 5 min later, all the chromosomes moved back to their initial positions, appeared clearly as six bivalents, and repeated the entire process (Fig. II.6, N and O). Similar RNAi phenotypes were observed for the two other ABI complex members: the survivin-related protein BIR-1 and the INCENP homologue ICP-1 (data not shown). These observations suggest that AIR-2 and the ABI complex are necessary for the separation of homologs in meiosis I and for the separation of sister chromatids in meiosis II.

4. AIR-2/ABI complex localizes to the junction between chromosomes in meiosis

The *air-2(RNAi)* phenotype suggests an important function for AIR-2 in chromosome separation during meiosis. A clue as to how AIR-2 might function in chromosome separation came from a detailed examination of the localization of AIR-2 during meiosis. The bulk of AIR-2 staining has been shown to remain cytoplasmic in oocytes until the -1 oocyte undergoes maturation (Schumacher et al., 1998a). Upon oocyte maturation, AIR-2 becomes chromatin-associated and remains so throughout the completion of meiosis, except during anaphase, when AIR-2 temporarily relocates to the microtubule midzone (Schumacher et al., 1998a).

We now show that chromatin-associated AIR-2 localizes to discrete sub-chromosomal foci in both meiotic divisions (Fig. II.7). In maturing oocytes, AIR-2 was localized to sub-chromosomal foci similar to H3P; however, H3P staining is observed earlier in the cell cycle (estimated to be more than 1 hr earlier). During the meiotic divisions, the AIR-2 foci corresponded to the equatorial axes of the bivalent (the inner ends of homologs) in metaphase I (Fig. II.7, D–F) and the inner ends of the end-to-end–joined sister chromatids in metaphase II

(Fig. II.7, J–L). At anaphase I, when homologs separate, AIR-2 was detected exclusively in the microtubule midzone between homologs (Fig. II.7, G–I).

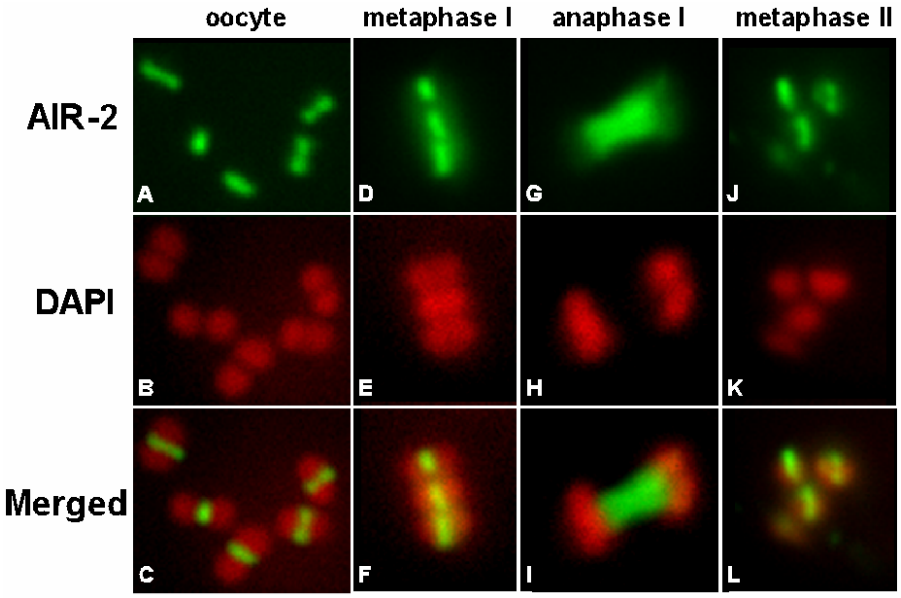

Figure II.7. AIR-2 localizes to the junction between chromosomes. (Reproduced from Rogers et al., 2002). Antibody staining is presented in green and DAPI staining in red. (A–L) Representative slice images of AIR-2 (A, D, G, and J), DAPI (B, E, H, and K), or merged (C, F, I, and L) staining in oocyte-derived nuclei at different meiotic stages. (M–P) Only merged images are shown for BIR-1 at corresponding stages. Arrows in A–F and M–N point to the staining between homologs in meiosis I; in J–L and P, they point to the staining between sister chromatids in meiosis II.

Both BIR-1 and ICP-1, which are ABI complex partners of AIR-2, exhibit a similar - if not identical - temporal and spatial antibody staining pattern to AIR-2 (Kaitna et al., 2002). We showed that BIR-1 and ICP-1 also localize to the inner ends between homologs in metaphase I and between sister chromatids in metaphase II (Fig. II.8), identical to the pattern observed with AIR-2. These foci represent the last points of contact between separating chromosomes, either homologs or sister chromatids. These results suggest the intriguing possibility that the ABI

complex targets AIR-2 to these foci to phosphorylate a chromosomal substrate(s) to trigger the release of chromosome cohesion.

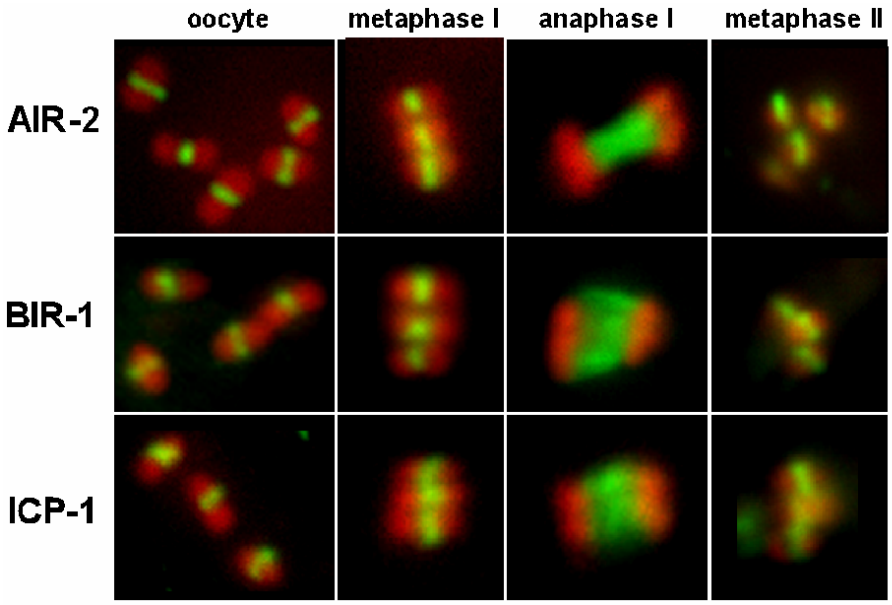

Figure II.8. BIR-1 and ICP-1 co-localize with AIR-2 between chromosomes. Antibody staining is presented in green and DAPI staining in red. Merged antibody and DNA staining in oocyte-derived nuclei at different meiotic stages. Only merged images are shown of AIR-2, BIR-1, and ICP-1.

5. Histone H3 is phosphorylated at the junction between chromosomes in meiosis

One substrate of AIR-2 is histone H3. Antibody staining of H3P during the meiotic divisions showed an interesting pattern. Intense H3P staining was detected on the inner ends between homologs in metaphase I (Fig. II.9, A and M) and between sister chromatids in metaphase II (Fig. II.9, F and R), coincident with the localization of AIR-2. These foci represent the last points of contact between separating chromosomes, either homologs or sister chromatids. At anaphase I, H3P was detected on the inner faces of the separating homologs (Fig. II.9, C and O), which although different from AIR-2, is expected for a chromosome-bound epitope. These

observations also support the idea that AIR-2 functions in the separation of homologs, perhaps playing a role in modulating chromatin structure via the phosphorylation of histone H3.

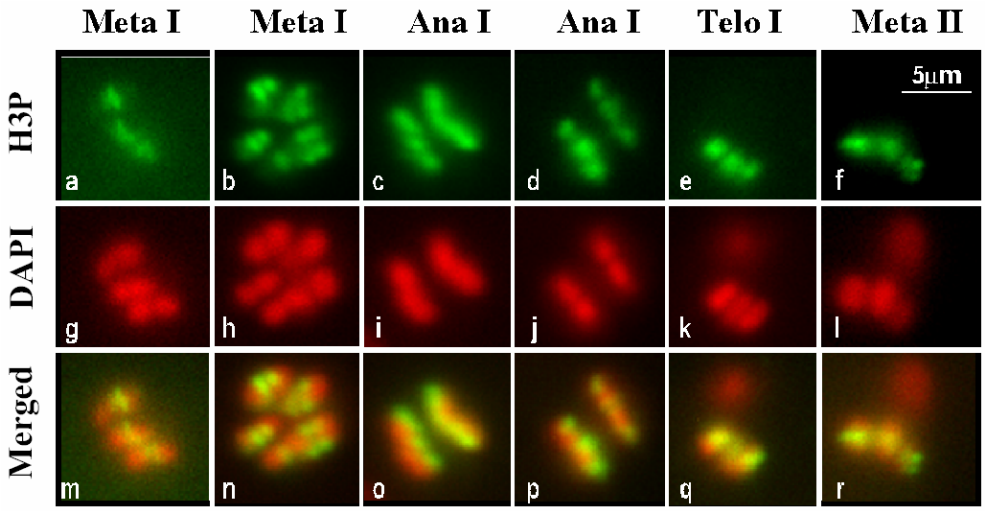

Figure II.9. Histone H3 is phosphorylated at the junction between chromosomes. Antibody staining is presented in green and DAPI staining in red. Representative slice images of H3P (a-f), DAPI (g-l), or merged (m-r) staining in oocyte-derived nuclei at different meiotic stages are provided.

6. The localization of AIR-2/ABI and H3P is dependent on chiasmata formation

Because there is, on average, one crossover per homolog pair in *C. elegans* meiosis (Brenner, 1974; Barnes et al., 1995), bivalents initially appear cross-shaped, with the center of each cross being the chiasma, and then become rod-shaped as chromosomes condense further. Because centromeres are located at the ends of meiotic chromosomes in *C. elegans,* the equatorial axes of the rod-shaped bivalent chromosomes correspond to the chromosome arms distal to the chiasmata and the poleward (or long) axis proximal in vertebrate and yeast chromosomes (Fig. 10, A and B; Albertson and Thomson, 1993).

The sub-chromosomal foci observed by the staining of AIR-2, BIR-1, ICP-1 and H3P appear to correspond to the chromosome arms distal to the chiasmata. Therefore, we asked whether these foci are dependent on chiasma formation. Mutations in *him-5* and *him-8* caused a high frequency of non-disjoined X chromosomes, resulting in five bivalents and two non-disjoined univalents that can be clearly observed in diakinetic oocytes (Hodgkin et al., 1979; Broverman and Meneely, 1994). In *him-5(e1490)* or *him-8(e1489)* mutants, we observed very intense staining of AIR-2, BIR-1, ICP-1, and H3P on all five bivalents, but no detectable staining was detected on either of the univalents (Fig. II.11, C and E; n=42 for AIR-2; n=11 for BIR-1).

We also examined the staining pattern of AIR-2, BIR-1, and H3P in *him-14*(*it44ts)* and *spo-11(ok79)* mutants in which crossing-over is defective for all chromosomes, resulting in 12 visible univalents in each oocyte (Dernburg et al., 1998; Zalevsky et al., 1999). In *spo-11(ok79)* and *him-14(it44ts)* mutants, approximately 50% of univalents had no detectable staining, whereas the other 50% had variable amounts of staining (Fig. II.11, E-F). The reasons for this are unknown. However, this result suggests that either the total number of univalents or the ratio of univalents to bivalents present in the nucleus affects the chromosomal localization of the AIR-2/ABI complex and the presence of H3P.

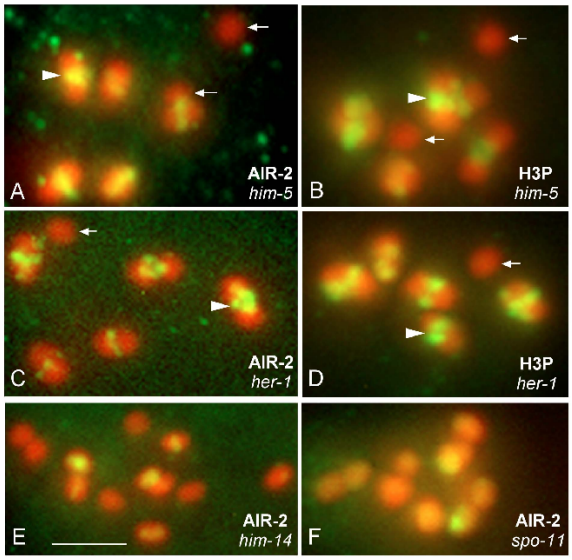

Figure II.10. The localization of AIR-2 and H3P requires chiasmata formation. Merged and projected images of chromosomes stained for either AIR-2 or H3P from the -1 oocytes of *him-5(e1490)* (A and B), *her-1(e1518)* XO (C and D), *him-14(it44ts)* (E), and *spo-11(ok79)* mutants (F). Arrows point to univalents, and arrowheads point to representative bivalents.

To demonstrate that the presence of these sub-chromosomal foci is dependent on chiasma formation instead of a direct requirement for the gene *him-5* or *him-8*, we examined the staining pattern for these proteins in *her-1*(*e1518*) XO animals. In *C. elegans*, sex is determined by the ratio of X chromosomes to autosomes (Madl and Herman, 1979). Wildtype hermaphrodites have two X chromosomes, whereas males have only one X chromosome. Mutations in the sex determination gene *her-1* result in XO animals developing into relatively normal, fertile hermaphrodites (Hodgkin, 1980). The single X chromosome appears as a non-paired univalent in oocytes of *her-1*XO animals. No staining of AIR-2, BIR-1 or H3P was observed on the univalents in oocytes of *her-1*(*e1518*) XO animals, whereas the staining on bivalents appeared wild type (Fig. II.11, D and F; n=14 for AIR-2; n=4 for BIR-1). These results demonstrate that the observed sub-chromosomal foci containing AIR-2, BIR-1, and H3P are dependent on chiasma formation, not on the function of specific genes required for meiotic crossover.

7. REC-8 remains detectable on both axes of bivalents in *air-2(RNAi)* zygotes

A potential candidate for AIR-2 phosphorylation at the chromosomal arms distal to chiasmata is the meiotic cohesin REC-8. The REC-8 protein was observed to localize to both poleward and equatorial axes of bivalents in metaphase I. At anaphase I, only the REC-8 on the poleward axis remained detectable, whereas the REC-8 on the equatorial axis was absent (Pasierbek et al., 2001). Comparison to the mitotic cohesion Scc1 in *S. cerivisae*, which is degraded more efficiently as a phospho-protein (Alexandru et al., 2001), suggests that selective removal of REC-8 at anaphase I in *C. elegans* could be regulated via differential phosphorylation by AIR-2.

If REC-8 is one of the in vivo substrates for AIR-2 and if phosphorylation of REC-8 plays an important role for its degradation, one would predict that REC-8 protein would remain localized along both axes of the bivalents in *air-2(RNAi)* zygotes. We stained meiotic chromosomes of wildtype and *air-2(RNAi)* zygotes with antibody to REC-8 (Pasierbek et al., 2001). In wildtype zygotes whose meiotic chromosomes appeared to be in metaphase I, 74% n=36) had detectable REC-8 clearly on both axes of bivalents (Fig. II.12 A). The other 26% had detectable REC-8 only on the poleward, but not the equatorial, axis of bivalents (Fig. II.12 B). We interpret that the 26% zygotes lacking equatorial REC-8 staining had initiated anaphase I and the removal of REC-8 distal to the chiasmata, but have not separated their homologs. Bivalents in *air-2(RNAi)* zygotes did not align normally at metaphase. Because no polar bodies were extruded in *air-2(RNAi)* zygotes, we could not distinguish the first from the second meiotic attempt. However, in zygotes where bivalents were clearly recognized, 100% had detectable REC-8. This suggests that REC-8 is not degraded in *air-2(RNAi)* zygotes and that its degradation requires AIR-2 activity.

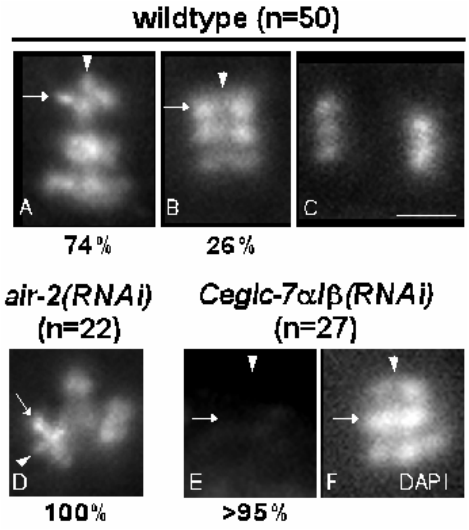

Figure II.11. REC-8 is detectable on both axes of bivalents in *air-2(RNAi)* zygotes. (Reproduced from Rogers et al., 2002.) Representative meiotic chromosomes stained with either REC-8 (A–E) antibody or DAPI (F). (A–C) Wildtype zygotes. In all zygotes examined whose homologues appeared connected, 74% had cross-shaped REC-8 staining as shown in A, whereas 26% had no detectable equatorial staining, similar to that shown in B. A representative REC-8 staining in anaphase is shown in C. (D) 100% of *air-2(RNAi*) embryos with recognizable bivalents had cross-shaped REC-8 staining. (E) 100% of *gsp-1/2(RNAi)* zygotes had very low or no detectable REC-8 staining. The corresponding DAPI staining is shown in F. The number of zygotes imaged and scored is indicated above each panel.

8. AIR-2 phosphorylates the meiotic cohesin REC-8 in vitro

Retention of REC-8 on meiotic chromosomes in *air-2(RNAi)* zygotes suggests that AIR-2 activity is required for the release of chromosomal REC-8 protein. It is possible that AIR-2 phosphorylates REC-8 directly to promote its release. We showed in vitro that recombinant AIR-2 could phosphorylate bacterially expressed REC-8. However, recombinant AIR-2 showed very low or no activity toward two other *C. elegans* Scc1-like molecules, COH-1 and COH-2 (Fig. II.13 A; Pasierbek et al., 2001). No phosphorylation of REC-8 was observed when a kinase-dead version of AIR-2 was used in the assay (Fig. II. 13 B). These results suggest that, in vitro, AIR-2 retains specificity for distinct substrates, such as REC-8.

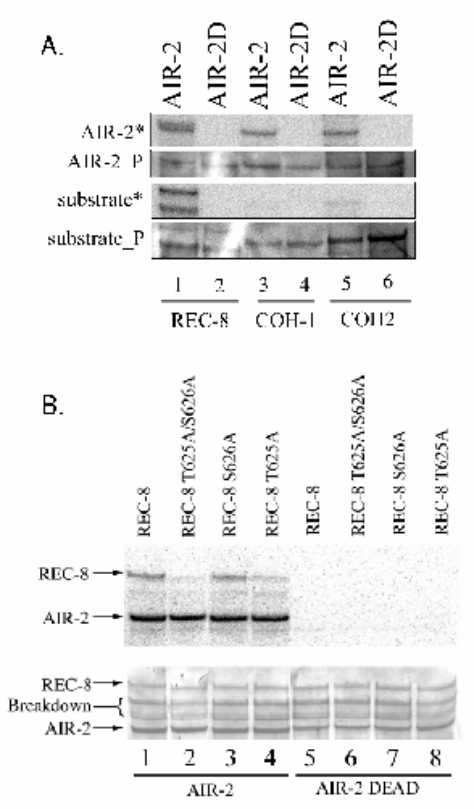

Figure II.12. AIR-2 phosphorylates the meiotic cohesin REC-8 in vitro. (Reproduced from Rogers et al., 2002.) (A) Kinase assays were performed with GST–AIR-2 (AIR-2; lanes 1, 3, and 5) or kinase-dead mutant GST–AIR-2 (AIR-2D; lanes 2, 4, and 6) using GST–REC-8 (lanes 1 and 2), GST–COH-1 (lanes 3 and 4), or GST–COH-2 (lanes 5 and 6) as substrates. Two bands were pulled down with glutathione beads in lanes 1 and 2. The slower migrating band is the full-length REC-8, whereas the faster migrating band is truncated REC-8. AIR-2,* GST–AIR-2 autophosphorylation; AIR-2_P, Ponceau staining of AIR-2 protein; substrate,* phosphorylation of corresponding test substrates; substrate_P, Ponceau staining of each substrate protein. (B) Kinase assays with either wildtype (lanes 1 and 5), T625A (lanes 2 and 6), S626A (lanes 3 and 7), or T625A/S626A (lanes 4 and 8) REC-8 using wildtype AIR-2 (lanes 1–4) or kinase-dead AIR-2 (lanes 5–8). The top half is phosphorimaging, and the bottom half is Ponceau staining. Full-length REC-8, AIR-2, and breakdown products are indicated at the left.

We then mapped the in vitro phosphorylation sites on REC-8 to determine if any specific site(s) was used. We predicted potential phosphorylation sites on REC-8, based on our previous experience with preferred substrate sites for AIR-2 (unpublished data). We mutated the following nine residues to alanines, either individually or in combination: S244, S248,

S244/S248, S395, S396, S395/S396, T625, S626, and T625/S626. We then assayed their phosphorylation by AIR-2.

The T625A and T625A/S626A mutations resulted in a 70% reduction of REC-8 phosphorylation, whereas the other mutations resulted in no significant change in the overall phosphorylation by AIR-2 in vitro (Fig. II.13 B; unpublished data). This result indicates that the majority of phosphorylation occurs on threonine 625. Because we still detected a low level of phosphorylation in T625A and T625A/S626A mutant REC-8, we cannot rule out the possibility that AIR-2 phosphorylates additional, non-preferred sites in our in vitro assay.

It remains to be investigated further whether T625 is phosphorylated by AIR-2 in vivo and whether phosphorylation of REC-8 at T625 facilitates its cleavage by separase. However, the sequence surrounding T625 is intriguing. Threonine 625 is two amino acids away from a consensus site, E-X-X-R, for separase cleavage, where X represents any amino acid. In yeast, phosphorylations that facilitate the cleavage of mitotic cohesion Scc1 have been shown to occur at sites a few residues away from the separase cleavage consensus site E-X-X-R (see Appendix E; Alexandru et al., 2001). Together with the above results, the in vitro kinase data support our hypothesis that AIR-2 promotes the release of chromosome cohesion by phosphorylating REC-8 at specific sub-chromosomal foci.

Recent studies have described requirements for the phosphorylation sites of various Aurora kinases. The human Aurora-B kinase is directed by positively charged residue located at the -2 position, two residues upstream of the phosphorylated residue (Sugiyama et al., 2002). This positively charged residue is most effectively fulfilled by an arginine, such as in histone H3 A-R-K-**S**-T-G-G (Sugiyama et al., 2002). In *S. cerivisae*, the Ipl1/Aurora consensus sequence

was shown to be (R/K)-X-(**T/S**)-(I/L/V), which demonstrates a preference for hydrophobic residues at the +1 position (Cheeseman et al., 2002). The sequence surrounding T625 in REC-8 (R-R-E-**T**-S-I-I) is consistent with this concept. The T625 phosphorylation site contains an arginine at -2 and also two hydrophobic isoleucines at +2 and +3. Also, Ipl1/Aurora was demonstrated to auto-phosphorylate at an important site that is conserved among Aurora kinases (Cheeseman et al., 2002). This conserved site in AIR-2 (T185) or possibly another similar site (T179) might represent the auto-phosphorylation observed in this kinase assay (Fig II. 13A-B). For a comprehensive list of Aurora-B substrates, see Appendix C.

9. NCC-1 is required for AIR-2 localization and histone H3 phosphorylation

Aurora kinases contain conserved activation sites predicted to be direct targets of Cdk1 (Giet and Prigent, 1999; Ubersax et al., 2003). In *C. elegans,* sequence analysis has revealed several homologues of Cdk1; however only one, named NCC-1 (nematode cell cycle), appears to be the primary functional homologue of Cdk1 (Boxem et al., 1999). In *ncc-1(RNAi)* animals, various germline defects, such as delayed oocyte maturation and defective meiotic chromosome segregation, were observed (Boxem et al., 1999). Despite the cell cycle delay, *ncc-1(RNAi)* oocytes eventually progress through oocyte maturation and are fertilized. The resulting *ncc-1(RNAi)* zygotes do not attempt meiosis; no polar bodies are formed, and dead polyploid embryos are observed arrested at the one-cell stage (Boxem et al., 1999). Interestingly, chromosome morphology appears normal in the diakinetic oocytes (Boxem et al., 1999).

Previously it had been reported that *ncc-1* is required for histone H3 phosphorylation in germline stem cells in the distal gonad (Boxem et al., 1999). We asked whether H3P staining in the diakinetic oocytes also requires NCC-1 activity. In *ncc-1(RNAi)* oocytes, we did not detect

any histone H3 phosphorylation. We observed a lack of AIR-2 staining in oocytes as well (Fig. II.14). Although, the lack of chromosome bound AIR-2 might be an indirect result of the delay in oocyte maturation, AIR-2 sometimes was detected in association with the chromosomes prior to oocyte maturation in wildtype. These results suggest that NCC-1/Cdk1 is required during meiosis for the activation of AIR-2, at least to phosphorylate histone H3, and also for the localization of AIR-2 to the bivalents. The appearance of wildtype looking chromosomes in oocytes of *ncc-1(RNAi)* animals again uncouples histone H3 phosphorylation from the process of chromosome condensation during meiosis.

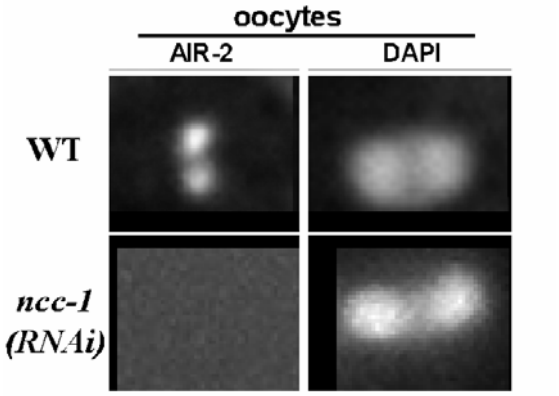

Figure II.13. NCC-1/Cdk1 is required for AIR-2 localization to bivalents. Close-up of individual bivalents taken from –1 oocytes of wildtype (top) and *ncc-1(RNAi)* animals (bottom). AIR-2 staining is shown in the left column, and corresponding DAPI images are shown in the right column.

10. RNAi of GSP-1/2 causes an increase in H3P and chromosome-bound AIR-2

Dr. Rueyling Lin and colleagues showed that two *C. elegans* PP1 phosphatases, GSP-1 and GSP-2, antagonize AIR-2 meiotic activity in vivo (Hsu et al., 2000). Here, we tested whether GSP-1/2 function is required for proper AIR-2 chromosomal localization. In wildtype gonads, AIR-2 was detected temporally in only the most proximal oocyte and spatially only at the chromosome arms distal to chiasmata (Fig. II.15, A, C, and D; Schumacher et al., 1998a).

In *gsp-1/2(RNAi)* animals, there was a striking increase in the number of oocytes containing chromosomal AIR-2 as well as the overall intensity of chromosomal AIR-2 staining. On average, four or five proximal oocytes had detectable chromosomal AIR-2 in *gsp-1/2(RNAi)* animals (Fig. II.15, A and B). The intensity of chromosomal AIR-2 staining always was greatest in the -1 oocyte and decreased in more distal oocytes. In all *gsp-1/2(RNAi)* gonads imaged, 100% had chromosomal AIR-2 in the -2 oocyte at a level equal to or greater than that in a wildtype -1 oocyte (n=30). Interestingly, in 67% of individual oocytes imaged, including all -1 oocytes, AIR-2 staining was detected on both equatorial and poleward axes of at least one bivalent (n=83; Fig. II.15, E and F). Similar results were obtained for BIR-1 and ICP-1 (Fig. II.16**)**

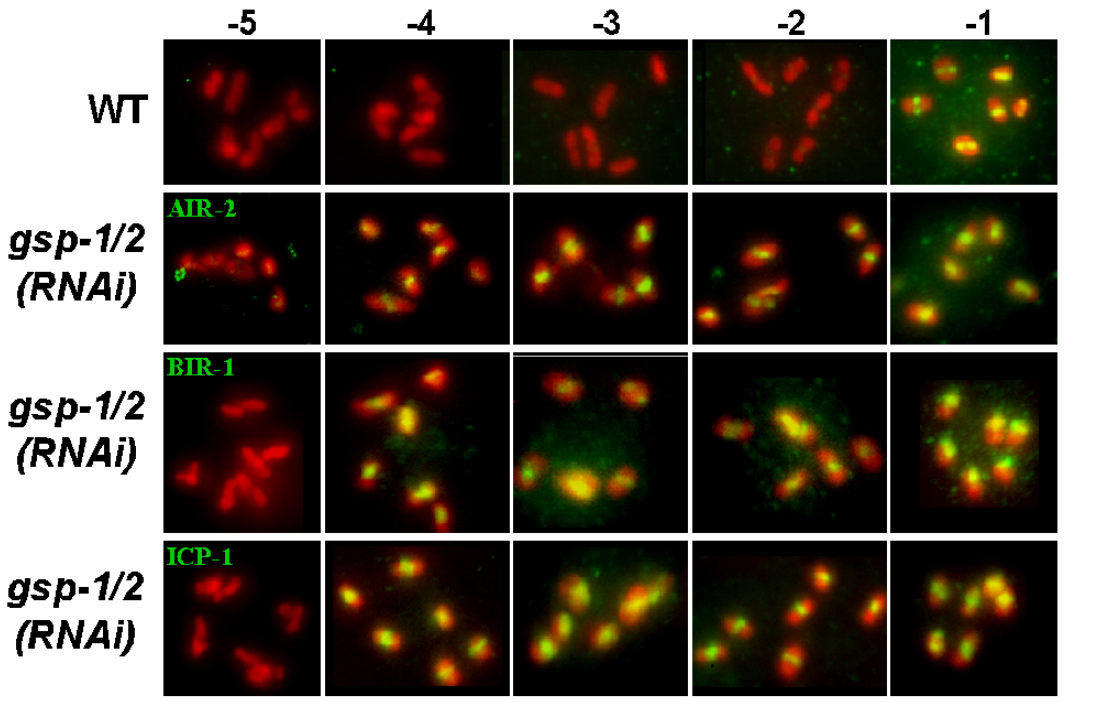

Figure II.14. *gsp-1/2(RNAi)* accelerates ABI localization to chromosomes in –4 oocytes. Merged images of fixed and stained images of proximal gonads from wildtype and gsp-1/2(RNAi) animals. Each image is a cropped oocyte nucleus, and the images are arranged in a line from –5 to –1. Each image in the line is taken from the same gonad. The top line shows a wildtype gonad stained with AIR-2 in green and DAPI in red. The following lines show AIR-2, BIR-1 and ICP-1 in *gsp-1/2(RNAi)* gonads.

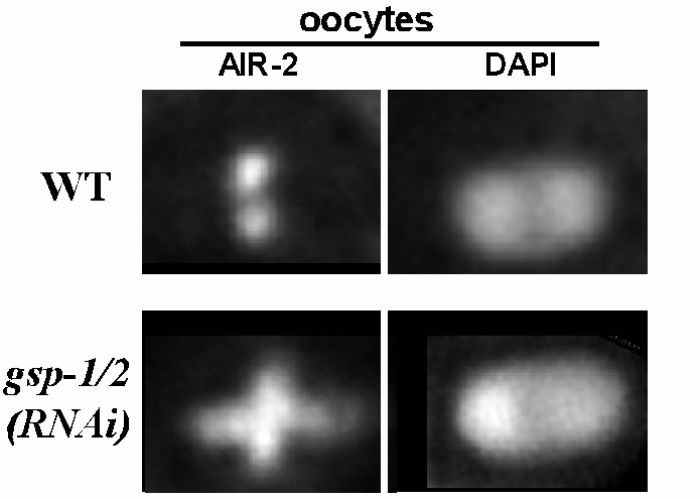

Figure II.15. AIR-2 is mis-localized throughout the bivalents in *gsp-1/2(RNAi)* oocytes. Close-up images of individual bivalents from wildtype and *gsp-1/2(RNAi)* oocytes. AIR-2 staining is shown in the left column, and corresponding DAPI stained images are provided in the right column.

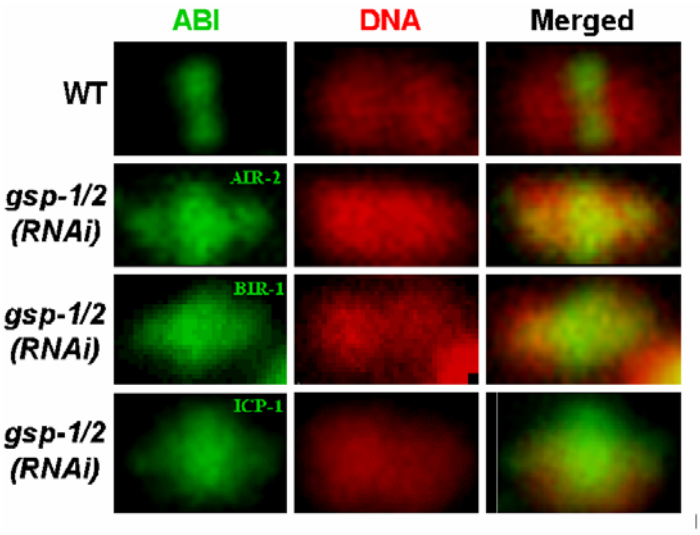

Figure II.16. The ABI complex is mis-localized throughout the bivalents in *gsp-1/2(RNAi)* oocytes. Close-up images of individual bivalents from wildtype (top) or *gsp-1/2(RNAi)* (bottom) –1 oocytes. AIR-2, BIR-1, and ICP-1 staining is shown green in the left column, corresponding DAPI stained images shown in red in the center column, and merged images in the right column.

We conclude from these observations that depletion of GSP-1/2 results in an increase, both spatially and temporally, in chromosomal AIR-2/ABI complex in the gonad. This increase could reflect either an increase in the overall level of AIR-2/ABI complex in the gonad or a redistribution of a greater amount of cytoplasmic AIR-2/ABI complex onto the chromosomes. At present, we cannot distinguish between these two possibilities. Because of the large cytoplasmic volume of oocytes, redistribution of cytoplasmic AIR-2/ABI complex to chromosomal foci could result in a significant increase in the intensity of chromosomal AIR-2 staining. However, the level of cytoplasmic AIR-2/ABI complex is very low, even in wildtype oocytes, making it difficult to determine if it is decreased in the *gsp-1/2* (*RNAi)* animals.

We also asked whether H3P localization is affected by inhibiting GSP1/2. It has been shown that *gsp-1(RNAi)* alone results in an increase in the overall intensity of H3P staining throughout the gonad and in embryos (Hsu et al., 2000). In *gsp-1/2(RNAi)* animals, the temporal pattern of H3P staining appeared similar to wildtype. However, the spatial pattern of H3P along the chromosomes was severely altered by GSP-1/2 RNAi. Almost all -1 oocytes exhibited H3P localized to both equatorial and poleward axes of the bivalents. In younger oocytes, the effect was variable. In *gsp-1/2(RNAi)* oocytes, the mis-localization of H3P to the proximal regions of the chromosome was similar to the mis-localization of AIR-2, although more intense.

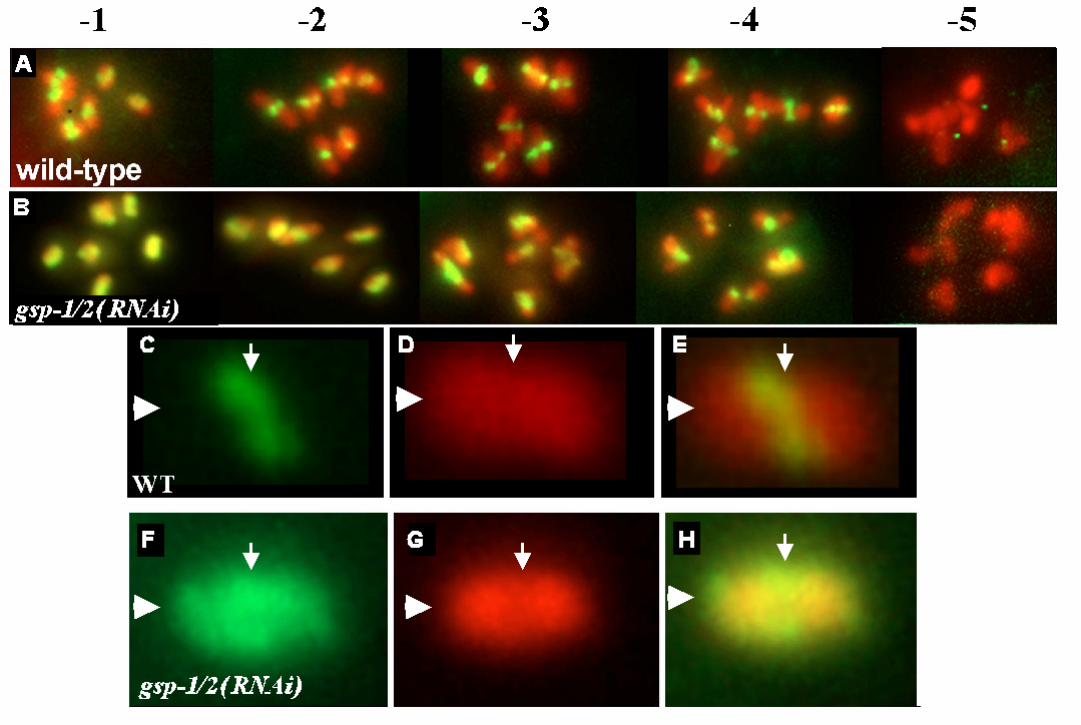

Figure II.17. *gsp-1/2(RNAi)* increases chromosomal H3P. Merged images of fixed and stained oocyte nuclei from either wiltype (A) or *gsp-1/2(RNAi)* (B) gonads. H3P staining is green, and DAPI staining is red. Close-up images of individual bivalents from either wildtype (C-E) or *gsp-1/2(RNAi)* (F-H) occytes. H3P staining is shown in green in the left column, DAPI staining is shown in red in the center column, and merged images in the right column.

These results demonstrate that not only does inhibition of GSP-1/2 result in an increase in the amount of total H3P but that there is also a loss of spatial restriction of H3P to the distal arms of the bivalents. The result is an equivalent amount of H3P on both equatorial and poleward axes of the bivalents in *gsp-1/2(RNAi)* oocytes. The increase in H3P could reflect a loss of global dephosphorylation of histone H3, or alternatively, it might reflect more complex regulation of the distal and proximal axes of the bivalents.

We attempted to produce phenotypes by injecting histone H3 tails into the germline, using an assay similar to the RNAi microinjection technique. We injected peptides corresponding to the N terminus of histone H3 with either (1) wildtype sequence with

phosphorylated serine 10 or (2) mutant sequence with serine 10 changed to alanine. Although this potentially might inhibit AIR-2 or affect a histone H3 related function, no defects were observed (data not shown).

11. RNAi of GSP-1/2 results in a decrease in chromosome-bound REC-8

We then asked whether the chromosomal localization of REC-8 is affected in *gsp-1/2(RNAi)* animals. Out of 80 *gsp-1/2(RNAi)* animals examined, we did not detect a single gonad or embryo that had wildtype REC-8 levels. In fact, 95% of meiotic zygotes had no detectable REC-8 staining at all (n=27; Fig. II.12 E). Although we were unable to detect REC-8, it is likely that a low level of REC-8 is present in *gsp-1/2*(*RNAi)* animals. This is because bivalents remain intact in these oocytes, unlike the case in *rec-8(RNAi)* oocytes where bivalents disintegrate to form single chromatids (Pasierbek et al., 2001). These results suggest that GSP-1/2 play a role in maintaining the steady-state level of chromosomal REC-8, either by increasing its chromosomal localization or by preventing its degradation.

We also asked whether meiotic cohesion is removed prior to the metaphase-anaphase transition. By using temperature-sensitive mutations in subunits of the APC, we are able to arrest meiosis at metaphase I (Golden et al., 2000; Davis et al., 2002). Under permissive conditions, these mutants do not show any noticeable defects. However, upon shifting to non-permissive temperature, the loss of APC function results in meiotic arrest at metaphase. Zygotes were examined at high temperature for REC-8 staining. We did not detect any difference between REC-8 staining in APC mutants and in wildtype (data not shown). This result demonstrates that there is no removal of cohesion prior to separase activation. Therefore, the reduction of REC-8 staining at metaphase in GSP-1/2 RNAi is not the result of an up-regulation

of a prophase-like cohesion removal pathway. Also of note, we did not detect any change in AIR-2/ABI staining or PLK-1 staining in metaphase arrest zygotes.

12. GSP-1/2 RNAi results in the precocious separation of sister chromatids

We then investigated whether meiotic chromosome separation is affected in *gsp-1/2RNAi)* zygotes by performing 4-D imaging. In *gsp-1/2(RNAi)* zygotes, bivalents were observed properly aligned and rotated at metaphase I (Fig. II.18). However, at a time equivalent to the onset of anaphase I and the separation of homologs in wildtype zygotes (Fig. II.18), the bivalents in *gsp-1/2(RNAi)* zygotes dissociated into as many as 24 DNA-staining structures (Fig. II.18). We interpret these small DNA structures to be the 24 individual sister chromatids that normally comprise the six bivalents. In all zygotes imaged (n=23), these small chromosomes never segregated into two groups and no polar bodies were extruded (Fig. II.18).

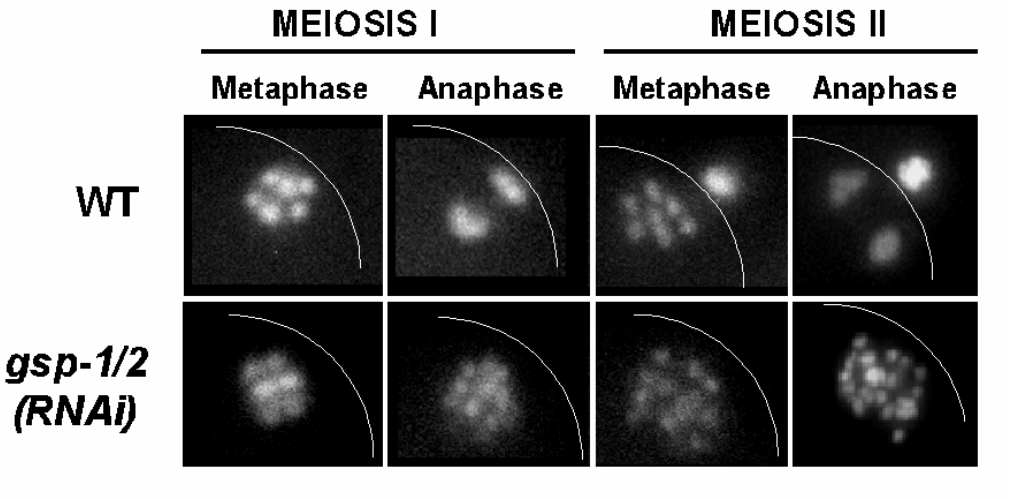

Figure II.18. GSP-2 or GSP-1/2 depletion results in precocious separation of sister chromatids. 4-D imaging of meiotic chromosome behavior. All images presented here are representative slices from each time point except for those of anaphase of meiosis II, which are both projections of multiple slices from the image stack of a single zygote. The edge of the zygote is indicated by a white curve.

We also examined the effects of depleting either GSP-1 or GSP-2 alone. Although GSP-1 and GSP-2 are partially redundant, we observed interesting differences in the contribution of each to the *gsp-1/2(RNAi)* phenotypes. The inhibition of GSP-2 alone displayed a weakly penetrant defect in regulating meiotic chromosome cohesion, similar to *gsp-1/2(RNAi)*. On the other hand, the inhibition of GSP-1 did not produce any meiotic chromosome cohesion defects. Instead, *gsp-1(RNAi)* caused a very penetrant meiotic cytokinesis defect (Fig. II.19). Although homolog separation appeared normal, the first polar body was reabsorbed into the zygote after anaphase I (Fig. II.19). Then 12 dyads were observed, which underwent a relatively normal meiosis II (Fig. II.19). During the second meiotic division cytokinesis was successful, resulting in a single polar body. The resulting embryos from *gsp-1(RNAi)* contained oocyte-derived nuclei with 2C DNA content.

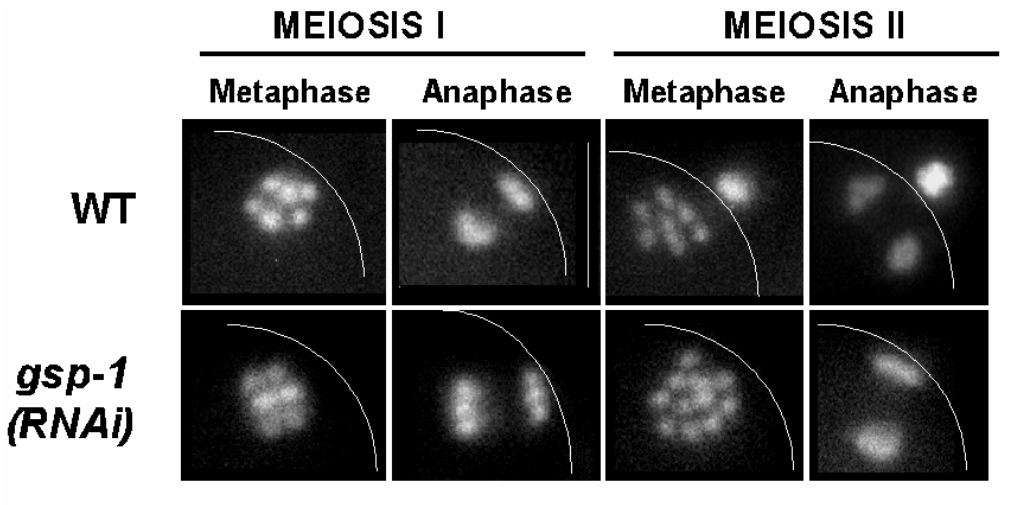

Figure II.19. GSP-1 depletion results in a meiosis I cytokinesis defect. 4-D imaging of meiotic chromosome behavior. All images presented here are representative slices from each time point except for those of anaphase of meiosis II, which are both projections of multiple slices from the image stack of a single zygote. The edge of the zygote is indicated by a white curve.

A comparison of *gsp-1/2(RNAi)* to separase/*sep-1(RNAi)* was made to confirm the timing of chromosome separation in *gsp-1/2(RNAi)*. In *sep-1(RNAi)* zygotes, the six bivalents remained in a metaphase I configuration for an extended period of time (Fig. II.20; Siomos et al., 2001). The homologs did not separate and meiosis I is delayed. Then this was repeated again in meiosis II, where there was no homolog separation as the chromosomes again appeared as if in metaphase I. In *gsp-1/2(RNAi); sep-1(RNAi)* zygotes, the chromosome behavior was similar to *sep-1(RNAi)* (Fig. II.20; Kaitna et al., 2002). These results demonstrate that separase is required for the precocious chromosome separation observed in *gsp-1/2(RNAi)* zygotes. These results also imply the regulation of chromosome cohesion is relatively normal until metaphase in *gsp-1/2(RNAi)* worms.

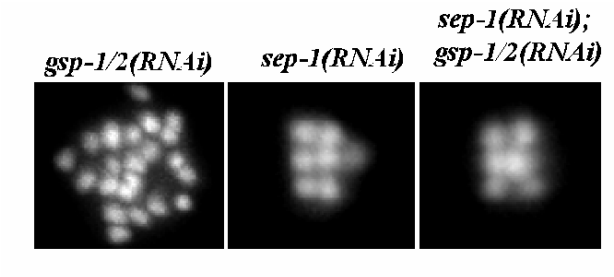

Figure II.20. *sep-1* is required for precocious chromosome separation in *gsp-1/2(RNAi)*. All images presented here are from zygotes fixed and then stained with DAPI. Representative images of metaphase are shown for each of the three mutants.

These results indicate that GSP-1/2 are required for the proper regulation of cohesion release during the metaphase I-anaphase I transition. This is likely a result of the reduced chromosomal REC-8 protein observed on both the equatorial and poleward axes of bivalents in *gsp-1/2(RNAi)* zygotes (Fig. II.12 E). This GSP-1/2 RNAi phenotype is dependent on AIR-2 activity, because *gsp-1/2(RNAi); air-2(RNAi)* zygotes are indistinguishable from *air-2(RNAi)* zygotes with respect to meiotic chromosome segregation (unpublished data). The dependence of *gsp-1/2(RNAi)* phenotype on AIR-2 activity suggests that GSP-1/2 might regulate the level of

chromosomal REC-8 by opposing AIR-2 activity toward the phosphorylation of REC-8. Alternatively, GSP-1/2 might regulate the level of chromosomal REC-8 simply by restricting chromosomal localization of AIR-2.

13. A *spo-11* mutant does not allow homolog segregation in *air-2(RNAi)* zygotes

If the only function of AIR-2 in meiosis is to release chromosome cohesion, the chromosome segregation defect in meiosis I should be suppressible by mutations in which homologs are not recombined to form chiasmata in the first place. Spo11 is required for the initiation of recombination during meiosis. Therefore in *spo-11* mutants, homologs do not become linked and oocytes contain 12 univalents.

We have made several attempts to suppress the *air-2(RNAi)* meiosis I phenotype with a mutation in *spo-11(ok79)* without success. In *spo-11(ok79); air-2(RNAi)* zygotes, the 12 univalents behaved similar to the six bivalents in *air-2(RNAi)* alone. In anaphase I of *spo-11(ok79); air-2(RNAi)* zygotes, the 12 univalents all moved together as one mass and were pressed against the outer membrane (Fig. II.21). Most importantly, in the period post-anaphase I but pre-anaphase II, all 12 univalents could be seen clearly in a single group (Fig. II.21). We performed serial dilution the of AIR-2 dsRNA for injection in attempt to titrate the *air-2(RNAi)* effect. However, all dilutions which affected wildtype meiosis had a similar effect on *spo-11(ok79)*, including a 1:300 dilution (approximately 10ng/μL). These results suggest that AIR-2 functions during meiosis in other processes, aside from its function in the separation of homologs.

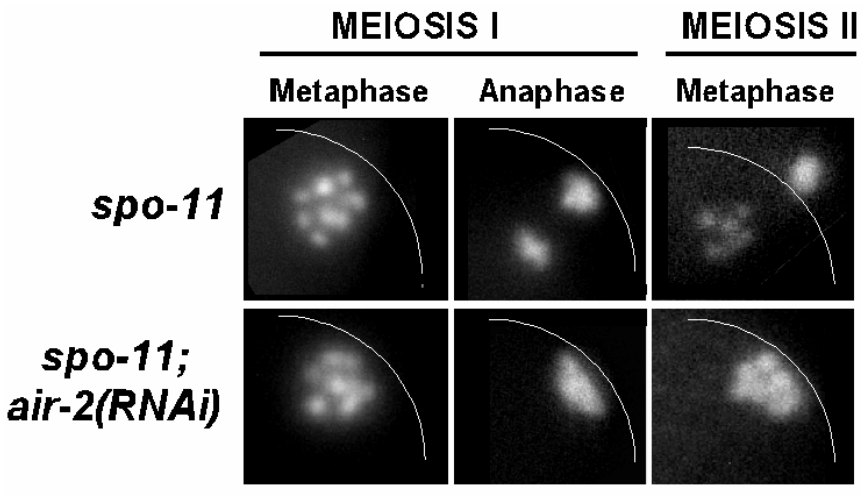

Figure II.21. *spo-11* does not suppress the failure of homolog separation in *air-2(RNAi)*. 4-D imaging of meiotic chromosome behavior. All images presented here are representative slices from each time point. The edge of the embryo is indicated by a white curve. In *spo-11* zygotes, univalent chromosomes randomly segregate during meiosis I; however, in *spo-11; air-2(RNAi)* zygotes, there is no evidence of chromosome segregation.

C. Discussion: The Aurora-B kinase AIR-2 regulates the selective release of chromosome cohesion during meiosis

Meiotic cells must ensure proper chromosome segregation during each cell division in order to form the immortal reproductive cycle. Without the careful regulation of chromosome segregation, meiotic cells could not maintain a constant chromosome number and sexual reproduction would not be possible.

We describe here the finding that the aurora-B kinase AIR-2 promotes the release of meiotic chromosome cohesion in *C. elegans*, likely through phosphorylation of the meiotic cohesin REC-8. Depletion of AIR-2 results in failure of chromosome separation during anaphase I and II. AIR-2 localizes to sub-chromosomal foci corresponding to the point of

contact between separating chromosomes in metaphase I and II, and the release of REC-8 from meiotic chromosomes depends on AIR-2 activity. In *vitro*, AIR-2 phosphorylates REC-8 at a specific amino acid. We also show that the GSP-1/2 phosphatases antagonize AIR-2 activity in chromosomal cohesion release, likely by restricting AIR-2 localization. In *gsp-1/2(RNAi)* zygotes, chromosomal AIR-2 is elevated, chromosomal REC-8 is decreased, and sister chromatids separate precociously at anaphase I. We propose that AIR-2 promotes the release of chromosome cohesion via phosphorylation of REC-8 at specific chromosomal locations and that GSP-1/2, directly or indirectly, antagonize AIR-2 activity.

1. Regulation of chromosome cohesion release in meiosis

Our observation that inhibition of AIR-2 and histone H3 phosphorylation does not affect chromosome condensation during meiosis led us to ask about the role of AIR-2 doing during meiosis. We found that AIR-2 promotes the release of meiotic chromosome cohesion in *C. elegans*, likely through phosphorylation of the meiotic cohesin REC-8. Depletion of AIR-2 results in failure of chromosome separation during anaphase I and II. AIR-2 localizes to sub-chromosomal foci corresponding to the point of contact between separating chromosomes in metaphase I and II, and the release of REC-8 from meiotic chromosomes depends on AIR-2 activity. In vitro, AIR-2 phosphorylates REC-8 at a specific amino acid, Thr625.

We also show that the GSP-1/2 phosphatases antagonize AIR-2 activity in chromosomal cohesion release, likely by restricting AIR-2 localization. In *gsp-1/2RNAi)* embryos, chromosomal AIR-2 is elevated, chromosomal REC-8 is decreased, and sister chromatids separate precociously at anaphase I. We propose that AIR-2 promotes the release of

chromosome cohesion via phosphorylation of REC-8 at specific chromosomal locations and that GSP-1/2, directly or indirectly, antagonize AIR-2 activity.

a. Aurora-B kinase functions during meiosis

Although mitotic and meiotic cell divisions share many features in common, they are very different with respect to chromosome behavior. Here we propose a novel mechanism for the selective release of chromosome cohesion during meiosis I and discuss how this regulation results from crossing over in *C. elegans* (Fig. II.22). Our results strongly support a model whereby the selective release of cohesion during meiosis I is, in part, regulated by selective localization of AIR-2 at chromosome arms distal to chiasmata, which results in the subsequent phosphorylation of REC-8 at these sites.

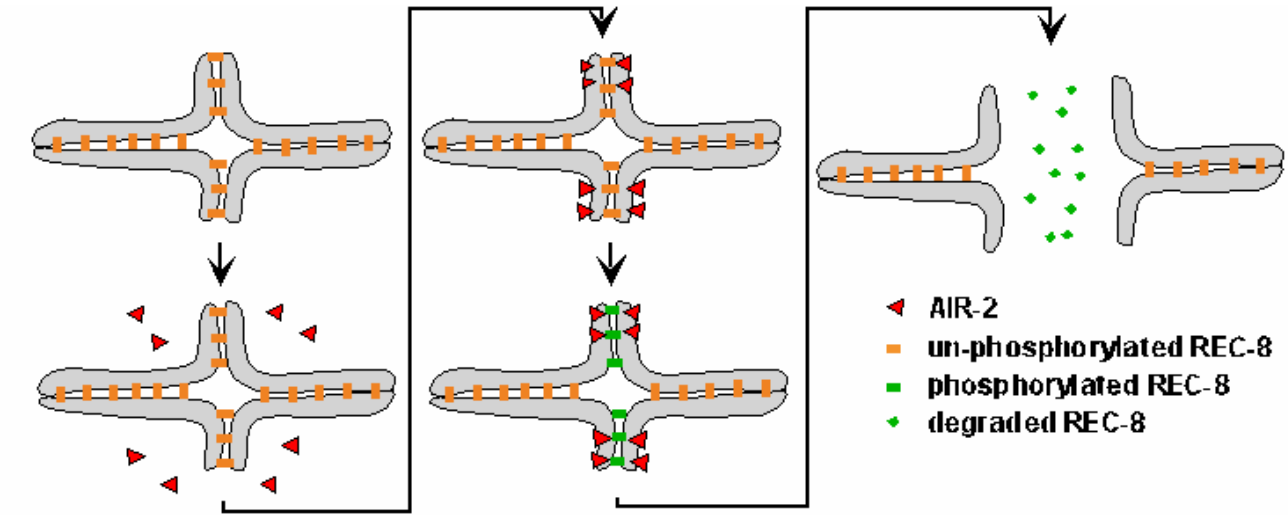

Figure II.22. Model for how AIR-2 regulates the selective release of chromosome cohesion. (Reproduced from Rogers et al., 2002.) A schematic model for how AIR-2 regulates the release of cohesion in meiosis I. Orange bar, unphosphorylated REC-8; red triangle, AIR-2; green bar, phosphorylated REC-8; and small green diamonds, degraded REC-8.

Cohesion between sister chromatids distal to chiasmata has been proposed to be responsible for holding homologs together (Maguire, 1974; Carpenter, 1994). Nasmyth and colleagues have further demonstrated that release of cohesion is only required for chromosome segregation in meiosis I if homologs recombine and form chiasmata (Buonomo et al., 2000). Therefore, the observed sub-chromosomal location of AIR-2 in metaphase I puts it at the right place and time for a function in selective release of a subset of cohesins responsible for holding homologs together.

In addition, we show that the sub-chromosomal AIR-2 localization in meiosis I is dependent on chiasmata formation and that release of REC-8 from meiotic chromosomes requires AIR-2 activity. REC-8 remains between chromosomal arms distal to chiasmata in *air-2(RNAi)* embryos. Finally, our in vitro data that AIR-2 phosphorylates REC-8 strongly suggest that AIR-2 functions through the phosphorylation of REC-8. Studies of yeast previously demonstrated Rec8 to be a phospho-protein in vivo, but the kinase responsible for this was unknown (Parisi et al., 1999; Watanabe and Nurse, 1999). Recent evidence demonstrates that Rec8 is phosphorylated in vivo in mammals as well (Lee et al., 2003). Based on our data, it is quite possible that Aurora kinases represent at least one group of Rec8 kinases.

Currently, we are unable to directly verify this model. To our knowledge, no one has been able to reconstitute a separase activity using the *C. elegans* separase. We attempted to monitor ceREC-8 cleavage by separase using a human separase assay; however, we found that ceREC-8 is not cleaved by human separase.

The fact that, in *S. cerivisae*, Scc1 cannot substitute for Rec8 during meiosis suggests an important difference between meiotic cohesions and mitotic cohesions (Toth et al., 2000). Scc1

can provide sister chromatid cohesion and monopolar attachment during meiosis I in *S. cerivisae* but cannot resist separase in the vicinity of centromeres. Rec8 clearly possesses special properties, which are lacking in Scc1 that enable it to be protected from separase in the vicinity of centromeres. Because Rec8, but not Scc1, is protected from degradation at meiosis I, the retention of proximal cohesion cannot be due to the general shielding of proximal cohesin complexes from access or modification by enzymes like separase, Aurora kinases, or Polo kinases.

b. Other models for the selective release of chromosome cohesion during meiosis

In the scientific literature, several other studies have recently attempted to answer similar questions about the step-wise release of Rec8 during meiosis. These studies most often involve studies of mutants in budding and fission yeast. Several mutants, such as Bub1, Slk19, Spo12, Spo13, and Sgo1, display precocious Rec8 removal during meiosis I. In Bub1 mutants, Rec8 is lost at centromeres during meiosis I; however, mono-orientation of sister kinetochores occurs normally. In yeast, Spo12, Spo13, Slk19, and Sgo1 all are required for mono-orientation and for protecting proximal chromosome cohesion. Of all these factors, only Spo13 and Sgo1 are specific to meiotic cells.

(1) Spo13 might function as a protector of proximal Rec8

Recent research has shown that the presence of Spo13 might be sufficient to protect Rec8 during mitotic growth in *S. cerivisae* (Lee et al., 2002; Shonn et al., 2002). Mis-expression of Spo13 transiently delays securin degradation and separase cleavage of cohesins. Also, mis-expression of Spo13 can prevent degradation of Scc1/Rec8 in the absence of securin. This inhibition can be

overcome by the over-expression of separase, which results in efficient Scc1/Rec8 cleavage in the presence of extra Spo13. The molecular mechanism of Spo13's ability to shield Rec8 is not clear, as Spo13 does not physically associate with Rec8 (Lee et al., 2002; Shonn et al., 2002). Homologues of Spo13 have yet to be identified in other species.

(2) Sgo1/Mei-S332 might function as a general protector of Rec8

Recently several reports have identified homologues of Mei-S322 from *Drosophila*. This factor has been named Shugoshin (Sgo1), which means "guardian spirit" in Japanese. Sgo1/Mei-S332 might be a general regulator of meiosis I in many organisms (Katis et al., 2004; Kitajima et al., 2004; Rabitsch et al., 2004; Salic et al., 2004). Sgo1/MEI-S332 associates with chromosomes in the proximity of the kinetochores from prometaphase I until the onset of anaphase II. In its absence, bivalents disjoin normally at anaphase I, but sister chromatids soon thereafter separate and mis-segregate at meiosis II. Sgo1/MEI-S332 behaves in similar fashion during mitotic divisions, associating with centromeric chromatin during prometaphase and dissociating at anaphase. It is not, however, required for chromosome segregation during mitosis. Sgo1/MEI-S332's absence from chromosomes until prometaphase suggests that it is not itself part of the sister chromatid cohesion apparatus. Moreover, its presence on chromosomes during metaphase II and during mitotic metaphases implies that it does not directly protect centromeric cohesion from its imminent destruction. Though present on chromosomes during both metaphase I and metaphase II, Sgo1/MEI-S332 protects cohesion only at the onset of anaphase I or during a short period thereafter.

(3) The utilization of different cohesin subtypes during meiosis

The existence of cohesin complexes composed of different subunits during meiosis in fission yeast and vertebrates suggest another potential mechanism for regulating the differential release of chromosome cohesion in meiosis (Prieto et al., 2001; Prieto et al., 2002; Kitajima et al., 2003a; Kitajima et al., 2003b). In *S. pombe*, there is preliminary evidence that different cohesin subtypes not only distribute to different locations on the chromosomes but also perform different functions (Kitajima et al., 2003a; Kitajima et al., 2003b). In *S. pombe*, cohesin complexes containing Psc3 bind sister chromatids together until meiosis II, and Psc3 is required for mono-oreintation during meiosis I. On the other hand, Rec11 cohesin complexes are removed from chromosome arms during meiosis I (Kitajima et al., 2003a; Kitajima et al., 2003b). Also, it is possible that different cohesin subtypes are targeted by kinases, such as Aurora-B and Polo kinases, either at different sub-chromosomal locations or at different times during meiosis.

a. Possible roles of Aurora-B and ABI/passenger complex in meosis

We have shown that AIR-2 likely phosphorylates REC-8 and promotes the release of chromosome cohesion during meiosis. This finding, combined with two previous findings – that AIR-2 functions in cytokinesis (Schumacher et al., 1998a; Severson et al., 2000) and AIR-2 is part of the chromosome passenger protein complex (Kaitna et al., 2000; Adams et al., 2000; Adams et al., 2001a) – suggests that the Aurora-B/ABI complex might play a critical role in coordinating chromosome separation and cytokinesis during cell division.

Recent studies have shown how the other components of the ABI complex might function. Both Survivin and INCENP now have been shown to be substrates of Aurora-B and

potentially can stimulate Aurora-B kinase activity (Kim et al., 1999; Bishop and Schumacher, 2002; Cheeseman et al., 2002; Honda et al., 2003). In both *Xenopus* and human cells, survivin is capable of binding Aurora-B and enhancing its kinase activity in vitro, although in *S. cerivisae* and *C. elegans*, Bir1/Survivin was reported to lack this capability in similar assays (Bolton et al., 2002; Bishop and Schumacher, 2002; Cheeseman et al., 2002; Honda et al., 2003). In *C. elegans*, ICP-1/INCENP clearly has the ability to stimulate AIR-2 kinase activity in vitro (Bishop and Schumacher, 2002; Honda et al., 2003). This stimulation requires the phosphorylation of ICP-1/INCENP by AIR-2 (Bishop and Schumacher, 2002; Honda et al., 2003).

These results suggest the function of survivin and INCENP are not only to localize AIR-2 to specific sub-cellular sites but also to stimulate kinase activity. This dual role of survivin and INCENP in both localization and activation of Aurora-B might provide important regulatory control of Aurora-B kinase activity. The observation that in *C. elegans* meiosis, histone H3 phosphorylation is delayed until the appearance of AIR-2/ABI localization might represent the function of BIR-1/survivin and ICP-1/INCENP in both the activation and localization of AIR-2 to the chromosome in order to target another substrate aside from histone H3.

Recently new components of the ABI complex have been discovered that might eventually lead to further understanding of the function of the passenger complex. In *C. elegans*, a novel factor called CSC-1 associates with BIR-1/survivin and ICP-1/INCENP (Romano et al, 2003). In vertebrates, three factors − Borealin, Dasra A, and Dasra B − recently have been described as new components of the ABI complex during mitosis (Gassmann et al., 2004;

Sampath et al., 2004). Unfortunately, identification of these new components has not revealed insight into the molecular functions of the ABI/passenger complex.

New information has been gained about ABI complex/passenger protein behavior. It appears that degradation of cyclin-B and down-regulation of Cdk1 play a critical role in the transit of Aurora-B from the chromosome to the spindle during mitosis (Murata-Hori et al., 2002a; Parry et al., 2003). Presumably, the ABI complex must transit to the spindle to coordinate cytokinesis with chromosome separation and entry into anaphase. This function might be accomplished by the ability of ABI complexes to travel along microtubules to different locations within the cell, such as towards the cell cortex (Murata-Hori and Wang, 2002b). This ability might allow the ABI complex to provide communication between the spindle and the cortex during anaphase. Theories that cytokinesis is regulated directly by the central spindle is supported by another recent study (Alsop and Zhang, 2003).

b. Unexplained functions of AIR-2 in *C. elegans* meiosis

Two observations suggest that AIR-2 has an additional, but poorly defined, role in meiotic chromosome segregation in *C. elegans*. First, if the sole function of AIR-2 in meiosis is to release chromosome cohesion, the chromosome segregation defect in meiosis I should be suppressible by mutations in which homologues are not recombined to form chiasmata in the first place. To no avail, we have made several attempts to suppress the *air-2(RNAi)* meiosis I phenotype with a mutation in *spo-11*. This suggests that, in addition to the release of chromosome cohesion, AIR-2 likely has another role in meiotic chromosome segregation. Similar attempts to suppress separase *sep-1(RNAi)* with *spo-11* mutations in *C. elegans* were reported to be unsuccessful (Siomos et al., 2001). This failure might be caused by the

requirement for either AIR-2 or separase for other essential functions. In particular, separase might be required in *C. elegans* for proper spindle stability during anaphase. Similarly, AIR-2 might play similar roles in regulating the anaphase spindle.

Second, at anaphase, all chromosomes in *air-2(RNAi)* embryos consistently move toward and are pressed against the edge of the embryo, as if a force from one spindle pole is overwhelming any forces from the other. Why this happens is unclear. This is interesting in light of a similar monopolar spindle phenotype observed in *Drosophila aurora* mutant embryos and Aurora-A depleted *Xenopus* oocytes (Glover et al., 1995; Giet et al., 1999). However, because bivalents in *air-2(RNAi)* embryos do not align normally at metaphase, it is difficult to interpret spindle phenotypes at or after metaphase.

The monopolar movement of homologs in *air-2(RNAi)* embryos is suggestive of spindle and/or kinetochore abnormalities. Similarly, the failure of bivalent alignment at metaphase in *air-2(RNAi)* embryos is suggestive of spindle and/or kinetochore abnormalities. Several recent studies have indicated that Aurora-B kinases are involved in regulating spindle attachments to the kinetochore at least in the context of mitosis. In human cells, Aurora-B is required for bi-orientation of the kinetochore and might function in a cell-cycle checkpoint in order to halt the cell cycle in response to incorrect kinetochore attachments (Ditchfield et al., 2003; Hauf et al., 2003; Straight et al., 2003; Lampson et al., 2004). Over-expression of a kinase-dead Aurora-B in mammalian cells perturbed the spindle attachment checkpoint and caused Mad2, dynenin, and CENP-E to be lost from kinetochores (Murata-Hori and Wang, 2002a). Similar observations were made in yeast species. In *S. pombe*, Aurora/Ark1 mutants show mis-regulation of Mad2 and defects in the spindle attachment checkpoint (Petersen and Hagan, 2003). In *S. cerivisae*,

Ipl1/Aurora was implicated in a mitotic spindle assembly checkpoint that senses tension (Biggins and Murray, 2001; Li et al., 2002).

Further research strongly supports a role for Ipl1/Aurora in regulating kinetochore attachments in order to achieve bi-orientation during mitosis (Cheeseman et al., 2002; Tanaka et al., 2002; Dewar et al., 2004). Also, recent work has implicated regulatory connections between the ABI complex and separase in *S. cerivisae*; however, the exact nature of this connection is unclear (Pereira and Schiebel, 2003). The abundance of continuing research on Aurora-B kinases likely will further understanding of Aurora-B kinases in the near future.

We observed defects in bivalent alignment during meiosis I in *air-2(RNAi)* zygotes. This phenotype cannot be explained by any evidence we have gathered, but this observation does suggest a role for AIR-2 in kinetochore attachments and/or spindle functions. In the 4-D analysis, *air-2(RNAi)* chromosomes have kinetic behavior, which presumably means that the kinetochores are attached to the meiotic spindle. However, the aberrant behavior of the bivalents at metaphase and anaphase in both *air-2(RNAi)* and in *air-2(RNAi); spo-11* zygotes, suggests the nature of the kinetochore-spindle attachments might be defective.

Currently, little is known about the spindle checkpoint in *C. elegans*. The antibody staining pattern of the spindle checkpoint factor, BUB-1, has been described (Oegema et al, 2001). In order to investigate checkpoint activation in relation to AIR-2 function, we examined the localization of BUB-1 in *air-2(RNAi)* embryos. Although we did not notice in obvious defect in BUB-1 localization during meiosis, the localization of BUB-1 in mitosis in *air-2(RNAi)* embryos (data not shown) resembled the localization of BUB-1 upon checkpoint activation by nocodazole treatment or anoxia (Stear and Roth, 2004). This result demonstrates *air-2(RNAi)*

activates the spindle checkpoint during mitosis, but currently there is no evidence of AIR-2 perturbing any checkpoints during meiosis.

Recently evidence has been acquired that Aurora-B kinases are involved directly with the kinesin related factor MCAK. MCAK is required for proper chromosome alignment and kinetochore-spindle attachments. Several studies implicate Aurora-B might co-localize with and phosphorylate MCAK in order to regulate chromosome congression in vertebrate cells (Ohi et al., 2003; Andrews et al., 2004; Lan et al., 2004; Ohi et al., 2004). Defects in MCAK function might explain the failure of homolog alignment in *air-2(RNAi)* zygotes and might also be involved in the monopolar spindle behavior at anaphase. Unfortunately, we have no evidence to support this hypothesis.

2. Histone H3 phosphorylation during cell division

According to the results of this thesis, the role of histone H3 phosphorylation during meiosis in *C. elegans* is unclear. Previous studies suggested the function of histone H3 phosphorylation during cell division was in the process of chromosome condensation (Wei et al., 1999; Hsu et al., 2000). Instead, we observed that phosphorylation occurs primarily in the short arms distal to the chiasmata in diakinetic oocytes. Although this correlates temporally with chromosome condensation, we observed no defect in chromosome condensation when histone H3 phosphorylation was blocked by *air-2(RNAi)*. The finding that AIR-2 is not required for chromosome condensation during meiosis is not altogether surprising in light of recent reports. Several studies also suggest Aurora kinases and histone H3 phosphorylation are dispensable for chromosome condensation during either mitosis or meiosis (Cobb et al., 1999a; Cobb et al., 1999b; Lavoie et al., 2002; Losada et al., 2002; Sumara et al., 2002).

There is a growing amount of evidence against histone H3 phosphorylation playing an essential role in chromosome condensation. In *Drosophila*, the inhibition of Aurora-B, which abolishes histone H3 phosphorylation, did not appear to perturb condensin recruitment and chromosome condensation (Giet and Glover, 2001). Also, the artificial induction of histone H3 phosphorylation was not sufficient to recruit condensins to the chromosome (Murnion et al., 2000). Similarly, experiments using in vitro extracts from *Xenopus* found no relationship between histone H3 phosphorylation and condensin function (Kimura and Hirano, 2000; MacCullum et al., 2002). The most persuasive result is the observation that condensin complexes can interact with nucleosomes composed of histone H3 that completely lack the N terminus (de la Barre, et al., 2001). Also the recent development of Aurora-B kinase inhibitors has shown inhibition of Aurora-B in human cells does not have a great effect on chromosome condensation (Ditchfield et al., 2003; Hauf et al., 2003). Instead, defects in spindle-kinetochore interactions, cell cycle checkpoints and cytokinesis were observed.

a. Histone H3 phosphorylation might facilitate the localization of AIR-2

One possible explanation for the role of histone H3 phosphorylation is to target the future binding of AIR-2 to specific sub-chromosomal sites. In all situations examined, the location of H3P presages the localization of AIR-2. In wildtype worms, H3P marks the distal arms in -4 oocytes about 1 hr before AIR-2 localizes to the same site in the -1 oocyte. Clearly, AIR-2 is able to phosphorylate histone H3 without stably associating with the chromosomes. In *ncc-1(RNAi)* animals, both H3P and AIR-2 are absent from the chromosome. In *gsp-1/2 (RNAi)* worms, the timing of histone H3 phosphorylation appears accelerated, resulting in a premature increase in overall histone H3 phosphorylation. Also, *gsp-1/2 (RNAi)* worms display an ectopic

appearance of histone H3 phosphorylation at more proximal regions of the bivalent, albeit at lower levels and delayed relative to distal regions. Correlated with the acceleration in histone H3 phosphorylation is the premature localization of AIR-2/ABI complex to the chromosomes and the ectopic localization of AIR-2/ABI complex to more proximal regions of the chromosomes, as presaged by the detection of histone H3 phosphorylation.

The localization of histone H3 phosphorylation presages where AIR-2/ABI complex will localize later during the cell cycle. This observation suggests that histone H3 phosphorylation might be involved in the localization of AIR-2/ABI complex to these chromosomal sites. A speculative model to propose is that although the AIR-2/ABI complex is initially only transiently associated with the chromosomes for this modification, as histone H3 phosphorylation increases, this somehow facilitates the stable recruitment of the AIR-2/ABI complex at these sites. Presumably at first, the AIR-2/ABI complex is associating transiently with the histone H3 tails in the distal chromosome arms. Then later, after some unknown event, the AIR-2/ABI complex begins to stably associate in these same chromosomal regions. In this model, the localization of AIR-2 to the chromosome is most likely for another purpose because histone H3 is already heavily phosphorylated. Presumably, AIR-2 is targeted to the distal arms to specifically phosphorylate another target, such as REC-8. How histone H3 phosphorylation might stabilize AIR-2/ABI complex binding to the chromosome is unknown. One possibility is that, once the amount of histone H3 phosphorylation reaches some threshold, this allows the AIR-2/ABI complex to be stabilized at those chromosomal regions. Also, more complex interactions between AIR-2, BIR-1, and ICP-1 might be involved.

b. Histone H3 phosphorylation might serve to 'open' the chromatin

Previously two potential functions for histone H3 phosphorylation have been offered. First, it has been suggested for a long time that histone H3 phosphorylation might alter the interactions of the nucleosomes with DNA. Phosphorylation of the histone H3 tail both increases its negative charge and might affect the secondary structure of the tail (Baneres et al., 1997; Mutskov et al., 1998; Sauve et al., 1999). Second, histone H3 phosphorylation might serve as a signal platform to recruit other factors, such as condensin complexes, to the chromatin (Wei et al., 1999). This concept is similar to the histone code model that is based on different factors recognizing the modifications on the N terminal tails of core histones. The histone code model is that combinations of histone acetylation, methylation, and phosphorylation of the core histone tails serve as binding sites to be recognized by transcriptional regulators (Jenuwein and Allis, 2000; Nowak and Corces, 2003).

The pattern of histone H3 phosphorylation during meiosis suggests another model. We observed histone H3 phosphorylation along the inner faces of dividing chromosomes. We also observed a correlation between defects in chromosome separation and the absence of histone H3 phosphorylation. Based on these observations, I favor a model in which histone H3 phosphorylation alters the structure of the chromatin to facilitate the separation of chromosomes. This function appears to begin in prophase, prior to division, at a time that is consistent with other localization studies (Kaszas and Cande, 2000). In this model, an unknown histone H3 phosphorylation-based mechanism would result in the 'loosening' or 'opening' of the chromatin to either enable the untangling of DNA linkages or to facilitate access to cohesins by enzymes such as kinases and separases. Alternatively, histone H3 phosphorylation might serve as a

docking site to recruit factors, such as kinases, topoisomerases, or separase. However, with so little evidence available, these possibilities are highly speculative.

c. Function of GSP-1 and GSP-2 phosphatases

The involvement of GSP-1/2 in the regulation of meiotic chromosome cohesion release is demonstrated best by our observation that, in *gsp-1/2*(*RNAi)* embryos, sister chromatids separate precociously at the onset of anaphase I. In addition, GSP-1/2 might function in the maintenance of chromosomal REC-8 before anaphase, given that we observed a dramatic decrease in the level of chromosomal REC-8 throughout the gonad in *gsp-1/2(RNAi)* animals. However, no phenotype was observed in *gsp-1/2(RNAi)* worms before the onset of anaphase I. It is possible that a decrease in chromosomal REC-8 is detrimental only when combined with a high separase activity in anaphase. Alternatively, this might be caused by incomplete penetrance of the *gsp-1/2(RNAi)* effect.

We believe that the function of GSP-1/2 in the regulation of meiotic cohesion is mediated through AIR-2. However, we cannot rule out the additional involvement of an AIR-2–independent mechanism. It is possible that GSP-1/2 antagonizes AIR-2 by: (a) directly inhibiting its activity, (b) restricting its chromosomal localization, (c) dephosphorylating REC-8, or (d) a combination of the preceding three mechanisms.

The first possibility is supported by recent work (Murnion et al., 2000) showing that a recombinant human PP1 phosphatase can inhibit directly the activity of *Xenopus* Aurora-B kinase in embryo extracts. Recent evidence suggests all Aurora kinases contain conserved domains that confer direct PP1 binding (Sugiyama et al., 2002). The second possibility is

supported by our observation of ectopic chromosomal AIR-2 in *gsp-1/2*(*RNAi)* animals. Alternatively, the *gsp-1/2(RNAi)* phenotype could be the result of a chromosome-wide increase in the phosphorylation of histone H3, a previously identified substrate for Aurora-B kinases and Glc7 phosphatases (Hsu et al., 2000). It is possible that an increase in phosphorylated histone H3 causes a change in chromatin organization that facilitates either the accessibility of AIR-2 to phosphorylate REC-8 or the accessibility of separase to degrade REC-8 (Fig. II.23). Because the functions of Aurora-B kinases and Glc7 phosphatases are essential for meiotic divisions in a variety of species, it is an intriguing possibility that they might play a role in the release of meiotic chromosome cohesion across diverse species.

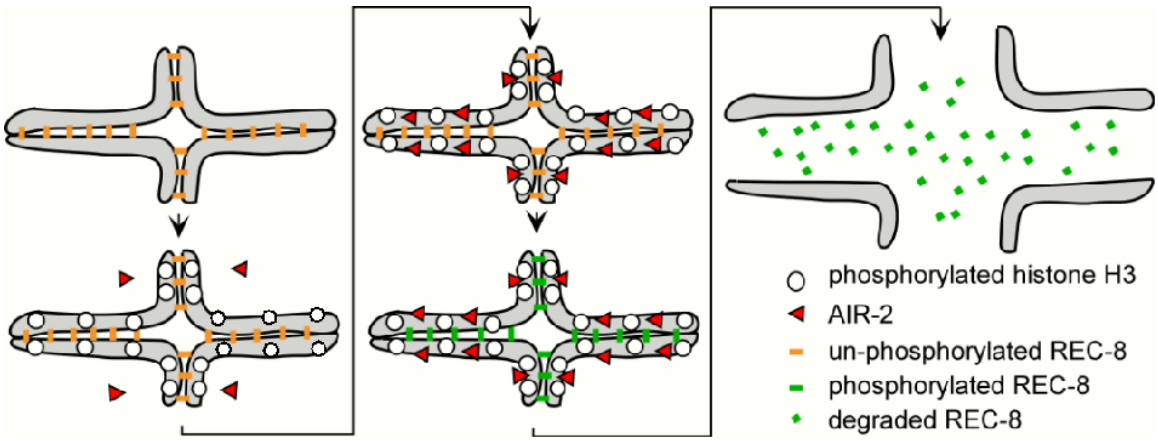

Figure II.23. Model for the mis-regulation of H3P, AIR-2, and REC-8 in *gsp-1/2(RNAi).* A schematic model for how the mis-regulation of histone H3 phosphorylation and AIR-2 localization result in the precocious separation of sister chromatids during meiosis I in *gsp-1/2(RNAi)* zygotes. White circle, phosphorylated histone H3; orange bar, unphosphorylated REC-8; red triangle, AIR-2; green bar, phosphorylated REC-8; and green diamonds, degraded REC-8.

It would be interesting to know the localization of GSP-1 and GSP-2. With human cells, a PP1 phosphatase, which is similar to Glc7, can be biochemically purified with chromosomal extracts (Murnion et al., 2000). Currently the localizations of either GSP-1 or GSP-2 in *C.*

elegans meiotic cells are unknown. Recently, an antibody to GSP-2 was generated (Ueda H. and Hosono R., pers. comm.). We found antibody staining to GSP-2 in oocytes showed a diffuse cytoplasmic, nuclear, and chromatin staining. There was no obvious GSP-2 staining at sub-chromosomal foci, nor was there an obvious deficiency of GSP-2 staining; instead we observed a general, low-level GSP-2 staining throughout the bivalent.

d. What selectively localizes AIR-2 to the distal arms?

In our model, the selective degradation of REC-8 during meiosis I requires the localization of AIR-2 specifically to the distal arms of the bivalents. This model begs the question of what selectively regulates AIR-2 localization to these sites. Although *gsp-1/2(RNAi)* affects the restricted localization of AIR-2/ABI complex to the distal arms of bivalents, other unknown mechanisms must target AIR-2/ABI complex to these sub-chromosomal foci.

Presumably there is some molecular information provided by the recombination machinery at or near the chiasmata. This could be in the form of a histone modification, such as H2AX phosphorylation, or it could be a response to a sensor of recombination – also known as the pachytene checkpoint – that might utilize either the activation of the cell cycle regulator MAPK, the DNA damage signal kinase Chk2, or even Plk1 (Mahadevaiah et al., 2001; Perez-Hidalgo et al., 2003; Tsvetkov et al., 2003). Interestingly in *S. cerivisae*, Glc7 is activated by the completion of recombination at pachytene, which might involve a MAPK signal pathway (Bailis and Roeder, 2000). Currently, the mechanism that selectively localizes AIR-2 is unknown, and furthermore, the processes by which the cell distinguishes distal versus proximal along the chromosomes once the chiasma is marked is not clear.

Recent, unpublished research in *C. elegans* shows that AIR-2 localization is first observed much earlier during diakinesis and is presaged by the localization of two synaptonemal components SYP-1 and SYP-2 (Nabeshima and Villeneuve, pers. comm.). Both SYP-1 and SYP-2 are required for synapsis and meiotic recombination (MacQueen et al., 2002; Colaiacovo et al., 2003). Although only preliminary, this observation suggests that bivalents might retain molecular information from the synaptonemal complex in the form of SYP-1/2 to signal where to load the AIR-2/ABI complex onto the bivalent. This could easily explain why univalents lack AIR-2 staining, because univalents do not exhibit SYP-1/2 localization.

3. Significance

Studies of anueploidy in human oocytes have shown that oocytes arrested in metaphase II prior to fertilization are far more likely to contain extra chromatids than they are to possess an extra pair of sister chromatids (Hassold and Hunt, 2001). This is probably the result of an equational division of a fraction of the chromosomes during meiosis I. This defect would occur if some chromosomes precociously lose cohesion throughout the bivalent prior to completing the first meiotic division, as occurs to all bivalents in *C. elegans* oocytes depleted of GSP-1/2. It is likely that the oocytes giving rise to trisomies might be the result of these aneuploid oocytes, because their pattern of aneuploidy resembles that found in aborted fetuses (Hassold and Hunt, 2001). In the future, the role of Aurora-B kinases and Glc7-type PP1 phosphatases might be important for understanding the mechanistic basis of the defects observed in aneuploid oocytes and fetuses.

All three subtypes of Aurora kinases are expressed in the germline of mammals. In particular, Aurora-C expression is restricted to the germline, suggesting a meiotic function for this Aurora kinase subtype. Recently, it has been shown that Aurora C kinases are functionally

similar to Aurora-B kinases, being able to rescue an Aurora-B mutant during mitosis (Sasai et al., 2004). It will be interesting to see in mammals whether Aurora-B and Aurora-C kinases regulate the unique chromosomal events of meiosis, such as the selective release of chromosome cohesion.

CHAPTER THREE: The Polo kinase PLK-1 functions in the release of chromosome cohesion during meiosis II

A. Introduction

Meiosis requires two rounds of chromosome cohesion release. In meiosis I, the cohesion between distal sister chromatid arms is released to allow homolog separation. In meiosis II, the remaining proximal/centromeric cohesion is released to permit sister chromatid separation. The preceding section described evidence that the aurora kinase AIR-2 regulates the release of chromosome cohesion.

Previously it was shown in budding yeast that Cdc5/Polo regulates the degradation of mitotic cohesion Scc1 via direct phosphorylation (Alexandru et al., 2001). During meiosis in budding yeast, Scc1 is replaced by its closest homologue, Rec8. An intriguing question is whether Polo kinases also regulate Rec8, especially with regard to the stepwise degradation of Rec8 during meiosis. In *C. elegans*, the Polo-like kinase PLK-1 is required for meiosis (Chase et al., 2000). This section describes an analysis of the function of PLK-1 with regard to chromosome cohesion release during meiosis.

Here, we show the second phase of chromosome cohesion release might require the Polo-like kinase PLK-1. We show that PLK-1 is required for sister chromatid separation and REC-8 release during meiosis II. Interestingly, PLK-1 appears dispensable for homolog disjunction and REC-8 release during meiosis I. In *plk-1(RNAi)* zygotes, REC-8 is removed during meiosis I,

allowing homolog separation. However, REC-8 is not removed during meiosis II; therefore, sister chromatids fail to separate in *plk-1(RNAi)* zygotes. The simultaneous depletion of REC-8 permits sister chromatid separation in *plk-1(RNAi)* animals. In *gsp-1/2(RNAi)* zygotes, simultaneous PLK-1 inhibition can suppress the precocious separation of sister chromatids, despite the coincident mis-regulation of AIR-2. We propose PLK-1 promotes the release of meiotic chromosome cohesion via regulating phosphorylation, specifically of the proximal/centromeric REC-8, thereby facilitating the disjunction of sister chromatids exclusively during meiosis II.

B. Results

1. In vivo 4-D analysis: PLK-1 is required for sister chromatid separation

In various organisms, Polo kinases are essential for meiosis; however, their precise molecular functions remain elusive, because of their pleiotropic phenotypes. In *Drosophila*, the failure of Polo mutants to execute meiosis was attributed primarily to spindle defects (Herrmann et al., 1998; Riparbelli et al., 1998). In budding yeast, a temperature-sensitive mutant of *cdc5/Polo* is defective in both meiosis I and II (Schild and Byers, 1980). More recent studies suggest Cdc5 functions both prior to the first meiotic division in regulating spindle-kinetochore attachments and in the resolution of chiasmata (Clyne et al., 2003; Lee et al., 2003). These studies argue Cdc5 functions in the release of meiotic chromosome cohesion via the regulation of Rec8 phosphorylation. We examined the function of the Polo-like kinase PLK-1 in the process of meiotic chromosome cohesion release in *C. elegans*.

We depleted PLK-1 by RNAi in a strain expressing a histone H2B::GFP reporter and then performed 4-D imaging of meiosis. In wildtype, oocytes undergo nuclear envelope breakdown and enter meiotic prometaphase during oocyte maturation and ovulation into the spermatheca. After fertilization, the oocyte nucleus enters metaphase I with each bivalent, composed of two axially arranged homologs, aligned near the surface of the embryo (Fig. III.1 A). At anaphase I, the homologous chromosomes separate into two groups of dyads, with the group closer to the surface of the embryo extruded as the first polar body (Fig. III.1, B and C). The remaining six dyads align at metaphase II and separate into two groups of single chromatids at anaphase II (Fig. III.1 D). The group of single chromatids closer to the surface of the zygote is extruded as the second polar body, and the fusion of haploid pronuclei ensues (Fig. III.1 E).

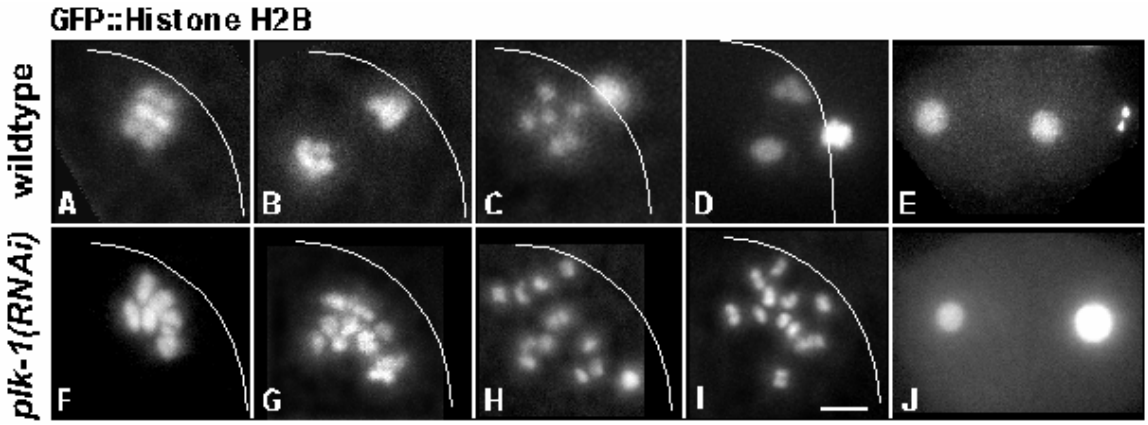

Figure III.1. Meiotic chromosome behavior in live *plk-1(RNAi)* zygotes. All images presented here are representative slices from each time point, except for that in I, which is a projection of the image stack of DAPI staining from a fixed embryo. The edge of the embryo is indicated by a white curve.

As noted previously, inhibition of PLK-1 by RNAi results in drastic and pleiotropic phenotypes: 100% embryonic lethality, single-cell arrest, and meiotic defects (Chase et al., 2000). The first defect we observed during 4-D imaging of *plk-1(RNAi)* animals was oocytes did not undergo nuclear envelope breakdown until 5-10 min after fertilization. In *plk-1(RNAi)*

zygotes, the bivalents appeared morphologically similar to wildtype bivalents but did not align properly (Fig. III.1 F). This likely was caused by the disorganization of the meiotic spindle we observed using a β-tubulin::GFP reporter (data not shown). However, approximately 6 min after nuclear envelope breakdown, the six bivalents synchronously separated into 12 dyads, suggesting the coordinated onset of anaphase I (Fig. III.1 G).

The meiotic chromosomes remained as 12 dyads (100%, *n*=78); they never separated into different groups or formed polar bodies for the remainder of meiosis, which lasted approximately 30 min (Fig. III.1 I). At the end of meiosis, the 4C oocyte pronucleus decondensed, which is clearly distinguishable from the 1C sperm pronucleus (Fig. III.1 J). These results demonstrate that, despite a delay in nuclear envelope breakdown and the presence of severe spindle defects, homologous chromosomes separate in *plk-1(RNAi)* zygotes. However, sister chromatids never separate in *plk-1(RNAi)* zygotes.

2. Assaying cell cycle progression in *plk-1(RNAi)* zygotes

To rule out the possibility that the failure of sister chromatid separation in *plk-1(RNAi)* zygotes is due to a failure to attempt meiosis II, we examined several GFP markers whose spatial/temporal movements during the cell cycle are well defined. First, the localization of the DYRK kinase, MBK-2, has been shown to change from uniform to punctate around the transition from meiosis I to meiosis II (Fig. III.2, *n*=30; Pellettieri, et al., 2003), and this change is dependent on the completion of meiosis I (Pellettieri et al., 2003). In *plk-1(RNAi)* zygotes, MBK-2::GFP behaved similarly to wildtype, initially present uniformly around the cortex and then redistributing to foci concurrent with the appearance of the 12-dyad phenotype (Fig. III.2, *n*=19).

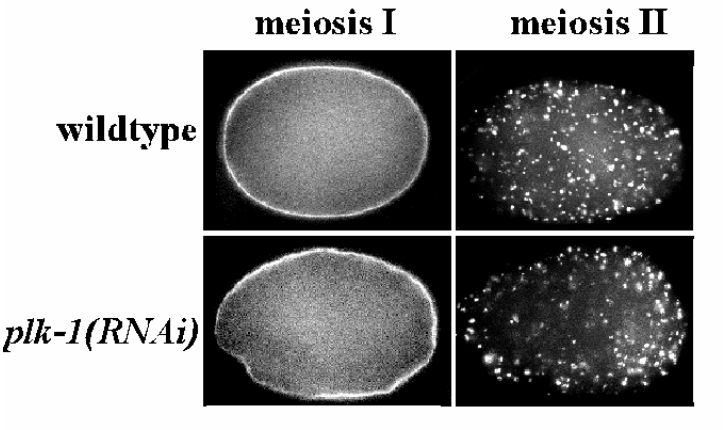

Figure III.2. Cell cycle progression in *plk-1(RNAi)*. Representative images of fixed GFP::MBK-2 zygotes, which have been projected. Both wildtype and *plk-1(RNAi)* embryos show similar localization patterns for MBK-2 during meiosis I and meiosis II.

Second, after the completion of meiosis II, two zinc-finger containing proteins, PIE-1 and MEX-5, polarize to the posterior and anterior cytoplasm, respectively, in wildtype embryos (Reese et al., 2000; Cuenca et al., 2003;). In *plk-1(RNAi)* embryos, proper polarization of PIE-1 and MEX-5 was observed (see Chapter Four). Third, the OMA-1 protein is degraded rapidly after the first mitotic division (Lin, 2003). In *plk-1(RNAi)* embryos, we also observed rapid OMA-1 degradation (data not shown). These results suggest that *plk-1(RNAi)* embryos, despite meiotic spindle defects, progress through meiosis I, enter meiosis II, and then execute post-meiotic processes. These conclusions are supported by the previous observation of multiple cell cycle repetitions of aborted cytokinesis attempts in *plk-1(RNAi)* embryos (Chase et al., 2000).

3. Disruption of spindle function does not block homolog separation in meiosis I

We believe that the observed defect in chromosome separation in *plk-1(RNAi)* zygotes is independent of its defect in spindle organization. Although spindle organization is an integral part of proper meiotic divisions, it does not appear to be required for the release of meiotic chromosome cohesion in meiosis I. In zygotes depleted of either an essential component of the

meiotic spindle, α-tubulin, or an essential regulator of meiotic spindle organization, MEI-1, two successful rounds of chromosome separation occurred, despite the lack of chromosome segregation during both divisions (Fig. III.3; Yang et al., 2003). In meiosis I, we observed 12 dyads in *tba-1(RNAi)* or *mei-1(RNAi)* zygotes. However in meiosis II, we never detected more than 12 DAPI-staining units in *tba-1(RNAi)* or *mei-1(RNAi)* zygotes (data not shown). These observations suggest meiotic spindle defects do not prevent chromosome cohesion release during meiosis I or meiosis II. The appearance of 12 dyads during meiosis I in *plk-1(RNAi)* zygotes suggests the regulation of chromosome cohesion is normal, whereas the failure of chromosome segregation resembles the effects of general meiotic spindle mutants. Therefore, it is not clear whether the failure in chromosome segregation during meiosis in *plk-1(RNAi)* zygotes is caused by defects in chromosome cohesion release and/or defects in meiotic spindle/kinetochore function.

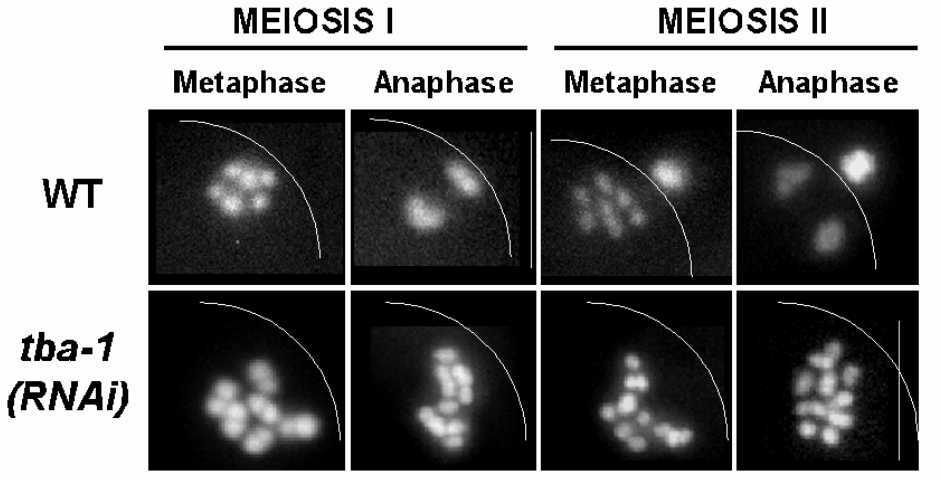

Figure III.3. The effect of tubulin depletion resembles the effect of *plk-1(RNAi)*. All images presented here are projections of image stacks of DAPI staining of the chromosomes from fixed zygote. The edge of the zygote is indicated by a white curve.

Based on these results, it is not clear if a second meiotic division is even attempted in *plk-1(RNAi)* zygotes. It is possible that both PLK-1 and a functional spindle are required for entry into meiosis II and to attempt the second meiotic division. This possibility suggests the existence of checkpoint that functions to bypass meiosis II in the case of spindle disruption by inhibition of either *tba-1* or *mei-1*. Similarly, the kinetochore and/or spindle defects in *plk-1(RNAi)* zygotes also might activate this checkpoint and bypass meiosis II completely.

4. Localization of PLK-1 during meiosis by antibody staining

Using an antibody to PLK-1, we analyzed the localization of PLK-1 during meiosis (Chase et al., 2000). The majority of PLK-1 was found associated with the chromosomes corresponding to the meiotic kinetochore (Fig. III.4 A), and a minority of PLK-1 was observed on the meiotic spindle (Fig. III.4 B). In oocytes, PLK-1 localization was throughout the bivalent (Fig. III.4 A), in contrast to the restricted localization of AIR-2 (Fig. III.4 E). At anaphase I, the localization of PLK-1 is very similar to AIR-2 (Fig. III.4, C and G). At metaphase II, PLK-1 was found between sister chromatids coinciding with the residual, proximally located REC-8 (Fig. III.4 D). The presence of PLK-1 staining between sister chromatids during meiosis II coincides with a role in REC-8 removal at this stage. Also, the presence of PLK-1 on the kinetochore and meiotic spindle correlates with roles for PLK-1 in meiotic spindle organization and kinetochore functions.

The inhibition of either PLK-1 or NCC-1/Cdk1 causes a cell cycle delay, particularly in the process of nuclear envelope breakdown in maturing oocytes. We tested whether NCC-1 is required for PLK-1 localization, especially noting the localization of PLK-1 to the nucleus during oocyte maturation. There was no observable change in PLK-1 localization in the gonads

of *ncc-1(RNAi)* worms (data not shown). This is consistent with published results suggesting that Plk1 activation is largely independent of Cdk1 activity (Abrieu et al., 1998; Karaiskou et al., 2003; Anger et al., 2004; Okano-Uchida et al., 2004).

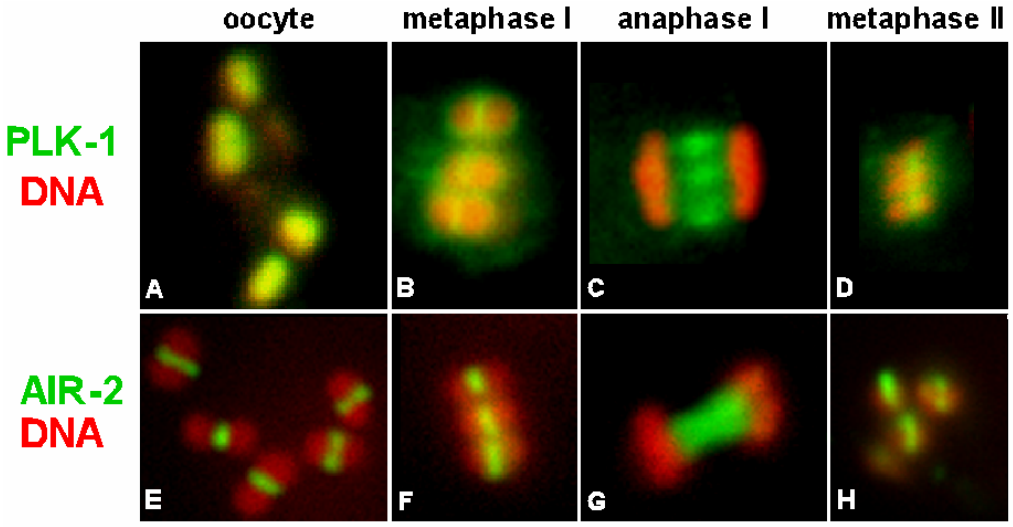

Figure III.4. PLK-1 is localized throughout the chromosome during meiosis. Antibody staining is presented in green, and DAPI staining is shown in red. Merged antibody and DNA staining is shown for wildtype meiotic chromosomes for PLK-1 (A-D) and AIR-2 (E-H) is presented.

5. REC-8 is retained on dyads in *plk-1(RNAi)* zygotes

To further investigate the observed defect in chromosome separation in *plk-1(RNAi)* zygotes, we examined the localization of the meiotic cohesin REC-8. During wildtype meiosis, REC-8 was readily detected by antibody staining on both the proximal and distal regions of the bivalent prior to metaphase (76%, *n*=41; Fig. III.5 A; Pasierbek, et al., 2001; Rogers et al., 2002). At anaphase I, the REC-8 on the distal arms of the bivalent was cleaved, allowing the separation of bivalent into two dyads, but the REC-8 on the proximal regions remained to hold the sister chromatids together (100%, *n*=18; Fig. III.5, B and C). In newly fertilized *plk-1(RNAi)* zygotes (representing meiosis I), we detected REC-8 on both distal and proximal arms of bivalents

similarly to wildtype zygotes (74%, *n*=19; Fig. III.5 E). However in *plk-1(RNAi)* zygotes containing 12 dyads (representing meiosis II), we observed 97% of zygotes (*n*=42) had detectable REC-8 staining. This is quite different from the 14% of wildtype zygotes (*n*=35) with detectable REC-8 staining at the stages between anaphase I and the end of meiosis. This result suggests that the defect in the separation of sister chromatids in *plk-1(RNAi)* zygotes might be due to a failure to degrade the meiotic cohesin REC-8 during meiosis II.

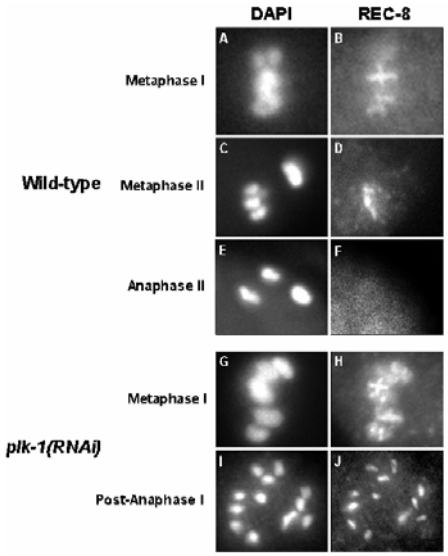

Figure III.5. REC-8 is retained on dyads in *plk-1(RNAi)* zygotes. REC-8 staining in wildtype (A-F) and *plk-1(RNAi)* zygotes (G-J). The left column shows DAPI staining, and the right column shows the corresponding REC-8 staining.

6. REC-8 is required to hold sister chromatids together in *plk-1(RNAi)* zygotes

To test the hypothesis that cohesion complexes are responsible for holding sister chromatids together in *plk-1(RNAi)* zygotes, we examined whether the *plk-1(RNAi)* phenotype is dependent on *rec-8*. When *plk-1* and *rec-8* were inhibited simultaneously by RNAi, more than 12 DAPI-staining units (*n*=3) were observed during meiosis, as opposed to the 12 dyads observed in all

plk-1(RNAi) zygotes. We also scored the number of DAPI-staining units in oocyte nuclei, because the chromosomes are more dispersed here and thus easier to count. Depletion of REC-8 alone by RNAi resulted in 32% (*n*=38) of the oocytes containing greater than 12 DAPI-staining units (Fig. III.6 A). We detected a similar percentage (36%, *n*=19) of oocytes containing greater than 12 DAPI-staining units when both REC-8 and PLK-1 were depleted (Fig. III.6 B). These results demonstrate that the maintenance of sister chromatid cohesion in *plk-1(RNAi)* zygotes depends on the meiotic cohesin REC-8. Next, we asked if *plk-1* is required for the precocious removal of REC-8 and the precocious separation of sister chromatids during meiosis I.

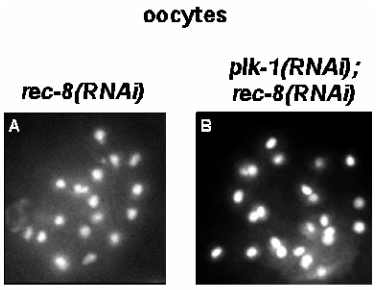

Figure III.6. REC-8 is required for the 12-dyad phenotype observed in *plk-1(RNAi).* Projected images of DAPI-stained oocytes from *rec-8(RNAi)* and *plk-1(RNAi); rec-8(RNAi)* animals.

7. PLK-1 is required for the precocious separation of sister chromatids in *gsp-1/2(RNAi)* zygotes

We showed previously that when two PP1 phosphatases, GSP-1/2, were depleted by RNAi, sister chromatids separated precociously at the onset of anaphase I, resulting in the formation of 24 single chromatids (Fig. III.7, A-D; Kaitna et al., 2002). This precocious separation of chromatids coincided with the ectopic localization of AIR-2 to the proximal arms of bivalents and a dramatic decrease in chromosomal REC-8. Here we show the phenotypes observed in *gsp-1(RNAi);gsp-2(RNAi)* zygotes are dependent on PLK-1 activity.

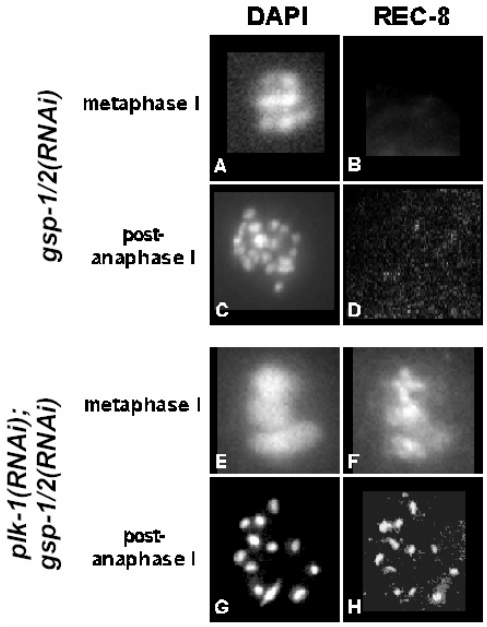

Figure III.7. PLK-1 is required for precocious sister chromatid separation and precocious REC-8 removal in *gsp-1/2(RNAi)* zygotes. Projected images of REC-8 staining in *gsp-1/2(RNAi)* (A-D) and *plk-1(RNAi); gsp-1/2(RNAi)* zygotes (E-H). DAPI staining is shown in the left column, with the corresponding REC-8 staining presented in the right column.

The first observable change in *plk-1(RNAi);gsp-1(RNAi);gsp-2(RNAi)* zygotes was that prophase I bivalents had detectable REC-8 staining similar to that observed in wildtype (Fig. III.7, E and F). The most dramatic change in the zygotes was the failure of precocious sister chromatid separation. In *plk-1(RNAi);gsp-1(RNAi);gsp-2(RNAi)* zygotes, we always observed a persistent 12-dyad phenotype (100%, n=28; Fig. III.7 G) that was indistinguishable from *plk-1(RNAi)* zygotes. Furthermore, REC-8 staining was detected on the dyads of *plk-1(RNAi); gsp-1(RNAi); gsp-2(RNAi)* zygotes (100%, n=14; Fig. III.7 H). It is important to note that in these triple RNAi zygotes, AIR-2 is detected ectopically on the proximal arms of bivalents (data not shown). These results suggest (1) ectopic AIR-2 localization is not sufficient to trigger the precocious separation of sister chromatids, and (2) PLK-1 is required for the release of REC-8

from proximal arms and separation of sister chromatids in the absence of the GSP-1/2 phosphatases.

We have described several RNAi situations that cause defects in meiotic chromosomes segregation (Table III.1). In *air-2(RNAi)*, *plk-1(RNAi),* and *gsp-1/2(RNAi)*, meiotic chromosome segregation completely failed and no polar bodies were produced. The end result of meiosis in all three situations is the oocyte-derived pronucleus retained a 4C DNA content; however, the connectivity of the chromosomes was very different. In *air-2(RNAi)* the chromosomes remained as six bivalents, in *plk-1(RNAi)* the chromosomes remained as twelve dyads, and in *gsp-1/2(RNAi)* the chromosomes separated into 24 individual chromatids. Combining either *air-2(RNAi)* or *plk-1(RNAi)* with *gsp-1/2(RNAi)* resulted in the phenotype of either *air-2(RNAi)* or *plk-1(RNAi)* alone (Table III.1).

Table III.1. Summary of Chromosome Segregation Defects.

Genotype	Chromosome Status at End of Meiosis
wildtype	1C : 1N
air-2(RNAi)	4C : 1N
plk-1(RNAi)	4C : 2N
gsp-1/2(RNAi)	4C : 4N
air-2(RNAi); gsp-1/2(RNAi)	4C : 1N
plk-1(RNAi); gsp-1/2(RNAi)	4C : 2N
gsp-1(RNAi)	2C : 2N

8. PLK-1 localization does not appear to be altered by *gsp-1/2(RNAi)*

The mis-regulation of REC-8 in *gsp-1/2(RNAi)* most likely is caused by the mis-localization of AIR-2. The phenotypes caused by PLK-1 depletion in both wildtype and *gsp-1/2(RNAi)* zygotes suggest that PLK-1 plays a role in regulating sister chromatid cohesion. As with AIR-2, it is possible that GSP-1/2 also regulate the localization or activation of PLK-1. However, we found no evidence that *gsp-1/2(RNAi)* perturbs PLK-1 localization in oocytes (Fig. III.8). Therefore, in contrast to AIR-2, PLK-1 localization is not effected by inhibition of the phosphatase GSP-1/2.

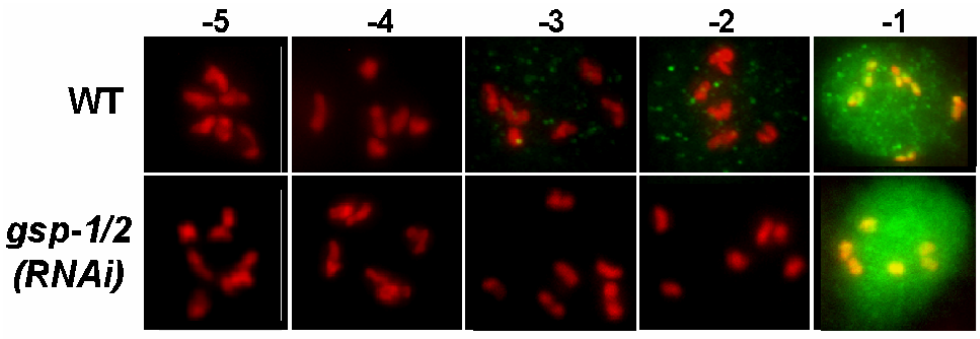

Figure III.8. *gsp-1/2(RNAi)* has no detectable effect on PLK-1 localization. Merged images of oocyte nuclei from wildtype (top) and *gsp-1/2(RNAi)* animals (bottom). PLK-1 antibody staining is shown in green, and DAPI staining is indicated in red.

9. Truncated PLK-1 phosphorylates REC-8, COH-1, and COH-2 in vitro

We tested whether PLK-1 can phosphorylate REC-8. When a full-length PLK-1 was purified from *E. coli*, there was no detectable kinase activity. However, if PLK-1 was truncated to the 333 amino acids at the N-terminal, the region compromising the kinase domain, then kinase activity was detected. This kinase domain constructs auto-phosphorylates and is capable of phosphorylating the three cohesins tested: REC-8, COH-1, and COH-2 (Fig. III.9).

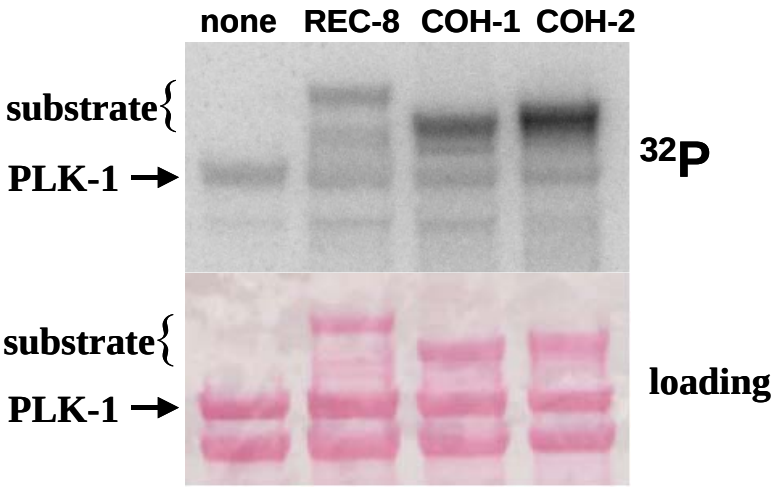

Figure III.9. The kinase domain of PLK-1 phosphorylates REC-8 in vitro. This kinase assay was performed with GST–PLK-1(N terminus fragment residues 1-333) using GST–REC-8 (lane 2), GST–COH-1 (lane 3), or GST–COH-2 (lane 4) as substrates. GST–PLK-1 auto-phosphorylation is observed in all lanes. The top half shows phosphor-imaging, and the bottom half shows Ponceau staining.

We did not map the PLK-1 phosphorylation site(s) in REC-8. However, based on a Cdc5/Polo substrate consensus sequence, potential sites can be predicted by sequence analysis. We predict that PLK-1 might phosphorylate REC-8 at the following five sites: T194 (K-E-I-**T**-M-H-S), T215 (M-H-S-**T**-F-V-E), T250 (E-I-**T**-L-G-E), S420 (E-D-P-**S**-F-A-I), and S626 (R-E-T-**S**-I-I-A). Interestingly, one predicted PLK-1 phosphorylation site in REC-8 (S626) is adjacent to the AIR-2 phosphorylation site (T625).

Although the PLK-1 truncation was capable of phosphorylating REC-8, the significance of this result is not clear. The fact that all three substrates tested were phosphorylated might suggest non-specific kinase activity. Perhaps removing the C-terminal half of PLK-1 could be the cause of this substrate specificity. Among the tested substrates, COH-2/SCC-1 was the most highly phosphorylated, which is consistent with the published function of Polo kinases in

regulating mitotic chromosome cohesion via the direct phosphorylation of Scc1 (Alexandru et al., 2001; Losada et al., 2002; Sumara et al., 2002). The phosphorylation of REC-8 is much weaker compared to COH-2/SCC-1; however, it is possible these results indicate that REC-8 is a direct target of PLK-1 in vivo.

C. Discussion: Polo kinases and chromosome cohesion release during meosis

Meiosis requires two rounds of chromosome cohesion release. In meiosis I, the cohesion between distal sister chromatid arms is released to allow homolog separation. This appears to involve the selective localization of the Aurora kinase AIR-2 to the distal arms and the differential phosphorylation of REC-8. In meiosis II, the remaining proximal/centromeric cohesion is released to permit sister chromatid separation. The second phase of chromosome cohesion release might require the Polo-like kinase PLK-1, although this is not clear.

We showed PLK-1 is required for sister chromatid separation and REC-8 release during meiosis II. Interestingly, PLK-1 appears dispensable for homolog disjunction and REC-8 release during meiosis I. We propose PLK-1 promotes the release of meiotic chromosome cohesion via regulating the phosphorylation of only the proximal/centromeric REC-8, thereby facilitating the disjunction of sister chromatids specifically during meiosis II (Fig. III.10). Conversely, PLK-1 might be required for entering into meiosis II to attempt the second meiotic division. Unfortunately, the data do not unambiguously support or dissuade either possibility.

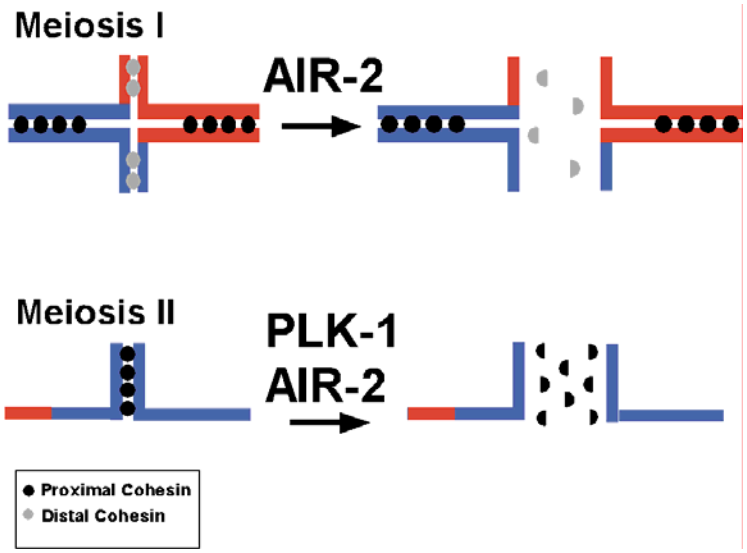

Figure III.10. PLK-1 regulates the release of chromosome cohesion during meiosis II. A diagram of how PLK-1 and AIR-2 regulate the chromosome segregation during meiosis. Homolgous chromosomes are shown in red and blue. The circles represent cohesin complexes, with black and grey circles corresponding to proximal and distal cohesion, respectively.

1. PLK-1 is required for the release of chromosome cohesion during meiosis II

This work demonstrates a specific role for PLK-1 in release of chromosome cohesion during meiosis II. Inhibition of PLK-1 by RNAi prevents sister chromatid separation and results in the maintenance of REC-8 staining between sister chromatids throughout meiosis. In addition, PLK-1 is found during metaphase II between sister chromatids, coinciding with the remaining, proximally located REC-8. In *rec-8(RNAi); plk-1(RNAi)* embryos, sister chromatids are capable of separating, demonstrating the maintenance of sister chromatid cohesion in *plk-1(RNAi)* embryos depends on REC-8. In *plk-1(RNAi)*; *gsp-1/2(RNAi)* embryos, both the precocious separation of sister chromatids and the precocious loss of REC-8 staining of sister chromatids are suppressed. All together, these results argue that PLK-1 is required for the removal of REC-8 to allow the separation of sister chromatids in either a normal meiosis II or an aberrant meiosis I.

Because of four indirect results, we also have implicated AIR-2 in the release of chromosome cohesion during meiosis II (Fig. III.10). First, AIR-2 localization is correlated with the position of cohesion release in meiosis II. Second, in *spo-11* embryos, which lack bivalent formation, AIR-2 is required for sister chromatid separation during meiosis II. Third, ectopic localization of AIR-2 to the entire bivalent is correlated with precocious loss of cohesion between sister chromatids in meiosis I. Fourth, AIR-2 is required for the precocious sister chromatid separation observed in *gsp-1/2(RNAi)* embryos.

Now we show in *plk-1(RNAi); gsp-1/2(RNAi)* embryos, the precocious removal of REC-8 and the precocious separation of sister chromatids is suppressed, even though AIR-2 is localized ectopically. These results suggest it is likely that both AIR-2 and PLK-1 function together to regulate the release of REC-8 during meiosis II. Results from *plk-1; gsp-1/2(RNAi)* suggest that GSP-1/2 regulates, at least, sister chromatid cohesion via PLK-1. In the absence of PLK-1, sister chromatids do not precociously separate in *gsp-1/2(RNAi)*. It is not clear what relationship exists between the GSP-1/2 phosphatases and PLK-1. GSP-1/2 inhibition does not affect PLK-1 localization, which suggests neither the activation nor the localization of PLK-1 is downstream of GSP-1/2. Interestingly, sequence gazing reveals putative Polo kinase binding site in both GSP-1 (T153) and GSP-2 (T152). If there is a functional interaction, it might suggest an intimate relationship between PLK-1 and the GSP-1/2 phosphatases.

Unfortunately, the sequence conservation between *C. elegans* REC-8 and budding yeast Rec8 does not reveal any conserved separase cleavage sites. Based on studies by Sullivan et al. (2004), a separase consensus site has been defined for both budding yeast and human forms of

separase. Using this information, we attempted to predict the separase cleavage sites in *C. elegans* REC-8 (Appendix E).

We have shown in vitro that AIR-2 can phosphorylate REC-8 but not the mitotic cohesions COH-1 and COH-2/SCC-1. This does not explain why all Scc1 is degraded during meiosis I in budding yeast. Based on our model, Scc1 should not be modified by Aurora kinase and therefore should not be targeted for degradation by separase. However, the exact opposite result was observed in yeast experiments (Toth et al., 2000). Perhaps when Scc1 is substituted for Rec8, the Polo kinase Cdc5 indiscriminately targets all the Scc1 to be degraded by separase during meiosis I, whereas normally Polo kinases do not target Rec8 for degradation during meiosis I. This is consistent with our observations that PLK-1 is not required for homolog separation in *C. elegans*. Another, alternative explanation is that different mechanisms operate in *S. cerivisae* and *C. elegans*.

2. PLK-1 does not appear to regulate chromosome cohesion release in meiosis I

Surprisingly, we observed PLK-1 function does not appear to be required for chromosome cohesion release during meiosis I. We proposed that AIR-2 regulates the selective release of REC-8 during meiosis I. Inhibition of AIR-2 prevents both homolog separation and REC-8 removal during meiosis I. However, inhibition of PLK-1 does not prevent homolog separation or the removal of REC-8 from the distal arms, despite a delay in nuclear envelope breakdown and development of drastic spindle organization defects.

Although *plk-1* RNAi restores REC-8 staining in *gsp-1/2(RNAi)* embryos, it does not delay or inhibit the separation of homologs in meiosis I. Also, PLK-1 localization is unaffected

by *air-2(RNAi)*, showing that the loss of AIR-2 phenotypes probably are not indirectly results of the mis-regulation. Similar results were reported by Speliotes et al. (2000). Similarly, PLK-1 localization is unaffected by *gsp-1/2(RNAi)*, which results in the mis-regulation of both AIR-2 and REC-8, resulting in the precocious separation of sister chromatids. Finally, in oocytes PLK-1 localizes to bivalent and univalents equally in contrast to the selective localization of AIR-2 to bivalents. Taken together, we believe these results suggest that AIR-2, and not PLK-1, is required for regulating the selective release of chromosome cohesion during meiosis I in *C. elegans* (Fig. III.10).

Recent studies using *S. cerivisae* addressed similar questions, yet they produced results contradictory to those of this study. Lee et al. (2003) demonstrated that *cdc5* is required for homolog segregation during meiosis I, possibly via Rec8 phosphorylation. Clyne et al. (2003) demonstrated that *cdc5* is required for proper recombination between homologs. Both studies concluded that *cdc5* is required for proper homolog segregation, but the reasons for this are not clear and might be due to recombination defects, kinetochore attachment defects, and/or chromosome cohesion defects.

We cannot explain why *cdc5/Polo* appears to be required for homolog separation in *S. cerivisae*, whereas *plk-1* appears dispensable for homolog separation in *C. elegans*. One possible explanation is inhibition of *plk-1* by RNAi is incomplete and therefore reveals only hypomorphic phenotypes. To address this possibility, we tried producing stronger RNAi phenotypes. Unfortunately, in this situation, *plk-1(RNAi)* oocytes failed to mature and arrested at diakinesis of prophase I, preventing the analysis of latter events. We would offer the simple hypothesis that, in this instance, different mechanisms might operate in different species.

PLK-1 is dispensable for meiosis I cohesion regulation regardless of why the first meiotic division fails. PLK-1 is required for the proper timing of the cell cycle and probably for kinetochore to attach to the spindle. In *plk-1(RNAi)*, the chromosomes appear to float randomly, without being directed by organized spindle forces. In summary, both AIR-2 and PLK-1 might regulate the spindle and cytokinesis during both meiosis I and meiosis II. AIR-2 appears to regulate chromosome cohesion during both meiosis I and meiosis II. PLK-1 appears to regulate chromosome cohesion exclusively during meiosis II.

3. The possible existence of a meiosis II-bypass checkpoint in *C. elegans*

Studies of meiotic spindle disruption suggest the existence of a meiosis II bypass checkpoint. In zygotes where the first meiotic division is inhibited by tubulin depletion, there was no evidence that a second meiotic division was attempted. However, the cell cycle preceded and the zygote entered into the mitotic cell cycle. Similar results were obtained when PLK-1 was depleted by RNAi. This is highly suggestive that the *plk-1(RNAi)* phenotype is predominantly a result of spindle disruption during the first meiotic division.

Cell division checkpoints have not been studied well in *C. elegans*. Homologues of the key spindle checkpoint protein Mad2 were cloned and named MDF-1 and MDF-2 (Kitagawa and Rose, 1999). MDF-1/2 have been studied by RNAi, which revealed various pleiotropic phenotypes; however, no meiotic arrest phenotypes were observed. The reasons for this are not clear, but it is possible that germline developmental defects might have obscured analysis of later functions for MDF-1/2 during the meiotic divisions.

Although there has not been any prior description of this type of meiotic checkpoint, these results are not surprising. Analysis of a temperature-sensitive mutant of Cdc5/Polo in budding yeast yielded similar results (Schild and Byers, 1980). In this conditional mutant, different temperature-shift regimes could be performed to produce different phenotypes. In some cases, the *cdc5* mutant underwent a single meiotic division and divided its chromosomes reductionally (Schild and Byers, 1980). More recently, analysis of a Cdc5 deletion mutant revealed that a small percentage of cells undergo a single meiotic division, after a long cell-cycle delay (Clyne et al., 2003). In both cases, only a single meiotic division was attempted, suggesting the existence of a bypass checkpoint that skips either the first or the second meiotic division.

4. The relationship between meiosis and mitosis

Meiosis is a specialized cell division. Meiosis I is unique because it is a reductional division involving the resolution of chiasmata, mono-orientation of the kinetochore, and the segregation of homologs. Therefore, some mechanisms regulating the selective release of chromosome cohesion during meiosis I must be distinct from those used in mitosis. However, meiosis II is an equational division – involving the release of centromeric cohesion, bi-orientation of the kinetochore, and the segregation of sister chromatids – that is directly analogous to mitosis. Therefore, mechanisms regulating meiosis II might be expected to be similar to mitotic mechanisms.

We propose here that both the Aurora-B kinase AIR-2 and the Polo kinase PLK-1 function together to release sister chromatid cohesion during meiosis II, a process that might reflect a shared mechanism with mitosis. This concept is supported by evidence that Aurora-B

kinase and Polo-like kinase Plx1/Plk1 function synergistically in the release of sister chromatid cohesion during mitosis in *Xenopus* and human cells (Losada et al., 2002; Sumara et al., 2002; Gimenez-Abian et al., 2004). These studies also suggest Cdk1 is not essential for cohesion removal, further supporting a role for these relatively novel cell cycle kinases in release of sister chromatid cohesion.

There is a growing suspicion that meiosis might be the ancestral process that gave rise to mitosis (Khodjakov et al., 2000; Megraw and Kaufman, 2000; Krylov et al., 2003; Murray, 2004). Also, it has been suggested that the recent evolution of Cdks implies many functions of Cdks have been subsumed from other kinases that originally performed these roles, perhaps Aurora and Polo kinases (Murray, 2004). The similarity in the functions of Polo kinases and Cdk1 is a prime example of apparently overlapping functions that might have diverged more recently. Polo kinases might have performed the ancestral roles of Cdk1. Now, Polo kinases might no longer be required for some of their ancestral functions, which instead are provided by Cdk1. Therefore, some Polo and Aurora kinase functions might be current-day avatisms of their ancestral functions. Similarly, some functions of Aurora or Polo kinases during meiosis might have been completely adopted by other factors, such as Cdk1, only during mitosis.

5. Possible involvement of Aurora-B kinases in mitotic chromosomal cohesion release

It has been demonstrated clearly in various organisms that Polo kinases facilitate the removal of chromosome cohesion during mitosis (Alexandru et al., 2001; Losada et al., 2002; Sumara et al., 2002; Gimenez-Abian et al., 2004). Whether Aurora-B kinases also play a role in the release of mitotic cohesion remains unclear. However, all studies done on invertebrates to date suggest that Aurora-B does not perform an essential function in release of cohesion during mitosis. But the

situation might be different in vertebrates, as suggested by studies using human cell lines (Gimenez-Abian et al., 2004).

First, in *S. cerivisae*, it has been reported that mitotic chromosome cohesion is unaffected in *ipl1* mutants (Biggins et al., 1999); instead the release of cohesion requires another kinase, Cdc5/Polo (Alexandru et al., 2001). Second, despite the polyploidy and chromosome segregation defects associated with the oocyte-derived nucleus, separation of sperm-derived chromosomes has been observed during mitosis in *air-2(RNAi)*, *bir-1(RNAi)*, and *icp-1(RNAi)* embryos (Oegema et al., 2001). Finally, we showed in vitro that AIR-2 phosphorylates REC-8 but not two other likely mitotic cohesions, COH-1 and COH-2/SCC-1, further suggesting that AIR-2 does not regulate cohesins during mitosis.

In vertebrates, the release of mitotic cohesion has been shown to involve two separate pathways: APC-separase-dependent and APC-separase-independent (Sumara et al., 2000; Waizenegger et al., 2000). Recent work using *Xenopus* extracts has shown a modest requirement for Aurora-B kinase in chromosome cohesion release during the prophase pathway; however, the mechanisms behind this function are unknown (Losada et al., 2002; Sumara et al., 2002). Studies using human cell lines suggest Aurora B is absolutely required for the release of chromosome cohesions during prophase (Gimenez-Abian et al., 2004). These studies suggest potential connections between Aurora-B kinases, histone H3 phosphorylation, and the proper regulation of chromosome cohesion during mitosis.

CHAPTER FOUR: Preliminary data implicating the Polo kinase PLK-1 in the regulation of cell polarity and asymmetric cell division

A. Introduction

In *C. elegans*, embryonic development begins with a series of asymmetric cell divisions that occur immediately following the completion of oocyte meiosis. Asymmetric cell division is a common mechanism utilized to generate different cell types during development and, later, by stem cells in adults. In general, asymmetric cell division describes the creation of non-identical daughter cells from a single mother cell. Prior to division, the mother cell performs two inter-related actions: (1) the asymmetric localization of various factors, and (2) the positioning of the mitotic spindle with respect to these factors. The coordination of these two events is necessary for both the proper segregation of cell fate determinants and the correct propagation of polarity information to the daughter cells. The result of these events is the creation of non-identical daughter cells.

1. The Par gene hierarchy regulates cell polarity

A powerful model for the study of asymmetric cell division is the first cleavage of the *C. elegans* embryo. Using the power of forward genetics in *C. elegans*, a complex hierarchy of polarity regulators composed of Par genes was described. The Par hierarchy provides a substantial framework for understanding the establishment of cellular polarity prior to the first cell division of the *C. elegans* embryo (for review see Cowan and Hyman, 2004). Later research

demonstrated that the Par genes are conserved throughout evolution and perform similar functions in other organisms (reviewed by Macara, 2004).

In *C. elegans*, eggs are symmetric and cell polarization is initiated by fertilization. Fertilization results in the introduction of the sperm asters into the egg cytoplasm, and the site of fertilization determines the position of the future posterior pole. Initially, the PAR proteins are localized symmetrically throughout the egg cortex. In response to the sperm aster signal, the zygote quickly becomes polarized along the anterior-posterior axis. Many of the PAR proteins redistribute into anterior and posterior cortical domains. A complex composed of PAR-3, PAR-6, and PKC-3 is localized to the anterior cortex, whereas both PAR-1 and PAR-2 are localized to a complementary cortical domain in the posterior. The establishment of these two Par domains is mutually exclusive, because both anterior PARs and posterior PARs inhibit the localization of the other. This opposition forms a strict boundary near the center of the cell where the anterior and posterior PAR domains meet. Once these two cortical domains are established, the PARs function to regulate the asymmetric localization of both cell-fate determinants and the mitotic spindle.

It is not known how the Par network executes downstream polarity events, nor is it known what other factors lie downstream of the Pars to perform these actions. Recently, five factors have been described which function downstream of the Par network to direct cell polarity. These factors (1,2) are the Zn-finger containing proteins MEX-5 and MEX-6 (Schubert et al., 2000); **(**3,4) the heterotrimeric, G-protein regulators GPR-1 and GPR-2 (Colombo et al., 2003; Gotta et al., 2003); and (5) the spindle regulator LET-99 (Tsou et al., 2002). All of these factors exhibit three common characteristics: (1) they are asymmetrically localized in response

to the Par hierarchy, (2) they do not regulate the initial polarization of the mother cell, and (3) they are required for at least a subset of downstream, PAR-regulated events.

2. Downstream effectors of the Par hierarchy

MEX-5 and MEX-6 are two, closely related, Zn-finger containing proteins with unknown molecular function (Schubert et al., 2000). The Par network regulates the asymmetric localization of MEX-5 and MEX-6 to the anterior cytoplasm of the embryo (Schubert et al., 2000; Cuenca et al., 2003). MEX-5 and MEX-6 subsequently are required for the asymmetric localization of both posterior cell fate determinants and the asymmetric positioning of the mitotic spindle (Schubert et al., 2000; Cuenca et al., 2003). In fact, recent evidence suggests that *mex-5/6* can be classified as Par genes, based on the common phenotypes of symmetric cell division and a loss of cytoplasmic polarity (Cuenca et al., 2003; Cheeks et al., 2004).

After asymmetric division, MEX-5/6 also function to promote the degradation of the small abundance of germline determinants that are inherited by the somatic blastomeres (DeRenzo et al., 2003). This somatic blastomere specific mechanism requires the activity of an ubiquitin ligase complex named the ECS, which is composed of Elongin C, CUL-2, ZIF-1, and RBX-1. There also is evidence that MEX-5/6 might regulate the polarization of the cytoplasm prior to division via a similar mechanism that promotes the degradation of posterior cytoplasmic factors by an anteriorly active degradation pathway (Cheeks et al., 2004).

GPR-1/2 are two, closely related proteins that contain GoLoco domains that regulate heterotrimeric G proteins via receptor-independent GTPase activation (Afshar et al., 2004; Couwenbergs et al., 2004; Hess et al., 2004). In the early embryo, heterotrimeric G proteins are

found uniformly at the cell cortex and at the centrosomes. The Par hierarchy is required to asymmetrically localize GPR-1/2 to the posterior cortex, where they locally activate heterotrimeric G protein signaling (Colombo et al., 2003; Gotta et al., 2003; Afshar et al., 2004; Couwenbergs et al., 2004; Hess et al., 2004). The inhibition of GPR-1/2 results in a symmetric spindle position but does not affect the localization of any of the PAR proteins or the localization of any of the cytoplasmic cell fate determinants.

LET-99, a DEP domain containing protein, is observed initially throughout the cell cortex. The Par hierachry functions to enrich LET-99 localization to an equatorial region of the cortex that is posteriorly displaced (Tsou et al., 2002). Like GPR-1/2, LET-99 is required for proper spindle placement but has no role in the localization of either the PARs or the cytoplasmic cell fate determinants (Rose and Kemphues, 1998; Tsou et al., 2002). Based on the presence of the DEP domain, it has been suggested that LET-99 might regulate heterotrimeric G protein signaling (Tsou et al., 2003).

Although these relatively new regulators of polarity have been described, how the Par hierarchy regulates cell polarity is relatively unknown. Recently, progress has been made into understanding how the par genes regulate the asymmetric forces acting on the spindle (Labbe et al., 2003; Severson and Bowerman, 2003). However, how the cytoplasm is divided into anterior and posterior domains prior to cell division is a mystery. Both PAR-1 and MEX-5/6 clearly play essential roles in this process; however, their molecular roles are not clear. Recently it has been suggested that MEX-5/6 and PAR-1 function via regulating protein stability of cell fate determinants, such as MEX-1, PIE-1, POS-1 and P granules, but the molecular mechanisms behind these processes are not known (Cheeks et al., 2004).

B. Results

We have discovered multiple lines of evidence suggesting the Polo-like kinase PLK-1 also functions as a mediator of the Par network during asymmetric cell division. PLK-1 fulfills the three criteria required of a downstream effector of the Par network: (1) PLK-1 is asymmetrically localized in response to the Par hierarchy, (2) PLK-1 is not required for the initial polariazation of the PAR proteins, and PLK-1 is required for at least some of the downstream Par-regulated polarity events.

1. PLK-1 is polarized in the cytoplasm prior to asymmetric cell division

A polyclonal antibody was created against a peptide corresponding to the N terminus of PLK-1 (Chase et al., 2000). This antibody was shown to be specific to PLK-1. Then, using this antibody, the sub-cellular localization of PLK-1 was described. PLK-1 was observed primarily at the centrosomes in mitotic cells from prophase until anaphase; also, PLK-1 was localized to the chromosomes and kinetochore during prometaphase and metaphase (Chase et al., 2000).

We performed a detailed analysis of PLK-1 antibody staining that resulted in additional observations. During the meiotic divisions of the zygote, PLK-1 was observed at a low level in the cytoplasm (Fig. IV.1 A). Then during the pronuclear migration stage, the cytoplasmic level of PLK-1 both increased and became enriched at the anterior pole (Fig. IV.1 B). At the time of pronuclear fusion, PLK-1 was asymmetrically localized to the anterior cytoplasm and was present on the centrosomes (Fig. IV.1 C). During prometaphase and metaphase, PLK-1 was localized to the kinetochore and the centrosomes (Fig. IV.1 D). At anaphase, PLK-1 was observed on the centrosomes and, in telophase, on the central spindle (Fig. IV.1 E). All

throughout this first division, PLK-1 was asymmetrically enriched in the anterior cytoplasm (Fig. IV.1 F). To prove the described staining pattern was specific to a PLK-1 epitope, we analyzed PLK-1 staining in *plk-1(RNAi)* embryos. In this situation, all of the described staining was abolished, which is consistent with previous controls for antibody specificity (Chase et al., 2000).

The first mitotic division in *C. elegans* is asymmetric. This division produces two daughter cells with different cell fates, the anterior daughter AB is a somatic blastomere and the posterior daughter P1 is a germline blastomere. This division is asymmetric with respect to cell size as well. AB is approximately 68% of the embryo whereas P2 comprises the remaining 32%. The asymmetry of the first division is characterized by the asymmetry of many markers and stereotyped behaviors. Both the spindle and the cleavage furrow are positioned 56% of the total length of the cell displaced toward the posterior. The spindle position is regulated by asymmetric forces, which act on the two centrosomes (Grill et al., 2003). These forces are generated at the cell cortex and regulated by the PARs.

The examination of PLK-1 staining in multicellular embryos also revealed an interesting expression pattern. Again as noted previously, PLK-1 was localized to the centrosomes and chromosomes in mitotic cells. However, PLK-1 was enriched in the cytoplasm of the somatic blastomere AB as compared to its germline sister P1 (Fig. IV.2, B and C). This pattern was presumably a result of the enrichment of PLK-1 to the anterior cytoplasm in the one-cell stage; therefore, the anterior sister AB inherited more cytoplasmic PLK-1 than did P1 (Fig. IV.2, B and C). Then P1 appeared to have generated more cytoplasmic PLK-1 de novo, which again was passed asymmetrically to its somatic daughter, EMS (Fig. IV.2, D-F). When P2 was dividing a

gradient of cytoplasmic PLK-1 was observed, whereas the level of cytoplasmic PLK-1 clearly was symmetric in the somatic blastomeres AB, and later in its daughters ABa and ABp. At the four-cell stage, sometimes EMS displayed slightly greater cytoplasmic PLK-1 staining than ABa or ABp (Fig IV.2 E). This might be the result of the degradation of cytoplasmic PLK-1 in older somatic blastomeres.

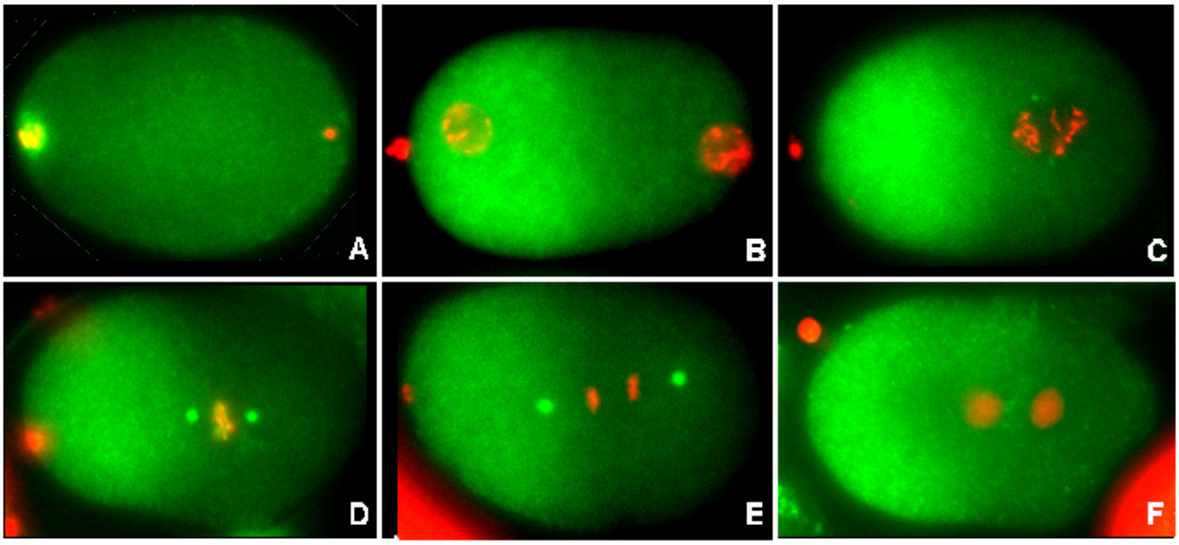

Figure IV.1. Antibody staining to PLK-1 reveals an asymmetric localization pattern. This figure displays merged images of PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. The panels display the following stages: meiosis I (A), pronuclei appearance (B), pronuclei fusion (C), metaphase (D), anaphase (E), and telophase (F).

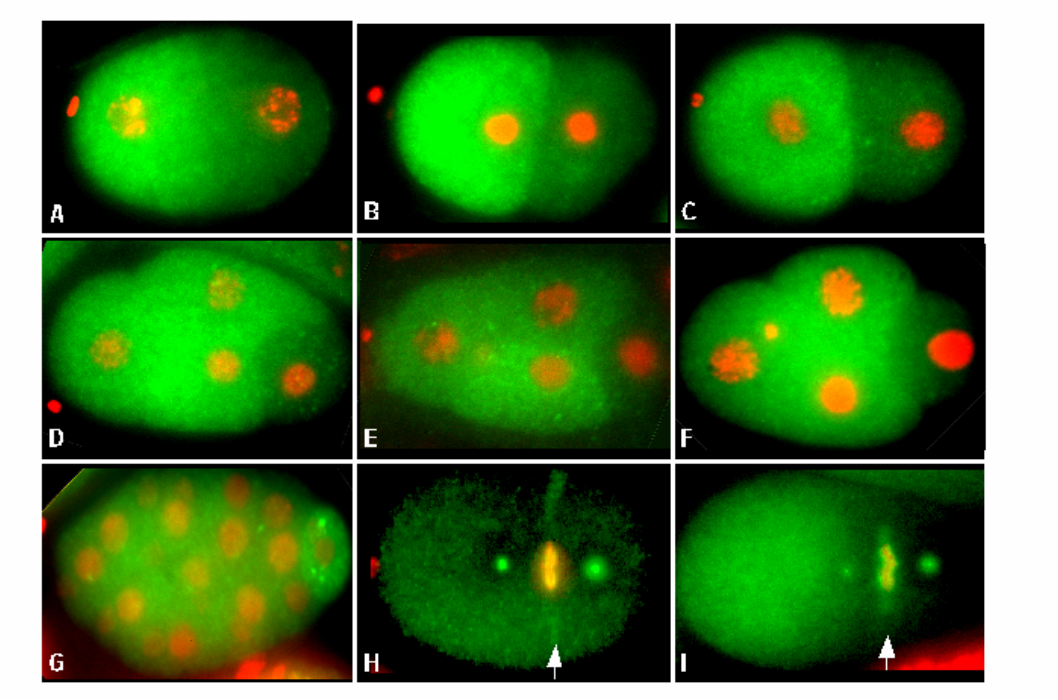

Figure IV.2. Antibody staining to PLK-1 revealed a soma/germline asymmetry. This figure displays merged images of PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. pronuclei stage (A), new 2-cell stage (B), middle 2-cell stage (C), new 4-cell stage (D), middle 4-cell stage (E), middle 4-cell stage (F), approximately 28-cell stage (G), and 1-cell stage, metaphase (H, I); the arrow marks the cytoplasmic band at the site of the future cleavage plane.

The soma/germline asymmetry of PLK-1 localization appeared to continue during later embryonic divisions. In multicellular embryos, PLK-1 localization was enriched in the dividing cells of the P lineage (Fig IV.2 G). Presumably this PLK-1 will be segregrated preferentially into its somatic daughter cell. One final observation of note was a cytoplasmic band of PLK-1 staining at the one-cell stage during metaphase (Fig IV.2, H and I). This band, which appeared to extend away from the chromosomes toward the cortex, might mark the location of the future cytokinetic furrow. In fact, the slight posterior displacement of the chromosomes placed this cytoplasmic band in alignment with the cortical band of LET-99 staining.

The expression pattern of PLK-1 in the cytoplasm resembles the pattern described for two other proteins, MEX-5 and MEX-6. Antibody staining showed MEX-5 was preferentially inherited by somatic blastomeres during germline blastomere divisions (Schubert et al., 2000). Analysis of a GFP fusion transgenic strain verified the localization of MEX-5 and allowed analysis of its behavior in live embryos (Cuenca et al., 2003). A partially redundant homologue of MEX-5, MEX-6, displayed an identical localization pattern when assayed in GFP fusion transgenic strain. The localization of MEX-5 and MEX-6 assayed by GFP analysis showed their movements in realtime. Initially, both MEX-5 and MEX-6 were distributed uniformly throughout the cytoplasm. Then during pronuclear migration and fusion, MEX-5 and MEX-6 were quickly enriched to the anterior side of the one-cell embryo in a PAR-dependent fashion (Cuenca et al., 2003).

Recently, a transgenic line expressing a GFP fusion to PLK-1 was constructed (Leidel and Gonczy, 2003). We examined GFP localization in this strain; however, we did not detect any polarized localization of GFP::PLK-1. The reason for the discrepancy between the results of the GFP fusion analysis and the antibody study are not clear. We did note that the GFP expression in the transgenic was very weak, although centrosomal localization was observed. Potentially, the intensity of the GFP might have prevented the detection of the cytoplasmic gradient at the one-cell stage. Experiments are underway to generate additional GFP::PLK-1 transgenics for future analyses. We are using constructs of PLK-1 that lack enzymatic activity, which might cause toxicity upon high expression, in hope of obtaining a transgenic with more increased GFP::PLK-1 expression.

2. The asymmetric localization of PLK-1 is dependent on the Par hierarchy and *mex-5/6*

The localization of MEX-5 and MEX-6 to the anterior cytoplasm was dependent on the Par hierarchy. Therefore, we tested whether the expression pattern of PLK-1 was dependent on the Par hierarchy as well. We examined PLK-1 antibody staining in several Par mutants (Fig. IV.3). We discovered that PLK-1 asymmetry was dependent on all the par genes tested: *par-1* (*n*=38), *par-2* (*n*=25), *par-3* (*n*=41), *par-4* (*n*=15), *par-5* (*n*=22), and *par-6* (*n*=7). Although cytoplasmic PLK-1 asymmetry was abolished in all of these Par mutants, it is important to note that PLK-1 remained localized to the centrosomes and chromosomes, as in wildtype (Fig. IV.3).

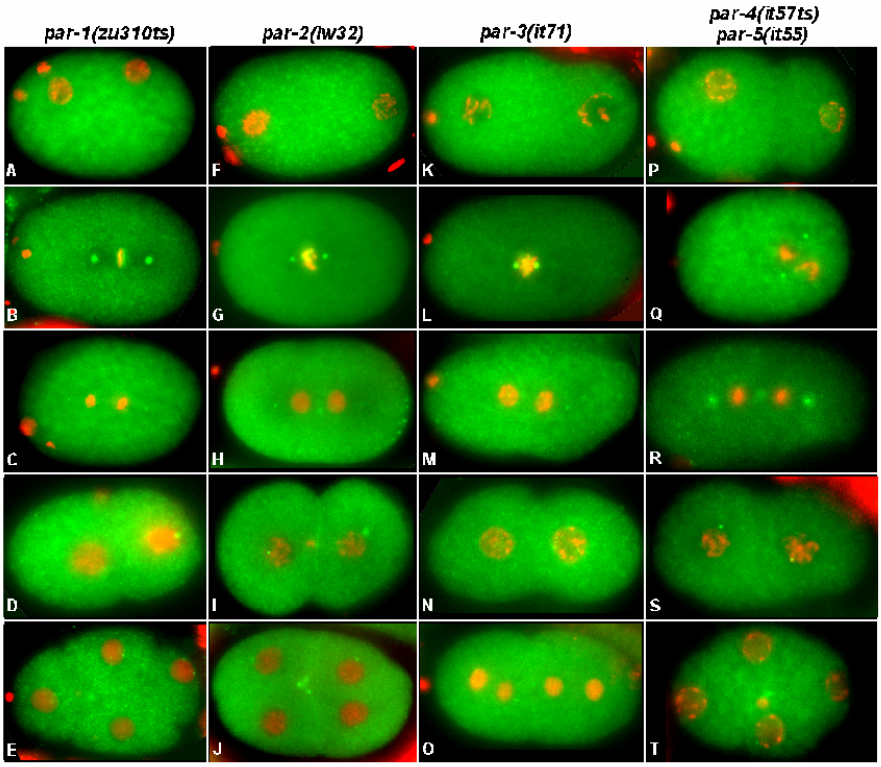

Figure IV.3. PAR mutants display symmetric PLK-1 staining. This figure displays merged images of PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. Selected embryos from the following mutants are shown *par-1(zu310ts)* (A-E), *par-2(lw32)* (F-J), *par-3(it71)* (K-O), and either *par-4(it57ts)* or *par-5(it55)* (P-T). Analysis of *par-6(zu222)* embryos yielded similar results to *par3(it71)*.

Many of the Par mutant embryos produce equal-sized cells from the first division. Also, because of polarity defects in many of the Par mutant embryos, the arrangement of the cells at the four-cell stage is abnormal. In *par-2* mutants, both of the cells at the two-cell stage divide synchronously and with transversely oriented spindles producing four equal-sized cells with a balanced arrangement (Fig. IV.3 J). In both *par-3* and *par-6* mutants, both of the cells at the two-cell stage divide synchronously and with longitudinally oriented spindles, producing four equal-sized cells arranged in a single row (Fig. IV.3 O). The embryos from the other Par mutants shown, *par-1*, *par-4*, and *par-5*, infrequently display division orientation defects similar to *par-2* mutant embryos.

These results suggest PLK-1 might function in the cytoplasm downstream of the PAR hierarchy. An important issue is to determine the dependency of PLK-1 asymmetry on MEX-5 and MEX-6. In *mex-5(RNAi); mex-6(pk440)* embryos, PLK-1 localization was similar to its localization in the Par mutants: the asymmetric localization of cytoplasmic PLK-1 was abolished (Fig. IV.4, D-F; n=18). The loss of cytoplasmic PLK-1 asymmetry was dependent on both *mex-5* and *mex-6*, because cytoplasmic PLK-1 staining in either *mex-5(RNAi)* (n=10) or *mex-6(pk440)* (n=21) embryos appeared similar to PLK-1 staining in wildtype embryos (Fig. IV.4, G-L).

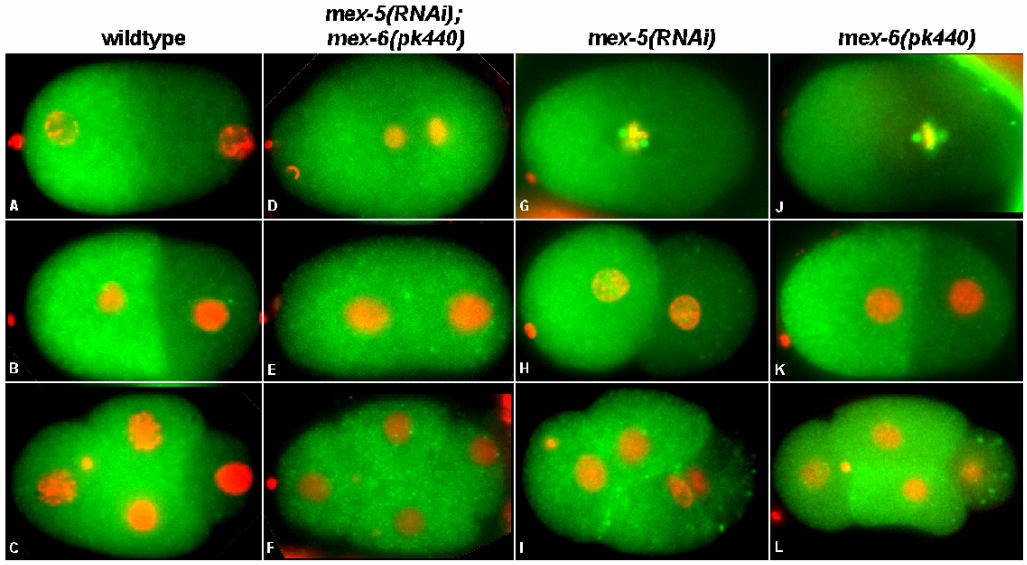

Figure IV.4. *mex-5* and *mex-6* are required redundantly for PLK-1 asymmetry. This figure displays merged images of PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. Selected embryos from the following genotypes are shown: wildtype(A-C), *mex-5(RNAi); mex-6(pk440)* (D-F), *mex-5(RNAi)* (G-I), and *mex-6(pk440)* (J-L).

The germline determinant, PIE-1, is localized to the posterior cytoplasm downstream of the Par hierachry and *mex-5/6*. We analyzed PLK-1 staining in *pie-1(zu154)* and *pie-1(RNAi)* embryos to determine whether PLK-1 cytoplasmic asymmetry is dependent on this downstream polarity factor. In *pie-1* mutant embryos, the localization pattern for PLK-1 was very similar to wildtype (Fig. IV.5, A-D; *n*=49). This result suggests that PLK-1 functions downstream of MEX-5/6 but upstream of PIE-1, as was demonstrated for MEX-5/6 (Schubert et al., 2000).

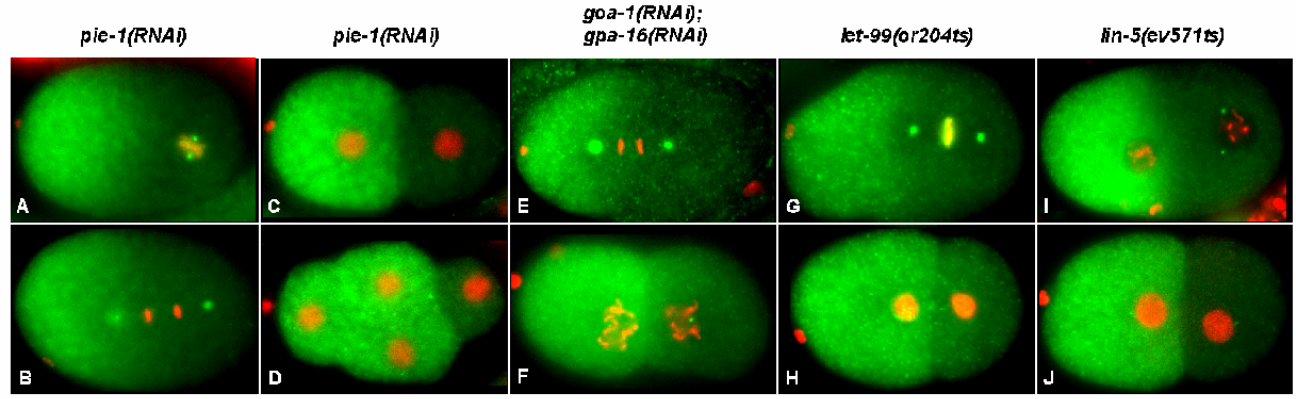

Figure IV.5. PLK-1 asymmetry appears normal in *pie-1* and G protein signaling mutants. This figure displays merged images, with PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. Selected embryos from the following genotypes are shown: *pie-1(RNAi)* (A-D), *goa-1(RNAi); gpa-16(RNAi)* (E-F), *let-99(or204ts)* (G-H), and *lin-5(ev571ts)* (I-J).

We also examined whether compromising heterotrimeric G protein signaling results in any perturbation in PLK-1 localization. Heterotrimeric G protein signaling is regulated downstream of the Par hierarchy and is required for some downstream polarity events. The $G\alpha_{i/o}$ subunits GOA-1 and GPA-16 are redundantly required for asymmetric cell division. We did not detect any change in PLK-1 localization in *goa-1(RNAi); gpa-16(RNAi)* embryos (Fig. IV.5, E-F; *n*=15). LIN-5 physically interacts with GOA-1, GPA-16, and GPR-1/2. LIN-5 mutants exhibit symmetric spindle placement (Lorson et al., 2000; Srinivasan et al., 2003). LIN-5 is predicted to regulate heterotrimeric G protein signaling perhaps via GPR-1/2 and GOA-1 and GPA-16 (Srinivasan et al., 2003). We did not detect any change in PLK-1 localization in *lin-5* mutant embryos (Fig. IV.5, I-J; *n*=13). LET-99 also might regulate G protein signaling during asymmetric cell division (Tsou et al., 2002). Again, we did not detect any change in PLK-1 localization in *let-99* mutant embryos (Fig. IV.5, G-H; *n*=12). These results demonstrate that PLK-1 asymmetry, like MEX-5 asymmetry, is established independently of G protein signaling.

3. Evidence that PLK-1 physically interacts with MEX-5 and MEX-6

Based on the co-localization of PLK-1 and MEX-5/6, we tested whether these proteins physically interact. Based on recent literature, we know the localization of Polo kinases can be regulated by the Polo box region's interaction with highly specific, phospho-dependent, binding motifs (Elia et al., 2003a; Elia et al., 2003b). Sequence analysis of MEX-5 and MEX-6 revealed a conserved, putative Polo kinase interaction motif. We found the Polo box domain of PLK-1 physically interacts with both MEX-5 and MEX-6 in a yeast two-hybrid assay, if this motif is mutated so as to mimic phosphorylation of the putative PBD interaction motif.

Structural analyses (Elia et al., 2003b) revealed that the two Polo boxes combine to form a larger functional domain termed the Polo box domain (PBD). Crystal structure analysis of human Plk1's PBD region bound to a peptide substrate uncovered how phosphorylated substrates interact with the PBD via numerous hydrogen bonds. The interaction between the PBD and artificial peptides is high-affinity, sequence-specific, and absolutely phosphorylation-dependent. The implication of these studies is that the PBD directs Polo kinase binding to protein partners via priming phosphorylation of docking sites (Elia et al., 2003b)

The consensus PBD interaction sequence was determined biochemically to be S-**pT**-P, with less stringent requirements for the surrounding sequences. This conserved, PBD-interaction motif resembles the consensus sequence for phosphorylation by the Cdks and other proline-directed kinases of the CMGC superfamily, such as MAPKs and GSKs (Elia et al., 2003b). Our examination of MEX-5 and MEX-6 revealed a putative PBD interaction site containing the conserved S-**T**-P motif. However, the full-length PLK-1 does not interact with either **MEX-5^{T186D}** or **MEX-6^{T190D}** in a yeast two-hybrid assay.

Studies of human Plk1 have shown that initially Plk1 is inactive, due to intramolecular inhibition of the kinase domain by the PBD. In theory, the naïve PLK-1 is locked in a closed position by this intra-molecular inhibition. This is likely to be the case with *C. elegans* PLK-1, because a recombinant, full-length PLK-1 purified from *E. coli* does not show any kinase activity in vitro (data not shown). We hypothesized that the structure of full-length PLK-1 might prevent the PBD from interacting with its targets, such as MEX-5 and MEX-6. Therefore, we truncated the yeast two-hybrid construct of PLK-1 so it contained only the PBD domain and surrounding sequences. This construct (**PLK-1^{PBD}**) was composed of the 330 C-terminal amino acids of PLK-1; this is roughly 50% of the protein sequence.

The **PLK-1^{PBD}** fragment did not interact with either wildtype MEX-5 or MEX-6. However, the **PLK-1^{PBD}** fragment did interact with **MEX-5^{T186D}** and **MEX-6^{T190D}**, suggesting specificity for the phosphorylation mimicking mutant constructs (Fig. IV.6). This interaction can be disrupted if complementary mutations are constructed in the residues of the PBD required for phospho-peptide binding. We constructed two different versions of **PLK-1^{PBD}** with compromised phospho-binding by mutating conserved residues in either the first or second polo box, **PLK-1$^{PBD\ polo\ box\ 1*}$** **PLK-1$^{PBDpolo\ box\ 2*}$**. Synonymous mutations in human Plk1 did not alter the overall protein structure (Elia et al., 2003b). Neither **PLK-1$^{PBD\ polo\ box\ 1*}$** or **PLK-1$^{PBDpolo\ box\ 2*}$** interacted with **MEX-5^{T186D}** or **MEX-6^{T190D}** (Fig. IV.6).

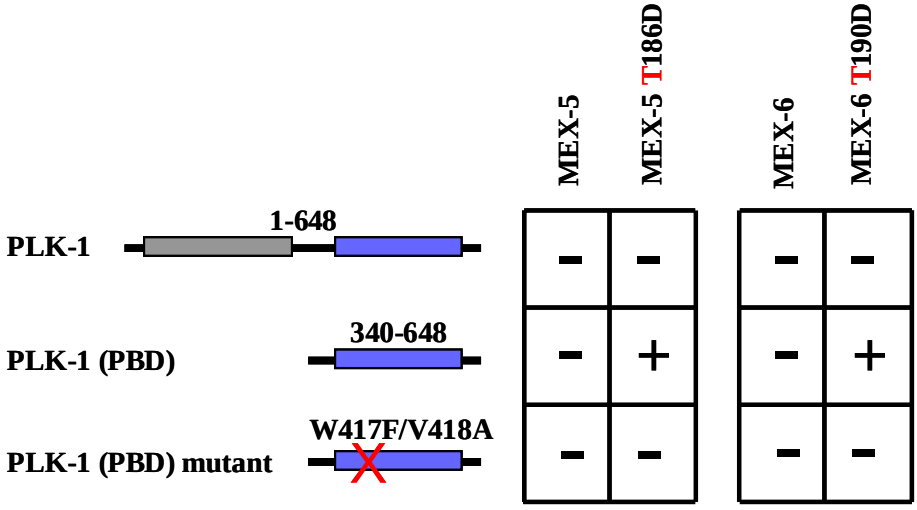

Figure IV.6. The PLK-1^{PBD} construct physically interacts with MEX-5^{T186D} and MEX-6^{T190D}. Yeast two-hybrid analysis of protein interaction. A plus denotes positive interaction, and a minus denotes no interaction, as compared to appropriate controls. Neither wildtype MEX-5 or MEX-6 interacted with any of the PLK-1 constructs tested. The **PLK-1^{PBD}** fragment did interact with **MEX-5^{T186D}** and **MEX-6^{T190D}.** This interaction was disrupted by mutations in polo box 1 W417F and V418A, or polo box 2 H542A and K544M.

These results suggest that PLK-1 physically binds phosphorylated MEX-5 and MEX-6 in vivo in order to asymmetrically localize PLK-1. In essence, our hypothesis is that PLK-1 'piggy backs' on MEX-5/6 for its asymmetric localization. Based on these results, we hypothesize that asymmetric localization of PLK-1 in vivo will depend on MEX-5T186 and MEX-6T190. In order to test this idea, we constructed a transgenic worm strain expressing a MEX-5T186A mutant GFP fusion protein (**Ppie-1::GFP::MEX-5^{T186A}**). This GFP fusion protein appeared to have wildtype localization, as compared to a wildtpye GFP::MEX-5 strain generated by Cuenca et al. (2003). Previously, it was demonstrated that a wildtype MEX-5 GFP fusion was able to rescue the *mex-5/6* mutant using a similar transgenic strain (Schubert et al., 2000).

We plan to test whether the T186 residue is required for this ability to rescue the *mex-5/6* phenotype. Currently, the result is not known. However, if T186 is essential, it implies that this residue is essential for MEX-5 function. If the anterior enrichment of PLK-1 is abnormal in this situation, it suggests the functional requirement of T186 is for targeting PLK-1 activity to the anterior cytoplasm.

4. PLK-1 asymmetry is predicted to depend on upstream kinases responsible for MEX-5/6 phosphorylation

Based on our yeast two-hybrid results and the published models for Plk1 localization (Elia et al., 2003b), we hypothesize that PLK-1 might bind MEX-5 and MEX-6 in vivo only when they are phosphorylated by an unknown kinase (or kinases). Kinases that phosphorylate PBD interaction S-T-P motifs are predicted to be from the CMGC super family. In *C. elegans*, there are over 30 kinases in this group; however, we chose to examine only the candidates required for embryonic viability. One likely candidate is NCC-1/Cdk1. However, *ncc-1(RNAi)* caused no perturbation of the PLK-1 asymmetry at the one-cell stage, despite the presence of numerous other defects (Fig. IV.7 E; *n*=11). Similarly, there was very little perturbation of PLK-1 localization caused by inhibition of two other candidates, *mbk-2* and *gsk-3* (Fig. IV.7; F and I; *n*=9 and *n*=10 respectively). However, temperature sensitive *mpk-1(ga111ts)* mutant embryos appeared to have lost PLK-1 asymmetry (Fig. IV.7, D and G; *n*=3).

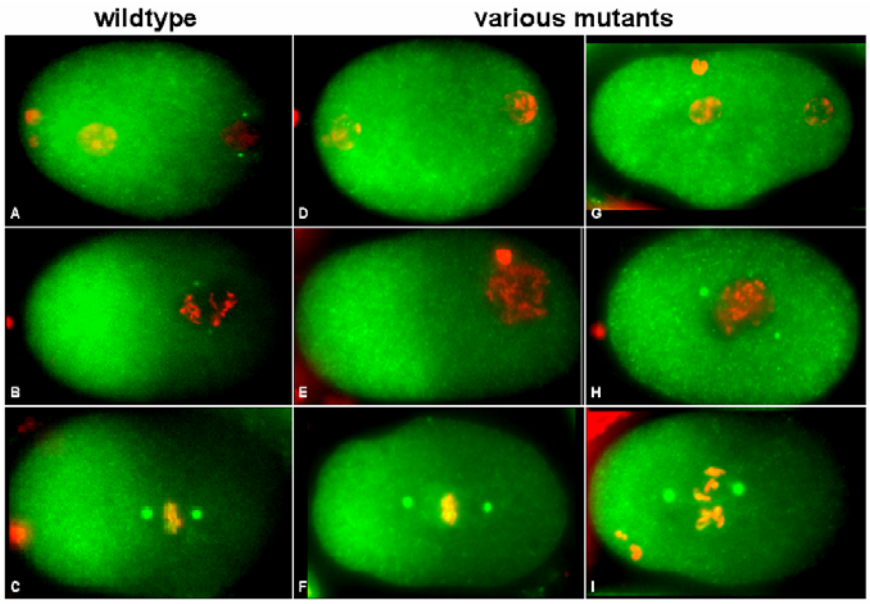

Figure IV.7. Analysis of PLK-1 asymmetry in several CMGC kinase mutants. This figure displays merged images with PLK-1 antibody staining in green and DAPI staining in red. All images are oriented with the anterior pole to the left. Selected embryos from the following genotypes are shown: wildtype (A-C), *mpk-1(ga111ts)* (D, G), *ncc-1(RNAi)* (E), *gsk-3(RNAi)* (F), *cul-2(RNAi)* (H), and *mbk-2(RNAi)* (I).

MPK-1 is a mitogen-activated kinase (MAPK) that has been characterized in *C. elegans* (Church et al., 1995; Ohmachi et al., 2002). Although there is no evidence that MPK-1 is activated at the one-cell stage, it is known that MPK-1 is activated in the germline and oocytes, where it performs essential functions (Church et al., 1995; Ohmachi et al., 2002). Perhaps one function of MPK-1 is to phosphorylate both MEX-5 and MEX-6 to regulate latter events during embryogenesis.

CUL-2 functions in an ECS complex to degrade polarity factors after the intial polarization of the PAR domains (DeRenzo et al., 2003). We observed a loss of PLK-1 asymmetry *cul-2(RNAi)* embryos (Fig. IV.7 H; n=5). The implications of this are not clear, however recently *cul-2(RNAi)* was shown to be required for proper AP polarity prior to the

establishment of PAR cortical polarity, at a time during meiosis II (Liu et al., 2004; Sonneville and Gonczy, 2004).

5. Inhibition of PLK-1 reveals minor polarity defects

The polarization of the zygote is initiated by the sperm asters and requires Par proteins. Establishment of two mutually exclusive Par domains begins after meiosis II, during the first S-phase. Initially, PAR-6/PAR-3/PKC-3 complex is everywhere; then upon the appearance of the pronuclei, the cortical Par domains become polarized. First, PAR-2 becomes enriched at the posterior cortex near the sperm asters. Then the posterior PAR-2 domain expands towards the anterior as the PAR-6/PAR-3/PKC-3 domain retracts away from the posterior. These two cortical PAR domains are mutually exclusive, such that as the posterior cortical domain expands the anterior cortical domain recedes. Once established at metaphase the two PAR domains can be observed oscillating slightly back and forth during a PAR polarity maintenance phase (Cuenca et al., 2003).

We examined PAR localization in *plk-1(RNAi)* embryos. Both the anterior and posterior cortical domains were localized with the proper polarity. Although the establishment of the cortical Par domains appeared normal in *plk-1(RNAi)* embryos, the maintenance of these domains was not (Fig. IV.8). In wildtype embryos, the PAR-3/6/PKC-3 domain extended slightly past the midpoint of the embryo towards the posterior, on average about 56% of the embryo length (Fig. IV.8; n=10). Also, the PAR-2 domain directly abutted the PAR-3/6/PKC-3 domain. In contrast, *plk-1(RNAi)* embryos exhibited a reduced PAR-3/6/PKC-3 domain that did not even reach to the midpoint of the cell, on average 44% of the embryo length (Fig. IV.8; n=44). Also, 66% of *plk-1(RNAi)* embryos displayed a posterior PAR-2 domain that extended

past the midpoint into the anterior cortex of the embryo (Fig. IV.8; *n*=44). Unlike the situation in wildtype embryos, the boundary between the anterior PAR-3/6/PKC-3 domain and the posterior PAR-2 domain was indistinct in *plk-1(RNAi)* embryos (Fig. IV.8; *n*=16).

We also examined *air-2(RNAi)* embryos as a control. Although *air-2* RNAi results in similar defects in both the meiotic and mitotic divisions as *plk-1* RNAi, there are no obvious defects in cellular polarity in *air-2(RNAi)* embryos. For example, PAR localization in *air-2(RNAi)* embryos appeared similar to wildtype embryos (Fig. IV.8; *n*=21). On average, the PAR-3/PKC-3 domain was positioned about 58% of the embryo length in *air-2(RNAi)* embryos (*n*=21). Also, the anterior and posterior PAR domains were in direct contact at their borders (Fig. IV.8). Only 10% of *air-2(RNAi)* embryos exhibited a PAR-2 domain that extended past the midpoint into the anterior cortex (*n*=21). The proper localization of PAR domains in *air-2(RNAi)* suggests that proper chromosome segregation and cytokinesis are not prerequisites for maintaining cellular polarity.

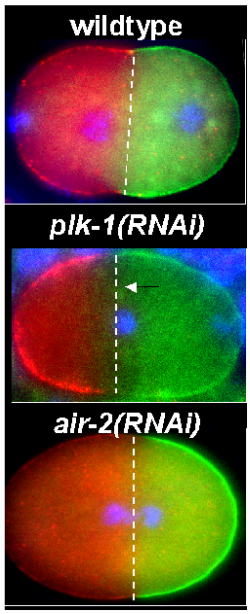

Figure IV.8. PAR localization in *plk-1(RNAi)* and *air-2(RNAi)* embryos. This figure shows merged images of fixed and stained embryos during the first mitotic division. All embryos are oriented with the anterior pole to the right. PKC-3 antibody staining, which marks the anterior cortical PAR domain, is shown in red. PAR-2 staining, which marks the posterior cortical PAR domain, is shown in green, and DAPI staining is presented in blue. A dashed line marks the position of the boundary between anterior and posterior Par domains. An arrow indicates the expansion of the posterior PAR domain in the *plk-1(RNAi)* embryo.

PIE-1 is a germline determinant that is enriched in the posterior cytoplasm prior to the first division. Subsequently, PIE-1 is preferentially inherited by the posterior P1 blastomere. MEX-5/6 are known to be required for PIE-1 polarization to the posterior cytoplasm. We believed it was likely that PLK-1 would be required for PIE-1 polarization as well. However, we discovered that *plk-1(RNAi)* had no overt effect on PIE-1 localization at the one-cell stage (Fig IV.9; *n*=17). Although both chromosome segregation and cytokinesis were defective in *plk-1(RNAi)* embryos, PIE-1 localization was surprisingly similar to its localization in wildtype embryos. This result suggests that PLK-1, unlike MEX-5/6, is not required to polarize the cytoplasmic factors such as PIE-1. Therefore, PLK-1 does not appear to function upstream of PIE-1.

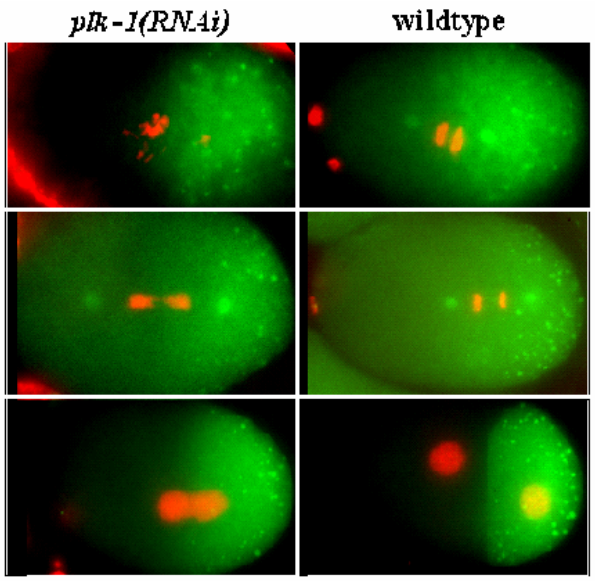

Figure IV.9. PIE-1 polarization appears normal in *plk-1(RNAi)* embryos. This figure shows merged images of fixed and stained embryos. PIE-1 staining is shown in green and DAPI staining is shown in red. Although chromosome morphology and segregation are defective in *plk-1(RNAi)* embryos, the localization of PIE-1 resembles that of wildtype embryos. The PIE-1 staining on the centrosomes reveals a more symmetric spindle in *plk-1(RNAi)* embryo. PIE-1 remains asymmetrically localized to the posterior pole in *plk-1(RNAi)* embryos, despite the failure in cytokinesis and chromosome segregation.

Another important polarity regulator is MEX-5. Because of our evidence that MEX-5 and PLK-1 physically interact, we thought it was possible that PLK-1 is required for the proper polarization of MEX-5. However, MEX-5 localization appeared normal in *plk-1(RNAi)* embryos (Fig. IV.10 B; *n*=4). Similar to the behavior of PIE-1, the localization of the P granules was unaffected in *plk-1(RNAi)* embryos (Fig. IV.8 D; *n*=14)

Another marker we examined was LET-99. Normally, LET-99 is localized to the cortex in band corresponding to the future cleavage plane. This LET-99 band is slightly displaced toward the posterior and aligns with the chromosomes at metaphase (Fig. IV.10 E). We observed abnormal LET-99 localization in *plk-1(RNAi)* embryos (Fig. IV.10, F and H; *n*=10). In most cases, LET-99 was localized to a central band, but the band was oriented at an oblique

angle to the equator of the cell (Fig. IV.10 F; *n*=7). In rare cases, LET-99 was found localized ectopically throughout the anterior cortex (Fig. IV.10 H; *n*=3).

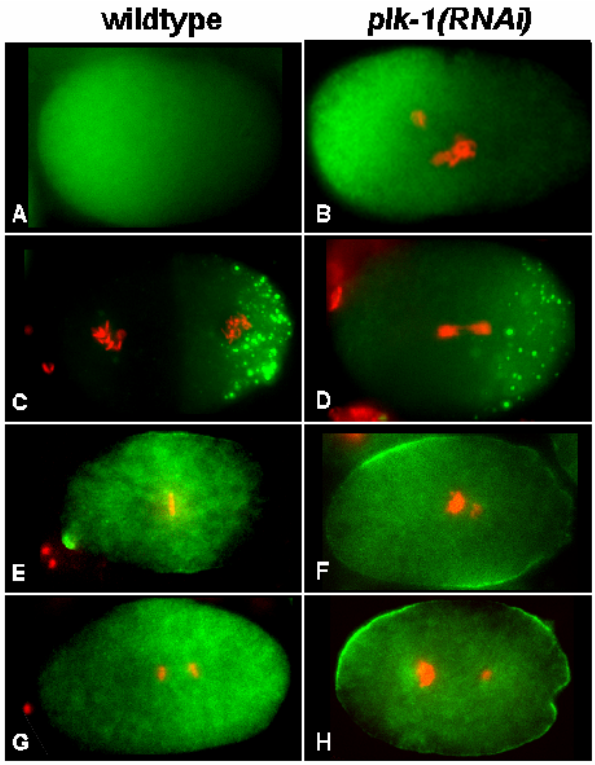

Figure IV.10. The localization of various polarity markers in *plk-1(RNAi)* embryos. This figure shows merged images of fixed and stained embryos. Antibody staining or GFP is shown in green, and DAPI staining is shown in red. Wildtype embryos are shown in the left column, and *plk-1(RNAi)* embryos are shown in the right column. MEX-5 localization (A-B), P granule localization (C-D), and LET-99 localization (E-H) are indicated.

Although no defects in cytoplasmic polarity were observed in *plk-1(RNAi)* embryos, we did observe spindle position defects during the first mitotic division. In both wildtype embryos, the spindle was displaced slightly towards the posterior, which ensures the unequal size of the daughter cells (Fig. IV.11). In *plk-1(RNAi)* embryos, the spindle remained centrally located (Fig. IV.11), which would result in equal-sized daughters, except for the fact that cytokinesis did not occur. We analyzed many embryos to calculate the mean spindle position (Table IV.1). The

deviation of the spindle position from the center of the cell was measured as a percentage of the length of the long axis of the cell. Consistent with measurements reported in the literature, the spindle position in wildtype embryos was 56%, displaced to the posterior. However, the spindle position was reduced to near 50% in *plk-1(RNAi)* embryos (Table IV.1). This analysis was performed without a marker for the anterior-posterior axis in *plk-1(RNAi)* embryos. We also analyzed *air-2(RNAi)* embryos, which revealed spindle positions very similar to wildtype embryos, despite chromosome segregation and cytokinesis defects (Table IV.1).

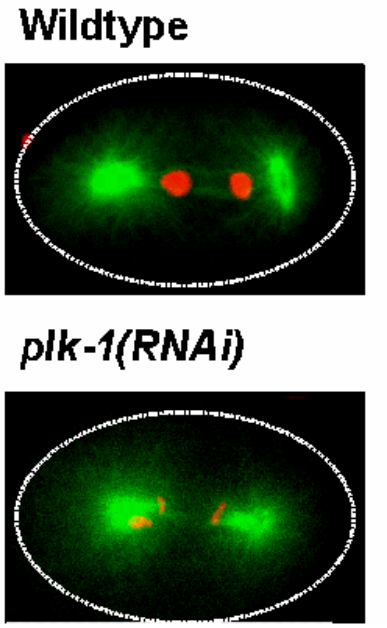

Figure IV.11. The spindle remains symmetric in *plk-1(RNAi)* embryos. The figure shows merged images of fixed and stained embryos; tubulin is shown in green, and DAPI staining is shown in red. Both images are oriented with the anterior pole to the left. The dashed line indicates the cell boundary. In wildtype embryos, the spindle is displaced slightly towards the posterior pole and the posterior centrosome has a characterstic flattened appearance. In *plk-1(RNAi)* embryos, the spindle remains symmetrically positioned and both centrosomes display a morphology similar to the anterior centrosome in wildtype embryos.

Table IV.1. Mean (SD) Spindle Position as Percentage of Diameter of Embryo Length

Genotype	% Embryo Length	Sample Size
Wildtype	**56.5 ± 1.7**	***n* = 35**
plk-1(RNAi)	**51.3 ± 1.0**	***n* = 42**
air-2(RNAi)	**55.6 ± 1.6**	***n* = 64**

Also, we observed in *plk-1(RNAi)* embryos that the posterior centrosome displayed a morphology similar to that of the anterior centrosome in wildtype embryos (Fig. IV.11). This phenotype was obserbed in *par-3*, *par-6*, and *pkc-3(RNAi)* mutant embryos. In these mutants, this phenotype was caused by a lack of posterior forces acting on the posterior centrosome. This result suggests that *plk-1(RNAi)* might result in defects in the function of the anterior cortical PAR complex, which is composed of PAR-3/6/PKC-3 and also utilizes CDC-42.

C. Discussion: PLK-1 regulates cell polarity downstream of the PAR genes

The early *C. elegans* embryo is a powerful tool for study of the process of cell polarity and asymmetric cell division (Cowan and Hyman 2004). The polarization of PLK-1 to the anterior cytoplasm during asymmetric cell division is intriguing. The localization of cytoplasmic PLK-1 by antibody staining mirrors the localization of two known polarity regulators, MEX-5 and MEX-6.

We propose PLK-1 functions downstream of the PAR hierarchy to regulate cellular polarization prior to asymmetric cell division (Fig. IV.10). The asymmetric localization of PLK-1 is dependent on all par genes tested and resembled the localization of MEX-5/6. Also, the initial polarization of the PAR proteins appears normal in *plk-1(RNAi)* embryos. These results suggest PLK-1 functions downstream of the PAR network. Finally, we showed that PLK-1 is mis-localized in *mex-5/6* mutants, suggesting PLK-1 also acts downstream of MEX-5/6 (Fig. IV.10).

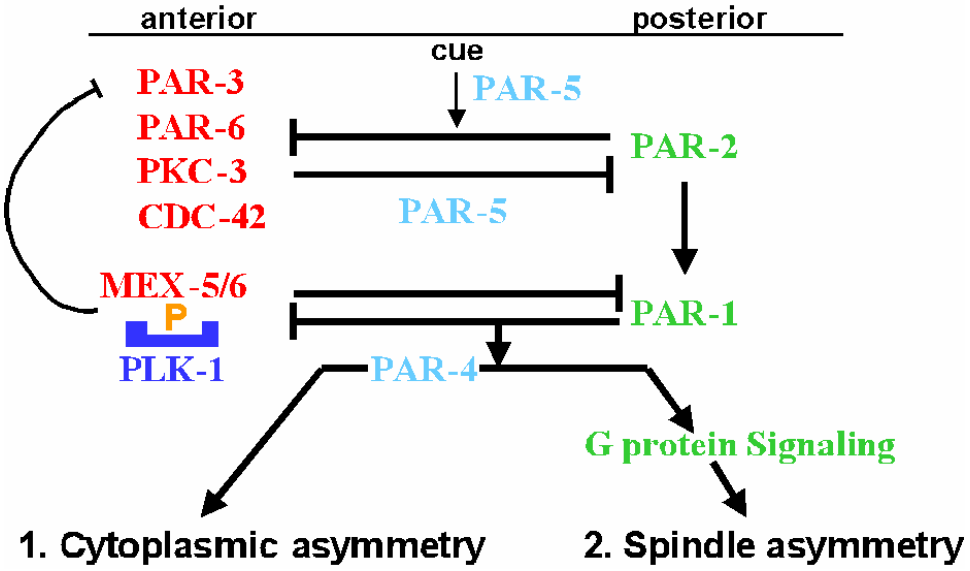

Figure IV.12. Model for the function of PLK-1 in asymmetric cell division. A model for the position of PLK-1 in a complex hierarchy of cell polarity regulators. PLK-1 cytoplasmic asymmetry is downstream of the PARs and MEX-5/6 but independent of PIE-1 and G protein signaling. PLK-1 might interact with MEX-5/6 to regulate downstream PAR events, such as spindle asymmetry and maintenance of PAR cortical polarities.

We also show PLK-1 is required for the proper maintenance of the cortical localization of the PARs and the normal asymmetric positioning of the mitotic spindle. This spindle position defect might result directly from the loss of PLK-1 or indirectly, via the failure of maintaining proper PAR localizations. It is intriguing that PLK-1 asymmetry resembles MEX-5/6 asymmetry and the loss-of-function phenotypes of PLK-1 somewhat resemble previously reported results for MEX-5/6 (Cuenca et al., 2003). Also, we have detected direct molecular interactions between PLK-1 and MEX-5 or MEX-6 using a yeast two-hybrid assay. This suggests that PLK-1 might function directly with phosphorylated MEX-5 and MEX-6 to regulate cell polarity and asymmetric cell division (Fig. IV.10).

Although, the functional connection between PLK-1 and cell polarity is not clear, I will speculate on several possibilities: (1) PLK-1 might function, along with MEX-5/6, in the maintenance of PAR localization to distinct cortical domains; (2) PLK-1 might be involved primarily in regulating microtubule and spindle dynamics during asymmetric cell division; (3) PLK-1 might function together with MEX-5/6 to target the degradation of germline factors, both prior to and after asymmetric cell division; and (4) PLK-1 might play as of yet unknown roles in somatic cells after asymmetric cell division.

1. PLK-1 and maintenance of PAR domains

Previously it was shown that MEX-5/6 were required to maintain proper cortical PAR polarity during the first mitotic division (Cuenca et al., 2003). Although MEX-5/6 localization is downstream of the Par hierarchy, MEX-5/6 appeared to regulate the maintenance of the localization of the cortical PAR domains. MEX-5/6 did not appear to regulate the establishment of the PAR domains, but instead regulated their maintenance latter during the cell cycle. In the

majority of *mex-5/6* mutant embryos, the anterior PAR domain extended abnormally far towards the posterior. The mechanism of this feedback regulation was unknown; however, *par-1* mutants exhibited an opposite phenotype (Cuenca et al., 2003). Perhaps PLK-1 is involved with either PAR-1 or MEX-5/6 in this mechanism.

In contrast to *mex-5/6* embryos, *plk-1(RNAi)* embryos exhibited an opposite phenotype. In *plk-1(RNAi)* embryos the posterior PAR domain extended abnormal far into the anterior. This observation suggests that PLK-1 and MEX-5/6 probably have an antagonistic relationship, with PLK-1 inhibiting MEX-5/6 function or vice versa. The PAR localization phenotypes observed in *plk-1(RNAi)* embryos are similar to the phenotypes observed in *par-1* mutant embryos.

2. PLK-1 and spindle placement

The defect in spindle position in *plk-1(RNAi)* embryos was highly penetrant. This defect could be caused by a change in the forces acting upon the spindle. Normally, the posterior centrosome is under stronger astral microtubule forces, which are pulling it towards the posterior pole. This force asymmetry is dependent on the PAR hierarchy, on two Gα subunits GOA-1 and GPA-16, and on the heterotrimeric G protein regulators GPR-1/2, LET-99, LIN-5 and RIC-8/RGS-7 (Tsou et al., 2003; Gotta et al., 2003; Afshar et al., 2004; Hess et al., 2004).

What role PLK-1 might play in spindle placement is unclear. The most likely case is the defect in cortical PAR domain maintenance might result in the abnormal spindle placement observed in *plk-1(RNAi)* embryos. An alternative explanation is that *plk-1(RNAi)* might disrupt the timeliness or coordination of the progression of the cell cycle. As noted, *plk-1(RNAi)*

embryos display severe delays in the progression of the cell cycle. Although not likely, it is possible that defects in the cell cycle might indirectly affect spindle polarity.

There were two subsets of PLK-1 in the cell: (1) PLK-1 that was localized symmetrically to the centrosomes, and (2) PLK-1 that was localized asymmetrically within the cytoplasm. The regulation of spindle position could be under the control of either subset or both. However, both PAR-1 and PAR-4 do not have penetrant spindle positioning defects; instead they have severe cytoplasmic polarity defects. In both of these mutants, PLK-1 asymmetry was abolished suggesting that a loss of PLK-1 asymmetry does not strictly coorelate with a loss of spindle asymmetry. This observation suggests that it is the subset of PLK-1 at the centrosomes that regulate spindle asymmetry. Other factors involved in heterotrimeric G protein signaling, such as LIN-5 and heterotrimeric G protein subunits, have been observed localized to the centrosomes as well.

3. PLK-1 and targeted protein degradation

Recently, it was shown that Plk1 regulates Cdk1 activation by directly targeting Wee1 for degradation by the SCF complex (Skp1, Cullin1 and F-box). Plk1 phosphorylates Wee1 in a motif that is recognized as a phosphorylation dependent degradation sequnece by the SCF complexed with βTrCP (Watanabe et al., 2004). Another recent study showed Plk1 phosphorylates another cell cycle regulator, Emi1, which in turn targets Emi1 for degradation (Hansen et al., 2004; Moshe et al., 2004). Again, Emi1 is degraded by the SCF complexed with βTrCP. The result of Emi1 degradation is the activation of the APC. These studies support a role for Polo kinases in targeting their substrates for degradation via a conserved βTrCP mediated phosphor-degron motif, D-pS-G-φ-X-pS (Wu et al., 2003). Perhaps, PLK-1 functions

in the early *C. elegans* embryo by by targeting factors for degradation via the SCF complex as well.

Recently several cases have been shown where the PBD binding partner is also a substrate of Plk1, such as in Nir2, Nlp, Cdc25, and Wee1 (Litvak et al., 2004). These studies raise the possibility that PLK-1 phosphorylates MEX-5 and MEX-6. However sequence analysis does not reveal any obvious Polo kinase phosphorylation site or phospho-degron in either MEX-5 or MEX-6. Another possibility is that MEX-5/6 target PLK-1 to phosphorylate an as of yet unknown substrate in order to target it for degradation by the SCF complex. Perhaps all the pleiotropic phenotypes associated with Polo kinases can be explained by a common molecular mechanism: the target degradation of various factors in coordination with the progression of the cell cycle.

4. Significance

Polo kinases have been studied extensively in various organisms for their roles in cell division and cancer formation. The possibility of Polo kinases functioning during asymmetric cell division, as described here in *C. elegans*, is entirely novel. It will be interesting to see if Polo kinases function in asymmetric cell division in other animals.

The implication of PLK-1 as a regulator of cellular polarity, asymmetric cell division, and cell fate in the early *C. elegans* embryo is intruiging. Previously, *air-1(RNAi)* was shown to perturb cellular polarity. However, this is probably an indirect result of centrosomal defects (Golden et al., 2000; Wallenfang and Seydoux, 2000; Shakes et al., 2003; Cowan and Hyman, 2004). Also, in *Drosophila*, Aurora-A was shown to be required for asymmetric cell division of

neuroblasts. In this case, the effect is suggested to be independent of centrosomal/microtubule defects (Berdnik and Knoblich, 2002). There is no evidence of Aurora-B kinases playing a role in asymmetric cell division or cell fates. Here we report that the polo kinase PLK-1 might be involved in asymmetric cell division in *C. elegans*.

Both Aurora and Polo kinases have been extensively studied for their roles in cell division. To date, most of these studies have been performed in the context of single cells. This is probably a result of their essential requirement for cellular life, which prevents analysis of multicellular stages. Also in cases were mutants exist, such as *Drosophila* and *C. elegans*, it is difficult to analyze their roles, because homozygotes grow using maternal supplies which are depleted slowly and in different cells at different times. We are attempting to use weaker RNAi phenotypes to obtain multicellular *C. elegans* embryos depleted for PLK-1, hoping to reveal new polarity or cell fate defects during embryogenesis. In the future, it will be interesting to see what roles both Aurora and Polo kinase play during asymmetric cell division in animal development.

APPENDIX A.

Mutants that do not separate chromosomes during cell division.

Mutant	Phenotype	Species
APC subunits	Chromosomes never separate in mitosis or meiosis	Vertebrate *C.e. S.c.* (Holloway et al., 1993; Irniger et al., 1995; Ciosk et al., 1998; Furata et al., 2000; Golden et al., 2000)
Cdc20	Chromosomes never separate in mitosis or meiosis	Vertebrate *D.m. S.c.* (Palmer et al., 1989; Dawson et al., 1995; Sigrist et al., 1995; Lorca et al., 1998)
Securin	Chromosomes never separate in mitosis or meiosis	Vertebrate *D.m. C.e. S.p.* (Funabiki et al., 1996; Stratmann and Lehner, 1996; Mei et al., 2001; Jallepalli et al., 2001; Kitagawa et al., 2002)
Separase	Chromosomes never separate in mitosis or meiosis	Vertebrate *C.e. S.p. S.c.* (Funabiki et al., 1996; Ciosk et al., 1998; Siomos et al., 2001)
Scc1 – non-degradable	Chromosomes never separate in mitosis	Vertebrate *S.p. S.c*. (Uhlmann et al., 1999; Hauf et al., 2001; Toyoda et al., 2002)
Rec8 – non-degradable	Chromosomes never separate in meiosis I or II	*S.c.* (Buonomo et al., 2000)
Securin– non-degradable	Chromosomes never separate in mitosis	Vertebrate (Zou et al., 1999; Zur and Brandeis, 2001; Hagting et al., 2002)
Aurora B	Chromosomes never separate in meiosis	*C.e*. (Rogers et al., 2002)
Plk1	Chromosomes never separate in mitosis	Vertebrate (Sumara et al., 2002)
Plk1 and Aurora B	Chromosomes never separate in mitosis	Vertebrate (Losada et al., 2002)
Plk1 and Aurora B	Chromosomes never separate in mitosis	Vertebrate (Gimenez-Abian et al., 2004)

***** *C.e*. is *Caenorhabditis elegans*, *D.m.* is *Drosophila melanogaster*, *S.c*. is *Saccharomyces cerivisae*, and *S.p.* is *Schizosaccharomyces pombe*

APPENDIX B.

Mutants that display precocious separation of sister chromatids during meiosis.

Mutant	Phenotype	Species
Rec8	Precocious separation	*C.e. S.c.* (Parisi et al., 1999; Klein et al., 1999; Watanabe and Nurse, 1999; Pasierbek et al., 2001)
Spo12	Precocious separation	*S.c.* (Shah et al., 2002)
Spo13	Precocious separation	*S.c.* (Shonn et al., 2002; Lee et al., 2002)
Slk19	Precocious separation	*S.c.* (Kamieniecki et al., 2000; Zeng and Saunders, 2000)
Cdc14	Precocious separation	*S.c.* (Sharon and Simchen, 1990a)
Bub1	Precocious separation	*S.p. S.c.* (Bernard et al., 2001; Yamaguchi et al., 2003)
Sgo1 Mei-S332	Precocious separation	*D.m. S.p. S.c.* (Tang et al., 1998; Moore et al., 1998; Kitajima et al., 2004; Rabitsch et al.,2004)
GSP-1/2	Precocious separation	*C.e.* (Rogers et al., 2002)

***** *C.e*. is *Caenorhabditis elegans*, *D.m.* is *Drosophila melanogaster*, *S c*. is *Saccharomyces cerivisae*, and *S p.* is *Schizosaccharomyces pombe*

APPENDIX C.

Known and putative Aurora-B kinase substrates.

Targets	Sequence	Situation	Function
Aurora B	S5 QRN**S**LVN T260 RRK**T**VCG	In vitro	Unknown (Biggins et al., 1999; Yang et al., 2000; Kang et al., 2001; Cheeseman et al., 2002)
Histone H3	S10 ARK**S**TGG S28 ARK**S**APS	In vivo, in vitro	Chromosome condensation (Hsu et al., 2000; Sugiyama et al., 2002)
CENP-A	S7 RRR**S**RKP	In vivo, in vitro	Cytokinesis (Zeitlin et al., 2001)
INCENP	S578RLKE**S**LAP S598KKRG**SS**AVW S894HKRT**S**SAV	In vivo, in vitro	Kinase stimulation (Kang et al., 2001; Bishop and Schumacher, 2002; Cheeseman et al., 2002; Honda et al., 2003)
Survivin	T117 AKE**T**NNK		(Wheatley et al., 2004)
REC-8	T625KRRE**T**SII	In vitro	Cohesin removal from chromosome (Rogers et al., 2002)
Dam1	S20 YRL**S**IGS S257 RRK**S**ILH S265 IRN**S**IAS S292 NRI**S**LGS	In vivo, in vitro	Phenocopies ipl1 mutant (Cheeseman et al., 2002; Li et al., 2002)
Ask1	S200KRKI**S**LL	In vivo, in vitro	Phenocopies ipl1 mutant (Cheeseman et al., 2002; Li et al., 2002)
Spc34	T199 RRK**T**IFV	In vivo, in vitro	Phenocopies ipl1 mutant (Cheeseman et al., 2002; Janke et al., 2002)
Ndc80	S100 SRL**S**INQ	In vitro	Unknown (Cheeseman et al., 2002)
RacGAP	S387 YRI**S**GCD	In vivo, in vitro	Cytokinesis (Minoshima et al., 2003)
GFAP	T7 RRRI**T**SAR S13 ARR**S**YVS S38 TRL**S**LAR	In vivo, in vitro	Cytokinesis (Kawajiri et al., 2003)
Desmin	S11 QRV**S**SYR T16 YRR**T**FGG S59 SRT**S**GGA	In vivo, in vitro	Cytokinesis (Kawajiri et al., 2003)
Vimentin	S72 RLR**S**SVP	In vitro	Unknown (Kawajiri et al., 2003)
MCAK	S92 KRR**S**VNS S106 GLR**S**RST S186 RRK**S**CIV	In vivo, in vitro	Unknown (Andrews et al., 2004; Lan et al., 2004)

APPENDIX D.

Known and putative Polo kinase substrates.

Targets	Sequence	Situation	Function
Plk1/Polo	S260 NEY**S**IPK S326 IDQ**S**LRK	In vivo, in vitro	Catalytic activation (Wind et al., 2002)
Scc1	WDT**S**LEV DDN**S**VEQ	In vivo, in vitro	Chromosome cohesion removal (Alexandru et al., 2001; Sumara et al., 2002; Losada et al., 2002)
Cyclin B	S133 MET**S**GCA S147 QAF**S**DVI	In vivo, in vitro	Cdk1/cyclin-B activation/nuclear entry (Toyoshima-Morimoto et al., 2001; Jackman et al., 2003;)
Cdc25	S198 MEF**S**LKD	In vivo, in vitro	Cdk1/cyclin-B activation (Toyoshima-Morimoto et al., 2002)
Cdc27/APC3	T220 PQD**T**IEL S427 IND**S**LEI S435 LDS**S**IIS	In vitro	APC activation (Golan et al., 2002; Kraft et al., 2003)
APC4	S770 LSE**S**EAE	In vitro	APC activation (Kraft et al., 2003)
APC7	S40 LTM**S**NNN S33 RLL**S**SLL S17 GLH**S**NVR	In vitro	APC activation (Kraft et al., 2003)
Tsg24/APC1	S47 LLG**S**LDE T520 PSL**T**MSN S608 GSL**S**PVI	In vitro	APC activation (Golan et al., 2002; Kraft et al., 2003)
Bfa1	T7 RPL**T**LNG S17 PET**S**FEE T24 LNT**T**LPR T34 ETL**T**LEE T46 STS**T**YIP S53 PPS**S**VGT T61 DTG**T**VFS S431 RWV**S**VSE S454 VGK**S**MKK	In vivo, in vitro	Inhibits GAP activity toward Tem1 during mitotic exit (Hu et al., 2001)
TCTP	S46 IDD**S**LIG S64 GTE**S**TVI	In vivo, in vitro	Microtubule stability (Yarm, 2002)
Net1/Cfi1	S48 GDA**S**LQY S64 FTP**S**YFN S242 GER**S**FLP T16 VPP**S**LQA T197 TTT**T**IRS T302 IMS**T**MTP	In vivo, in vitro	Regulates Bfa1 and mitotic exit (Shou et al., 2002)
Myt1	S426 LDS**S**LSS S495 FED**T**LDP	In vivo, in vitro	Cdk1/cyclin-B activation (Nakajima et al., 2003)

NudC	S275 PENSKLS S326 MDFSKAK	In vitro	Dynein/dynactin complex involved in cytokinesis (Zhou et al., 2003)
Emi1	S123 GSSSPVK S145 YEDSGYS S149 GYSSFSL S934 VSSSFLT	In vitro	Phospho-dependent degradation (Hansen et al., 2004; Moshe et al., 2004)
Wee1A	S53 GEDSAFG	In vivo, in vitro	Phospho-dependent degradation (Watanabe et al., 2004)
Ninein and Ninein-like protein Nlp	S88 EDESSYLE T161 S686	In vivo, in vitro	Microtubule anchoring at centrosome (Casenghi et al., 2003)
Claspin	S934 QDASPVA	In vivo, in vitro	Unknown (Yoo et al., 2004)

APPENDIX E.

Protein sequence features in *C.e.*REC-8.

Possible Separase sites in *C. elegans* REC-8:

*C.e.*REC-8 sites based on core [D/E]-X-X-R	Likelihood of sites based on peptide studies (Sullivan et al., 2004)
R9	Maybe (P2I)
R115	Not likely* (P5Q and P2Q)
R135	Not likely* (P5E and P2D)
R355	Maybe (P5D)
R389	Excellent
R561	Not likely (P5E and P2P)
R623	Maybe
R657	Excellent
R678	Not likely (P5E and P2T)
R689	Not likely (P6F, P5E, and P2E)
R691	Not likely (P5E and P2T)

"Not likely" denotes sites that are not likely to be Separase cleavage sites, based on the analysis by Sullivan et al., 2004. In parentheses are listed the position and the residues that should not be in a Separase site. "Maybe" denotes sites with low conservation with the consensus sequence determined by Sullivan et al. (2004). "Excellent" refers to high conservation with the consensus sequence determined by Sullivan et al. (2004).

Predicted phosphorylation sites in REC-8 by Scansite (MIT):

*C.e.*REC-8 sites	Kinase site
S98	Potential AIR-2 site
T194	Potential PLK-1 site
S395	Potential AIR-2 site
S522	Potential Casein kinase site
T625	Confirmed AIR-2 site

There are many other possible phosphorylation sites determined by sequence-gazing, as well.
Potential PLK-1 sites: S44, T194, T198, T233, T329, S420, T553, and S626.
Potential AIR-2 sites: S56, S98, S248, S395, and T625.

Conservation with Rec8 in other species:
ScRec8 has an Aurora kinase site at 455 (just next to verified Separase site 453) and perhaps at 224. HsRec8 has an Aurora kinase site at 432 (just next to putative Separase site) and perhaps at 66. MmRec8 has Aurora kinase site at 482 (which aligns with hsRec8432) just next to putative Separase site and perhaps at position 66 as well.

APPENDIX F.

Potential Polo kinase binding partners and substrates involved in cell polarity.

Polo binding motifs:

Consensus: P-φ-X-S-$_P$[S/T]-P (Elia et al., 2003b)

Searched: [L/E]-X-T-S-[S/T]-P-[G/L]-P

Polarity Proteins containing putative PBD binding

MEX-5/6	**LTSSTPLP**
MEX-1	**TSTSSPVQ**
MEX-3	**EMSSSPFG**
PAR-3	**GGPSTPIA**
PAR-3	**LRVSTPKP**
GPR-1/2	**AIDSSPDP**
ZYG-11	**SLSSSPVR**
PAL-1	**ESSSTPSP**
POD-1	**several**
NSY-1	**FSSSSPVP**
NSY-1	**SQPSSPIV**
GLP-1	**TTTSTPNR**
AEX-3	**DRRSTPKD**
AEX-3	**EYESTPVS**
AEX-3	**MLLSSPVP**

Polo kinase substrates:

Polarity proteins contain putative Polo kinase target motifs

GPR-1/2
RNP-1
PGL-3
PAR-3
ZYG-11
OOC-3
NOS-2
DNC-1
Actin
Myosin

APPENDIX G.

Materials and methods.

Alleles and strains:

The N2 Bristol strain was used as the wildtype strain. The genetic markers used were: **LGI**: *par-6(zu222)*; **LGII**: *him-14(it44ts)*, *lin-5(ev571ts)*, *mex-6(pk440)*, *par-2(lw32)*, *par-3(it71)*; **LGIII**: *mpk-1(ga111ts)*, *pie-1(zu154)*; **LGIV**: *him-8(e1489)*, *let-99(or204ts)*, *par-5(it55)*, *spo-11(ok79)*; and **LGV**: *her-1(e1518)*, *him-5(e1490)*, *par-1(zu310ts)*, *par-4(it57ts)*.

Strains used for PLK-1 staining: **KK822** (*par-1*), **KK747** (*par-2*), **KK571** (*par-3*), **KK300** (*par-4*), **KK299** (*par-5*), **KK818** (*par-6*), **SD939** (*mpk-1*), **EU363** (*let-99*), **SV124** (*lin-5*), **JJ532** (*pie-1*), **JJ1237** (*mex-6*), and **JJ1244** (*mex-6*; *mex-5*).

GFP Reporter Strains:

AZ212	Ppie-1::GFP::histone H2B
WH204	Ppie-1::GFP::β-tubulin
GZ336	Ppie-1::GFP::PLK-1
JH227	Ppie-1::GFP::PIE-1
JH1244	Ppie-1::GFP::PAR-6
JH1447	Ppie-1::GFP::MEX-6
JH1448	Ppie-1::GFP::MEX-5
JH1473	Ppie-1::GFP::PAR-2 and Ppie-1::GFP::α-tubulin
JH1572	Ppie-1::GFP::MBK-2
SS747	Ppie-1::GFP::PGL-1
TX277	Ppie-1::GFP::histone H2B and Ppie-1::GFP::β-tubulin
TX492	Poma-1::GFP::OMA-1
TX660-664	Pheatshock::PLK-1
TX665-671, 708-710	Ppie-1::GFP::PLK-1 PBD
TX672-682	Ppie-1::GFP::MEX-5 mutant (T186A)
TX698-706	Ppie-1::GFP::PLK-2
TX707	Ppie-1::GFP::PLK-2 PBD

Antibody staining:

All antibody staining was performed using freeze-cracking method as described previously (Lin et al., 1998). AIR-2 (1:200, 2% PFA, DMF), BIR-1 (1:100, 2% PFA, DMF), BUB-1 (1:200, methanol 20 min), GSP-2 (1:100, 2% PFA, DMF), phospho-Ser10 histone H3 (1:25,000, 2% PFA, DMF), ICP-1 (1:1000, 2% PFA, DMF), LET-99 (1:50, 2% PFA, DMF), MEX-5 (no dilution, 2% PFA, DMF), PAR-1 (1:10, 2% PFA, DMF), PAR-2 (1:5, 2% PFA, DMF), PAR-3 (1:20, 2% PFA, DMF), PIE-1 (1:10, 2% PFA, DMF), PGL-1 (1,10,000, 2% PFA, DMF), PKC-3 (1:100, 2% PFA, DMF), PLK-1 (1:2000, 2% PFA, DMF), and REC-8 (1:200, M9, MetOH, EtOH).

RNA interference:

Double stranded RNA were injected at concentrations of 1-3 mg/ml. Injections were performed as described (Hsu et al., 2000). Feeding RNAi was performed as described (Fraser et al., 2000). Plasmids used for RNAi are listed below.

air-2	yk483g8; also feeding pRL983
bir-1	yk100gf03; also feeding pRL1035
coh-1	yk226d1
***coh-2* (*scc-1*)**	yk632f7
gsp-1	yk393h9; also feeding pRL1036
gsp-2	yk150g8; also feeding pRL1037
icp-1	yk329a11; also feeding pRL1038
plk-1	yk128g1; also feeding pRL930
plk-2	yk840g05; also feeding pRL1427
rec-8	pRL1062
sep-1	yk429h5
ncc-1	yk104h8
pie-1	feeding pRL1141 from Seydoux Lab
gpa-16	feeding from Wood Lab
mbk-2	feeding pJP1.03 from Seydoux Lab
cul-2	feeding pRL1184 from Kipreos Lab
zif-1	feeding pRL1324 from Seydoux Lab

RNAi of *tba-1*, *tbb-2*, and *mei-1* was done using dsRNA prepared from Ahringer's feeding RNAi plasmid library. Also feeding RNAi was performed using Ahringer's RNAi plasmids for the following genes: *air-1*, *par-1*, *par-2*, *par-3*, *goa-1*, *gsk-3*, *let-99*, and *mex-5*.

Other important plasmids:

Entry clones:

pRL686 – REC-8
pRL1016– Histone H3
pRL799 – Histone H3 N terminus aa 1-45
pRL753 – PLK-1
pRL1083 – PLK-1 mutant (T194D/T198D)
pRL1084 – PLK-1 mutant (N166A)
pRL772 – PLK-1 truncation aa 1-335 (kinase domain)
pRL1108 – PLK-1 truncation aa 340-648 (PBD)
pRL1186 – PLK-1 truncation aa 340-648 (PBD) mutant (W417F/V418A)
pRL1187 – PLK-1 truncation aa 340-648 (PBD) mutant (H542A/K544M)
pRL1150 – MEX-5 mutant (T186D)
pRL1185 – MEX-5 mutant (T186A)
pRL866 – MEX-6
pRL1207 – MEX-6 mutant (T190D)
pRL1214 – MEX-6 mutant (T190A)
pRL1299 – MEX-6 mutant (T177D)
pRL1390 – PLK-2
pRL1391 – PLK-2 truncation aa 1-331 (kinase domain)
pRL1392 – PLK-2 truncation aa 335-632 (PBD)

pRL1479 – OMA-1 mutant (T339E)
pRL1480 – OMA-2 mutant (T327E)
pRL1481 – MEX-5 mutant (T186E)
pRL1482 – MEX-6 mutant (T190E)

GST fusions and heatshock constructs:
pRL586 – GST::REC-8
pRL554 – GST::COH-1
pRL552 – GST::COH-2/SCC-1
pRL1089 – GST::PLK-1
pRL1090 – GST::PLK-1 mutant (T194D/T198D) constitutive active
pRL1091 – GST::PLK-1 mutant (N166A) kinase dead
pRL1206 – GST::PLK-1 truncation (kinase domain)
pRL1297 – GST::PLK-1 truncation (kinase domain) mutant (N166A)
pRL1163 – Pheat shock78::PLK-1
pRL1164 – Pheat shock83::PLK-1

Yeast two-hybrid plasmids: (all based on pRL864 and pRL865)
pRL1078 – PLK-1 bait
pRL1085 – PLK-1 prey
pRL1094 – PLK-1 mutant (T194D/T198D) bait
pRL1109 – PLK-1 truncation (PBD) prey
pRL1110 – PLK-1 truncation (PBD) bait
pRL868 – PLK-1 truncation (PBD) mutant (W417F/V418A)
pRL869 – PLK-1 truncation (PBD) mutant (H542A/K544M)
pRL1323 – PLK-1 truncation (kinase domain) prey
pRL888 – MEX-5 prey
pRL1151 – MEX-5 mutant (T186D) prey
pRL1212 – MEX-6 mutant (T 190D) prey
pRL1314 – MEX-6 mutant (T177D) prey
pRL1319 – OMA-1 prey
pRL1320 – OMA-2 prey
pRL1399 – PLK-2 bait
pRL1394 – PLK-2 truncation (PBD) bait
pRL1398 – PLK-2 truncation (kinase domain) prey
pRL1479 – OMA-1 mutant (T339E) prey
pRL1480 – OMA-2 mutant (T327E) prey
pRL1481 – MEX-5 mutant (T186E) prey
pRL1482 – MEX-6 mutant (T190E) prey

Ppie-1::GFP fusions for bombardment
pRL711 – Ppie-1::GFP::REC-8 (no transgenics recovered)
pRL765 – Ppie-1::GFP::REC-8 mutant (T625A) (no bombardment attempted)
pRL766 – Ppie-1::GFP::PLK-1 (no transgenics recovered)

pRL1216 – Ppie-1::GFP::PLK-1 truncation (PBD)
pRL1315 – Ppie-1::GFP::PLK-1 truncation (PBD) mutant (W417F/V418A) (no bombardment attempted)
pRL1400 – Ppie-1::GFP::MEX-5 mutant (T186A)
pRL1403 – Ppie-1::GFP::PLK-2
pRL1404 – Ppie-1::GFP::PLK-2 truncation (PBD)
pRL1414 – Ppie-1::GFP::MEX-5 (no bombardment attempted)

BIBLIOGRAPHY

Abrieu, A., T. Brassac, et al. (1998). "The Polo-like kinase Plx1 is a component of the MPF amplification loop at the G2/M-phase transition of the cell cycle in *Xenopus* eggs." *Journal of Cell Science* **111**(Pt 12): 1751-7.

Abrieu, A., J. A. Kahana, et al. (2000). "CENP-E as an essential component of the mitotic checkpoint in vitro." *Cell* **102**(6): 817-26.

Abrieu, A., L. Magnaghi-Jaulin, et al. (2001). "Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint." *Cell* **106**(1): 83-93.

Adams, R. R., A. A. Tavares, et al. (1998). "pavarotti encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis." *Genes & Development* **12**(10): 1483-94.

Adams, R. R., S. P. Wheatley, et al. (2000). "INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow." *Current Biology* **10**(17): 1075-8.

Adams, R. R., M. Carmena, et al. (2001a). "Chromosomal passengers and the (aurora) ABCs of mitosis." *Trends in Cell Biology* **11**(2): 49-54.

Adams, R. R., H. Maiato, et al. (2001b). "Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation." *Journal of Cell Biology* 153(4): 865-80.

Adams, R. R., D. M. Eckley, et al. (2001c). "Human INCENP colocalizes with the Aurora-B/AIRK2 kinase on chromosomes and is overexpressed in tumour cells." *Chromosoma* **110**(2): 65-74.

Afshar, K., F. Willard, et al. (2004). "RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of *C. elegans* embryos." *Cell* **119**(2): 219-30.

Ainsztein, A. M., S. E. Kandels-Lewis, et al. (1998). "INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1." *Journal of Cell Biology* **143**(7): 1763-74.

Albertson, D. G. and J. N. Thomson (1993). "Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*." *Chromosome Research* **1**(1): 15-26.

Alexandru, G., F. Uhlmann, et al. (2001). "Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast." *Cell* **105**(4): 459-72.

Alfa, C. E., R. Booher, et al. (1989). "Fission yeast cyclin: subcellular localisation and cell cycle regulation." *Journal of Cell Science - Supplement* **12**: 9-19.

Alfa, C. E., B. Ducommun, et al. (1990). "Distinct nuclear and spindle pole body population of cyclin-cdc2 in fission yeast." *Nature* **347**(6294): 680-2.

Alsop, G. B. and D. Zhang (2003). "Microtubules are the only structural constituent of the spindle apparatus required for induction of cell cleavage." *Journal of Cell Biology* **162**(3): 383-90.

Ambrosini, G., C. Adida, et al. (1997). "A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma." *Nature Medicine* **3**(8): 917-21.

Andreassen, P. R., F. B. Lacroix, et al. (1997). "Chromosomes with two intact axial cores are induced by G2 checkpoint override: evidence that DNA decatenation is not required to template the chromosome structure." *Journal of Cell Biology* **136**(1): 29-43.

Andrews, P. D., E. Knatko, et al. (2003). "Mitotic mechanics: the auroras come into view." *Current Opinion in Cell Biology* **15**(6): 672-83.

Andrews, P. D., Y. Ovechkina, et al. (2004). "Aurora B regulates MCAK at the mitotic centromere." *Developmental Cell* **6**(2): 253-68.

Anger, M., J. Klima, et al. (2004). "Timing of Plk1 and MPF activation during porcine oocyte maturation." *Molecular Reproduction & Development* **69**(1): 11-6.

Ankeny, R. A. (2001). "The natural history of *Caenorhabditis elegans* research." *Nature Reviews Genetics* **2**(6): 474-9.

Arnaud, L., J. Pines, et al. (1998). "GFP tagging reveals human Polo-like kinase 1 at the kinetochore/centromere region of mitotic chromosomes." *Chromosoma* **107**(6-7): 424-9.

Bailis, J. M. and G. S. Roeder (2000). "Pachytene exit controlled by reversal of Mek1-dependent phosphorylation." *Cell* **101**(2): 211-21.

Bailis, J. M., A. V. Smith, et al. (2000). "Bypass of a meiotic checkpoint by overproduction of meiotic chromosomal proteins." *Molecular & Cellular Biology* **20**(13): 4838-48.

Bailly, E., M. Doree, et al. (1989). "p34cdc2 is located in both nucleus and cytoplasm; part is centrosomally associated at G2/M and enters vesicles at anaphase." *EMBO Journal* **8**(13): 3985-95.

Baneres, J. L., A. Martin, et al. (1997). "The N tails of histones H3 and H4 adopt a highly structured conformation in the nucleosome." *Journal of Molecular Biology* **273**(3): 503-8.

Barnes, T. M., Y. Kohara, et al. (1995). "Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*." *Genetics* **141**(1): 159-79.

Basto, R., R. Gomes, et al. (2000). "Rough deal and Zw10 are required for the metaphase checkpoint in *Drosophila*." *Nature Cell Biology* **2**(12): 939-43.

Belmont, L. D., A. A. Hyman, et al. (1990). "Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts." *Cell* **62**(3): 579-89.

Berdnik, D. and J. A. Knoblich (2002). "*Drosophila* Aurora-A is required for centrosome maturation and actin-dependent asymmetric protein localization during mitosis." *Current Biology* **12**(8): 640-7.

Bernard, P., Hardwick K, et al. (1998). "Fission yeast bub1 is a mitotic centromere protein essential for the spindle checkpoint and the preservation of correct ploidy through mitosis." *Journal of Cell Biology* **143**(7): 1775-87.

Bernard, P., J. F. Maure, et al. (2001). "Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation." *Nature Cell Biology* **3**(5): 522-6.

Bernstein, H. S. and S. R. Coughlin (1998). "A mammalian homolog of fission yeast Cdc5 regulates G2 progression and mitotic entry." *Journal of Biological Chemistry* **273**(8): 4666-71.

Bhat, M. A., A. V. Philp, et al. (1996). "Chromatid segregation at anaphase requires the barren product, a novel chromosome-associated protein that interacts with Topoisomerase II." *Cell* **87**(6): 1103-14.

Bhatt, A. M., C. Lister, et al. (1999). "The DIF1 gene of Arabidopsis is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family." *Plant Journal* **19**(4): 463-72.

Biggins, S. and A. W. Murray (1999). "Sister chromatid cohesion in mitosis." *Current Opinion in Genetics & Development* **9**(2): 230-6.

Biggins, S. and A. W. Murray (2001). "The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint." *Genes & Development* **15**(23): 3118-29.

Biggins, S., F. F. Severin, et al. (1999). "The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast." *Genes & Development* **13**(5): 532-44.

Biggins, S., N. Bhalla, et al. (2001). "Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerivisae*." *Genetics* **159**(2): 453-70.

Bischoff, J. R. and G. D. Plowman (1999). "The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis." *Trends in Cell Biology* **9**(11): 454-9.

Bischoff, J. R., L. Anderson, et al. (1998). "A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers." *EMBO Journal* **17**(11): 3052-65.

Bishop, J. D. and J. M. Schumacher (2002). "Phosphorylation of the carboxyl terminus of inner centromere protein (INCENP) by the Aurora B Kinase stimulates Aurora B kinase activity." *Journal of Biological Chemistry* **277**(31): 27577-80.

Blat, Y. and N. Kleckner (1999). "Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region." *Cell* **98**(2): 249-59.

Bobinnec, Y., M. Fukuda, et al. (2000). "Identification and characterization of *Caenorhabditis elegans* gamma-tubulin in dividing cells and differentiated tissues." *Journal of Cell Science* **113 Pt 21**: 3747-59.

Bolton, M. A., W. Lan, et al. (2002). "Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation." *Molecular Biology of the Cell* **13**(9): 3064-77.

Boveri, T. (1887). *Morph. Physiol. Munchen **3**: 153.*

Bowerman, B., M. K. Ingram, et al. (1997). "The maternal par genes and the segregation of cell fate specification activities in early *Caenorhabditis elegans* embryos." *Development* **124**(19): 3815-26.

Bowman, A. B., R. S. Patel-King, et al. (1999). "*Drosophila* roadblock and Chlamydomonas LC7: a conserved family of dynein-associated proteins involved in axonal transport, flagellar motility, and mitosis." *Journal of Cell Biology* **146**(1): 165-80.

Boxem, M., D. G. Srinivasan, et al. (1999). "The *Caenorhabditis elegans* gene ncc-1 encodes a cdc2-related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase." *Development* **126**(10): 2227-39.

Bradbury, E. M., P. D. Cary, et al. (1973). "Conformations and interactions of histones and their role in chromosome structure." *Annals of the New York Academy of Sciences* **222**: 266-89.

Brenner, S. (1974). "The genetics of *Caenorhabditis elegans*." *Genetics* **77**(1): 71-94.

Broverman, S. A. and P. M. Meneely (1994). "Meiotic mutants that cause a polar decrease in recombination on the X chromosome in *Caenorhabditis elegans*." *Genetics* **136**(1): 119-27.

Brown, K. D., R. M. Coulson, et al. (1994). "Cyclin-like accumulation and loss of the putative kinetochore motor CENP-E results from coupling continuous synthesis with specific degradation at the end of mitosis." *Journal of Cell Biology* **125**(6): 1303-1312.

Brown, J. R., K. K. Koretke, et al. (2004). "Evolutionary relationships of Aurora kinases: Implications for model organism studies and the development of anti-cancer drugs." *BMC Evolutionary Biology* **4**(1): 39-49.

Buendia, B., G. Draetta, et al. (1992). "Regulation of the microtubule nucleating activity of centrosomes in *Xenopus* egg extracts: role of cyclin A-associated protein kinase." *Journal of Cell Biology* **116**(6): 1431-42.

Buonomo, S. B., R. K. Clyne, et al. (2000). "Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin." *Cell* **103**(3): 387-98.

Cahill, D. P., C. Lengauer, et al. (1998). "Mutations of mitotic checkpoint genes in human cancers." *Nature* **392**(6673): 300-303.

Cai, X., F. Dong, et al. (2003). "The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing." *Journal of Cell Science* **116**(Pt 14): 2999-3007.

Cameron, L. A., P. S. Maddox, et al. (2003). "Direct observation of microtubule dynamics at kinetochores in *Xenopus* extract spindles: implications for spindle mechanics." *Nature* **424**(6952): 1074-8.

Canman, J. C., N. Sharma, et al. (2002). "Anaphase onset does not require the microtubule-dependent depletion of kinetochore and centromere-binding proteins." *Journal of Cell Science* **115**(Pt 19): 3787-95.

Carmena, M. and W. C. Earnshaw (2003). "The cellular geography of aurora kinases." *Nature Reviews Molecular Cell Biology* **4**(11): 842-54.

Carmena, M., M. G. Riparbelli, et al. (1998). "*Drosophila* polo kinase is required for cytokinesis." *Journal of Cell Biology* **143**(3): 659-71.

Carpenter, A. T. (1994). "Chiasma function." *Cell* **77**(7): 957-62.

Casenghi, M., P. Meraldi, et al. (2003). "Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation." *Developmental Cell* **5**(1): 113-25.

Cassimeris, L., S. Inoue, et al. (1988). "Microtubule dynamics in the chromosomal spindle fiber: analysis by fluorescence and high-resolution polarization microscopy." *Cell Motility & the Cytoskeleton* **10**(1-2): 185-96.

Castro, A., Y. Arlot-Bonnemains, et al. (2002). "APC/Fizzy-Related targets Aurora-A kinase for proteolysis." *EMBO Reports* **3**(5): 457-462.

Celerin, M., S. T. Merino, et al. (2000). "Multiple roles of Spo11 in meiotic chromosome behavior." *EMBO Journal* **19**(11): 2739-50.

Champagne, C., M. Roy, et al. (2002). "Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1." *Molecular & Cellular Biology* **22**(1): 41-56.

Chan, C. S. and D. Botstein (1993). "Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast." *Genetics* **135**(3): 677-91.

Chan, G. K., S. A. Jablonski, et al. (2000). "Human Zw10 and ROD are mitotic checkpoint proteins that bind to kinetochores." *Nature Cell Biology* **2**(12): 944-7.

Chan, R. C., A. Chan, et al. (2003). "Chromosome cohesion is regulated by a clock gene paralogue TIM-1." *Nature* **423**(6943): 1002-9.

Chang, E. J., V. Archambault, et al. (2004). "Analysis of protein phosphorylation by hypothesis-driven multiple-stage mass spectrometry." *Analytical Chemistry* **76**(15): 4472-83.

Charles, J. F., S. L. Jaspersen, et al. (1998). "The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerivisae*." *Current Biology* **8**(9): 497-507.

Chase, D., C. Serafinas, et al. (2000). "The polo-like kinase PLK-1 is required for nuclear envelope breakdown and the completion of meiosis in *Caenorhabditis elegans*." *Genesis: the Journal of Genetics & Development* **26**(1): 26-41.

Cheeks, R. J., J. C. Canman, et al. (2004). "*C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes." *Current Biology* **14**(10): 851-62.

Cheeseman, I. M., S. Anderson, et al. (2002). "Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p." *Cell* **111**(2): 163-72.

Chen, S. H. and T. K. Tang (2002). "Mutational analysis of the phosphorylation sites of the Aie1 (Aurora-C) kinase in vitro." *DNA & Cell Biology* **21**(1): 41-6.

Chen, R. H., A. Shevchenko, et al. (1998). "Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores." *Journal of Cell Biology* **143**(2): 283-95.

Chen, R. H., D. M. Brady, et al. (1999). "The spindle checkpoint of budding yeast depends on a tight complex between the Mad1 and Mad2 proteins." *Molecular Biology of the Cell* **10**(8): 2607-18.

Cheng, L., L. Hunke, et al. (1998). "Cell cycle regulation of the *Saccharomyces cerivisae* polo-like kinase cdc5p." *Molecular & Cellular Biology* **18**(12): 7360-70.

Cheng, K. Y., E. D. Lowe, et al. (2003). "The crystal structure of the human polo-like kinase-1 polo box domain and its phospho-peptide complex." *EMBO Journal* **22**(21): 5757-68.

Choi, T., F. Aoki, et al. (1991). "Activation of p34cdc2 protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos." *Development* **113**(3): 789-95.

Chu, T., G. Henrion, et al. (2001). "Cortex, a *Drosophila* gene required to complete oocyte meiosis, is a member of the Cdc20/fizzy protein family." *Genesis: the Journal of Genetics & Development* **29**(3): 141-52.

Church, D. L., K. L. Guan, et al. (1995). "Three genes of the MAP kinase cascade, mek-2, mpk-1/sur-1 and let-60 ras, are required for meiotic cell cycle progression in *Caenorhabditis elegans*." *Development* **121**(8): 2525-35.

Ciosk, R., W. Zachariae, et al. (1998). "An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast." *Cell* **93**(6): 1067-76.

Clay, F. J., M. R. Ernst, et al. (1997). "The mouse Plk gene: structural characterization, chromosomal localization and identification of a processed Plk pseudogene." *Gene* **198**(1-2): 329-39.

Clem, R. J., M. Fechheimer, et al. (1991). "Prevention of apoptosis by a baculovirus gene during infection of insect cells." *Science* **254**(5036): 1388-90.

Clyne, R. K., V. L. Katis, et al. (2003). "Polo-like kinase Cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis I." *Nature Cell Biology* **5**(5): 480-5.

Cobb, J., B. Cargile, et al. (1999a). "Acquisition of competence to condense metaphase I chromosomes during spermatogenesis." *Developmental Biology* **205**(1): 49-64.

Cobb, J., M. Miyaike, et al. (1999b). "Meiotic events at the centromeric heterochromatin: histone H3 phosphorylation, topoisomerase II alpha localization and chromosome condensation." *Chromosoma* **108**(7): 412-25.

Cohen-Fix, O., J. M. Peters, et al. (1996). "Anaphase initiation in *Saccharomyces cerivisae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p." *Genes & Development* **10**(24): 3081-93.

Colaiacovo, M. P., A. J. MacQueen, et al. (2003). "Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination." *Developmental Cell* **5**(3): 463-74.

Collas, P. (1999). "Sequential PKC- and Cdc2-mediated phosphorylation events elicit zebrafish nuclear envelope disassembly." *Journal of Cell Science* **112**(Pt 6): 977-87.

Collins, I. and C. S. Newlon (1994). "Meiosis-specific formation of joint DNA molecules containing sequences from homologous chromosomes." *Cell* **76**(1): 65-75.

Colombo, K., S. W. Grill, et al. (2003). "Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos." *Science* **300**(5627): 1957-61.

Cooke, C. A., M. M. Heck, et al. (1987). "The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis." *Journal of Cell Biology* **105**(5): 2053-67.

Cosma, M. P., K. Wirth, et al. (1999). "*Drosophila* polo kinase is required for cytokinesis." *Cell* **98**(6): 847-58.

Courtwright, A. M. and X. He (2002). "Dam1 is the right one: phosphoregulation of kinetochore biorientation." *Developmental Cell* **3**(5): 610-1.

Couteau, F., K. Nabeshima, et al. (2004). "A component of *C. elegans* meiotic chromosome axes at the interface of homolog alignment, synapsis, nuclear reorganization, and recombination." *Current Biology* **14**(7): 585-92.

Couwenbergs, C., A. C. Spilker, et al. (2004). "Control of embryonic spindle positioning and Galpha activity by *C. elegans* RIC-8." *Current Biology* **14**(20): 1871-6.

Cowan, C. R. and A. A. Hyman (2004). "Asymmetric cell division in *C. elegans*: cortical polarity and spindle positioning." *Annual Review of Cell and Developmental Biology* **20**: 427-53.

Crosio, C., G. M. Fimia, et al. (2002). "Mitotic phosphorylation of histone H3: spatio-temporal regulation by mammalian Aurora kinases." *Molecular & Cellular Biology* **22**(3): 874-85.

Cruzalegui, F. H., D. Fesquet, et al. (1993). "The MO15 gene encodes the catalytic subunit of a protein kinase that activates cdc2 and other cyclin-dependent kinases (CDKs) through phosphorylation of Thr161 and its homologues." *Nature* **366**(6452): 270-3.

Cuenca, A. A., A. Schetter, et al. (2003). "Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases." *Development* **130**(7): 1255-65.

Cutts, S. M., K. J. Fowler, et al. (1999). "Defective chromosome segregation, microtubule bundling and nuclear bridging in inner centromere protein gene (Incenp)-disrupted mice." *Human Molecular Genetics* **8**(7): 1145-55.

Dai, W. and J. P. Cogswell (2003). "Polo-like kinases and the microtubule organization center: targets for cancer therapies." *Progress in Cell Cycle Research* **5**: 327-34.

Davis, L. and G. R. Smith (2003). "Nonrandom homolog segregation at meiosis I in *Schizosaccharomyces pombe* mutants lacking recombination." *Genetics* **163**(3): 857-74.

Davis, E. S., L. Wille, et al. (2002). "Multiple subunits of the *Caenorhabditis elegans* anaphase-promoting complex are required for chromosome segregation during meiosis I." *Genetics* **160**(2): 805-13.

Dawson, I. A., S. Roth, et al. (1993). "Mutations of the fizzy locus cause metaphase arrest in *Drosophila* melanogaster embryos." *Development* **117**(1): 359-76.

Dawson, I. A., S. Roth, et al. (1995). "The *Drosophila* cell cycle gene fizzy is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of *Saccharomyces cerivisae*." *Journal of Cell Biology* **129**(3): 725-37.

de la Barre, A. E., D. Angelov, et al. (2001). "The N-terminus of histone H2B, but not that of histone H3 or its phosphorylation, is essential for chromosome condensation." *EMBO Journal* **20**(22): 6383-93.

DeRenzo, C., K. J. Reese, et al. (2003). "Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation." *Nature* **424**(6949): 685-9.

Dernburg, A. F., K. McDonald, et al. (1998). "Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis." *Cell* **94**(3): 387-98.

Desai, D., H. C. Wessling, et al. (1995). "Effects of phosphorylation by CAK on cyclin binding by CDC2 and CDK2." *Molecular & Cellular Biology* **15**(1): 345-50.

Descombes, P. and E. A. Nigg (1998). "The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in *Xenopus* egg extracts." *EMBO Journal* **17**(5): 1328-35.

Deveraux, Q. L. and J. C. Reed (1999). "IAP family proteins—suppressors of apoptosis." *Genes & Development* **13**(3): 239-52.

Dewar, H., K. Tanaka, et al. (2004). "Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle." *Nature* **428**(6978): 93-97.

Ditchfield, C., V. L. Johnson, et al. (2003). "Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores." *Journal of Cell Biology* **161**(2): 267-80.

do Carmo Avides, M., A. Tavares, et al. (2001). "Polo kinase and Asp are needed to promote the mitotic organizing activity of centrosomes." *Nature Cell Biology* **3**(4): 421-4.

Dobles, M., V. Liberal, et al. (2000). "Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2." *Cell* **101**(6): 635-45.

Donaldson, M. M., A. A. Tavares, et al. (2001). "Metaphase arrest with centromere separation in polo mutants of *Drosophila*." *Journal of Cell Biology* **153**(4): 663-76.

Donzelli, M., M. Squatrito, et al. (2002). "Dual mode of degradation of Cdc25 A phosphatase." *EMBO Journal* **21**(18): 4875-84.

Dove, W. (2003). "Aurora and the hunt for cancer-modifying genes." *Nature Genetics* **34**(4): 353-4.

Draetta, G., F. Luca, et al. (1989). "Cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF." *Cell* **56**(5): 829-38.

Dunphy, W. G. and K. A. (1991). "The cdc25 protein contains an intrinsic phosphatase activity." *Cell* **67**(1): 189-96.

Eaker, S., A. Pyle, et al. (2001). "Evidence for meiotic spindle checkpoint from analysis of spermatocytes from Robertsonian-chromosome heterozygous mice." *Journal of Cell Science* **114**(Pt 16): 2953-65.

Eijpe, M., C. Heyting, et al. (2000). "Association of mammalian SMC1 and SMC3 proteins with meiotic chromosomes and synaptonemal complexes." *Journal of Cell Science* **113**(Pt 4): 673-82.

Eijpe, M., H. Offenberg, et al. (2003). "Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1beta and SMC3." *Journal of Cell Biology* **160**(5): 657-70.

Elia, A. E., L. C. Cantley, et al. (2003a). "Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates." *Science* **299**(5610): 1228-31.

Elia, A. E., P. Rellos, et al. (2003b). "The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain." *Cell* **115**(1): 83-95.

Erikson, R. L. and K. S. Lee (1997). "Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1." *Molecular & Cellular Biology* **17**(6): 3408-17.

Evans, T., E. T. Rosenthal, et al. (1983). "Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division." *Cell* **33**(2): 389-96.

Fang, G., H. Yu, et al. (1998). "The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation." *Genes & Development* **12**(12): 1871-83.

Farmer, J. and J. Moore (1905). "The maiotic (sic) phase in animals and plants." *Quarterly Journal of Microscopic Science* **48**: 489-557.

Fenton, B. and D. M. Glover (1993). "A conserved mitotic kinase active at late anaphase-telophase in syncytial *Drosophila* embryos." *Nature* **363**(6430): 637-40.

Fesquet, D., A. L. Johnson, et al. (1998). "Activation of the human anaphase-promoting complex by proteins of the CDC20/Fizzy family." *EMBO Journal* **17**(2): 498-506.

Finley, D., A. Ciechanover, et al. (2004). "Ubiquitin as a central cellular regulator." *Cell* **116**(2 Suppl): S29-32.

Fisher, R. P., P. Jin, et al. (1995). "Alternative mechanisms of CAK assembly require an assembly factor or an activating kinase." *Cell* **83**(1): 47-57.

Flemming, W. (1875). "Studien in der Entwicklungsgeschichte der Najaden." *Sitzungsber. Kaiserl. Akad. Wiss.* **71**: 81-212.

Flemming, W. (1879). "Beitrage zur Kermtnisse der Zelle und ihrer Lebenserscheinungen." *Arch. f. Mikrosk Anatomie* **18**: 151.

Flemming, W. (1882). *Cell, Substance, Nucleus, and Cell Division.*

Freeman, L., L. Aragon-Alcaide, et al. (2000). "The condensin complex governs chromosome condensation and mitotic transmission of rDNA." *Journal of Cell Biology* **149**(4): 811-24.

Fuchs, J., J. Loidl, et al. (2000). "Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins." *Nature Cell Biology* **2**(8): 492-9.

Funabiki, H. and M. A. W. (2000). "The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement." *Cell* **102**(4): 399-402.

Funabiki, H., K. Kumada, et al. (1996). "Fission yeast Cut1 and Cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes." *EMBO Journal* **15**(23): 6617-28.

Furuta, T., S. Tuck, et al. (2000). "EMB-30: an APC4 homologue required for metaphase-to-anaphase transitions during meiosis and mitosis in *Caenorhabditis elegans*." *Molecular Biology of the Cell* **11**(4): 1401-19.

Gallant, P. and E. A. Nigg (1992). "Cyclin B2 undergoes cell cycle-dependent nuclear translocation and, when expressed as a non-destructible mutant, causes mitotic arrest in HeLa cells." *Journal of Cell Biology* **117**(1): 213-24.

Gassmann, R., A. Carvalho, et al. (2004). "Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle." *Journal of Cell Biology* **166**(2): 179-91.

Gautier, J., M. J. Solomon, et al. (1991). "cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2." *Cell* **67**(1): 197-211.

Geley, S., E. Kramer, et al. (2001). "Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint." *Journal of Cell Biology* **153**(1): 137-48.

Giet, R. and C. Prigent (1999). "Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases." *Journal of Cell Science* **112**(Pt 21): 3591-601.

Giet, R. and D. M. Glover (2001). "*Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis." *Journal of Cell Biology* **152**(4): 669-82.

Giet, R., R. Uzbekov, et al. (1999). "The *Xenopus* laevis aurora-related protein kinase pEg2 associates with and phosphorylates the kinesin-related protein XlEg5." *Journal of Biological Chemistry* **274**(21): 15005-13.

Giet, R., D. McLean, et al. (2002). "*Drosophila* Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules." *Journal of Cell Biology* **156**(3): 437-51.

Gimenez-Abian, J. F., D. J. Clarke, et al. (1995). "A postprophase topoisomerase II-dependent chromatid core separation step in the formation of metaphase chromosomes." *Journal of Cell Biology* **131**(1): 7-17.

Gimenez-Abian, J. F., I. Sumara, et al. (2004). "Regulation of sister chromatid cohesion between chromosome arms." *Current Biology* **14**(13): 1187-93.

Glotzer, M., A. W. Murray, et al. (1991). "Cyclin is degraded by the ubiquitin pathway." *Nature* **349**(6305): 132-8.

Glover, D. M., M. H. Leibowitz, et al. (1995). "Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles." *Cell* **81**(1): 95-105.

Glover, D. M., I. M. Hagan, et al. (1998). "Polo-like kinases: a team that plays throughout mitosis." *Genes & Development* **12**(24): 3777-87.

Golan, A., Y. Yudkovsky, et al. (2002). "The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly activated by protein kinases Cdk1-cyclin B and Plk." *Journal of Biological Chemistry* **277**(18): 15552-7.

Golden, A., P. L. Sadler, et al. (2000). "Metaphase to anaphase (mat) transition-defective mutants in *Caenorhabditis elegans*." *Journal of Cell Biology* **151**(7): 1469-82.

Golsteyn, R. M., S. J. Schultz, et al. (1994). "Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila* polo and *Saccharomyces cerivisae* Cdc5." *Journal of Cell Science* **107**(Pt 6): 1509-17.

Golsteyn, R. M., K. E. Mundt, et al. (1995). "Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function." *Journal of Cell Biology* **129**(6): 1617-28.

Gotta, M., Y. Dong, et al. (2003). "Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo." *Current Biology* **13**(12): 1029-37.

Gould, K. L. and P. Nurse (1989). "Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis." *Nature* **342**(6245): 39-45.

Grallert, B. and M. Sipiczki (1990). "Dissociation of meiotic and mitotic roles of the fission yeast cdc2 gene." *Molecular & General Genetics* **222**(2-3): 473-5.

Gregan, J., A. Schleiffer, et al. (2004). "Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis." *Current Biology* **14**(4): 287-301.

Grelon, M., D. Vezon, et al. (2001). "AtSPO11-1 is necessary for efficient meiotic recombination in plants." *EMBO Journal* **20**(3): 589-600.

Grenfell, T. Z., F. R. Yarm, et al. (1998). "Plk is a functional homolog of *Saccharomyces cerivisae* Cdc5, and elevated Plk activity induces multiple septation structures." *Proceedings of the National Academy of Sciences of the United States of America* **95**(16): 9301-6.

Griffin, D. K. (1996). "The incidence, origin, and etiology of aneuploidy." *International Review of Cytology* **167**: 263-96.

Grill, S. W., J. Howard, et al. (2003). "The distribution of active force generators controls mitotic spindle position." *Science* **301**(5632): 518-21.

Grossberger, R., C. Gieffers, et al. (1999). "Characterization of the DOC1/APC10 subunit of the yeast and the human anaphase-promoting complex." *Journal of Biological Chemistry* **274**(20): 14500-7.

Gruneberg, U., R. Neef, et al. (2004). "Relocation of Aurora B from centromeres to the central spindle at the metaphase to anaphase transition requires MKlp2." *Journal of Cell Biology* **166**(2): 167-72.

Guacci, V., D. Koshland, et al. (1997). "A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerivisae*." *Cell* **91**(1): 47-57.

Guo, S. and K. J. Kemphues (1995). "par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed." *Cell* **81**(4): 611-20.

Gurley, L., Walters RA, et al. (1974). "Cell cycle-specific changes in histone phosphorylation associated with cell proliferation and chromosome condensation." *Journal of Cell Biology* **60**(2): 356-64.

Hagstrom, K. A., V. F. Holmes, et al. (2002). "*C. elegans* condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis." *Genes & Development* **16**(6): 729-42.

Hagting, A., N. Den Elzen, et al. (2002). "Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1." *Journal of Cell Biology* **157**(7): 1125-37.

Hamanaka, R., M. R. Smith, et al. (1995). "Polo-like kinase is a cell cycle-regulated kinase activated during mitosis." *Journal of Biological Chemistry* **270**(36): 21086-91.

Hames, R. S., S. L. Wattam, et al. (2001). "APC/C-mediated destruction of the centrosomal kinase Nek2A occurs in early mitosis and depends upon a cyclin A-type D-box." *EMBO Journal* **20**(24): 7117-27.

Hannak, E., M. Kirkham, et al. (2001). "Aurora-A kinase is required for centrosome maturation in *Caenorhabditis elegans*." *Journal of Cell Biology* **155**(7): 1109-16.

Hansen, D., Loktev AV, et al. (2004). "Plk1 Regulates Activation of the Anaphase Promoting Complex by Phosphorylating and Triggering SCFβ TrCP-dependent Destruction of the APC Inhibitor Emi1." *Molecular Biology of the Cell.* **15**(12): 5623-34.

Hartwell, L. H., J. Culotti, et al. (1974). "Genetic control of the cell division cycle in yeast." *Science* **183**(120): 46-51.

Hassold, T. and P. Hunt (2001). "To err (meiotically) is human: the genesis of human aneuploidy." *Nature Reviews Genetics* **2**(4): 280-91.

Hauf, S., R. W. Cole, et al. (2003). "The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint." *Journal of Cell Biology* **161**(2): 281-94.

Hauf, S., I. C. Waizenegger, et al. (2001). "Cohesin cleavage by separase required for anaphase and cytokinesis in human cells." *Science* **293**(5533): 1320-3.

Hays, T. S., R. B. Nicklas, et al. (2000). "Tension on chromosomes increases the number of kinetochore microtubules but only within limits." *Journal of Cell Biology* **151**(4): 739-48.

He, X., D. R. Rines, et al. (2001). "Molecular analysis of kinetochore-microtubule attachment in budding yeast." *Cell* **106**(2): 195-206.

Heald, R. and F. McKeon (1990). "Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis." *Cell* **61**(4): 579-89.

Hendzel, M. J., Y. Wei, et al. (1997). "Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation." *Chromosoma* **106**(6): 348-60.

Herman, R. K., J. E. Madl, et al. (1979). "Duplications in *Caenorhabditis elegans*." *Genetics* **92**(2): 419-35.

Herrmann, S., I. Amorim, et al. (1998). "The POLO kinase is required at multiple stages during spermatogenesis in *Drosophila* melanogaster." *Chromosoma* **107**(6-7): 440-51.

Hershko, A., D. Ganoth, et al. (1991). "Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts." *Journal of Biological Chemistry* **266**(25): 16376-9.

Hess, H. A., J. C. Roper, et al. (2004). "RGS-7 completes a receptor-independent heterotrimeric g protein cycle to asymmetrically regulate mitotic spindle positioning in *C. elegans*." *Cell* **119**(2): 209-218.

Hiebert, S. W. and G. E. Ward (1999). "Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C." *Journal of Cellular Biochemistry* **Suppl 32-33**: 51-8.

Hildebrandt, E. R. and M. A. Hoyt (2001). "Cell cycle-dependent degradation of the *Saccharomyces cerivisae* spindle motor Cin8p requires APC(Cdh1) and a bipartite destruction sequence." *Molecular Biology of the Cell* **12**(11): 3402-16.

Hilioti, Z., Y. S. Chung, et al. (2001). "The anaphase inhibitor Pds1 binds to the APC/C-associated protein Cdc20 in a destruction box-dependent manner." *Current Biology* **11**(17): 1347-52.

Hirano, T. and T. J. Mitchison (1994). "A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro." *Cell* **79**(3): 449-58.

Hirano, T., R. Kobayashi, et al. (1997). "Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a *Xenopus* homolog of the *Drosophila* Barren protein." *Cell* **89**(4): 511-21.

Hird, S. N. and J. G. White (1993). "Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*." *Journal of Cell Biology* **121**(6): 1343-55.

Hodgkin, J. (1980). "More sex-determination mutants of *Caenorhabditis elegans*." *Genetics* **96**(3): 649-64.

Hodgkin, J., H. Horvitz, et al. (1979). "Nondisjunction mutants of the nematode *Caenorhabditis elegans*." *Genetics* **91**: 67-94.

Hoffman, D. B., C. G. Pearson, et al. (2001). "Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores." *Molecular Biology of the Cell* **12**(7): 1995-2009.

Hoffmann, I., P. R. Clarke, et al. (1993). "Phosphorylation and activation of human cdc25-C by cdc2--cyclin B and its involvement in the self-amplification of MPF at mitosis." *EMBO Journal* **12**(1): 53-63.

Holloway, S. L., M. Glotzer, et al. (1993). "Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor." *Cell* **73**(7): 1393-402.

Holm, C., T. Goto, et al. (1985). "DNA topoisomerase II is required at the time of mitosis in yeast." *Cell* **41**(2): 553-563.

Honda, K., H. Mihara, et al. (2000). "Degradation of human Aurora2 protein kinase by the anaphase-promoting complex-ubiquitin-proteasome pathway." *Oncogene* **19**(24): 2812-9.

Honda, R., R. Korner, et al. (2003). "Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis." *Molecular Biology of the Cell* **14**(8): 3325-41.

Hoque, M. T. and F. Ishikawa (2001). "Human chromatid cohesin component hRad21 is phosphorylated in M phase and associated with metaphase centromeres." *Journal of Biological Chemistry* **276**(7): 5059-67.

Hoque, M. T. and F. Ishikawa (2002). "Cohesin defects lead to premature sister chromatid separation, kinetochore dysfunction, and spindle-assembly checkpoint activation." *Journal of Biological Chemistry* **277**(44): 42306-14.

Hornig, N. C., P. P. Knowles, et al. (2002). "The dual mechanism of separase regulation by securin." *Current Biology* **12**(12): 973-82.

Howe, M., K. L. McDonald, et al. (2001). "HIM-10 is required for kinetochore structure and function on *Caenorhabditis elegans* holocentric chromosomes." *Journal of Cell Biology* **153**(6): 1227-38.

Hoyt, M. A., L. Totis, et al. (1991). "*S. cerivisae* genes required for cell cycle arrest in response to loss of microtubule function." *Cell* **66**(3): 507-17.

Hsu, J. Y., Z. W. Sun, et al. (2000). "Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes." *Cell* **102**(3): 279-91.

Hu, F., Y. Wang, et al. (2001). "Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints." *Cell* **107**(5): 655-65.

Huang, J. Y. and J. W. Raff (1999). "The disappearance of cyclin B at the end of mitosis is regulated spatially in *Drosophila* cells." *EMBO Journal* **18**(8): 2184-95.

Huang, J. Y. and J. W. Raff (2002). "The dynamic localisation of the *Drosophila* APC/C: evidence for the existence of multiple complexes that perform distinct functions and are differentially localised." *Journal of Cell Science* **115**(Pt 14): 2847-56.

Huang, J. Y., J. Raff, et al. (2000). "The disappearance of cyclin B at the end of mitosis is regulated spatially in *Drosophila* cells." *Current Biology* **10**(21): 1367-70.

Hubbard, S. R. (1997). "Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog." *EMBO Journal* **16**(18): 5572-81.

Huchon, D., H. Rime, et al. (1993). "Control of metaphase I formation in *Xenopus* oocyte: effects of an indestructible cyclin B and of protein synthesis." *Biology of the Cell* **77**(2): 133-41.

Hudson, J. W., A. Kozarova, et al. (2001). "Late mitotic failure in mice lacking Sak, a polo-like kinase." *Current Biology* **11**(6): 441-6.

Hwang, L. H., L. F. Lau, et al. (1998). "Budding yeast Cdc20: a target of the spindle checkpoint." *Science* **279**(5353): 1041-1044.

Hwang, S. Y., B. Oh, et al. (2001). "Expression of genes involved in mammalian meiosis during the transition from egg to embryo." *Molecular Reproduction & Development* **59**(2): 144-58.

Inoue, S. and H. Sato (1967). "Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement." *Journal of General Physiology* **50**(6): Suppl 259-92.

Inoue, S. and E. D. Salmon (1995). "Force generation by microtubule assembly/disassembly in mitosis and related movements." *Molecular Biology of the Cell* **6**(12): 1619-40.

Irniger, S. and K. Nasmyth (1997). "The anaphase-promoting complex is required in G1 arrested yeast cells to inhibit B-type cyclin accumulation and to prevent uncontrolled entry into S-phase." *Journal of Cell Science* **110**(Pt 13): 1523-31.

Irniger, S., S. Piatti, et al. (1995). "Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast." *Cell* **81**(2): 269-78.

Izumi, T. and J. L. Maller (1993). "Elimination of cdc2 phosphorylation sites in the cdc25 phosphatase blocks initiation of M-phase." *Molecular Biology of the Cell* **4**(12): 1337-50.

Jackman, M., C. Lindon, et al. (2003). "Active cyclin B1-Cdk1 first appears on centrosomes in prophase." *Nature Cell Biology* **5**(2): 143-8.

Jaffrey, R. G., S. C. Pritchard, et al. (2000). "Genomic instability at the BUB1 locus in colorectal cancer, but not in non-small cell lung cancer." *Cancer Research* **60**(16): 4349-52.

Jallepalli, P. V. and C. Lengauer (2001). "Chromosome segregation and cancer: cutting through the mystery." *Nature Reviews. Cancer* **1**(2): 109-17.

Jallepalli, P. V., I. C. Waizenegger, et al. (2001). "Securin is required for chromosomal stability in human cells." *Cell* **105**(4): 445-57.

Jang, Y. J., C. Y. Lin, et al. (2002). "Functional studies on the role of the C-terminal domain of mammalian polo-like kinase." *Proceedings of the National Academy of Sciences of the United States of America* **99**(4): 1984-9.

Janke, C., J. Ortiz, et al. (2002). "Four new subunits of the Dam1-Duo1 complex reveal novel functions in sister kinetochore biorientation." *EMBO Journal* **21**(1-2): 181-93.

Janssens, F. (1909). “La théorie de la chiasmatypie. Nouvelle interpretation des cinèses de maturation.” *Cellule* **22**: 387-411.

Jantsch, V., P. Pasierbek, et al. (2004). “Targeted gene knockout reveals a role in meiotic recombination for ZHP-3, a Zip3-related protein in *Caenorhabditis elegans*.” *Molecular & Cellular Biology* **24**(18): 7998-8006.

Jaspersen, S. L., J. F. Charles, et al. (1998). “A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerivisae*.” *Molecular Biology of the Cell* **9**(10): 2803-17.

Jensen, S., M. Segal, et al. (2001). “A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1.” *Journal of Cell Biology* **152**(1): 27-40.

Jenuwein, T. and C. D. Allis (2000). “Translating the histone code.” *Nature* **406**(6796): 593-9.

Johnson, L. N., M. E. Noble, et al. (1996). “Active and inactive protein kinases: structural basis for regulation.” *Cell* **85**(2): 149-58.

Juang, Y. L., J. Huang, et al. (1997). “APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle.” *Science* **275**(5304): 1311-4.

Kaitna, S., M. Mendoza, et al. (2000). “Incenp and an aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis.” *Current Biology* **10**(19): 1172-81.

Kaitna, S., P. Pasierbek, et al. (2002). “The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous Chromosomes during meiosis.” *Current Biology* **12**(10): 798-812.

Kamieniecki, R. J., R. M. Shanks, et al. (2000). “Slk19p is necessary to prevent separation of sister chromatids in meiosis I.” *Current Biology* **10**(19): 1182-90.

Kang, J. S., C. S. Chan, et al. (1999). “Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerivisae*.” *Journal of Cell Biology* **145**(7): 1381-94.

Kang, J., I. M. Cheeseman, et al. (2001). “Functional cooperation of Dam1, Ipl1, and the inner centromere protein (INCENP)-related protein Sli15 during chromosome segregation.” *Journal of Cell Biology* **155**(5): 763-74.

Kang, D., J. Chen, et al. (2002). “The checkpoint protein Chfr is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G2 to M transition.” *Journal of Cell Biology* **156**(2): 249-59.

Karaiskou, A., C. Jessus, et al. (1999). “Phosphatase 2A and polo kinase, two antagonistic regulators of cdc25 activation and MPF auto-amplification.” *Journal of Cell Science* **112**(Pt 21): 3747-56.

Karaiskou, A., A. C. Lepretre, et al. (2004). “Polo-like kinase confers MPF autoamplification competence to growing *Xenopus* oocytes.” *Development* **131**(7): 1543-52.

Karashima, T., A. Sugimoto, et al. (2000). “*Caenorhabditis elegans* homologue of the human azoospermia factor DAZ is required for oogenesis but not for spermatogenesis.” *Development* **127**(5): 1069-79.

Kaszas, E. and W. Z. Cande (2000). "Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin." *Journal of Cell Science* **113**(Pt 18): 3217-26.

Katis, V. L., M. Galova, et al. (2004). "Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332." *Current Biology* **14**(7): 560-72.

Kato, J. Y., R. P. Fisher, et al. (1994). "A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase." *Molecular & Cellular Biology* **14**(11): 7265-75.

Kawajiri, A., Y. Yasui, et al. (2003). "Functional significance of the specific sites phosphorylated in desmin at cleavage furrow: Aurora-B may phosphorylate and regulate type III intermediate filaments during cytokinesis coordinatedly with Rho-kinase." *Molecular Biology of the Cell* **14**(4): 1489-500.

Kawasaki, A., I. Matsumura, et al. (2001). "Downregulation of an AIM-1 kinase couples with megakaryocytic polyploidization of human hematopoietic cells." *Journal of Cell Biology* **152**(2): 275-87.

Keeney, S., C. N. Giroux, et al. (1997). "Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family." *Cell* **88**(3): 375-84.

Kelm, O., M. Wind, et al. (2002). "Cell cycle-regulated phosphorylation of the *Xenopus* polo-like kinase Plx1." *Journal of Biological Chemistry* **277**(28): 25247-56.

Kemphues, K. J., N. Wolf, et al. (1986). "Two loci required for cytoplasmic organization in early embryos of *Caenorhabditis elegans*." *Developmental Biology* **113**(2): 449-60.

Kerrebrock, A. W., W. Y. Miyazaki, et al. (1992). "The *Drosophila* mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation." *Genetics* **130**(4): 827-41.

Khodjakov, A., R. W. Cole, et al. (2000). "Centrosome-independent mitotic spindle formation in vertebrates." *Current Biology* **10**(2): 59-67.

Kiat, L. S., K. M. Hui, et al. (2002). "Aurora-A kinase interacting protein (AIP), a novel negative regulator of human Aurora-A kinase." *Journal of Biological Chemistry* **277**(47): 45558-65.

Kim, S. H., D. P. Lin, et al. (1998). "Fission yeast Slp1: an effector of the Mad2-dependent spindle checkpoint." *Science* **279**(5353): 1045-7.

Kim, J. H., J. S. Kang, et al. (1999). "Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerivisae*." *Journal of Cell Biology* **145**(7): 1381-94.

Kim, S. K., J. Lund, et al. (2001). "A gene expression map for *Caenorhabditis elegans*." *Science* **293**(5537): 2087-92.

Kimura, K. and T. Hirano (1997). "ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation." *Cell* **90**(4): 625-34.

Kimura, K. and T. Hirano (2000). "Dual roles of the 11S regulatory subcomplex in condensin functions." *Proceedings of the National Academy of Sciences of the United States of America* **97**(22): 11972-7.

Kimura, K., M. Hirano, et al. (1998). "Phosphorylation and activation of 13S condensin by Cdc2 in vitro." *Science* **282**(5388): 487-90.

Kimura, K., V. V. Rybenkov, et al. (1999a). "13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation." *Cell* **98**(2): 239-48.

Kimura, M., K. Matsunaga, et al. (1999b). "Cell cycle-dependent expression and centrosome localization of a third human aurora/Ipl1-related protein kinase, AIK3." *Cancer Research* **59**(9): 2041-4.

Kimura, K., O. Cuvier, et al. (2001). "Chromosome condensation by a human condensin complex in *Xenopus* egg extracts." *Journal of Biological Chemistry* **276**(8): 5417-20.

King, R. W., J. M. Peters, et al. (1995). "A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B." *Cell* **81**(2): 279-88.

King, J. M. and R. B. Nicklas (2000). "Tension on chromosomes increases the number of kinetochore microtubules but only within limits." *Journal of Cell Science* **113 Pt 21**: 3815-23.

King, J. M., T. S. Hays, et al. (2000). "Dynein is a transient kinetochore component whose binding is regulated by microtubule attachment, not tension." *Journal of Cell Biology* **151**(4): 739-48.

Kirschner, M. W. and T. Mitchison (1986). "Microtubule dynamics." *Nature* **324**(6098): 621.

Kitada, K., A. L. Johnson, et al. (1993). "A multicopy suppressor gene of the *Saccharomyces cerivisae* G1 cell cycle mutant gene dbf4 encodes a protein kinase and is identified as CDC5." *Molecular & Cellular Biology* **13**(7): 4445-57.

Kitagawa, R. and A. M. Rose (1999). "Components of the spindle-assembly checkpoint are essential in *Caenorhabditis elegans*." *Nature Cell Biology* **1**(8): 514-21.

Kitagawa, R., E. Law, et al. (2002). "The Cdc20 homolog, FZY-1, and its interacting protein, IFY-1, are required for proper chromosome segregation in *Caenorhabditis elegans*." *Current Biology* **12**(24): 2118-23.

Kitajima, T. S., Y. Miyazaki, et al. (2003a). "Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast." *EMBO Journal* **22**(20): 5643-53.

Kitajima, T. S., S. Yokobayashi, et al. (2003b). "Distinct cohesin complexes organize meiotic chromosome domains." *Science* **300**(5622): 1152-5.

Kitajima, T. S., S. A. Kawashima, et al. (2004). "The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis." *Nature* **427**(6974): 510-7.

Kitamura, K., Maekawa H, et al. (1998). "Fission yeast Ste9, a homolog of Hct1/Cdh1 and Fizzy-related, is a novel negative regulator of cell cycle progression during G1-phase." *Molecular Biology of the Cell.* **9**(5): 1065-80.

Klein, F., P. Mahr, et al. (1999). "A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis." *Cell* **98**(1): 91-103.

Knecht, R., R. Elez, et al. (1999). "Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck." *Cancer Research* **59**(12): 2794-7.

Kneisel, L., K. Strebhardt, et al. (2002). "Expression of polo-like kinase (PLK1) in thin melanomas: a novel marker of metastatic disease." *Journal of Cutaneous Pathology* **29**(6): 354-8.

Kominami, K., H. Seth-Smith, et al. (1998). "Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast." *EMBO Journal* **17**(18): 5388-99.

Kornberg, R. D. and Y. Lorch (1999). "Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome." *Cell* **98**(3): 285-94.

Koshland, D., A. Strunnikov, et al. (1997). "Centromere position in budding yeast: evidence for anaphase A." *Cell* **91**(1): 47-57.

Kotani, S., S. Tugendreich, et al. (1998). "PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression." *Molecular Cell* **1**(3): 371-380.

Kraft, C., F. Herzog, et al. (2003). "Mitotic regulation of the human anaphase-promoting complex by phosphorylation." *EMBO Journal* **22**(24): 6598-609.

Kramer, E. R., C. Gieffers, et al. (1998). "Activation of the human anaphase-promoting complex by proteins of the CDC20/Fizzy family." *Current Biology* **8**(22): 1207-10.

Kramer, E. R., N. Scheuringer, et al. (2000). "Mitotic regulation of the APC activator proteins CDC20 and CDH1." *Molecular Biology of the Cell* **11**(5): 1555-69.

Krawchuk, M. D., L. C. DeVeaux, et al. (1999). "Meiotic chromosome dynamics dependent upon the rec8(+), rec10(+) and rec11(+) genes of the fission yeast Schizosaccharomyces pombe." *Genetics* **153**(1): 57-68.

Krylov, D. M., K. Nasmyth, et al. (2003). "Evolution of eukaryotic cell cycle regulation: stepwise addition of regulatory kinases and late advent of the CDKs." *Current Biology* **13**(2): 173-7.

Kufer, T. A., H. H. Sillje, et al. (2002). "Human TPX2 is required for targeting Aurora-A kinase to the spindle." *Journal of Cell Biology* **158**(4): 617-23.

Kumada, K., M. Yanagida, et al. (1996). "Fission yeast Cut1 and Cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes." *EMBO Journal* **15**(23): 6617-28.

Kumagai, A. and W. G. Dunphy (1991). "The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system." *Cell* **67**(1): 189-96.

Kumagai, A. and W. G. Dunphy (1996). "Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts." *Science* **273**(5280): 1377-80.

Labbe, J. C., J. P. Capony, et al. (1989). "MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B." *EMBO Journal* **8**(10): 3053-8.

Labbe, J., E. K. McCarthy, et al. (2003). "The forces that position a mitotic spindle asymmetrically are tethered until after the time of spindle assembly." *Journal of Cell Biology* **167**(2): 245-56.

Lake, R. J. and W. R. Jelinek (1993). "Cell cycle- and terminal differentiation-associated regulation of the mouse mRNA encoding a conserved mitotic protein kinase." *Molecular & Cellular Biology* **13**(12): 7793-801.

Lamoureux, J. S., D. Stuart, et al. (2002). "Structure of the sporulation-specific transcription factor Ndt80 bound to DNA." *EMBO Journal* **21**(21): 5721-32.

Lampson, M. A., K. Renduchitala, et al. (2004). "Correcting improper chromosome-spindle attachments during cell division." *Nature Cell Biology* **6**(3): 232-7.

Lan, W., X. Zhang, et al. (2002). "Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity." *Current Biology* **14**(4): 273-86.

Lane, H. A. and E. A. Nigg (1996). "Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes." *Journal of Cell Biology* **135**(6 Pt 2): 1701-13.

Lavoie, B. D., K. M. Tuffo, et al. (2000). "Mitotic chromosome condensation requires Brn1p, the yeast homologue of Barren." *Molecular Biology of the Cell* **11**(4): 1293-304.

Lavoie, B. D., E. Hogan, et al. (2002). "In vivo dissection of the chromosome condensation machinery: reversibility of condensation distinguishes contributions of condensin and cohesin." *Journal of Cell Biology* **156**(5): 805-15.

Lee, K. S. and R. L. Erikson (1997). "Plk is a functional homolog of *Saccharomyces cerivisae* Cdc5, and elevated Plk activity induces multiple septation structures." *Molecular & Cellular Biology* **17**(6): 3408-17.

Lee, K. S., Y. L. Yuan, et al. (1995). "Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1." *Molecular & Cellular Biology* **15**(12): 7143-51.

Lee, K. S., T. Z. Grenfell, et al. (1998). "Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk." *Proceedings of the National Academy of Sciences of the United States of America* **95**(16): 9301-6.

Lee, K. S., S. Song, et al. (1999). "The polo-box-dependent induction of ectopic septal structures by a mammalian polo kinase, plk, in *Saccharomyces cerivisae*." *Proceedings of the National Academy of Sciences of the United States of America* **96**(25): 14360-5.

Lee, S. E., S. Jensen, et al. (2001). "The Bub2-dependent mitotic pathway in yeast acts every cell cycle and regulates cytokinesis." *Journal of Cell Science* **114**(Pt 12): 2345-54.

Lee, B. H., A. Amon, et al. (2002). "Spo13 regulates cohesin cleavage." *Genes & Development* **16**(13): 1672-81.

Lee, J., T. Iwai, et al. (2003). "Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis." *Journal of Cell Science* **116**(Pt 13): 2781-90.

Lee, J. Y., K. J. Dej, et al. (2004). "Control of centromere localization of the MEI-S332 cohesion protection protein." *Current Biology* **14**(14): 1277-83.

Lehner, C. F. and O. Leismann (2003). "*Drosophila* securin destruction involves a D-box and a KEN-box and promotes anaphase in parallel with Cyclin A degradation." *Journal of Cell Science* **116**(Pt 12): 2453-60.

Leidel, S. and P. Gonczy (2003). "SAS-4 is essential for centrosome duplication in C elegans and is recruited to daughter centrioles once per cell cycle." *Developmental Cell* **4**(3): 431-9.

Leismann, O. and C. F. Lehner (2003). "*Drosophila* securin destruction involves a D-box and a KEN-box and promotes anaphase in parallel with Cyclin A degradation." *Journal of Cell Science* **116**(Pt 12): 2453-60.

Leismann, O., A. Herzig, et al. (2000). "Degradation of *Drosophila* PIM regulates sister chromatid separation during mitosis." *Genes & Development* **14**(17): 2192-205.

Leiss, D., M. A. Felix, et al. (1992). "Association of cyclin-bound p34cdc2 with subcellular structures in *Xenopus* eggs." *Journal of Cell Science* **102**(Pt 2): 285-97.

Lengauer, C., K. W. Kinzler, et al. (1998). "Genetic instabilities in human cancers." *Nature* **396**(6712): 643-9.

Leung, G. C., J. W. Hudson, et al. (2002). "The Sak polo-box comprises a structural domain sufficient for mitotic subcellular localization." *Nature Structural Biology* **9**(10): 719-24.

Li, R. and A. W. Murray (1991). "Feedback control of mitosis in budding yeast." *Cell* **66**(3): 519-31.

Li, Y., J. Bachant, et al. (2002). "The mitotic spindle is required for loading of the DASH complex onto the kinetochore." *Genes & Development* **16**(2): 183-97.

Liao, H., G. Li, et al. (1994). "Mitotic regulation of microtubule cross-linking activity of CENP-E kinetochore protein." *Science* **265**(5170): 394-8.

Lieb, J. D., M. R. Albrecht, et al. (1998). "MIX-1: an essential component of the *C. elegans* mitotic machinery executes X chromosome dosage compensation." *Cell* **92**(2): 265-77.

Lim, H. H., P. Y. Goh, et al. (1998). "Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M phase in budding yeast." *Current Biology* **8**(4): 231-4.

Lin, R. (2003). "A gain-of-function mutation in oma-1, a *C. elegans* gene required for oocyte maturation, results in delayed degradation of maternal proteins and embryonic lethality." *Developmental Biology* **258**(1): 226-39.

Littlepage, L. E. and J. V. Ruderman (2002). "Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit." *Genes & Development* **16**(17): 2274-85.

Litvak, V., R. Argov, et al. (2004). "Mitotic phosphorylation of the peripheral Golgi protein Nir2 by Cdk1 provides a docking mechanism for Plk1 and affects cytokinesis completion." *Molecular Cell* **14**(3): 319-30.

Liu, D., M. M. Matzuk, et al. (1998). "Cyclin A1 is required for meiosis in the male mouse." *Nature Genetics* **20**(4): 377-80.

Liu, J., S. Vasudevan, et al. (2004). "CUL-2 and ZYG-11 promote meiotic anaphase II and the proper placement of the anterior-posterior axis in *C. elegans*." *Development* **131**(15): 3513-25.

Llamazares, S., A. Moreira, et al. (1991). "polo encodes a protein kinase homolog required for mitosis in *Drosophila*." *Genes & Development* **5**(12A): 2153-65.

Lorca, T., A. Castro, et al. (1998). "Fizzy is required for activation of the APC/cyclosome in *Xenopus* egg extracts." *EMBO Journal* **17**(13): 3565-75.

Lorson, M. A., H. R. Horvitz, et al. (2000). "LIN-5 is a novel component of the spindle apparatus required for chromosome segregation and cleavage plane specification in *Caenorhabditis elegans*." *Journal of Cell Biology* **148**(1): 73-86.

Losada, A. and T. Hirano (2001). "Intermolecular DNA interactions stimulated by the cohesin complex in vitro: implications for sister chromatid cohesion." *Current Biology* **11**(4): 268-72.

Losada, A., M. Hirano, et al. (1998). "Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion." *Genes & Development* **12**(13): 1986-97.

Losada, A., T. Yokochi, et al. (2000). "Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes." *Journal of Cell Biology* **150**(3): 405-16.

Losada, A., M. Hirano, et al. (2002). "Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis." *Genes & Development* **16**(23): 3004-16.

Lowe, M., C. Rabouille, et al. (1998). "Cdc2 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis." *Cell* **94**(6): 783-93.

Luca, F. C. and J. V. Ruderman (1989). "Control of programmed cyclin destruction in a cell-free system." *Journal of Cell Biology* **109**(5): 1895-909.

Lundgren, K., N. Walworth, et al. (1991). "mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2." *Cell* **64**(6): 1111-22.

Macara, I. G. (2004). "Parsing the polarity code." *Nature Reviews Molecular Cell Biology* **5**(3): 220-31.

MacCallum, D. E., A. Losada, et al. (2002). "ISWI remodeling complexes in *Xenopus* egg extracts: identification as major chromosomal components that are regulated by INCENP-aurora B." *Molecular Biology of the Cell* **13**(1): 25-39.

Mackay, A. M., A. M. Ainsztein, et al. (1998). "A dominant mutant of inner centromere protein (INCENP), a chromosomal protein, disrupts prometaphase congression and cytokinesis." *Journal of Cell Biology* **140**(5): 991-1002.

MacQueen, A. J., M. P. Colaiacovo, et al. (2002). "Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*." *Genes & Development* **16**(18): 2428-42.

Madl, J. E. and R. K. Herman (1979). "Polyploids and sex determination in *Caenorhabditis elegans*." *Genetics* **93**(2): 393-402.

Maguire, M. P. (1974). "Letter: The need for a chiasma binder." *Journal of Theoretical Biology* **48**(2): 485-7.

Mahadevaiah, S. K., J. M. Turner, et al. (2001). "Recombinational DNA double-strand breaks in mice precede synapsis." *Nature Genetics* **27**(3): 271-276.

Mahadevan, D., D. J. Bearss, et al. (2003). "Structure-based design of novel anti-cancer agents targeting aurora kinases." *Current Medicinal Chemistry - Anti-Cancer Agents* **3**(1): 25-34.

Mahaffey, D., Y. Yoo, et al. (1993). "Ubiquitin metabolism in cycling *Xenopus* egg extracts." *Journal of Biological Chemistry* **268**(28): 21205-11.

Maller, J. L. (1993). "On the importance of protein phosphorylation in cell cycle control." *Molecular & Cellular Biochemistry* **127-128**: 267-81.

Maller, J., J. Gautier, et al. (1989). "Maturation-promoting factor and the regulation of the cell cycle." *Journal of Cell Science - Supplement* **12**: 53-63.

Maney, T., A. W. Hunter, et al. (1998). "Mitotic centromere-associated kinesin is important for anaphase chromosome segregation." *Journal of Cell Biology* **142**(3): 787-801.

Martinez-Exposito, M. J., K. B. Kaplan, et al. (1999). "Retention of the BUB3 checkpoint protein on lagging chromosomes." *Proceedings of the National Academy of Sciences of the United States of America* **96**(15): 8493-8.

Masui, Y. and C. L. Markert (1971). "Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes." *Journal of Experimental Zoology* **177**(2): 129-45.

Matsubara, N., M. Yanagisawa, et al. (1995). "Murine polo like kinase 1 gene is expressed in meiotic testicular germ cells and oocytes." *Molecular Reproduction & Development* **41**(4): 407-15.

Mayer, M. L., S. P. Gygi, et al. (2001). "Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in *S. cerivisae*." *Molecular Cell* **7**(5): 959-70.

McCarter, J., B. Bartlett, et al. (1999). "On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*." *Developmental Biology* **205**(1): 111-28.

McEwen, B. F., G. K. Chan, et al. (2001). "CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells." *Molecular Biology of the Cell* **12**(9): 2776-89.

McGowan, C. H. and P. Russell (1993). "Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15." *EMBO Journal* **12**(1): 75-85.

McNeill, P. A. and M. W. Berns (1981). "Chromosome behavior after laser microirradiation of a single kinetochore in mitotic PtK2 cells." *Journal of Cell Biology* **88**(3): 543-53.

Megraw, T. L. and T. C. Kaufman (2000). "The centrosome in *Drosophila* oocyte development." *Current Topics in Developmental Biology* **49**: 385-407.

Mei, J., X. Huang, et al. (2001). "Securin is not required for cellular viability, but is required for normal growth of mouse embryonic fibroblasts." *Current Biology* **11**(15): 1197-201.

Mendoza, M., V. Jantsch-Plunger, et al. (2000). "Incenp and an aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis." *Current Biology* **10**(19): 1172-81.

Meneely, P. M., A. F. Farago, et al. (2002). "Crossover distribution and high interference for both the X chromosome and an autosome during oogenesis and spermatogenesis in *Caenorhabditis elegans*." *Genetics* **162**(3): 1169-77.

Metzler-Guillemain, C. and B. de Massy (2000). "Identification and characterization of an SPO11 homolog in the mouse." *Chromosoma* **109**(1-2): 133-8.

Michaelis, C., R. Ciosk, et al. (1997). "Cohesins: chromosomal proteins that prevent premature separation of sister chromatids." *Cell* **91**(1): 35-45.

Michel, L. S., V. Liberal, et al. (2001). "MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells." *Nature* **409**(6818): 355-9.

Mihara, H., Y. Kato, et al. (2000). "Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast." *Oncogene* **19**(24): 2812-9.

Minoshima, Y., T. Kawashima, et al. (2003). "Phosphorylation by aurora B converts MgcRacGAP to a RhoGAP during cytokinesis." *Dev Cell* **4**(4): 549-60.

Mitchison, T. and M. Kirschner (1984). "Dynamic instability of microtubule growth." *Nature* **312**(5991): 237-42.

Mitchison, T. J. and E. D. Salmon (2001). "Mitosis: a history of division." *Nature Cell Biology* **3**(1): E17-21.

Mito, Y., A. Sugimoto, et al. (2003). "Distinct developmental function of two *Caenorhabditis elegans* homologs of the cohesin subunit Scc1/Rad21." *Molecular Biology of the Cell* **14**(6): 2399-409.

Molnar, M., J. Bahler, et al. (1995). "The rec8 gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis." *Genetics* **141**(1): 61-73.

Molnar, M., J. Bahler, et al. (2001). "Live observation of fission yeast meiosis in recombination-deficient mutants: a study on achiasmate chromosome segregation." *Journal of Cell Science* **114**(Pt 15): 2843-53.

Molnar, M., E. Doll, et al. (2003). "Linear element formation and their role in meiotic sister chromatid cohesion and chromosome pairing." *Journal of Cell Science* **116**(Pt 9): 1719-31.

Moore, L. A., M. Van Doren, et al. (1998). "The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate." *Development* **125**(4): 655-66.

Morton, D. G., D. C. Shakes, et al. (2002). "The *Caenorhabditis elegans* par-5 gene encodes a 14-3-3 protein required for cellular asymmetry in the early embryo." *Developmental Biology* **241**(1): 47-58.

Moshe, Y., J. Boulaire, et al. (2004). "Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome." *Proceedings of the National Academy of Sciences of the United States of America* **101**(21): 7937-42.

Moutinho-Santos, T., P. Sampaio, et al. (1999). "In vivo localisation of the mitotic POLO kinase shows a highly dynamic association with the mitotic apparatus during early embryogenesis in *Drosophila*." *Biology of the Cell* **91**(8): 585-96.

Mueller, P. R., T. R. Coleman, et al. (1995). "Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15." *Science* **270**(5233): 86-90.

Muhlrad, P. J. and S. Ward (2002). "Spermiogenesis initiation in *Caenorhabditis elegans* involves a casein kinase 1 encoded by the spe-6 gene." *Genetics* **161**(1): 143-55.

Mundt, K. E., A. M. Fry, et al. (1995). "Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function." *Journal of Cell Biology* **129**(6): 1617-28.

Munro, E., J. Nance, et al. (2004). "Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo." *Developmental Cell* **7**(3): 413-24.

Murata-Hori, M., Tatsuka M, et al. (2002a). "Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis." *Molecular Biology of the Cell.* **13**(4): 1099-108.

Murata-Hori, M. and Y. L. Wang (2002b). "Both midzone and astral microtubules are involved in the delivery of cytokinesis signals: insights from the mobility of aurora B." *Journal of Cell Biology* **159**(1): 45-53.

Murnion, M. E., K. Fumoto, et al. (2000). "Chromatin-associated protein phosphatase 1 regulates aurora-B and histone H3 phosphorylation." *Journal of Biochemistry* **128**(6): 903-7.

Murray, A. W. (1989). "Cyclin synthesis and degradation and the embryonic cell cycle." *Journal of Cell Science* **12**(Sup): 65-76.

Murray, A. W. (2004). "Recycling the cell cycle: cyclins revisited." *Cell* **116**(2): 221-34.

Murray, A. W., M. J. Solomon, et al. (1989). "The role of cyclin synthesis and degradation in the control of maturation promoting factor activity." *Nature* **339**(6222): 280-6.

Murray, J. C., H. F. Willard, et al. (1991). "Feedback control of mitosis in budding yeast." *Genomics* **11**(4): 991-6.

Musacchio, A. and K. G. Hardwick (2002). "The spindle checkpoint: structural insights into dynamic signalling." *Nature Reviews Molecular Cell Biology* **3**(10): 731-41.

Muszynski, M. G., G. Kelly, et al. (1999). "Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos." *Journal of Cell Biology* **145**(3): 425-35.

Mutskov, V., D. Gerber, et al. (1998). "Persistent interactions of core histone tails with nucleosomal DNA following acetylation and transcription factor binding." *Molecular & Cellular Biology* **18**(11): 6293-304.

Mutucumarana, V. P., D. L. Straight, et al. (2003). "Determining the position of the cell division plane." *Journal of Biological Chemistry* **278**(46): 45468-75.

Nakajima, H., F. Toyoshima-Morimoto, et al. (2003). "Identification of a consensus motif for Plk (Polo-like kinase) phosphorylation reveals Myt1 as a Plk1 substrate." *Journal of Biological Chemistry* **278**(28): 25277-80.

Nasmyth, K. (2001). "Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis." *Annual Review of Genetics* **35**: 673-745.

Neef, R., C. Preisinger, et al. (2003). "Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis." *Journal of Cell Biology* **162**(5): 863-75.

Newport, J. W. and M. W. Kirschner (1984). "Regulation of the cell cycle during early *Xenopus* development." *Cell* **37**(3): 731-42.

Nicklas, R. B. and S. C. Ward (1994). "Elements of error correction in mitosis: microtubule capture, release, and tension." *Journal of Cell Biology* **126**(5): 1241-53.

Nigg, E. A. (1992). "Assembly-disassembly of the nuclear lamina." *Current Opinion in Cell Biology* **4**(1): 105-9.

Nigg, E. A. (1998). "Polo-like kinases: positive regulators of cell division from start to finish." *Current Opinion in Cell Biology* **10**(6): 776-83.

Nigg, E. A. (2001). "Mitotic kinases as regulators of cell division and its checkpoints." *Nature Reviews Molecular Cell Biology* **2**(1): 21-32.

Nowak, S. J. and V. G. Corces (2004). "Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation." *Trends Genet* **20**(4): 214-20.

Nurse, H. M. and B. Grallert (1989). "Dissociation of meiotic and mitotic roles of the fission yeast cdc2 gene." *American Journal of Medicine* **86**(2): 183-6.

Nurse, P. (1990). "Universal control mechanism regulating onset of M-phase." *Nature* **344**(6266): 503-8.

Oegema, K., A. Desai, et al. (2001). "Functional analysis of kinetochore assembly in *Caenorhabditis elegans*." *Journal of Cell Biology* **153**(6): 1209-26.

Ohi, R., T. Sapra, et al. (2004). "Differentiation of cytoplasmic and meiotic spindle assembly MCAK functions by Aurora B-dependent phosphorylation." *Molecular Biology of the Cell* **15**(6): 2895-906.

Ohkura, H., Hagan IM, et al. (1995). "The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells." *Genes & Development* **9**(9): 1059-73.

Ohmachi, M., Rocheleau CE, et al. (2002). "*C. elegans* ksr-1 and ksr-2 have both unique and redundant functions and are required for MPK-1 ERK phosphorylation." *Current Biology* **12**(5): 427-33.

Ohsumi, K., C. Katagiri, et al. (1993). "Chromosome condensation in *Xenopus* mitotic extracts without histone H1." *Science* **262**(5142): 2033-5.

Okano-Uchida, T., E. Okumura, et al. (2003). "Distinct regulators for Plk1 activation in starfish meiotic and early embryonic cycles." *EMBO Journal* **22**(20): 5633-42.

Ono, T., L. A., et al. (2003). "Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells." *Cell* **115**(1): 109-121.

Ota, T., S. Suto, et al. (2002). "Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability." *Cancer Research* **62**(18): 5168-77.

Ouspenski, II, O. A. Cabello, et al. (2000). "Chromosome condensation factor Brn1p is required for chromatid separation in mitosis." *Molecular Biology of the Cell* **11**(4): 1305-13.

Ouyang, B., H. Pan, et al. (1997). "Human Prk is a conserved protein serine/threonine kinase involved in regulating M phase functions." *Journal of Biological Chemistry* **272**(45): 28646-51.

Ouyang, B., Y. Wang, et al. (1999). "*Caenorhabditis elegans* contains structural homologs of human prk and plk." *DNA Sequence* **10**(2): 109-13.

Pahlavan, G., Z. Polanski, et al. (2000). "Characterization of polo-like kinase 1 during meiotic maturation of the mouse oocyte." *Developmental Biology* **220**(2): 392-400.

Page, S. L. and R. S. Hawley (2003). "Chromosome choreography: the meiotic ballet." *Science* **301**(5634): 785-9.

Paliulis, L. V. and R. B. Nicklas (2000). "The reduction of chromosome number in meiosis is determined by properties built into the chromosomes." *Journal of Cell Biology* **150**(6): 1223-32.

Palmer, R. E., M. Koval, et al. (1989). "The dynamics of chromosome movement in the budding yeast *Saccharomyces cerivisae*." *Journal of Cell Biology* **109**(6 (Pt 2)): 3355-66.

Parisi, S., M. J. McKay, et al. (1999). "Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans." *Molecular & Cellular Biology* **19**(5): 3515-28.

Parker, L. L., S. Atherton-Fessler, et al. (1992). "p107wee1 is a dual-specificity kinase that phosphorylates p34cdc2 on tyrosine 15." *Proceedings of the National Academy of Sciences of the United States of America* **89**(7): 2917-21.

Parra, M. T., A. Viera, et al. (2004). "Involvement of the cohesin Rad21 and SCP3 in monopolar attachment of sister kinetochores during mouse meiosis I." *Journal of Cell Science* **117**(Pt 7): 1221-34.

Parry, D. H., G. R. Hickson, et al. (2003). "Cyclin B destruction triggers changes in kinetochore behavior essential for successful anaphase." *Current Biology* **13**(8): 647-53.

Pasierbek, P., M. Jantsch, et al. (2001). "A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction." *Genes & Development* **15**(11): 1349-60.

Pei, L. and S. Melmed (1997). "Isolation and characterization of a pituitary tumor-transforming gene (PTTG)." *Molecular Endocrinology* **11**(4): 433-41.

Pellettieri, J., V. Reinke, et al. (2003). "Coordinate activation of maternal protein degradation during the egg-to-embryo transition in *C. elegans*." *Developmental Cell* **5**(3): 451-62.

Pellman, D., M. Bagget, et al. (1995). "Two microtubule-associated proteins required for anaphase spindle movement in *Saccharomyces cerivisae*." *Journal of Cell Biology* **130**(6): 1373-85.

Pelttari, J., M. R. Hoja, et al. (2001). "A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells." *Molecular & Cellular Biology* **21**(16): 5667-77.

Pereira, G. and E. Schiebel (2003). "Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14." *Science* **302**(5653): 2120-4.

Peter, M., J. Nakagawa, et al. (1990). "In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase." *Cell* **61**(4): 591-602.

Peters, J. M., R. W. King, et al. (1996). "Identification of BIME as a subunit of the anaphase-promoting complex." *Science* **274**(5290): 1190-201.

Petersen, J. and I. M. Hagan (2003). "*S. pombe* aurora kinase/survivin is required for chromosome condensation and the spindle checkpoint attachment response." *Current Biology* **13**(7): 590-7.

Petersen, J., J. Paris, et al. (2001). "The *S. pombe* aurora-related kinase Ark1 associates with mitotic structures in a stage dependent manner and is required for chromosome segregation." *Journal of Cell Science* **114**(Pt 24): 4371-84.

Petronczki, M., M. F. Siomos, et al. (2003). "Un menage a quatre: the molecular biology of chromosome segregation in meiosis." *Cell* **112**(4): 423-40.

Pfleger, C. M. and M. W. Kirschner (2000). "The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1." *Genes & Development* **14**(6): 655-65.

Pfleger, C. M., E. Lee, et al. (2001). "Substrate recognition by the Cdc20 and Cdh1 components of the anaphase-promoting complex." *Genes & Development* **15**(18): 2396-407.

Pines, J. (1999). "Four-dimensional control of the cell cycle." *Nature Cell Biology* **1**(3): E73-9.

Praitis, V., E. Casey, et al. (2001). "Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*." *Genetics* **157**(3): 1217-26.

Prieto, I., J. A. Suja, et al. (2001). "Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I." *Nature Cell Biology* **3**(8): 761-6.

Prieto, I., N. Pezzi, et al. (2002). "STAG2 and Rad21 mammalian mitotic cohesins are implicated in meiosis." *EMBO Reports* **3**(6): 543-50.

Prieto, I., C. Tease, et al. (2004). "Cohesin component dynamics during meiotic prophase I in mammalian oocytes." *Chromosome Research* **12**(3): 197-213.

Prinz, S., E. S. Hwang, et al. (1998). "The regulation of Cdc20 proteolysis reveals a role for APC components Cdc23 and Cdc27 during S phase and early mitosis." *Current Biology* **8**(13): 750-60.

Qian, Y. W., E. Erikson, et al. (1998a). "Purification and cloning of a protein kinase that phosphorylates and activates the polo-like kinase Plx1." *Science* **282**(5394): 1701-4.

Qian, Y. W., E. Erikson, et al. (1998b). "Activated polo-like kinase Plx1 is required at multiple points during mitosis in *Xenopus* laevis." *Molecular & Cellular Biology* **18**(7): 4262-71.

Qian, Y. W., E. Erikson, et al. (1999). "Mitotic effects of a constitutively active mutant of the *Xenopus* polo-like kinase Plx1." *Molecular & Cellular Biology* **19**(12): 8625-32.

Rabitsch, K. P., A. Toth, et al. (2001). "A screen for genes required for meiosis and spore formation based on whole-genome expression." *Current Biology* **11**(13): 1001-9.

Rabitsch, K. P., M. Petronczki, et al. (2003). "Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I." *Developmental Cell* **4**(4): 535-48.

Rabitsch, K. P., J. Gregan, et al. (2004). "Two fission yeast homologs of *Drosophila* Mei-S332 are required for chromosome segregation during meiosis I and II." *Current Biology* **14**(4): 287-301.

Rachidi, N., C. Janke, et al. (2002). "Degradation of human Aurora2 protein kinase by the anaphase-promoting complex-ubiquitin-proteasome pathway." *Cell* **108**(3): 317-29.

Raff, J. W., K. Jeffers, et al. (2002). "The roles of Fzy/Cdc20 and Fzr/Cdh1 in regulating the destruction of cyclin B in space and time." *Journal of Cell Biology* **157**(7): 1139-49.

Rajagopalan, S. and M. K. Balasubramanian (2002). "*Schizosaccharomyces pombe* Bir1p, a nuclear protein that localizes to kinetochores and the spindle midzone, is essential for chromosome condensation and spindle elongation during mitosis." *Genetics* **160**(2): 445-56.

Rappleye, C. A., A. Tagawa, et al. (2002). "The anaphase-promoting complex and separin are required for embryonic anterior-posterior axis formation." *Developmental Cell* **2**(2): 195-206.

Reese, K. J., M. A. Dunn, et al. (2000). "Asymmetric segregation of PIE-1 in *C. elegans* is mediated by two complementary mechanisms that act through separate PIE-1 protein domains." *Molecular Cell* **6**(2): 445-55.

Reich, A., A. Yanai, et al. (1999). "Cloning, mapping, and expression of ial, a novel *Drosophila* member of the Ipl1/aurora mitotic control kinase family." *DNA & Cell Biology* **18**(7): 593-603.

Revenkova, E., G. P. di Sansebastiano, et al. (2001). "Novel meiosis-specific isoform of mammalian SMC1." *Genes & Development* **15**(6): 699-709.

Riabowol, K. T., M. Z. Gilman, et al. (1989). "The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells." *Molecular & Cellular Biology* **9**(10): 4272-81.

Riedel, C. G., J. Gregan, et al. (2004). "Is chromatin remodeling required to build sister-chromatid cohesion?" *Trends in Biochemical Sciences* **29**(8): 389-92.

Rieder, C. L. and S. P. Alexander (1990). "Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells." *Journal of Cell Biology* **110**(1): 81-95.

Rieder, C. L. and A. Khodjakov (2003). "Mitosis through the microscope: advances in seeing inside live dividing cells." *Science* **300**(5616): 91-6.

Rimmington, G., B. Dalby, et al. (1994). "Expression of N-terminally truncated cyclin B in the *Drosophila* larval brain leads to mitotic delay at late anaphase." *Journal of Cell Science* **107**(Pt 10): 2729-38.

Riparbelli, M. G., G. Minestrini, et al. (1998). "pavarotti encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis." *Journal of Cell Biology* **143**(3): 659-71.

Rogers, E., J. D. Bishop, et al. (2002). "The aurora kinase AIR-2 functions in the release of chromosome cohesion in *Caenorhabditis elegans* meiosis." *Journal of Cell Biology* **157**(2): 219-29.

Romanienko, P. J. and R. D. Camerini-Otero (2000). "The mouse Spo11 gene is required for meiotic chromosome synapsis." *Molecular Cell* **6**(5): 975-87.

Romano, A., A. Guse, et al. (2003). "CSC-1: a subunit of the Aurora B kinase complex that binds to the survivin-like protein BIR-1 and the incenp-like protein ICP-1." *Journal of Cell Biology* **161**(2): 229-36.

Rose, L. S. and K. Kemphues (1998). "The let-99 gene is required for proper spindle orientation during cleavage of the *C. elegans* embryo." *Development* **125**(7): 1337-46.

Rudner, A. D. and A. W. Murray (2000). "Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex." *Journal of Cell Biology* **149**(7): 1377-90.

Russell, P. and P. Nurse (1987). "Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog." *Cell* **49**(4): 559-67.

Saitoh, N., I. G. Goldberg, et al. (1994). "ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure." *Journal of Cell Biology* **127**(2): 303-18.

Saka, Y., T. Sutani, et al. (1994). "Fission yeast cut3 and cut14, members of a ubiquitous protein family, are required for chromosome condensation and segregation in mitosis." *EMBO Journal* **13**(20): 4938-52.

Salah, S. M. and K. Nasmyth (2000). "Destruction of the securin Pds1p occurs at the onset of anaphase during both meiotic divisions in yeast." *Chromosoma* **109**(1-2): 27-34.

Salic, A., J. C. Waters, et al. (2004). "Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis." *Cell* **118**(5): 567-78.

Salmon, E. D., R. J. Leslie, et al. (1984). "Spindle microtubule dynamics in sea urchin embryos: analysis using a fluorescein-labeled tubulin and measurements of fluorescence redistribution after laser photobleaching." *Journal of Cell Biology* **99**(6): 2165-74.

Sammak, P. J. and G. G. Borisy (1988). "Direct observation of microtubule dynamics in living cells." *Journal of Cell Biology* **106**(4): 1185-92.

Sampath, S. C., R. Ohi, et al. (2004). "The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly." *Cell* **118**(2): 187-202.

Samuel, A. D., V. N. Murthy, et al. (2001). "Calcium dynamics during fertilization in *C. elegans*." *BMC Developmental Biology* **1**(1): 8.

Sanford, C. and M. D. Perry (2001). "Asymmetrically distributed oligonucleotide repeats in the *Caenorhabditis elegans* genome sequence that map to regions important for meiotic chromosome segregation." *Nucleic Acids Research* **29**(14): 2920-6.

Sasagawa, Y., T. Urano, et al. (2003). "*Caenorhabditis elegans* RBX1 is essential for meiosis, mitotic chromosomal condensation and segregation, and cytokinesis." *Genes to Cells* **8**(11): 857-72.

Sassoon, I., F. F. Severin, et al. (1999). "Regulation of *Saccharomyces cerivisae* kinetochores by the type 1 phosphatase Glc7p." *Genes & Development* **13**(5): 545-55.

Saunders, W. S. and X. Zeng (2000). "Slk19p is a centromere protein that functions to stabilize mitotic spindles." *Genetics* **155**(2): 577-87.

Sausville, E. A. (2004). "Aurora kinases dawn as cancer drug targets." *Nature Medicine* **10**(3): 234-5.

Sauve, D. M., H. J. Anderson, et al. (1999). "Phosphorylation-induced rearrangement of the histone H3 NH2-terminal domain during mitotic chromosome condensation." *Journal of Cell Biology* **145**(2): 225-35.

Saxton, W. M., D. L. Stemple, et al. (1984). "Tubulin dynamics in cultured mammalian cells." *Journal of Cell Biology* **99**(6): 2175-86.

Schaar, B. T., G. K. Chan, et al. (1997). "CENP-E function at kinetochores is essential for chromosome alignment." *Journal of Cell Biology* **139**(6): 1373-82.

Schedl, T. (1997). Developmental genetics of the germ line. *C. elegans II*. D. Riddle, Blumenthal, T., Meyer, B., Priess, J. Plainview, NY, Cold Spring Harbor Laboratory Press**:** 241-270.

Schild, D. and B. Byers (1980). "Diploid spore formation and other meiotic effects of two cell-division-cycle mutations of *Saccharomyces cerivisae*." *Genetics* **96**(4): 859-76.

Schleicher, W. (1879). "Die Knorpelzellteilung. Ein Beitrag zur Lehre der Teilung von Gewebszellen." *Arch. Mikrosk. Anat.* **16**: 248-300.

Schneider, U., T. Mini, et al. (1999). "Phosphorylation of the major *Drosophila* lamin in vivo: site identification during both M-phase (meiosis) and interphase by electrospray ionization tandem mass spectrometry." *Biochemistry* **38**(14): 4620-32.

Schubert, C., Lin R, et al. (2000). "MEX-5 and MEX-6 function to establish soma/germline asymmetry in early *C. elegans* embryos." *Mol. Cell* **5**(4): 671-82.

Schultz, S. J., J. Bartek, et al. (1994). "Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila* polo and *Saccharomyces cerivisae* Cdc5." *Journal of Cell Science* **107**(Pt 6): 1509-17.

Schumacher, J. M., A. Golden, et al. (1998a). "AIR-2: An Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos." *Journal of Cell Biology* **143**(6): 1635-46.

Schumacher, J. M., N. Ashcroft, et al. (1998b). "A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in *Caenorhabditis elegans* embryos." *Development* **125**(22): 4391-402.

Schwab, U. S., E. S. Sarkkinen, et al. (1997). "Yeast Hct1 is a regulator of Clb2 cyclin proteolysis." *Metabolism: Clinical & Experimental* **46**(6): 666-72.

Schwab, M., M. Neutzner, et al. (2001). "Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC." *EMBO Journal* **20**(18): 5165-75.

Schwacha, A. and N. Kleckner (1994). "Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis." *Cell* **76**(1): 51-63.

Seong, Y. S., K. Kamijo, et al. (2002). "A spindle checkpoint arrest and a cytokinesis failure by the dominant-negative polo-box domain of Plk1 in U-2 OS cells." *Journal of Biological Chemistry* **277**(35): 32282-93.

Severin, F., A. A. Hyman, et al. (2001). "Correct spindle elongation at the metaphase/anaphase transition is an APC-dependent event in budding yeast." *Journal of Cell Biology* **155**(5): 711-8.

Severson, A. F. and B. Bowerman (2003). "Myosin and the PAR proteins polarize microfilament-dependent forces that shape and position mitotic spindles in *Caenorhabditis elegans*." *Journal of Cell Biology* **161**(1): 21-6.

Severson, A. F., D. R. Hamill, et al. (2000). "The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis." *Current Biology* **10**(19): 1162-71.

Shah, J. V. and D. W. Cleveland (2000). "Waiting for anaphase: Mad2 and the spindle assembly checkpoint." *Cell* **103**(7): 997-1000.

Shakes, D. C., P. L. Sadler, et al. (2003). "Developmental defects observed in hypomorphic anaphase-promoting complex mutants are linked to cell cycle abnormalities." *Development* **130**(8): 1605-20.

Shang, C., T. R. Hazbun, et al. (2003). "Kinetochore protein interactions and their regulation by the Aurora kinase Ipl1p." *Molecular Biology of the Cell* **14**(8): 3342-55.

Shannon, K. B., J. C. Canman, et al. (2002). "Mad2 and BubR1 function in a single checkpoint pathway that responds to a loss of tension." *Molecular Biology of the Cell.* **13**(10): 3706-19.

Sharon, G. and G. Simchen (1990a). "Mixed segregation of chromosomes during single-division meiosis of *Saccharomyces cerivisae*." *Genetics* **125**(3): 475-85.

Sharon, G. and G. Simchen (1990b). "Centromeric regions control autonomous segregation tendencies in single-division meiosis of *Saccharomyces cerivisae*." *Genetics* **125**(3): 487-94.

Sharp, D. J., G. C. Rogers, et al. (2000). "Cytoplasmic dynein is required for poleward chromosome movement during mitosis in *Drosophila* embryos." *Nature Cell Biology* **2**(12): 922-930.

Shelby, R. D., K. M. Hahn, et al. (1996). "Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells." *Journal of Cell Biology* **135**(3): 545-57.

Shen, X., L. Yu, et al. (1995). "Linker histones are not essential and affect chromatin condensation in vivo." *Cell* **82**(1): 47-56.

Shirayama, M., W. Zachariae, et al. (1998). "The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerivisae*." *EMBO Journal* **17**(5): 1336-49.

Shirayama, M., A. Shevchenko, et al. (2000). "Identification of cohesin association sites at centromeres and along chromosome arms." *Molecular Cell* **5**(2): 243-54.

Shonn, M. A., R. McCarroll, et al. (2000). "Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis." *Science* **289**(5477): 300-3.

Shonn, M. A., R. McCarroll, et al. (2002). "Spo13 protects meiotic cohesin at centromeres in meiosis I." *Genes & Development* **16**(13): 1659-71.

Shou, W., R. Azzam, et al. (2002). "Cdc5 influences phosphorylation of Net1 and disassembly of the RENT complex." *BMC Molecular Biology* **3**(1): 3.

Sigrist, S., H. Jacobs, et al. (1995). "Exit from mitosis is regulated by *Drosophila* fizzy and the sequential destruction of cyclins A, B and B3." *EMBO Journal* **14**(19): 4827-38.

Sigrist, S. J. and C. F. Lehner (1997). "*Drosophila* fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles." *Cell* **90**(4): 671-81.

Silke, J. and D. L. Vaux (2001). "Two kinds of BIR-containing protein - inhibitors of apoptosis, or required for mitosis." *Journal of Cell Science* **114**(Pt 10): 1821-7.

Simizu, S. and H. Osada (2000). "Mutations in the Plk gene lead to instability of Plk protein in human tumour cell lines." *Nature Cell Biology* **2**(11): 852-4.

Siomos, M. F., A. Badrinath, et al. (2001). "Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*." *Current Biology* **11**(23): 1825-35.

Skibbens, R. V., L. B. Corson, et al. (1999). "Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery." *Genes & Development* **13**(3): 307-19.

Skoufias, D. A., P. R. Andreassen, et al. (2001). "Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints." *Proceedings of the National Academy of Sciences of the United States of America* **98**(8): 4492-7.

Smith, A. V. and G. S. Roeder (1997). "The yeast Red1 protein localizes to the cores of meiotic chromosomes." *Journal of Cell Biology* **136**(5): 957-67.

Smith, L. and R. Ecker (1971). "The interaction of steroids with Rana pipiens oocytes in the induction of maturation." *Developmental Biology* **25**(2): 232-47.

Smits, V. A., R. Klompmaker, et al. (2000). "Polo-like kinase-1 is a target of the DNA damage checkpoint." *Nature Cell Biology* **2**(9): 672-6.

Song, S., R. L. Erikson, et al. (1999). "Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk." *Proceedings of the National Academy of Sciences of the United States of America* **96**(25): 14360-5.

Song, S., T. Z. Grenfell, et al. (2000). "Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures." *Molecular & Cellular Biology* **20**(1): 286-98.

Song, S. and K. S. Lee (2001). "A novel function of *Saccharomyces cerivisae* CDC5 in cytokinesis." *Journal of Cell Biology* **152**(3): 451-69.

Sonneville, R. and P. Gonczy (2004). "Zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in *C. elegans*." *Development* **131**(15): 3527-43.

Sonoda, E., T. Matsusaka, et al. (2001). "Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells." *Developmental Cell* **1**(6): 759-70.

Sorensen, C. S., C. Lukas, et al. (2000). "Nonperiodic activity of the human anaphase-promoting complex-Cdh1 ubiquitin ligase results in continuous DNA synthesis uncoupled from mitosis." *Molecular Cell Biol* **20**(20): 7613-23.

Speliotes, E. K., A. Uren, et al. (2000). "The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone." *Molecular Cell* **6**(2): 211-23.

Srayko, M., D. W. Buster, et al. (2000). "MEI-1/MEI-2 katanin-like microtubule severing activity is required for *Caenorhabditis elegans* meiosis." *Genes & Development* **14**(9): 1072-84.

Srinivasan, D. G., R. M. Fisk, et al. (2003). "A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in C elegans." *Genes & Development* **17**(10): 1225-39.

Starr, D. A., B. C. Williams, et al. (1998). "ZW10 helps recruit dynactin and dynein to the kinetochore." *Journal of Cell Biology* **142**(3): 763-74.

Stear, J. H. and M. B. Roth (2004). "The *Caenorhabditis elegans* kinetochore reorganizes at prometaphase and in response to checkpoint stimuli." *Molecular Biology of the Cell* **15**(11): 5187-96.

Steen, R. L., F. Cubizolles, et al. (2000). "A kinase-anchoring protein (AKAP)95 recruits human chromosome-associated protein (hCAP)-D2/Eg7 for chromosome condensation in mitotic extract." *Journal of Cell Biology* **149**(3): 531-6.

Steffensen, S., P. A. Coelho, et al. (2001). "A role for *Drosophila* SMC4 in the resolution of sister chromatids in mitosis." *Current Biology* **11**(5): 295-307.

Stemmann, O., H. Zou, et al. (2001). "Dual inhibition of sister chromatid separation at metaphase." *Cell* **107**(6): 715-26.

Stern, B. M. and A. W. Murray (2001). "Lack of tension at kinetochores activates the spindle checkpoint in budding yeast." *Current Biology* **11**(18): 1462-7.

Straight, A., F. Wang, et al. (2003). "Dissecting temporal and spatial control of cytokinesis with a myosin II Inhibitor." *Science* **299**(5613): 1743-7.

Stratmann, R. and C. Lehner (1996). "Separation of sister chromatids in mitosis requires the *Drosophila* pimples product, a protein degraded after the metaphase/anaphase transition." *Cell* **84**(1): 25-35.

Strausfeld, U., J. C. Labbe, et al. (1991). "Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein." *Nature* **351**(6323): 242-5.

Strausfeld, U., A. Fernandez, et al. (1994). "Activation of p34cdc2 protein kinase by microinjection of human cdc25C into mammalian cells. Requirement for prior phosphorylation of cdc25C by p34cdc2 on sites phosphorylated at mitosis." *Journal of Biological Chemistry* **269**(8): 5989-6000.

Strome, S. and W. B. Wood (1983). "Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos." *Cell* **35**(1): 15-25.

Strunnikov, A. V., E. Hogan, et al. (1995). "SMC2, a *Saccharomyces cerivisae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family." *Genes & Development* **9**(5): 587-99.

Sudakin, V., D. Ganoth, et al. (1995). "The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis." *Molecular Biology of the Cell* **6**(2): 185-97.

Sugiyama, K., K. Sugiura, et al. (2002). "Aurora-B associated protein phosphatases as negative regulators of kinase activation." *Oncogene* **21**(20): 3103-11.

Sullivan, M., N. C. Hornig, et al. (2004). "Studies on substrate recognition by the budding yeast separase." *Journal of Biological Chemistry* **279**(2): 1191-6.

Sullivan, M., Lehane C, et al. (2001). "Orchestrating anaphase and mitotic exit: separase cleavage and localization of Slk19." *Nat. Cell Biol.* **9**: 771-7.

Sumara, I., E. Vorlaufer, et al. (2000). "Characterization of vertebrate cohesin complexes and their regulation in prophase." *Journal of Cell Biology* **151**(4): 749-62.

Sumara, I., E. Vorlaufer, et al. (2002). "The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase." *Molecular Cell* **9**(3): 515-25.

Sumiyoshi, E., A. Sugimoto, et al. (2002). "Protein phosphatase 4 is required for centrosome maturation in mitosis and sperm meiosis in *C. elegans*." *Journal of Cell Science* **115**(Pt 7): 1403-10.

Sunkel, C. E. and D. M. Glover (1988). "polo, a mitotic mutant of *Drosophila* displaying abnormal spindle poles." *Journal of Cell Science* **89**(Pt 1): 25-38.

Suprynowicz, F. A. and L. Gerace (1986). "A fractionated cell-free system for analysis of prophase nuclear disassembly." *Journal of Cell Biology* **103**(6 Pt 1): 2073-81.

Surana, U., A. Amon, et al. (1993). "Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast." *EMBO Journal* **12**(5): 1969-78.

Sutani, T., T. Yuasa, et al. (1999). "Fission yeast condensin complex: essential roles of non-SMC subunits for condensation and Cdc2 phosphorylation of Cut3/SMC4." *Genes & Development* **13**(17): 2271-83.

Swedlow, J. R., J. W. Sedat, et al. (1993). "Multiple chromosomal populations of topoisomerase II detected in vivo by time-lapse, three-dimensional wide-field microscopy." *Current Opinion in Cell Biology* **5**(3): 412-6.

Swenson, K. I., K. M. Farrell, et al. (1986). "The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes." *Cell* **47**(6): 861-70.

Takahashi, T., B. Sano, et al. (2003). "Polo-like kinase 1 (PLK1) is overexpressed in primary colorectal cancers." *Cancer Science* **94**(2): 148-52.

Tanaka, T., M. P. Cosma, et al. (1999). "Identification of cohesin association sites at centromeres and along chromosome arms." *Cell* **98**(6): 847-58.

Tanaka, T., J. Fuchs, et al. (2000). "Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation." *Nature Cell Biology* **2**(8): 492-9.

Tanaka, T. U., N. Rachidi, et al. (2002). "Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections." *Cell* **108**(3): 317-29.

Tang, T. T., S. E. Bickel, et al. (1998). "Maintenance of sister-chromatid cohesion at the centromere by the *Drosophila* MEI-S332 protein." *Genes & Development* **12**(24): 3843-56.

Tang, Z., R. Bharadwaj, et al. (2001). "Mad2-Independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1." *Dev Cell.* **1**(2): 162-164.

Taniguchi, E., F. Toyoshima-Morimoto, et al. (2002). "Nuclear translocation of plk1 mediated by its bipartite nuclear localization signal." *Journal of Biological Chemistry* **277**(50): 48884-8.

Tassan, J. P., S. J. Schultz, et al. (1994). "Cell cycle analysis of the activity, subcellular localization, and subunit composition of human CAK (CDK-activating kinase)." *Journal of Cell Biology* **127**(2): 467-78.

Tatsuka, M., H. Katayama, et al. (1998). "Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells." *Cancer Research* **58**(21): 4811-6.

Tavares, A. A., D. M. Glover, et al. (1996). "The conserved mitotic kinase polo is regulated by phosphorylation and has preferred microtubule-associated substrates in *Drosophila* embryo extracts." *EMBO Journal* **15**(18): 4873-83.

Tavares, A. A., A. Salzberg, et al. (1998). "Metaphase arrest with centromere separation in polo mutants of *Drosophila*." *Genes & Development* **12**(10): 1483-94.

Tavares, A. A., H. Ohkura, et al. (2001). "Mouse polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation." *Journal of Cell Biology* **153**(4): 663-76.

Taylor, S., D. Hussein, et al. (2001). "Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells." *Journal of Cell Science* **114**(24): 4385-95.

Terada, Y., M. Tatsuka, et al. (1998). "AIM-1: a mammalian midbody-associated protein required for cytokinesis." *EMBO Journal* **17**(3): 667-76.

Tham, W. H., H. Shah, et al. (2004). "The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis." *Science* **303**(5662): 1367-70.

Toczyski, D. P., D. J. Galgoczy, et al. (1997). "CDC5 and CKII control adaptation to the yeast DNA damage checkpoint." *Cell* **90**(6): 1097-106.

Tomonaga, T., K. Nagao, et al. (2000). "Characterization of fission yeast cohesin: essential anaphase proteolysis of Rad21 phosphorylated in the S phase." *Genes & Development* **14**(21): 2757-70.

Tong, C., H. Y. Fan, et al. (2002). "Polo-like kinase-1 is a pivotal regulator of microtubule assembly during mouse oocyte meiotic maturation, fertilization, and early embryonic mitosis." *Biology of Reproduction* **67**(2): 546-54.

Toth, A., R. Ciosk, et al. (1999). "Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication." *Genes & Development* **13**(3): 320-33.

Toth, A., K. P. Rabitsch, et al. (2000). "Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I." *Cell* **103**(7): 1155-68.

Tournebize, R., A. Popov, et al. (2000). "Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in *Xenopus* egg extracts." *Nature Cell Biology* **2**(1): 13-19.

Toyoda, Y., K. Furuya, et al. (2002). "Requirement of chromatid cohesion proteins rad21/scc1 and mis4/scc2 for normal spindle-kinetochore interaction in fission yeast." *Current Biology* **12**(5): 347-58.

Toyoshima-Morimoto, F., E. Taniguchi, et al. (2001). "Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase." *Nature* **410**(6825): 215-20.

Toyoshima-Morimoto, F., E. Taniguchi, et al. (2002). "Plk1 promotes nuclear translocation of human Cdc25C during prophase." *EMBO Reports* **3**(4): 341-8.

Tseng, T. C., C. K. Chuang, et al. (1998). "Protein kinase profile of sperm and eggs: cloning and characterization of two novel testis-specific protein kinases (AIE1, AIE2) related to yeast and fly chromosome segregation regulators." *DNA & Cell Biology* **17**(10): 823-33.

Tsou, M. F., A. Hayashi, et al. (2002). "LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in *C. elegans* embryos." *Development* **129**(19): 4469-81.

Tsvetkov, L., X. Xu, et al. (2003). "Polo-like kinase 1 and Chk2 interact and co-localize to centrosomes and the midbody." *Journal of Biological Chemistry* **278**(10): 8468-75.

Ubersax, J. A., E. L. Woodbury, et al. (2003). "Targets of the cyclin-dependent kinase Cdk1." *Nature* **425**(6960): 859-64.

Uemura, T., H. Ohkura, et al. (1987). "DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in S. pombe." *Cell* **50**(6): 917-25.

Uhlmann, F., Lottspeich F, et al. (1999). "Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1." *Nature* **400**(6739): 37-42.

Uhlmann, F., D. Wernic, et al. (2000). "Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast." *Cell* **103**(3): 375-86.

Uren, A. G., T. Beilharz, et al. (1999). "Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division." *Proceedings of the National Academy of Sciences of the United States of America* **96**(18): 10170-5.

Uren, A. G., L. Wong, et al. (2000). "Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype." *Current Biology* **10**(21): 1319-28.

Vagnarelli, P. B. and W. C. Earnshaw (2001). "INCENP loss from an inactive centromere correlates with the loss of sister chromatid cohesion." *Chromosoma* **110**(6): 393-401.

Vagnarelli, P., C. Morrison, et al. (2004). "Analysis of Scc1-deficient cells defines a key metaphase role of vertebrate cohesin in linking sister kinetochores." *EMBO Reports* **5**(2): 167-71.

van Benenden, E. (1883). *Arch. Biol.* **4**(265).

Verde, F., J. C. Labbe, et al. (1990). "Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs." *Nature* **343**(6255): 233-8.

Virchow, R. (1858). *Die Cellularpathologie*.

Visintin, R., S. Prinz, et al. (1997). "CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis." *Science* **278**(5337): 460-3.

Wagener, C., F. Marinoni, et al. (2000). "Mitotic regulation of the APC activator proteins CDC20 and CDH1." *Genes & Development* **14**(18): 2330-43.

Waizenegger, I. C., S. Hauf, et al. (2000). "Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase." *Cell* **103**(3): 399-410.

Waizenegger, I., J. F. Gimenez-Abian, et al. (2002). "Regulation of human separase by securin binding and autocleavage." *Current Biology* **12**(16): 1368-78.

Walczak, C. E., T. J. Mitchison, et al. (1996). "XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly." *Cell* **84**(1): 37-47.

Wallenfang, M. R. and G. Seydoux (2000). "Polarization of the anterior-posterior axis of *C. elegans* is a microtubule-directed process." *Nature* **408**(6808): 89-92.

Walter, A. O., W. Seghezzi, et al. (2000). "The mitotic serine/threonine kinase Aurora2/AIK is regulated by phosphorylation and degradation." *Oncogene* **19**(42): 4906-16.

Wang, Q., C. Moyret-Lalle, et al. (2003). "Alterations of anaphase-promoting complex genes in human colon cancer cells." *Oncogene* **22**(10): 1486-90.

Ward, G. E. and M. W. Kirschner (1990). "Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C." *Cell* **61**(4): 561-77.

Warren, W. D., S. Steffensen, et al. (2000). "The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis." *Current Biology* **10**(22): 1463-6.

Watanabe, Y. and P. Nurse (1999). "Cohesin Rec8 is required for reductional chromosome segregation at meiosis." *Nature* **400**(6743): 461-4.

Watanabe, N., H. Arai, et al. (2004). "M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFbeta-TrCP." *Proceedings of the National Academy of Sciences of the United States of America* **101**(13): 4419-24.

Waters, J. C., E. D. Salmon, et al. (2001). "Mad2 binding by phosphorylated kinetochores links error detection and checkpoint action in mitosis." *Journal of Cell Science* **114**(Pt 23): 4173-83.

Watts, J. L., D. G. Morton, et al. (2000). "The *C. elegans* par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry." *Development* **127**(7): 1467-75.

Wei, Y., C. A. Mizzen, et al. (1998). "Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in Tetrahymena." *Proceedings of the National Academy of Sciences of the United States of America* **95**(13): 7480-4.

Wei, Y., L. Yu, et al. (1999). "Phosphorylation of histone H3 is required for proper chromosome condensation and segregation." *Cell* **97**(1): 99-109.

Weinstein, J. (1997). "Cell cycle-regulated expression, phosphorylation, and degradation of p55Cdc. A mammalian homolog of CDC20/Fizzy/slp1." *Journal of Biological Chemistry* **272**(45): 28501-11.

Weismann, A. (1887). "On the signification of the polar globules." *Nature* **36**: 607-9.

Whangbo, J., J. Harris, et al. (2000). "Multiple levels of regulation specify the polarity of an asymmetric cell division in *C. elegans*." *Development* **127**(21): 4587-98.

Wheatley, S. P., A. Carvalho, et al. (2001). "INCENP is required for proper targeting of Survivin to the centromeres and the anaphase spindle during mitosis." *Current Biology* **11**(11): 886-90.

Wheatley, S. P., A. J. Henzing, et al. (2004). "Aurora-B phosphorylation in vitro identifies a residue of survivin that is essential for its localization and binding to inner centromere protein (INCENP) in vivo." *Journal of Biological Chemistry* **279**(7): 5655-60.

Wianny, F., A. Tavares, et al. (1998). "Mouse polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation." *Chromosoma* **107**(6-7): 430-9.

Wilson, E. B. (1928). Protoplasm. It's composition and structure. *The Cell in Development and Heredity*. New York, Macmillan Company.

Wind, M., O. Kelm, et al. (2002). "Identification of phosphorylation sites in the polo-like kinases Plx1 and Plk1 by a novel strategy based on element and electrospray high resolution mass spectrometry." *Proteomics* **2**(11): 1516-23.

Wolf, G., R. Elez, et al. (1997). "Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer." *Oncogene* **14**(5): 543-9.

Wu, G., X. G, et al. (2003). "Structure of a beta-TrCP1-Skp1-beta-catenin complex: destruction motif binding and lysine specificity of the SCF(beta-TrCP1) ubiquitin ligase." *Molecular Cell* **11**(6): 1445-56.

Yamaguchi, S., A. Decottignies, et al. (2003). "Function of Cdc2p-dependent Bub1p phosphorylation and Bub1p kinase activity in the mitotic and meiotic spindle checkpoint." *EMBO Journal* **22**(5): 1075-87.

Yamamoto, A. and Y. Hiraoka (2003). "Monopolar spindle attachment of sister chromatids is ensured by two distinct mechanisms at the first meiotic division in fission yeast." *EMBO Journal* **22**(9): 2284-96.

Yamamoto, A., V. Guacci, et al. (1996). "Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s)." *Journal of Cell Biology* **133**(1): 99-110.

Yang, H. Y., K. McNally, et al. (2003). "MEI-1/katanin is required for translocation of the meiosis I spindle to the oocyte cortex in C elegans." *Developmental Biology* **260**(1): 245-59.

Yang, S. C., C. H. Huang, et al. (2000). "Functional implication of human serine/threonine kinase, hAIK, in cell cycle progression." *Journal of Biomedical Science* **7**(6): 484-93.

Yao, X., A. Abrieu, et al. (2000). "CENP-E forms a link between attachment of spindle microtubules to kinetochores and the mitotic checkpoint." *Nature Cell Biology* **2**(8): 484-91.

Yarm, F. R. (2002). "Plk phosphorylation regulates the microtubule-stabilizing protein TCTP." *Molecular Cell Biol* **22**(17): 6209-21.

Yeong, F. M. (2004). "Anaphase-promoting complex in *Caenorhabditis elegans*." *Molecular & Cellular Biology* **24**(6): 2215-25.

Yokobayashi, S., M. Yamamoto, et al. (2003). "Cohesins determine the attachment manner of kinetochores to spindle microtubules at meiosis I in fission yeast." *Molecular & Cellular Biology* **23**(11): 3965-73.

Yoo, H. Y., A. Kumagai, et al. (2004). "Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase." *Cell* **117**(5): 575-88.

Yu, H., R. W. King, et al. (1996). "Identification of a novel ubiquitin-conjugating enzyme involved in mitotic cyclin degradation." *Current Biology* **6**(4): 455-66.

Yu, H., J. M. Peters, et al. (1998). "Identification of a cullin homology region in a subunit of the anaphase-promoting complex." *Science* **279**(5354): 1219-22.

Yu, K. R., J. C. Sisson, et al. (1999). "CENP-E forms a link between attachment of spindle microtubules to kinetochores and the mitotic checkpoint." *Nature Cell Biology* **1**(1): 51-4.

Yuan, Y. L., R. Kuriyama, et al. (1995). "The polo-box-dependent induction of ectopic septal structures by a mammalian polo kinase, plk, in *Saccharomyces cerivisae*." *Molecular & Cellular Biology* **15**(12): 7143-51.

Yuan, J., F. Eckerdt, et al. (2002). "Cooperative phosphorylation including the activity of polo-like kinase 1 regulates the subcellular localization of cyclin B1." *Oncogene* **21**(54): 8282-92.

Yucel, J. K., J. D. Marszalek, et al. (2000). "CENP-meta, an essential kinetochore kinesin required for the maintenance of metaphase chromosome alignment in *Drosophila*." *Journal of Cell Biology* **150**(1): 1-11.

Zachariae, W. and K. Nasmyth (1996). "TPR proteins required for anaphase progression mediate ubiquitination of mitotic B-type cyclins in yeast." *Molecular Biology of the Cell* **7**(5): 791-801.

Zachariae, W. and K. Nasmyth (1999). "Whose end is destruction: cell division and the anaphase-promoting complex." *Genes & Development* **13**(16): 2039-58.

Zachariae, W., T. H. Shin, et al. (1996). "Identification of subunits of the anaphase-promoting complex of *Saccharomyces cerivisae*." *Science* **274**(5290): 1201-4.

Zachariae, W., M. Schwab, et al. (1998). "Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex." *Science* **282**(5394): 1721-4.

Zalevsky, J., A. J. MacQueen, et al. (1999). "Crossing over during *Caenorhabditis elegans* meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast." *Genetics* **153**(3): 1271-83.

Zeitlin, S. G., C. M. Barber, et al. (2001). "Differential regulation of CENP-A and histone H3 phosphorylation in G2/M." *Journal of Cell Science* **114**(Pt 4): 653-61.

Zeng, X. and W. S. Saunders (2000). "The *Saccharomyces cerivisae* centromere protein Slk19p is required for two successive divisions during meiosis." *Genetics* **155**(2): 577-87.

Zhang, X., G. A. Horwitz, et al. (1999). "Structure, expression, and function of human pituitary tumor-transforming gene (PTTG)." *Molecular Endocrinology* **13**(1): 156-66.

Zhou, J., D. Panda, et al. (2002). "Minor alteration of microtubule dynamics causes loss of tension across kinetochore pairs and activates the spindle checkpoint." *Journal of Biological Chemistry* **277**(19): 17200-8.

Zhou, T., J. P. Aumais, et al. (2003). "A role for Plk1 phosphorylation of NudC in cytokinesis." *Developmental Cell* **5**(1): 127-38.

Zickler, D. and N. Kleckner (1998). "The leptotene-zygotene transition of meiosis." *Annual Review of Genetics* **32**: 619-97.

Zou, H., T. J. McGarry, et al. (1999). "Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis." *Science* **285**(5426): 418-422.

Zou, H., O. Stemman, et al. (2002). "Anaphase specific auto-cleavage of separase." *FEBS Letters* **528**(1-3): 246-50.

Zur, A. and M. Brandeis (2001). "Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis." *EMBO Journal* **20**(4): 792-801.

VITAE

Eric Jason Rogers was born in San Antonio, Texas, on October 13, 1975, the son of Linda Jean Rogers and Walter Russel Rogers. After graduating from Tom C. Clark High School, San Antonio, Texas in 1994, he entered the Univeristy of Texas at Austin as a member of the Dean's Scholars Program. He received the degree of Bachelor of Science with a major in molecular biology from the University of Texas at Austin College of Natural Sciences in May, 1998. During the following year he was employed as a research technician at the Biochemistry Department of the Univeristy of Texas Health Science Center at San Antonio. In September, 1999 he entered the Graduate School of Biomedical Sciences at the University of Texas Southwestern Medical Center at Dallas and joined the lab of Rueyling Lin, PhD in the summer of 2000.

Permanent Address: 2614 Inwood View Drive
San Antonio, TX 78248