REGULATION OF SISTER CHROMATID COHESION BY THE

ACETYLTRANSFERASE NAA50

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Dedicated To My Parents

For their love and support

REGULATION OF SISTER CHROMATID COHESION BY THE ACETYLTRANSFERASE NAA50

by

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REGULATION OF SISTER CHROMATID COHESION BY THE ACETYLTRANSFERASE NAA50

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During the cell cycle, sister-chromatid cohesion tethers sister chromatids together from S phase to the metaphase–anaphase transition and ensures accurate chromosome segregation of chromatids into daughter cells. N-terminal acetylation is one of the most prevalent protein covalent modifications in eukaryotes and is mediated by a family of N-terminal acetyltransferases (NAT). Naa50 (also called San or NatE) has previously been shown to play a role in sister-chromatid cohesion in metazoans. The mechanism by which Naa50 contributes to cohesion is not understood, however.

Here, I show that depletion of Naa50 in HeLa cells weakens the interaction between cohesin and its positive regulator sororin and causes cohesion defects in interphase, consistent with a role of Naa50 in cohesion establishment or maintenance. Strikingly, co-depletion of NatA, a heterodimeric NAT complex that physically interacts with Naa50, rescues the sister-chromatid cohesion defects and the resulting mitotic arrest caused by Naa50 depletion, indicating that NatA and Naa50 play antagonistic roles in cohesion. Purified recombinant NatA and Naa50 do not affect each other's NAT activity *in vitro*. Because NatA and Naa50 exhibit distinct substrate specificity, I propose that they modify different effectors and regulate sister-chromatid cohesion in opposing ways.

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List of Abbreviations

ABC ATP-binding cassette

APC/C anaphase promoting complex/cyclosome

ATP adenosine triphosphate

BSA bovine serum albumin

C-terminal carboxy terminal

CDK cyclin dependent kinase

cDNA complimentary deoxyribonucleic acid

CoA coenzyme A

DAPI 4',6-diamidino-2-phenylindole

DMEM Dulbecco's modified Eagle's medium

DNA deoxyribonucleic acid

DTT dithiothreitol

Esco1/2 Establishment of Sister Chromatid Cohesion N-Acetyltransferase 1/2

FACS Fluorescence-activated cell sorting

FISH Fluorescence in Situ Hybridization

FRAP Fluorescence recovery after photobleaching

G1 phase Gap 1 phase

GFP Green fluorescent protein

GNAT Gcn5-related N-acetyltransferase

GST glutathione-S-transferase

HEAT Huntington/elongation/A subunit/Tor

iMet initiator Methionine

IPTG isopropyl-β-D-thiogalactopyranoside

KAT lysine acetyltransferase

KD kilodaltons

MetAP1/2 Methionine aminopeptidase 1/2

Naa50 N-alpha-acetyltransferase 50

NAT N-terminal acetyltransferase

NBD nucleotide-binding domain

Nt-acetylation N-terminal acetylation

PBS Phosphate Buffered Saline

PCR polymerase chain reaction

Pds5 precocious dissociation of sisters protein 5

Plk1 Polo-like kinase 1

PP2A protein phosphatase 2A

pre-RC pre-replication complex

Rad21 radiation-sensitive 21

RNAi RNA interference

RNase A ribonuclease A

S phase synthesis phase

SA2 stromal antigen 2

Scc1 sister chromatid cohesion protein 1

SDS-PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sgo1 Shugoshin-like 1

siRNA short interfering ribonucleic acid

SMC Structural Maintenance of Chromosome

SSC Saline-sodium citrate buffer

Wapl wings apart-like protein

Chapter 1

Introduction

The equal distribution of genetic material into two identical daughter cells is important to maintain genome stability. Any defects during this period may cause aneuploidy, leading to cell death and tumorigenesis (Aniek et al., 2011; Schvartzman et al., 2010). To ensure the accurate segregation of chromosomes, two sister chromatids are linked together after DNA replication from S phase until separation in mitosis. This sister chromatid cohesion is essential to maintain sister chromatid linkage.

1.1 Sister chromatid cohesion

A protein complex named cohesin is formed to maintain sister chromatid cohesion. Cohesin is highly conserved from yeast to human, and is composed of multiple subunits (Fig 1). The core members of cohesin were first identified in *Saccharomyces cerevisiae*: Smc1, Smc3, and Scc1 (Michaelis et al., 1997). Later, this observation was confirmed by an improved screening approach, and a new gene called Scc3 was also identified (Toth et al., 1999).

Smc1 and Smc3 belong to the SMC (structural maintenance of chromosomes) family of chromosomal ATPases. Each SMC protein contains two nucleotide-binding motifs, Walker A and Walker B, at opposing ends of the polypeptide. A hinge region is at the center of the peptide. A 50-nm-long antiparallel coiled-coil is situated

between the binding motifs creating a rod-shaped protein with a globular hinge domain at one end and an ATP-binding domain at the other (Melby et al., 1998; Haering et al., 2002). This ATP binding domain is structurally related to the NBD (nucleotide-binding domain) of ABC (ATP-binding cassette) transporters. The interaction between the hinge domains of Smc1 and Smc3 facilitates the formation of a V-shaped heterodimer (Anderson et al., 2002).

In order to form a closed ring structure, Scc1 binds both Smc1 and Smc3. The C-terminal domain of Scc1 interacts with the NBD of Smc1, whereas the N-terminal domain associates with the NBD of Smc3 (Haering et al., 2002). Scc1 belongs to the kleisin protein family. The formation of tripartite ring could trap the chromatin fibers (Schleiffer et al., 2003).

Scc3, the fourth subunit of cohesin, associates with the complex via interaction with Scc1. Scc3 is essential for cell growth in yeast and is required for proper cohesion processes, even though Scc3 mutants still have the intact Smc1, Smc3, and Scc1 tripartite ring (Toth et al., 1999; Arumugam et al., 2003). Scc3 may affect the association of the cohesin ring with chromosomes (Arumugam et al., 2003; Hu et al., 2011). There is also evidence showing Scc3 interacts with some cohesion regulatory proteins (Nasmyth et al., 2005; Roig et al., 2014). These findings suggest the function of Scc3 is in promoting the loading of cohesin onto chromosomes or in maintaining the stability of interaction of cohesion rings with chromosomes.

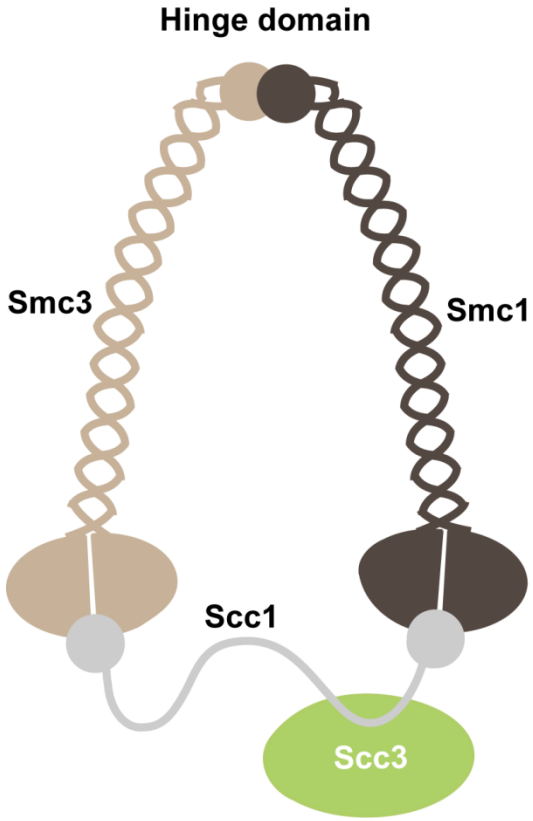


Figure 1: Architecture of the cohesin complex. Cohesin is composed of four main subunits: Smc1, Smc3, Scc1 and Scc3. Smc1 and Smc3 form a heterodimer via their hinge domains. The C-terminus of Scc1 associates with Smc1, whereas the N-terminus associates with Smc3. A closed ring structure is formed. Scc3, the fourth subunit, binds directly to Scc1.

In telophase/G1, cohesins are loaded onto chromosomes. ATPase activity is required during this time. Cohesin rings composed of Smc1 or Smc3 mutants that can bind but not hydrolyze ATP fail to associate stably with chromosomes (Arumugam et al., 2003; Weitzer et al., 2003). Scc2 and Scc4 are two important factors in facilitating the association of cohesin with chromosomes (Ciosk et al., 2000; Toyoda et al., 2002; Watrin et al., 2006). In addition to Scc2/Scc4, studies in Xenopus revealed the involvement of Cdc7/Drf1 kinase, an essential component of pre-replication complexes (pre-RCs), in cohesin loading by recruiting Scc2/Scc4 to chromatin

(Takahashi et al., 2004; Takahashi et al., 2008).

During DNA replication in S phase, Smc3 is acetylated by Esco1/2 and promotes the establishment of sister chromatid cohesion (Rolef et al., 2008; Zhang et al., 2008; Beckouët et al., 2010). In human cells, Sororin and Pds5 are also important in this phase: the acetylated Smc3 recruits Sororin to interact with Pds5, thus antagonizing the release of cohesin by Wapl (Rankin et al., 2005; Schmitz et al., 2007; Nishiyama et al., 2010).

In vertebrate cells, sister chromatid cohesion is resolved in two steps (Fig 2) (Waizenegger et al., 2000). In prophase, cohesins on chromosome arms are dissociated. This process is regulated by Wapl, Plk1, and other mitotic kinases (Nishiyama et al., 2003; Hauf et al., 2005; Gandhi et al., 2006). Phosphorylation of SA2, a subunit of cohesion, by Plk1 is required during this time (Gandhi et al., 2006). Sororin is phosphorylated by Aurora B and Cdk1 at multiple sites. This modification leads to the release of Sororin from Pds5, allowing Wapl to interact with Pds5 and promote the resolution of cohesion on chromosome arms (Nishiyama et al., 2003; Dreier et al., 2011). During this time, the centromeric cohesion is protected by Sgo1/PP2A (Kitajima et al., 2006; Tang et al., 2006). Cdk1 mediated phosphorylation of Sgo1 enables the binding of Sgo1 with centromeric cohesin and recruits PP2A to centromere. PP2A inhibits the phosphorylation of Sororin and maintains the interaction between Sororin and Pds5. Thus hypophosphorylated Sororin competes with Wapl to destabilize centromeric cohesion (Liu et al., 2013).

In anaphase, centromeric cohesins are removed through cleavage of Scc1

subunits by separase. The cleavage allows the final separation of sister chromatids (Uhlmann et al., 1999; Hauf et al., 2001). From yeast to human, separase is protected by physically association with securin before anaphase. In higher organisms, the activity of separase is also inhibited by phosphorylation of Cdk1/cyclin B. In anaphase, APC/C is activated which leads to the degradation of both securin and Cdk1/cyclin B to allow the activation of separase and sister chromatid separation (Hornig et al., 2002; Waizenegger et al., 2002).

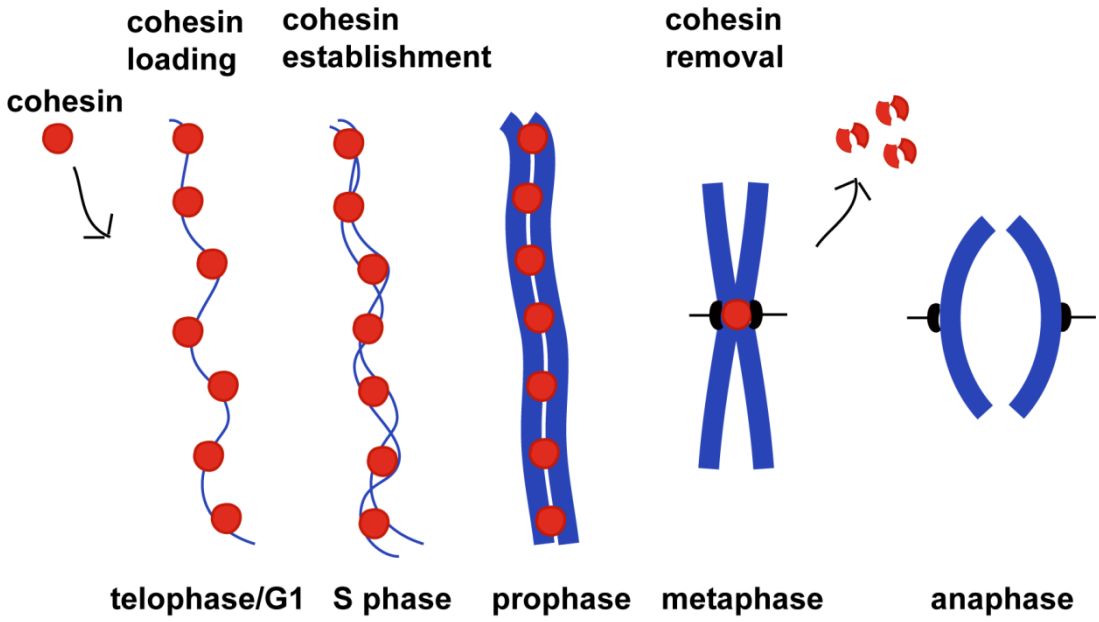


Figure 2: Cohesin throughout the cell cycle in vertebrate cells. After cohesin loading and cohesion establishment, cohesins are removed from chromosomes in two steps: in prophase and in anaphase.

1.2 Protein acetylation

Acetylation is a chemical reaction involving the addition of an acetyl group to acceptor compounds. It is one of the important modifications of proteins in cells. It is

thought that acetylation neutralizes positive charges, thereby changing the chemical properties of proteins. Acetylation of proteins occurs both co-translationally and post-translationally. Unlike other modifications, this modification can be reversible or irreversible (Verdin et al., 2015).

One group of acetyltransferases is the lysine acetyltransferases (KATs). They catalyze the transfer of an acetyl group from acetyl coenzyme A (acetyl-CoA) to the terminal amine on the side chain of lysine residues. Lysine acetylation is a posttranslational modification which is reversible. The best known substrates of KAT are histones. Acetylation and deacetylation of histones are important for gene expression (Sterner et al., 2000). However, histones are not the only proteins to undergo lysine acetylation. Some other proteins also have well-characterized lysine acetylation sites, including the tumor suppressor protein p53 and the tubulin components of the cytoskeleton (Glozak et al., 2005; Brooks et al., 2011).

Another group of acetyltransferases are N-terminal acetyltransferases (NATs). Unlike lysine acetylation, N-terminal acetylation occurs cotranslationally at ribosomes. It catalyzes the transfer of an acetyl group from acetyl-CoA to the α-amino group of the first amino acid of the protein. N-terminal acetylation is one of the most common modifications in eukaryotic cells (Silva et al., 2015; Varland et al., 2015). In yeast, nearly half of the proteins are modified by NAT. In human cells, around 85% proteins are N-terminal acetylated by NAT.

NATs belong to the GNAT superfamily of acetyltransferases (Polevoda et al., 1999). This superfamily is characterized by their GCN5-related N-acetyltransferase

(GNAT) domain, which forms a structurally conserved fold composed of four conserved sequence motifs (A-D). These four motifs form the Ac-CoA binding site (Dyda et al., 2000; Vetting et al., 2005).

Previously, four NATs have been identified to be responsible for the majority of N-terminal acetylation events in *Saccharomyces cerevisiae:* NatA, NatB, NatC and NatD (Polevoda et al., 1999). Different NAT complexes have differences in subunit composition (Table 1) and substrate specificity (Starheim et al., 2012). NatE is also found in yeast and is conserved to higher eukaryotes. However, the biological function of NatE in yeast is still not clear (Gautschi et al., 2003). In addition, another NAT, NatF, has been found (Van Damme et al., 2011). Till now, NatF is only found to be expressed in higher eukaryotes.

N-terminal acetyltransferases are recognized to play important roles in diverse cellular processes such as apoptosis, cell proliferation, sister chromatid cohesion, and chromatin silencing (Arnesen 2011; Starheim et al., 2012). Defects in N-terminal acetylation are linked to the development of rare genetic disorders and cancer (Kalvik et al., 2013).

An increasing number of studies suggest that N-terminal acetylation (Nt-acetylation) is crucial for regulation of proteins, affecting processes like protein degradation, protein interaction, protein localization, and protein folding. Stabilization of proteins by Nt-acetylation has also been examined in some reports (Ciechanover and Ben, 2004; Kuo et al., 2004). One possible explanation is that Nt-acetylation of a polypeptide chain may inhibit the ubiquitination of N-termini of proteins, thus to

protect proteins from being degraded (Arnesen et al., 2011). However, N-terminal acetylation has also been found to be involved in creating degradation signals on proteins (Hwang et al., 2010; Varshavsky, 2011; Eiyama and Okamoto, 2015).

The function of Nt-acetylation in affecting interaction of proteins has been suggested by some findings. For example, in 2011, a group showed that NatC mediated Nt-acetylation of Ubc12 increased its affinity for Dcn1 (Scott et al., 2011). There are also reports suggesting Nt-acetylation of Tfs1 affects its inhibition of CPY through blocking their interaction (Caesar and Blomberg, 2011).

Localization of proteins can also be affected by Nt-acetylation. Trm1-II is observed to be localized to the inner nuclear membrane. However, Trm1-II which cannot be acetylated at the N-terminus is located in nucleoplasm (Murthi and Hopper, 2005). Two Golgi proteins have also been reported to mislocalize to the cytoplasm in the absence of Nt-acetylation: Arl3 (Behnia et al., 2004) and Grh1 (Behnia et al., 2007).

There are also evidences to show the importance of Nt-acetylation in protein folding. Holmes and colleagues showed the loss of Nt-acetylation of Sup35 proteins caused the accumulation of misfolded prions and induced a stress response in the cells (Holmes et al., 2014).

NAT	Subunits
NatA	Naa10, Naa15
NatB	Naa20, Naa25
NatC	Naa30, Naa35
NatD	Naa40
NatE	Naa50, (Naa10, Naa15)
NatF	Naa60

Table 1: Compositions of different N-terminal acetyltransferases. Six types of NAT complex are found in eukaryotic cells. Catalytic subunits are shown in red. NatA, B, C, D, and E are identified from yeast to human, while NatF has only been found in higher eukaryotes. NatE is considered to be composed of Naa50, Naa10, Naa15 in some studies, while Naa50 alone can also be called NatE in other studies.

1.3 Naa50

Many proteins have been identified to be involved in mediating sister chromatid cohesion. Interestingly, a study in *Drosophila* revealed an important acetyltransferase called Naa50 (Williams et al., 2003). Naa50 is a small protein which is around 19 kilodaltons (KD). In interphase cells, Naa50 is mainly localized in the cytosol, but not the nucleus (Hou et al., 2007). Mutation of *Drosophila melanogaster* Naa50 disrupts sister chromatid cohesion and leads to mitotic arrest or delay (Williams et al., 2003). Depletion of Naa50 in HeLa cells also exhibits obvious cohesion defects such as premature separation of sister chromatids (Hou et al., 2007). However, deletion of NAT5, the closest homologue of Naa50 in *S. cerevisiae*, causes no detectable cohesion phenotype (Gautschi et al., 2003). Therefore, Naa50 is required for sister chromatid cohesion in metazoans but not in budding yeast. The amino acid differences between

Naa50 in budding yeast and that in other higher eukaryotes may explain this observation.

As an acetyltransferase, Naa50 displays both KAT and NAT activity (Arnesen et al., 2006; Evjenth et al., 2009). From yeast to human, Naa50 is associated with Naa10 and Naa15, both of which are subunits of the NatA complex. However, unlike Naa50, depletions of one of the other NatA subunits has no apparent sister chromatid cohesion defects (Hou et al., 2007).

By using an acetyltransferase defective mutant, a study in HeLa cells showed that the acetyltrasferase activity of Naa50 is required in regulating sister chromatid cohesion (Hou et al., 2007). Some substrates of Naa50 have been identified. Naa50 acetylates itself at three lysine sites (Evjenth et al., 2009). Tubulin is acetylated by Naa50 at K256 (Chu et al., 2011). Naa50 prefers to acetylate peptides with a methionine followed by hydrophobic amino acid (Liszczak et al., 2011; Evjenth et al., 2012). However, none of these substrates have been identified to be important for sister chromatid cohesion. The mechanisms of how Naa50 regulates sister chromatid cohesion still needs to be elucidated.

1.4 Goal of the project

Overall Goal : Investigate the mechanisms by which Naa50 regulates sister chromatid cohesion.

Specific Aim 1: Confirm that Naa50 affects sister chromatid cohesion through cohesin-related pathway and explore the stage at which Naa50 plays its role in

regulating sister chromatid cohesion.

Specific Aim 2: Determine if Naa50 affects the interaction of cohesion related proteins or whether the dynamics of cohesin are influenced by Naa50.

Specific Aim 3: Verify the importance of the acetyltransferase activity of Naa50 for sister chromatid cohesion. Attempt to identify the substrates of Naa50 that are important during this process.

Specific Aim 4: Understand if the NatA complex plays a role in regulating sister chromatid cohesion.

Chapter 2

Materials and methods

2.1 Cells culture, cell cycle synchronization and cellular fractionation

HeLa Tet-On (Invitrogen) cells were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 1% Penicillin-Streptomycin. For synchronization, cells were arrested at G1/S by 2 mM thymidine (Sigma) treatment for 18 h. After release cells from thymidine, 4 h later, S phase cells were collected. 7 h later, G2 cells were collected. 8 h later, 300 nM nocodazole (Sigma) was added for another 3 h and mitotic cells were collected. 11 h later, telophase cells were collected. The cellular fractionation was performed as follows. Cell pellet was resuspended in Buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M Sucrose, 10 % Glycerol, 0.1% Triton X-100), 1 mM DTT, and protease inhibitor mixture (Roche). After incubation on ice for 8 mins, the nuclear fraction was collected as a pellet by centrifugation (1,300 x g, 4 ℃, for 5 min) and resuspended in SDS sample buffer. The cytoplasmic fraction was collected in the supernatant.

2.2 Generation of site-directed mutants

Naa50 F27A mutant was generated by PCR amplification using pCS2-Myc containing the Naa50 siRNA resistant form of full length Naa50 cDNA as template. To make pCS2-Myc Pds5B 7KR mutant, pUC57 Pds5B 7KR fraction 1 and pUC57

Pds5B 7KR fraction 2 were synthesized by Genescript. After digestion with XbaI, vector (pUC57 Pds5B 7KR fraction 1) and inserted fragment (Pds5B 7KR fraction 2) were ligated to make pUC57 Pds5B 7KR. Since one more XbaI site was in this plasmid, by using it as template, PCR was performed to delete this site with the following primers: Pds5B-7KR-1-XbaI

5'- CTTCGAGCCCCTCCACAAGAG TCTAGA TCCGTCAAACCTGGAACACC –'3 (sense strand) and

5'- GGTGTTCCAGGTTTGACGGA TCTAGA CTCTTGTGGAGGGGCTCGAAG -'3 (anti-sense strand). Then Pds5B 7KR cDNA was obtained from this pUC57 Pds5B 7KR by PCR amplify with the following primers: Pds5B-7KR-1-FseI back

5'-AAGGCCGGCCAATGGCACACTCAAAAACCCGCAC -'3 (sense strand) and Pds5B-AscI-for

5'- AAGGCGCGCCTTAGCGGCGTTCACGTTTTGCGGA -'3 (anti-sense strand). The obtained cDNA was then ligated into pCS2-Myc vector.

To make Sororin K187RK197R mutant, pCS2-Myc containing the Sororin siRNA resistant form of full length Sororin cDNA was used as template. PCR mutagenesis was done with the following primers: Sororin K187R 5'-

GAGTCTCGCCAGTGGTGTGCTCCAGACTCACCGAGGTCCCCAGGGTTTG -'3 (sense strand), 5'-

CAAACCCTGGGGACCTCGGTGAGTCTGGAGCACACCACTGGCGAGACTC -3' (anti-sense strand), K197R 5'-

CCGAGGTCCCCAGGGTTTGTGCAAGGCCCTGGGCCCCAGACATGACTCT

-‘3 (sense strand), 5’-CCGAGGTCCCCAGGGTTTGTGCAAGGCCCTGGGCCCCAGACATGACTCT -3’ (anti-sense strand)

2.3 Plasmid transfection and siRNA oligonucleotides transfection

Effectene reagent (Qiagen) was used for plasmid transfection according to the manufacturer's protocols. Myc-Sgo1 WT and Smc1-GFP stable cell line were made by transfecting HeLa Tet-On cells with pTRE2 vectors encoding RNAi-resistant Myc-Sgo1 WT and Smc1-GFP WT and selected with hygromycin (Invitrogen). The surviving clones were picked and screened.

For RNAi experiments, Lipofectamine RNAiMax (Invitrogen) was used to transfect HeLa Tet-On cells. The siRNA oligonucleotides used in this study are: Naa50 siRNA (5’-GCUACAAUGACAAGUUCUAdTdT-3’), WapL siRNA (5’-CGGACUACCCUUAGCACAAdTdT-3’), Scc1 siRNA (5’-GGAAGAAGCATTTGCATTGdTdT-3’), Esco1 siRNA (Dharmacon ON-TARGETplus Set of 4), Esco2 siRNA (Dharmacon ON-TARGETplus Set of 4), Naa10 siRNA (siGENOME D-009606-02, D-009606-03, Thermo Scientific), Naa15 siRNA (siGENOME D-012847-01, D-012847-03, Thermo Scientific), Naa20 siRNA (siGENOME D-008944-03, D-008944-04, Thermo Scientific), Naa30 siRNA (siGENOME D-009961-01, D-009961-06, Thermo Scientific), MetAP1 siRNA (siGENOME D-008693-01, D-008693-02, Dharmacon), MetAP2 siRNA (siGENOME D-005943-06, D-005943-07, Dharmacon),

2.4 Antibodies, immunoblotting and immunoprecipitation

The polyclonal rabbit antibody to human Naa50 was raised by Genemed Synthesis, Inc. using His-tagged recombinant Naa50 as the antigen. The resulting crude serum was affinity-purified before use. Antibody to acetylated N-termini of Scc1 was raised by UTSW Animal Resources Center using the acetylated form of Scc1 N-terminal peptide (MFYAHFVLS) synthesized by the Protein Chemistry Technology Core at UTSW. The resulting crude serum was affinity-purified before use. Antibodies to Sgo1, Rabbit polyclonal eGFP and Sororin were raised at Yenzym. Antibody to Myc (Roche 11667203001) was produced at Roche. The following antibodies were purchased as indicated: Naa10 (Santa Cruz sc-373920), Naa15 (Santa Cruz sc-365931), Scc1 (Bethyl A300-080A), Smc1 (Bethyl A300-055A), Smc3, ac-Smc3 (MBL PD040), Esco2 (Bethyl A301-689A), tubulin (Sigma-Aldrich T9026), Pds5A (Bethyl A300-089A), MPM-2 (Millipore 05-368).

For immunoblotting, commercial antibodies were used at 1:300, 1:500 or 1:1,000 dilution according to its concentration or at 1 μg/ml for purified and monoclonal antibodies.

For immunoprecipitation, anti-Smc1, anti-Myc or anti-Sororin antibodies were coupled with Affi-Prep Protein A beads (Bio-Rad) at a concentration of 1 mg/ml. Cells were lysed with lysis buffer (25 mM Tris HCl at pH 7.5, 75 mM NaCl, 5 mM MgCl2, 5 mM NaF, 0.1% NP-40, 10mM β-glycerophosphate), 0.5 mM okadaic acid, 0.3 mM Na_3VO_4, 1 mM DTT, protease inhibitor mixture (Roche), and 50 units/ml Turbo-nuclease (Accelagen). Then, the lysate was incubated on ice for 2 h and then at 37 ℃ for 10 min. Then the lysate was centrifuged at 4 ℃ for 20 min at 13200 rpm.

The supernatant was incubated with the antibody beads for 2 h at 4 ℃. The beads were washed three times with lysis buffer. Samples were added with SDS sample buffer and boiled for 10 min, thus the bound proteins were eluted. Then, samples were separated by SDS-PAGE and blotted with the appropriate antibodies.

2.5 Protein production and purification

The cDNAs encoding human N-termini of Scc1 (MFYAHFVLSKR), Naa50 WT and F27A mutant were cloned into the pGEX6p-1_FseI/AscI vector (with N-terminal GST tag). These vectors were transformed into BL21 cells. The transformed cells were cultured at 37 ℃ until the cell OD595 reached around 0.7. Protein expression was induced by IPTG addition (0.2mM, 16 ℃, overnight). Cells pellets were harvested by centrifugation and lysed in lysis buffer (50 mM Tris HCl at pH 7.2, 100 mM NaCl, 3 mM KCl, 1% Triton X-100, 5% glycerol) with1 mM DTT. After sonication, recombinant GST-tagged N-Scc1, Naa50 WT, and F27A proteins were purified with Glutathione Sepharose 4B resin (GE Healthcare). Proteins were eluted and concentrated with Amicon Ultra-15 Centrifugal Filter Units. Then, PD-10 column were used to change the elution buffer to storage buffer (50 mM Tris HCl at pH 7.5, 100 mM NaCl, 5% glycerol) with1 mM DTT. Fractions containing protein were judged by SDS-PAGE and the concentration was measured by Bradford Assay. Then proteins were stored at -80 ℃ until used.

2.6 In vitro acetylation assay

2 μg GST-tagged Naa50 proteins (WT or F27A) and same amount of proteins that I needed to test were incubated in the acetylation buffer (50 mM Tris HCl at pH

7.5, 100 mM NaCl, and 10% glycerol) with 70 μM 14C-acetyl-CoA (4 mCi/mmol; Perkin Elmer, NEC313010UC) at 37 ℃ for 1 h. For experiments that did not need radiolabeled signals, 70 μM nonradioactive acetyl-CoA (Sigma) was added instead. The total volume of each reaction was 10 μl. The reaction was terminated by the addition of SDS sample buffer. Samples were resolved on SDS-PAGE followed by staining with Gelcode blue stain reagent (Fisher Scientific). Acetylation signals were detected by exposing the dried gel to a phosphorimage screen.

2.7 Chromosome spreads and cohesin loading assay

After synchronization, mitotic cells were collected by shake off. For chromosome spreads, mitotic cells were swelled in 55 mM KCl and fixed with methanol/acetic acid (v:v=3:1) for 20 min at room temperature. Cells were dropped onto microscope slides, dried at room temperature, and stained with 1 μg/ml DAPI for 5 min. After the final washes, the slides were mounted then sealed with nail polish. The slides were observed using a 100× objective on a DeltaVision fluorescence microscope (GE Healthcare). Image processing and analysis were made with ImageJ.

For the cohesin-loading assay, cells were seeded in four-well chamber slides (LabTeck). After transfection of GFP-SA2 plasmids for 7 h, cells were transfected with siNaa50 oligo. Cells were first extracted with PHEM buffer (25 mM HEPES at pH 7.5, 10 mM EGTA at pH 8.0, 60 mM PIPES at pH 7.0, 2 mM $MgCl_2$) containing 0.5% Triton X-100 for 5 min and then were fixed in 4% paraformaldehyde for 15 min. After washing with PBS, cells were blocked in PBS containing 3% BSA for 1 h. Then cells were incubated with the anti-GFP and anti-tubulin antibodies in PBS containing

0.2% Triton X-100 and 3% BSA overnight at 4 ℃. After washing three times with PBS containing 0.05% Tween 20, cells were incubated with fluorescent secondary antibodies in PBS containing 0.2% Triton X-100 and 3% BSA for 1 h at room temperature. The cells were again washed three times with PBS containing 0.05% Tween 20 and stained with 1 μg/ml DAPI in PBS for 5 min. After final washes, slides were mounted, sealed, viewed, and analyzed as mentioned above.

2.8 Fluorescence in Situ Hybridization (FISH)

A commercial FISH probe was ordered from Empire Genomics. (BAC clone RP11-446L19 from the RPCI collection Locus 21q22.3). Cells were synchronized at S phase, fixed with methanol/acetic acid (v:v=3:1) and dropped onto microscope slides. After being dried at room temperature, slides were treated successively with denaturation buffer (70% formamide, 2 × SSC, pH 7.0-8.0) at 73 ℃, 70%, 85%, 100% ethanol at room temperature. Then, slides were dried at 45 ℃. Cells were incubated with the probe in a sealed humidified chamber with 50% formamide in 2×SSC as humidity control at 37 ℃ for 16 h and subsequently treated with WS1 (0.4× SSC/0.3% NP-40) at 73 ℃ and WS2 (2 × SSC/0.1% NP-40) at room temperature. DNA was counterstained and mounted with DAPI (ProLong Gold Antifade Mountant with DAPI, Thermo Scientific) and slides were visualized with a 100 × objective on a DeltaVision fluorescence microscope. Distance measurements were performed in Image J.

2.9 Fluorescence-activated cell sorting (FACS) analysis

Cells were harvested and fixed in 70% pre-chilled ethanol. After being washed

with PBS, cells were permeabilized with PBS containing 0.25% Triton X-100 on ice for 5 min. Then cells were incubated with the MPM2 antibody in PBS containing 1% BSA for 3 h at room temperature. After being washed with PBS containing 1% BSA, cells were incubated with a fluorescent secondary antibody (Invitrogen) for 30 min. After being washed with PBS, cells were resuspended in PBS containing RNase A and propidium iodide, and then analyzed with a flow cytometer. Data were processed with FlowJo.

2.10 Fluorescence recovery after photobleaching (FRAP)

HeLa Tet-On Smc1-GFP stable cell line was used in this experiment. Cells were seeded in 4-well Lab Tek II chambered coverglass (Fisher Scientific). The expression of Smc1 was induced by adding 2mg/ml doxycycline and Naa50 was depleted by siRNA transfection. Cells were synchronized in thymidine for 17 hours. Then cells were observed under SDC/TIRF (BioVision) confocal microscope using a 100x objective. One image was acquired before bleaching (405-nm laser at 100% intensity). Thirty images were acquired afterwards at 20 s intervals after bleaching. Average intensities of signals were measured at bleached site by ImageJ. Data was processed, plateau and half-life ($t_{1/2}$) of recovery in each sample was calculated by PRISM as described (Hong et al., 2015).

Chapter 3

Confirming the involvement of Naa50 in sister chromatid cohesion by cohesin-related pathway and determining the stage at which it functions

3.1 Introduction

Premature separation of sister chromatids is induced by the defects of sister chromatid cohesion. This process is not only affected by cohesin, but also many other factors like the spindle assembly checkpoint and histones (Magnaghi-Jaulin et al., 2007; Musacchio A and Salmon ED, 2007). Wapl has been found to be a negative regulator of sister chromatid cohesion. The association of Wapl and Pds5B promotes the dissociation of Sororin and Pds5B, and causes the release of cohesin from chromosome arms during prophase. If Naa50 regulates sister chromatid cohesion through a cohesin-related pathway, it is expected that the defects caused by Naa50 depletion would be rescued by Wapl deletion. To verify the function of Naa50 in cohesin related pathway, a double depletion of Naa50 and Wapl was performed in cells

To determine the mechanism by which Naa50 affects sister chromatid cohesion, it is important to understand the stage at which it functions. In eukaryotic cells, there are three main processes cohesin undergoes during the cell cycle: loading of cohesins onto chromosomes, establishment of cohesion, and the release of cohesin from

chromosomes. In this study, I used different methods to test at which stage Naa50 begins to play its role in regulating sister chromatid cohesion.

3.2 Results

3.2.1 Depletion of Wapl rescues mitotic arrest and premature separation caused by Naa50 knockdown.

In order to determine if Naa50 affects sister chromatid cohesion in a cohesin-related manner, a double depletion of both Naa50 and Wapl was performed. After depletion of Naa50 itself or Naa50 and Wapl together, cells were collected and analyzed for mitotic indices by FACS. Compared with single depletion of Naa50, double depletion decreased the mitotic index (Fig. 3A).

Cells were also collected for chromosome spreads analysis in mitosis. Both paired and unpaired chromatids were observed in this experiment (Fig. 3B). For each sample, at least 30 mitotic cells were counted. This quantitative data in Figure 3C shows that in mock cells, only a few mitotic cells had unpaired sister chromatids. Naa50 depletion largely increased the percentage of prematurely separated sister chromatids in mitotic cells, while double depletion of Naa50 and Wapl greatly reduced the percentage of premature separation. Accordingly, we can conclude that Naa50 regulates sister chromatid cohesion through cohesin-related pathway.

3.2.2 Naa50 is not required for loading of cohesin.

Previous data using a cell line stably expressed C-terminal GFP-tagged Smc1 indicated that localization of cohesin in interphase was not affected by Naa50 (Hou et

al., 2007). To further understand if Naa50 has an effect on cohesin loading, I transfected HeLa Tet-On cells with GFP-SA2 plasmids. Cells were then transfected with siNaa50 oligo or not. Cells were arrested at telophase as mentioned in the methods. The morphology of tubulin revealed by immunofluorescence was used to distinguish different cell cycle stages. DAPI and GFP signals were measured upon microscopy observation (Fig. 4A). SA2, the homologue of *Scc3* in vertebrate cells, is one of the subunits of cohesin. The colocalization of GFP and DAPI signals represents the interaction of GFP-SA2 with DNA. As expected, similar percentage of colocalization was shown in mock and Naa50 depleted cells (Fig. 4B). The total expression level of GFP-SA2 in mock and Naa50 depleted cells was similar as shown by immunoblotting in Figure 4C. This confirms that Naa50 is not required for loading of cohesin on chromosomes.

3.2.3 Naa50 is important for cohesion establishment in S phase.

Since Naa50 depletion does not affect cohesin loading, next, I tested if Naa50 is required for the establishment of cohesion. HeLa Tet-On cells were depleted of Naa50 and arrested in S phase with thymidine treatment. Cells were collected and used for chromosome spreads. The FISH assay was performed. Sister chromatids were visualized as two paired dots under the microscope (Fig. 5A). The distance between two paired FISH dots was measured by Image J. After quantification analysis, it was clear that the distance between two paired dots increased in Naa50 depleted cells (Fig. 5B). This result indicates that a premature separation of two sister chromatids already exists from S phase. Thus, Naa50 is important for sister chromatid cohesion

establishment in S phase.

In S phase, Esco1/Esco2 acetylates Smc3 and promotes the establishment of sister chromatid cohesion (Rolef et al., 2008; Zhang et al., 2008; Beckouët et al., 2010). Naa50 also has acetyltransferase activity. Therefore I tested if Naa50 plays a role in establishment by affecting Smc3 acetylation. Naa50 was depleted and siEsco1 plus siEsco2 was used as a positive control. Cells were arrested in S phase and collected. By using fractionation assay, cells were fractionated to cytosol and nuclear fraction. Through detecting by corresponding antibodies, I confirmed the reduction of acetylation level of Smc3 by Esco1 and Esco2 depletion. However, the acetylation signal of Smc3 was not influenced by Naa50 depletion (Fig. 5C). Therefore, Naa50 does not alter cohesion establishment by affecting Smc3 acetylation.

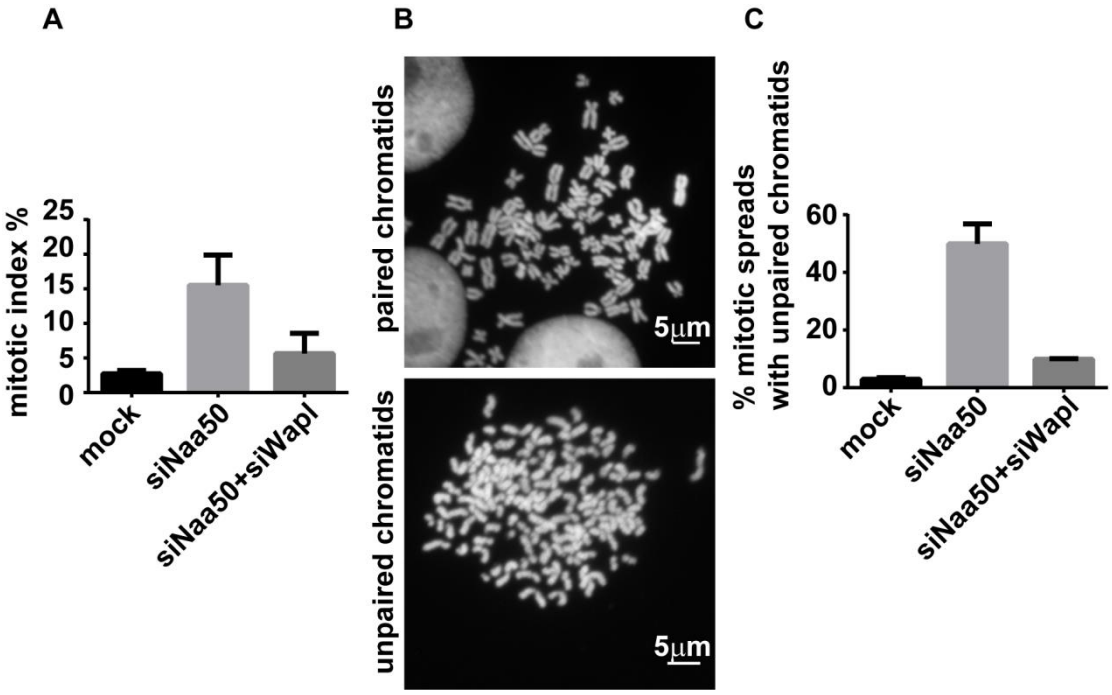


Figure 3: Depletion of Wapl rescues the mitotic defects caused by Naa50 depletion. (A) Naa50 and Wapl were depleted by siRNA in HeLa Tet-On cells. FACS analysis was used to quantify the mitotic indices. Mean±SD, n=3 independent experiments. (B) Cells were treated with nocodazole for 3 h and subjected to chromosome spreads. Paired (top) and unpaired (bottom) sister chromatids in mitosis were observed. (C) Quantification of percentage of premature separated chromatids in mitotic spreads (Mean±SD). Data were collected from two independent trials. (n>30 for each sample)

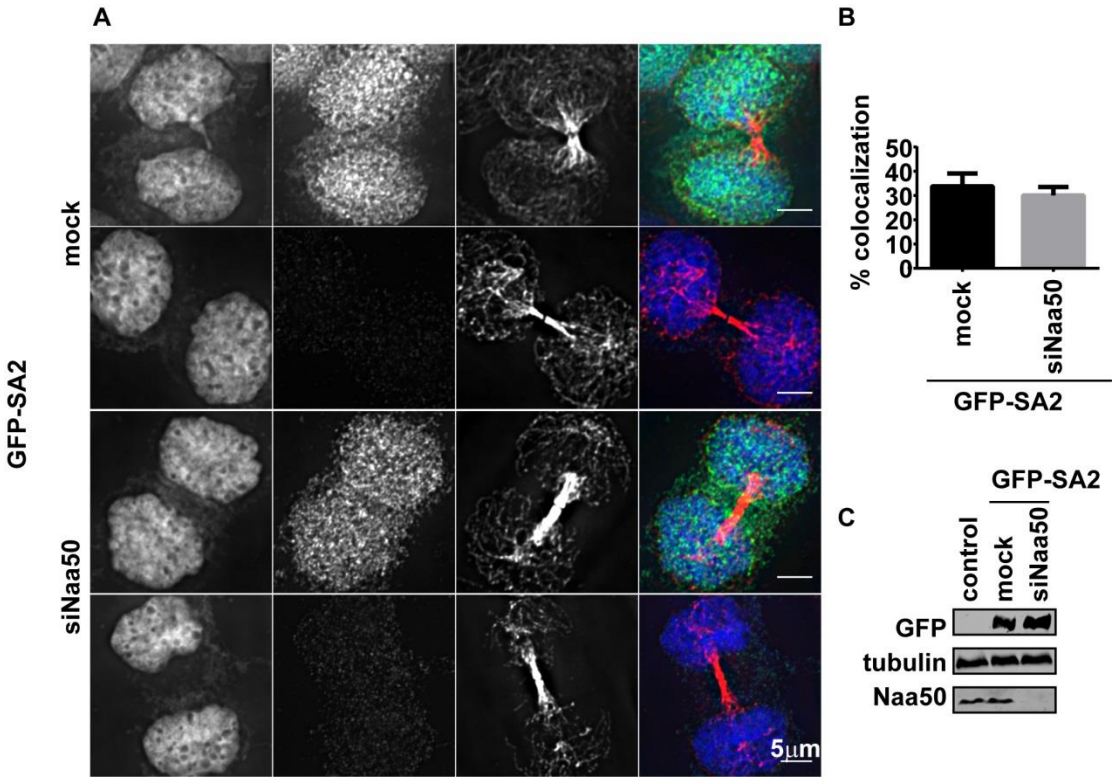


Figure 4: Loading of cohesin is not affected by Naa50 depletion. (A) Transiently transfected GFP-SA2 HeLa Tet-On cells were depleted with Naa50 or not. Cells were arrested in telophase and collected for analysis. After staining with DAPI (blue), anti-GFP (green) and anti-tubulin (red), telophase/G1 cells were observed under microscope. (B) Quantification analysis of the percentage of GFP-SA2 signals colocalized with DAPI in telophase/G1 cells (Mean±SD). Data were collected from two independent trials. For each sample, more than 40 telophase/G1 cells were analyzed. (C) The expression level of GFP-SA2 and the depletion efficiency of Naa50 were determined by immunoblotting assay with anti-GFP antibody. The level of α-tubulin was blotted as a loading standard.

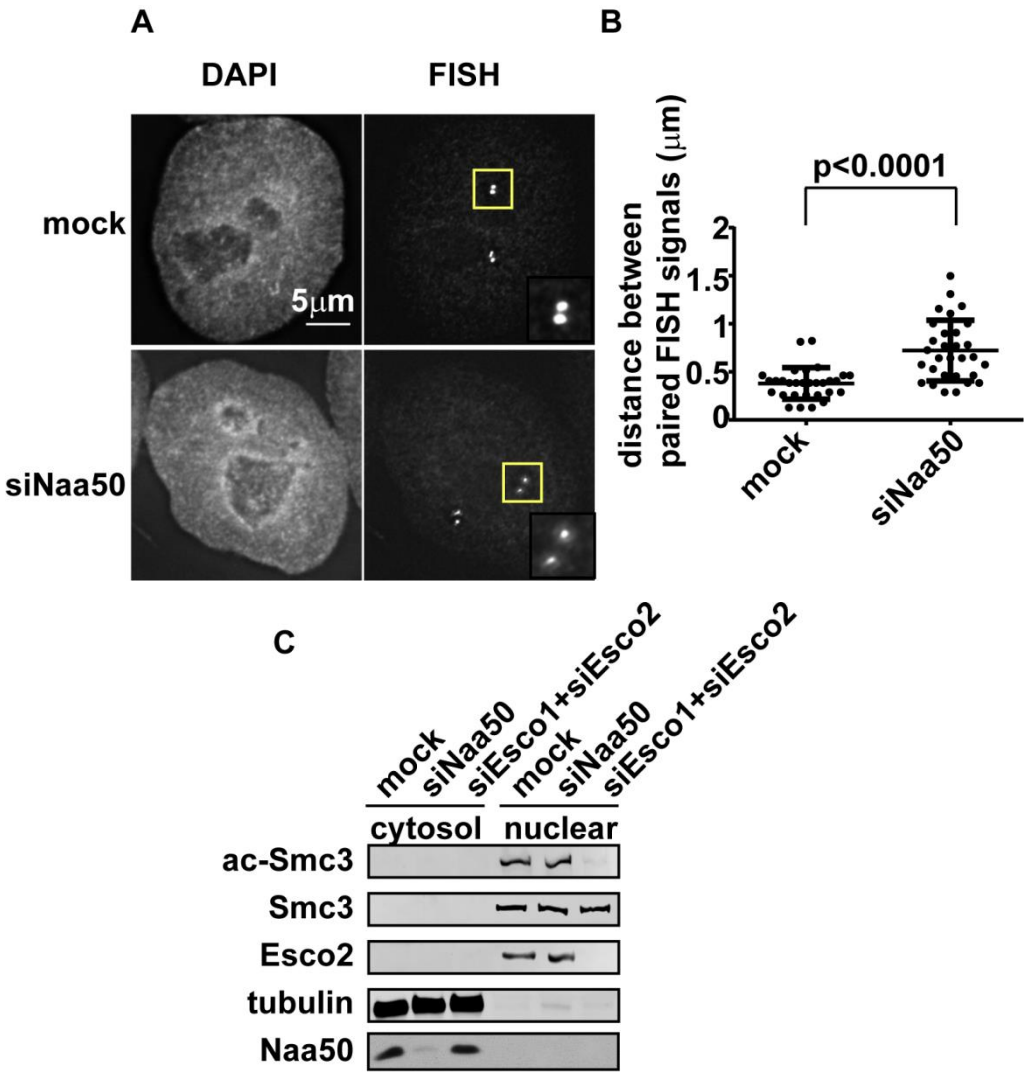


Figure 5: Naa50 is required for sister chromatid cohesion establishment. (A) The distances between two paired FISH signals were observed. In mock cells, the paired dots were very close in most of the cells (top). In Naa50 depleted cells, some of the paired dots were separated. (B) Quantification of distances between two paired FISH dots in interphase cells. The means and the SDs (represented by error bars) of the percentage of the mitotic indices were shown. Data were collected from two independent trials (n=30 for each sample). (C) Smc3 acetylation level in cells. After Naa50 siRNA or Esco1/2 siRNA was made, cells were arrested at S phase. Then cells were collected, lysed and analyzed by indicated antibodies. Tubulin was blotted as the loading control.

3.3 Discussion

Co-depletion of Wapl rescues the defects induced by Naa50 single depletion, indicating that Naa50 and Wapl have opposite roles in mediating sister chromatid cohesion. Since Wapl associates with cohesin and promotes the removal of cohesin from chromosomes, it also suggests the loss of cohesion in Naa50 depleted cells is induced by affecting a cohesin-related pathway.

The role of Naa50 in a cohesin-related pathway led us to think about at which stage of cohesin cycle Naa50 begins to be important. By performing a cohesin loading assay, it suggests Naa50 is not required during loading of cohesin. Next, I tried to test if Naa50 is important for cohesion establishment. As demonstrated before, a FISH experiment was performed to observe paired FISH signals in S phase cells. The longer distance between two paired dots in Naa50 depleted cells indicates that Naa50 begins to play its role during cohesion establishment in S phase.

Previous studies already revealed the importance of Smc3 acetylation by Esco1/2 in cohesion establishment. Esco1/2 depletion greatly reduced the Smc3 acetylation level as expected. However, Smc3 acetylation remained the same level even after Naa50 was depleted. This result suggests another mechanism of Naa50-mediated establishment of sister chromatid cohesion may exist.

Chapter 4

Effects of Naa50 on cohesin interaction and dynamics

4.1 Introduction

Besides the four main cohesin subunits, a variety of proteins interact with cohesin ring. Among them, Sororin and Sgo1 are two key regulators of sister chromatid cohesion. Depletion of Sororin or Sgo1 leads to the increase of mitotic index and premature separation of sister chromatids (Rankin S et al., 2005; Liu et al., 2013). Sororin is considered to be already required during establishment of cohesion in S phase (Nishiyama et al., 2010). It also acts in maintaining centromeric cohesion in prophase by counteracting Wapl with the help of Sgo1/PP2A (Liu et al., 2013). To understand if Naa50 affects interaction of cohesin subunits and regulators of cohesion, immunoprecipitation experiments were performed.

Cohesin proteins are dynamic in association with chromatin. After cohesion establishment, the cohesin ring needs to open and close, thus to allow cohesin loading on and releasing from chromosomes. It has been suggested that this dynamic is controlled by proteins such as Pds5, Wapl and Sororin (Losada et al., 2005; Nishiyama et al. 2010). To figure out if Naa50 has an effect on cohesin dynamics, a FRAP assay was performed.

4.2 Results

4.2.1 Naa50 affects the interaction among Sororin/cohesin/Pds5 but not the interaction of Smc1/Scc1 or Sgo1/cohesin.

The rescue results of Naa50 and WapL double depletion already suggest that Naa50 may regulate sister chromatid cohesion by a cohesin-related pathway. Along with the four main subunits of cohesin, Sororin and Sgo1 have also been found to be very important during this process.

The similarity of Naa50 and Sororin in opposing Wapl to establish cohesion during S phase leads to the hypothesis that interaction among Sororin/cohesin/Pds5 may be disrupted in cells depleted of endogenous Naa50. HeLa Tet-On cells were depleted of Naa50 and arrested in S phase. After collecting cells, lysates were immunoprecipitated by anti-Sororin or anti-Smc1 to analyze the corresponding bound proteins. As Figure 6A presents, in Naa50 depleted cells, the amounts of Sororin bound to endogenous Smc1 and Scc1 proteins was greatly reduced, and the interaction of Pds5A and Sororin was weaker. When cells were immunoprecipitated by anti-Smc1, the binding of Scc1 to Smc1 was not affected by Naa50 depletion. Smc1 bound to Pds5A was also reduced in Naa50 depleted cells. Therefore, Naa50 is important in maintaining the interaction between Sororin/cohesin/Pds5, but not in maintaining the interaction between Scc1 and Smc1.

To exclude the possibility that the differences in Sororin/cohesin/Pds5 interaction resulted from changes in the cell cycle, FACS analysis was performed as in Figure 6B. Both samples exhibited a similar cell cycle profile. Thus the disruption of interaction

among Sororin/cohesin/Pds5 by Naa50 depletion is not due to altering cell cycle progression.

I then examined if Naa50 has effect on the interaction between Sgo1 and cohesin. A Myc-tagged Sgo1 stable cell line was used in this experiment. Doxycycline was added to induce the expression of Myc-Sgo1. Cells were synchronized in mitosis by thymidine release. Mitotic cells were harvested by shaking-off. Cells lysates were then to use for immunoprecipitation with anti-Myc (Fig. 6C). However, even though Naa50 was well depleted, endogenous Smc1 or Scc1 still bound with Myc-Sgo1. This indicates that Naa50 depletion cannot weaken Sgo1 and cohesin interaction, which is consistent with previous findings exhibiting that the localization of Sgo1 still remained the same when Naa50 was depleted (Hou et al., 2007).

4.2.2 Naa50 does not affect the recovery of Smc1.

Although Naa50 is dispensable for cohesion loading in telophase/G1, there are still possibilities that Naa50 might be involved in cohesin dynamics. To examine if Naa50 affects dynamic binding of cohesin with chromosomes, a HeLa Tet-On C-terminal GFP-tagged Smc1 stable cell line was used to perform FRAP assay. After bleaching with laser, Smc1-GFP diffusion was observed (Fig 7A). According to the analysis, the plateau and half-life ($t_{1/2}$) of Smc1-GFP recovery was unchanged between control and Naa50 depleted cells (Fig 7B). Naa50 depletion efficiency was exhibited by immunoblotting (Fig 7C). Therefore, Naa50 does not affect the dynamics of cohesin on chromosomes.

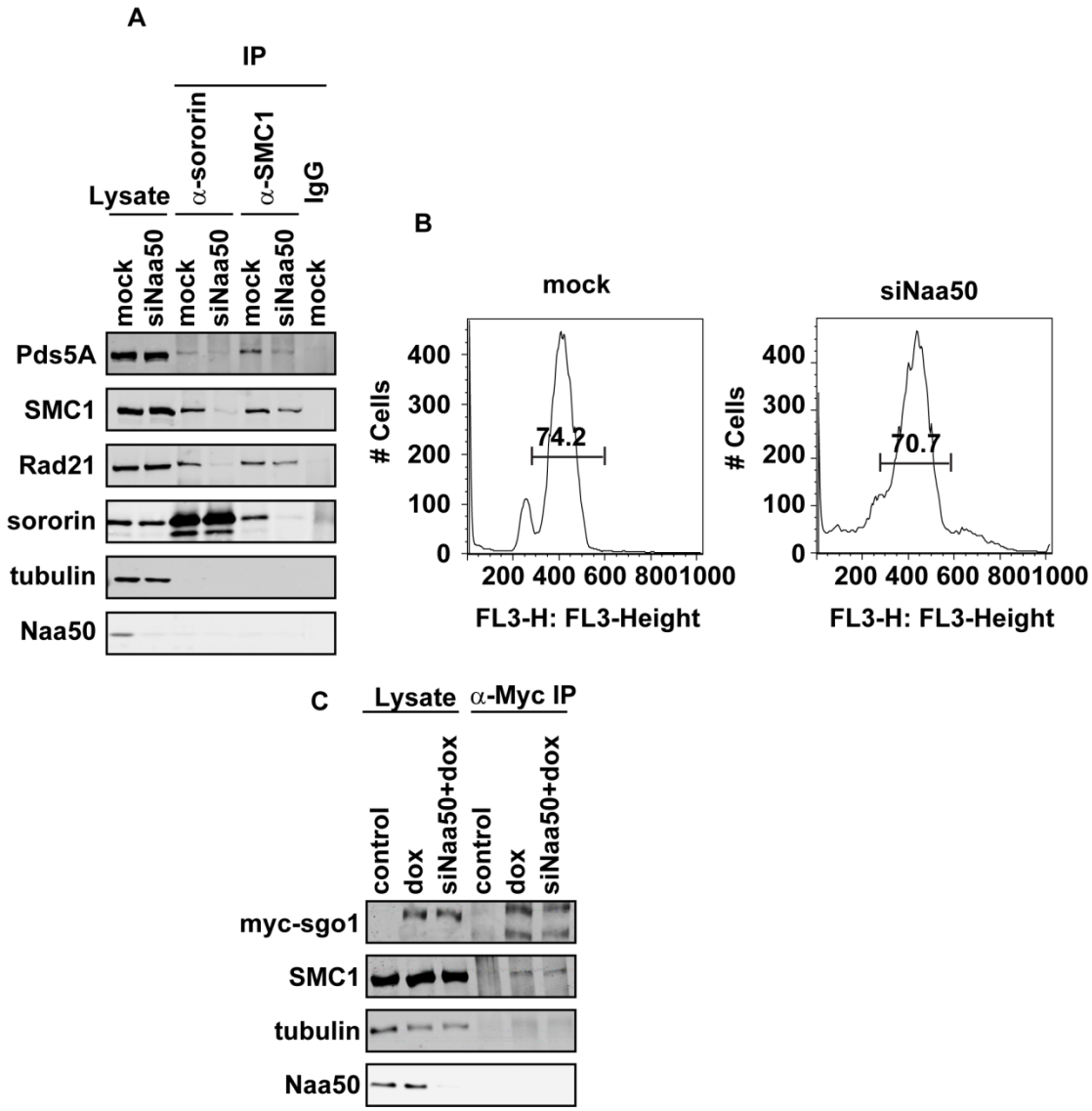
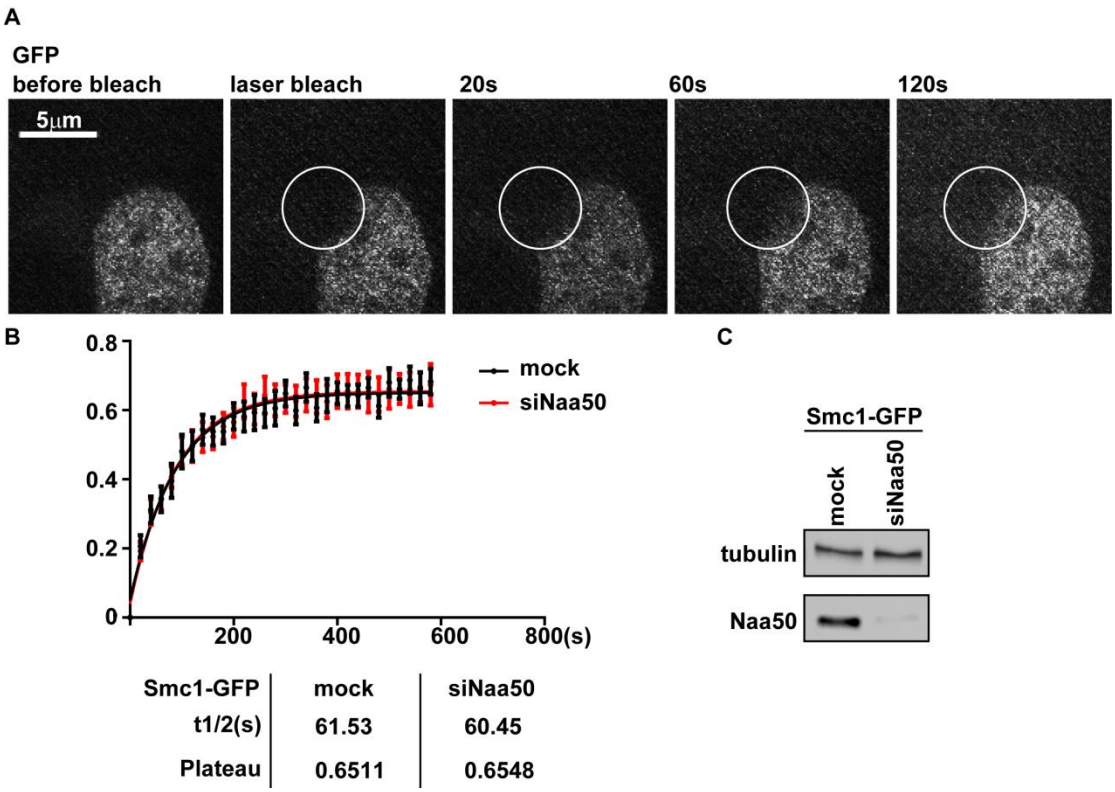


Figure 6: Naa50 affects the interaction of Sororin/cohesin/Pds5 but not that of Sgo1/cohesin in cells. (A) Naa50 affects interaction among Sororin, Pds5 and cohesin subunits. HeLa Tet-On cells were arrested in S phase. Lysates of cells were immunoprecipitated with IgG or anti-Sororin or anti-Smc1. The total cell lysates (Input) and IgG /anti-Sororin /anti-Smc1 immunoprecipitate (IP) were blotted with the indicated antibodies. (B) FACS analysis of mock and Naa50 RNAi cells. Similar cell cycle profiles were obtained. (C) Naa50 does not affect interaction of Sgo1 and cohesin. HeLa Tet-On cells stably expressing Myc-Sgo1 WT were used. Cells were arrested in mitosis as shown in materials and methods, mitotic cells were collected by shaking off and lysed with Turbo Nuclease. The total cell lysates (Input) and anti-Myc immunoprecipitates were blotted with the indicated antibodies.



Smc1-GFP	mock	siNaa50
t1/2(s)	61.53	60.45
Plateau	0.6511	0.6548

Figure 7: Smc1 dynamics are not affected in cells depleted of Naa50. (A) HeLa Tet-On cells stably expressing Smc1-GFP were used. Cells were synchronized in G1/S phase and representative images of FRAP experiments are shown. White circles point to the region of bleaching. (B) Quantification of fluorescent signals and non-linear fitted curve from (A). Before bleach, immunofluorescence intensity of the bleached region in nucleus was set as 1. After bleach, it was set as 0. Immunofluorescence intensities of this region at different time points were normalized accordingly. Half-life ($t_{1/2}$), plateau and SDs were analyzed. Data were collected from 9 cells for each group in three independent trials. (C) Immunoblotting of Naa50 and tubulin. Tubulin was detected as a loading control.

4.3 Discussion

Previously, it was shown that in Naa50 depleted cells, the nuclear localization of Sgo1 at centromeres in mitosis remains the same. My data by using immunoprecipitation with anti-Myc-Sgo1 also demonstrates that Naa50 has no effect on the interaction of Sgo1 and cohesin. Thus, these results suggest that Naa50 is dispensable for the localization of Sgo1 at centromeres and binding of Sgo1 to cohesin. On the other hand, Sororin binding to cohesin is disrupted by Naa50 depletion. FACS analysis was performed to show in this experiment, through thymidine release treatment, Naa50 depleted cells had a similar cell cycle profile as control. However, there are still several explanations for this result: Naa50 may directly modify proteins involved in Sororin interaction with cohesin, it may have an indirect role in maintaining Sororin interaction with cohesin, or the results might be a consequence of premature separation of sister chromatids or other defects caused by Naa50 depletion.

For the FRAP assay, a C-terminal GFP-tagged Smc1 stable cell line was used as a reporter for cohesin dynamics. However, there were no differences in both plateau and half-life of recovery between control and Naa50 depleted cells, indicating the kinetics of cohesin turnover on chromatin are not affected by Naa50

Chapter 5

Acetyltransferase activity of Naa50 in regulating cohesion

5.1 Introduction

Naa50 has both NAT and KAT activity. Previous studies already revealed that the Y124F mutant of Naa50, which had reduced acetyltransfease activity, did not rescue sister chromatid cohesion as effectively as wild type, although the expression level of mutant was even more than that of WT (Hou et al., 2007). Tyrosine-124 is a conserved site that is required for catalysis among several well-characterized acetyltransferases. To confirm the importance of acetyltransferase activity of Naa50 for sister chromatid cohesion, another site was chosen for my study.

In 2011, a structural analysis of Naa50 with its substrate peptide and CoA exhibited some residues that contact the peptide backbone. Phe-27, a highly conserved site in Naa50 orthologs, is one of the residues that contacted the initiator Methionine (iMet) of the peptide. Using an acetyltransferase activity assay with substrate peptide and radiolabeled CoA, the catalytic efficiency of the F27A mutant was measured and it demonstrated no detectable N-terminal acetyltransferase activity (Liszczak et al., 2011). Based on these findings, I used a F27A mutant of Naa50 to determine if it had defects in rescuing mitotic arrest caused by Naa50 depletion. The importance of the acetyltransferase activity was verified.

The requirement of enzymatic activity led me to think about identifying

substrates of Naa50 for sister chromatid cohesion. Previously, an immunoprecipitation experiments showed some interacting proteins with Naa50 including tubulin. The lysine acetylation site has been identified. This novel acetylation of β-tubulin at K252 by Naa50 slows down tubulin incorporation into MTs (Chu et al., 2011). Acetylation of histone H4 by Naa50 was also observed (Evjenth et al., 2009). However, there was no evidence showing any of the identified substrates are important for sister chromatid cohesion.

In this study, different purified recombinant cohesion related proteins were tested by an in vitro acetylation assay. Preliminary data with the in vitro acetylation assay showed some possible candidates. Further investigation was performed by mass spectrometry analysis, and by making mutations of possible acetylation sites to see if any candidates are substrates of Naa50 that plays a role in sister chromatid cohesion.

5.2 Results

5.2.1 Acetyltransferase activity of Naa50 is important for sister chromatid cohesion.

As stated above, NAT activity of Naa50 is abolished in Naa50 F27A mutant. The in vitro acetylation assay was performed, recombinant protein of GST tagged Naa50 WT or F27A was incubated with ^{14}C-acetyl-CoA. Autoacetylation of Naa50 was observed at three lysine sites: K34, K37 and K140. This modification was suggested to facilitate its enzymatic activity by increasing the specificity of substrates (Evjenth et al., 2009; Chu et al., 2011). As expected, the autoacetylation signal of Naa50 F27A was significantly reduced compared with that of Naa50 WT as determined by ^{14}C

incorporation (Fig 8A). Thus, Naa50 F27A has defects in total acetyltransferase activity.

To further confirm if the acetyltransferase activity of Naa50 is required for sister chromatid cohesion, a rescue experiment was performed. After depletion of Naa50 in HeLa Tet-On cells, the same amount of Myc-tagged Naa50 WT or Naa50 F27A plasmids were transfected. Three days later, cells were collected and used to perform MPM-2 staining for FACS (Fig 8B). There was an increase of mitotic index in cells depleted of Naa50. Expression of Naa50 WT rescued this mitotic arrest defect, while the Naa50 F27A mutant only partially relieved the defects. Depletion efficiency of Naa50 is shown in Figure 8C. The expression level of Naa50 WT and Naa50 F27A is comparable but a little less than endogenous Naa50. These results demonstrate that the Naa50 F27A mutant cannot rescue cohesion defects caused by Naa50 depletion as efficiently as Naa50 WT. This indicates the acetyltransferase activity of Naa50 is required for sister chromatid cohesion.

5.2.2 Attempting to identify the cohesion substrate of Naa50.

Based on the data from Soonjoung Kim in my lab, the N-terminus of Scc1 is important for Scc1 function in sister chromatid cohesion. Compared with untagged Scc1, N-terminal Myc-tagged Scc1 cannot rescue defects caused by Scc1 depletion (Fig 9B). The alignment of Scc1 reveals N-termini of Scc1 are conserved from fission yeast to human (Fig 9A). The N-terminus of Scc1 is Methionine followed by a hydrophobic amino acid, Phenylalanine, which is suited to the substrate character of Naa50 as a NAT (Liszczak et al., 2011; Evjenth et al., 2012). The difference of the

N-terminus in budding yeast also correlates with the findings that depletion of Naa50 has no observed defects in cohesion in budding yeast. Also, there was evidence showing the existence of Nt-acetylation of Scc1 in cell crude lysates (Gauci et al., 2009). Taken together, these data led us to hypothesize that Scc1 may be a NAT substrate of Naa50.

In order to address this question, an antibody to the acetylated form of Scc1's N-terminus was produced by injecting rabbits with the corresponding peptide. The serum was harvested and purified. The Scc1 N-terminus (MFYAHFVL) with C-terminal GST tag was produced in bacteria. Proteins were affinity purified and eluted into three fractions. As judged by SDS-PAGE, each fraction contained proteins (Fig 9C). Concentration of proteins was determined by Bradford assay. Then an in vitro acetylation assay with acetyl-CoA was performed to test if the N-terminus of Scc1 was acetylated by Naa50 in vitro. GST proteins were used as a negative control to rule out the possible influence of the GST tag on acetylation. The same amount of Naa50 was added into all samples. As shown in Figure 9D, GST itself had no detectable acetylation signal by using the Ac-N-Scc1 antibody. N-Scc1-GST had increased level of acetylation when it was treated with Naa50 and acetyl-CoA.

Next, I tested if endogenous Scc1 N-terminal acetylation was affected by Naa50 or not. Cells were fractionated into cytosol and nuclear portions. Scc1 should be in nuclear fraction but not in the cytosol fraction, however even in the cytosol fraction, the Ac-N-Scc1 antibody still detected signals. This indicated issues with the specificity of antibody. In nuclear fractions, the Ac-N-Scc1 signal was very weak, and

there were no major differences in mock and Naa50 depleted cells. Scc1 depletion was tested as a negative control, which seemed to abolish the acetylation signals in cell lysates made from nuclear fractions, as expected. Thus, although Nt-acetylation of Scc1 by Naa50 was determined in vitro, acetylation may be too weak to detect in cells or the modification may not be influenced by Naa50 in cells.

Preliminary data by screening cohesion related recombinant proteins as substrates of Naa50 with the in vitro acetylation assay provided a potential substrate: Pds5B. As mentioned, Naa50 affects the association among Sororin/cohesin/Pds5. There are possibilities that disruption of these interactions may be caused by direct modification of Pds5B or Sororin by Naa50. Pds5B 1-1120 and Sororin 91-252(Δ214-222) were tested by in vitro acetylation (Pds5B and Sororin recombinant proteins were provided by Zhuqing Ouyang in my lab). An acetylation signal appeared in Pds5B treated with ^{14}C-acetyl-CoA and Naa50 WT (Fig 10). Pds5B with ^{14}C-acetyl-CoA and Naa50 F27A had a much weaker acetylation signal. However, Sororin in any conditions exhibited an acetylation band. The acetylation signals of Sororin in all samples might be explained by some contamination of acetyltransferase which can interact with and acetylate Sororin in the protein prep. These data indicate that Naa50 may acetylate Pds5B, but it does not acetylate Sororin in vitro.

At first, I focused on Pds5B acetylation by Naa50. After treatment of Pds5B 1-1120 with Naa50 and acetyl CoA, samples were resolved by SDS-PAGE. The target protein band was sliced out and sent for mass spectrometry. Seven acetylated lysine

sites were discovered (K212, K282, K523, K528, K683, K964 and K1103). Pds5B 7KR mutant gene was synthesized by Genescript. K to R mutation is routinely used as an unacetylated mimic of a protein. Pds5B 7KR cDNA was cloned and insert into pCS2-Myc (Nt) vector. Sequence was validated by DNA sequencing.

Although all seven lysine sites were mutated, compared with Pds5B WT, Pds5B 7KR did not have reduced levels of acetylation upon Naa50 and ^{14}C-acetyl-CoA treatment, indicating they may not be the primary sites modified by Naa50 (Fig 11).

An immunoprecipitation assay was performed to check if 7KR disrupted interaction between Pds5B and Sororin. Myc-tagged Pds5B WT or 7KR was expressed in cells with or without Naa50 depletion. However, the amount of Pds5B 7KR coimmunoprecipitated Sororin was indistinguishable from that of Pds5B WT (Fig 11B). The reduction of Sororin in Naa50 depleted cells was also not changed in cells expressing Pds5B 7KR. These results suggest that Pds5B 7KR mutant may still be functional for cohesion.

Later, by using different conditions and different truncations of Sororin, I finally found that Sororin 131-252(Δ214-222) was acetylated by Naa50 in vitro (Fig 12). This protein was incubated with Naa50 and acetyl-CoA. After separation by SDS-PAGE, the corresponding protein band was cut and analyzed by mass spectrometry. According to the results, two lysine acetylation sites were obtained, K187 and K197 (Fig 13A).

Sororin 131-252(Δ214-222) WT or KKRR recombinant proteins exhibited similar level of acetylation signals (Fig 13B). Plasmids with siRNA resistant form of

Sororin WT or KKRR were also constructed. A rescue experiment was made to determine if Sororin KKRR had any functional defects (Fig 13C). As expected, Sororin depletion significantly increase the mitotic index compared with mock. However, the expression of Sororin KKRR mutant rescued this defect to the extent that was comparable to Sororin WT. Thus, the results indicate that Sororin KKRR has no detectable defects for sister chromatid cohesion, suggesting Sororin may not a KAT substrate of Naa50 in cells.

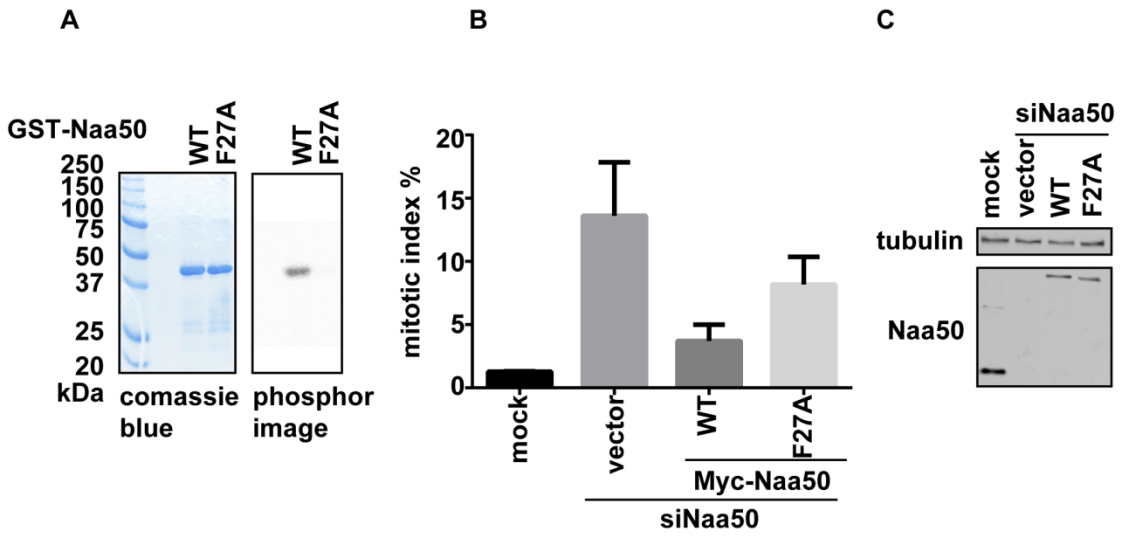


Figure 8: Naa50 F27A mutant cannot rescue cohesion defects caused by Naa50 depletion as efficiently as WT. (A) Autoacetylation level of Naa50 WT and F27A mutant was detected in the presence of ^{14}C-acetyl-CoA. Gelcode blue staining and the phosphor image are demonstrated. (B) FACS analysis and quantification of the mitotic indices of cells (Mean±SD, n=2 independent experiments). Cells were transfected with the indicated siRNAs and Myc-Naa50 WT or F27A plasmids. (C) Immunoblot showing the levels of endogenous Naa50 and the expression level of Myc-tagged rescue constructs in (B).

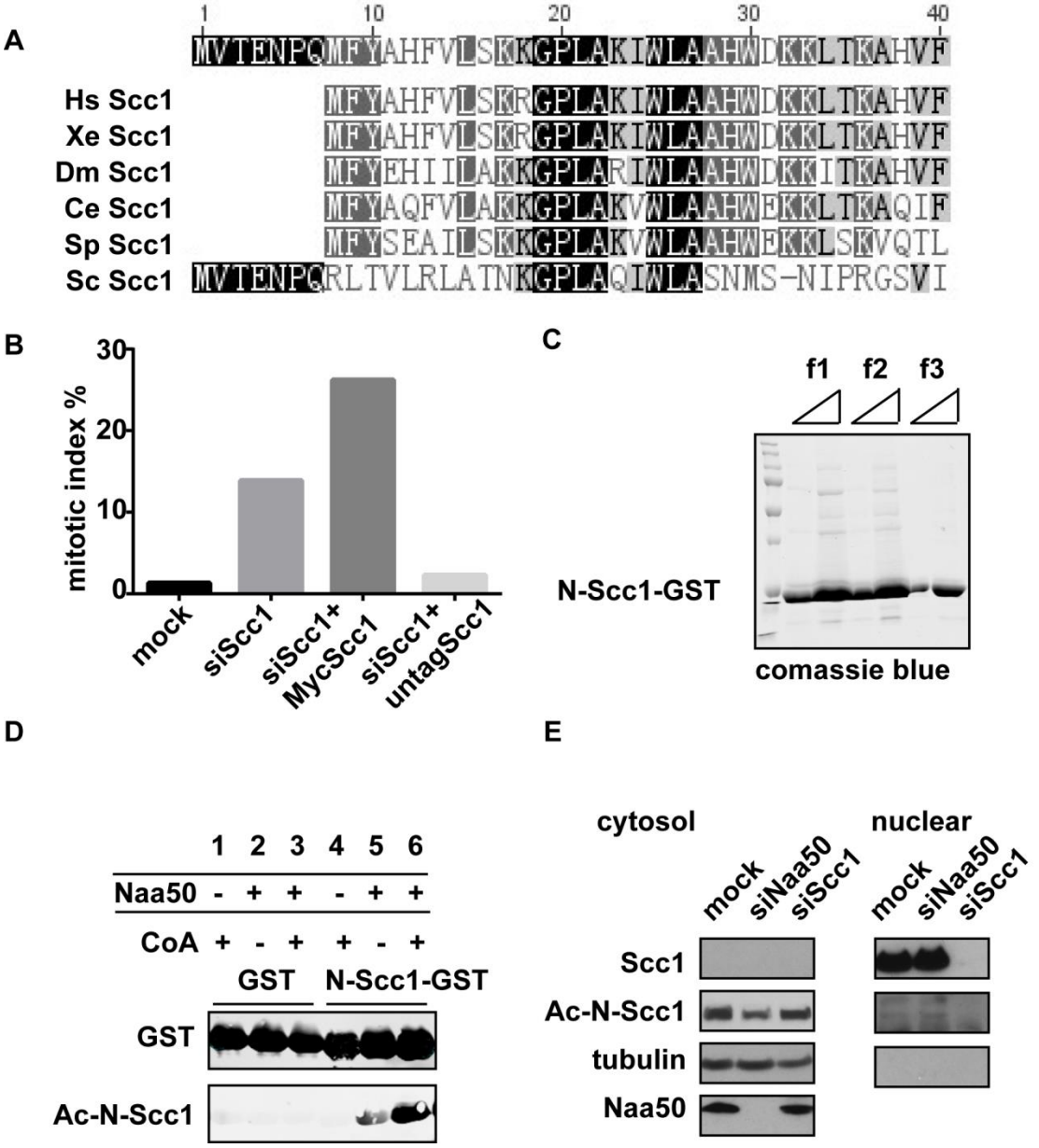


Figure 9: N-terminal acetylation of Scc1. (A) Alignment of the N-terminus of Scc1 in different species. (B) FACS analysis and quantification of the mitotic indices of the rescue effect by Myc-Scc1 and untagged-Scc1. (C) Production and purification of GST-tagged (C-terminal) Scc1 N-terminus recombinant proteins. Proteins in different fractions (f1-3) of elution were judged by SDS-PAGE. (D) Acetylation of N-Scc1-GST by Naa50 in vitro. Purified Ac-N-Scc1 antibody was used to detect acetylation signals. (E) Endogenous Scc1 N-terminal acetylation in cells transfected with the indicated siRNAs. Cells lysates were fractionated into cytosol and nuclear portions. Proteins were blotted with corresponding antibodies.

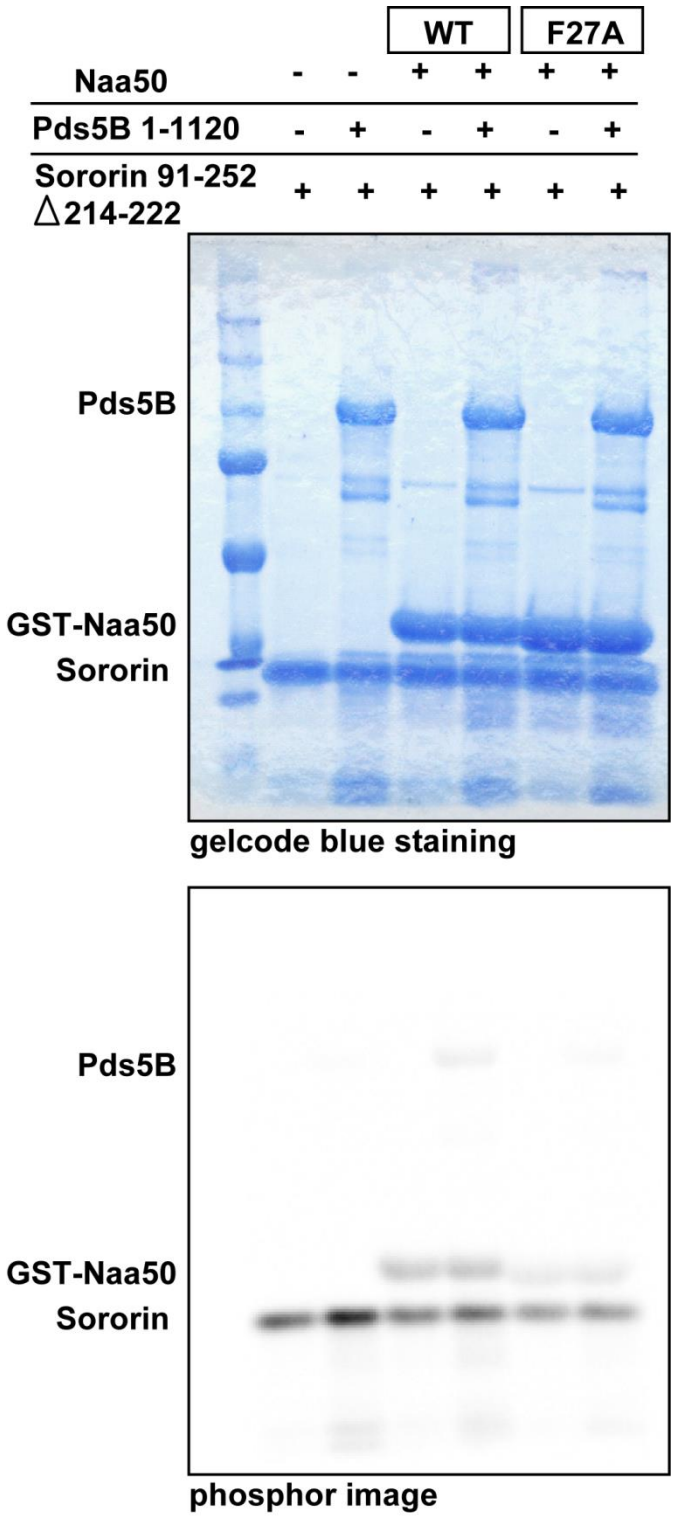


Figure 10: Pds5B is acetylated by Naa50 in vitro. Pds5B 1-1120 and Sororin 91-252(Δ214-222) were tested. ^{14}C-acetyl-CoA was added into all mixtures. Samples were treated without Naa50, with Naa50 WT or with Naa50 F27A. Gelcode blue staining of proteins and phosphor image of acetylation signals are shown.

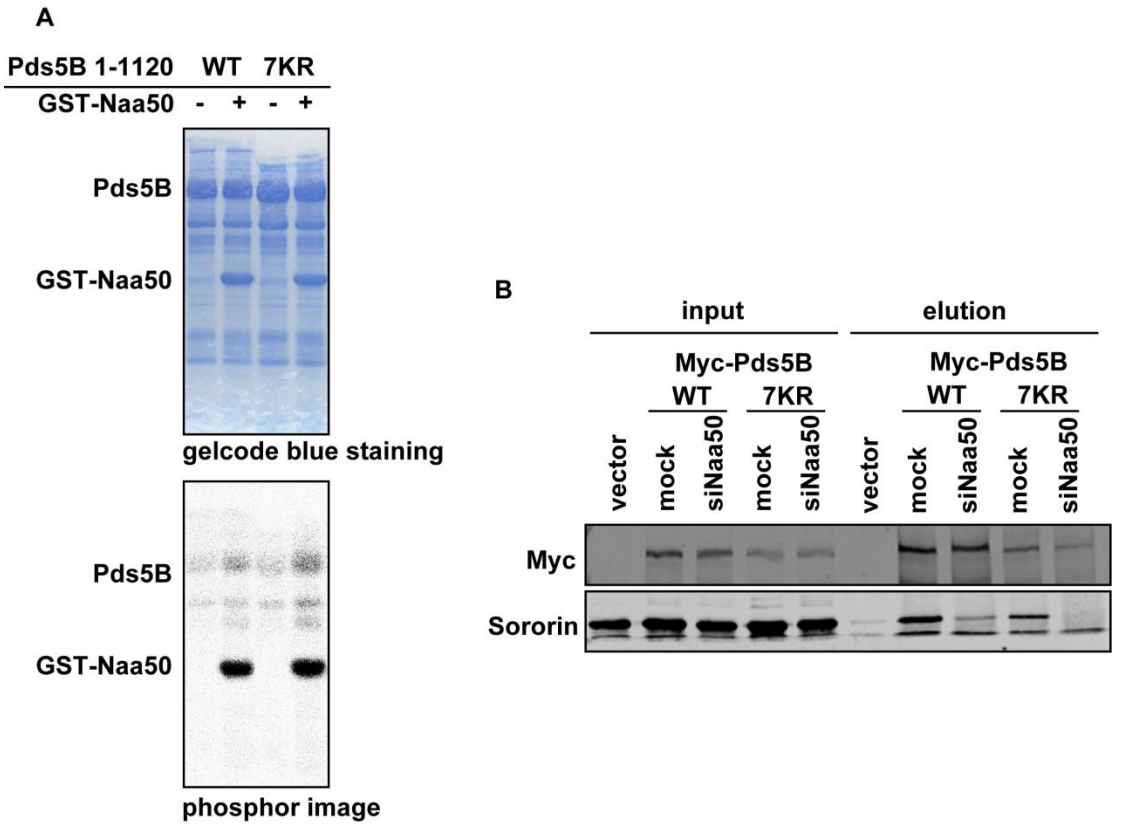


Figure 11: Pds5B 7KR mutant has no detectable defects. (A) Acetylation of Pds5B WT and Pds5B 7KR with Naa50 in vitro. Pds5B 1-1120 WT and 7KR was used in this experiment. ^{14}C-acetyl-CoA was added into all samples. Naa50 were added or not. In vitro acetylation assay was performed. Gelcode blue staining of proteins and phosphor image of acetylation signals are shown. (B) Interaction of Pds5B WT and Pds5B 7KR mutant with Sororin. HeLa Tet-On cells were transfected with empty vector, Myc-Pds5B WT or Myc-Pds5B 7KR mutant plasmid. Then cells were depleted with Naa50 or not. Cell lysates were collected and immunoprecipitated with anti-Myc. Proteins were blotted with indicated antibodies.

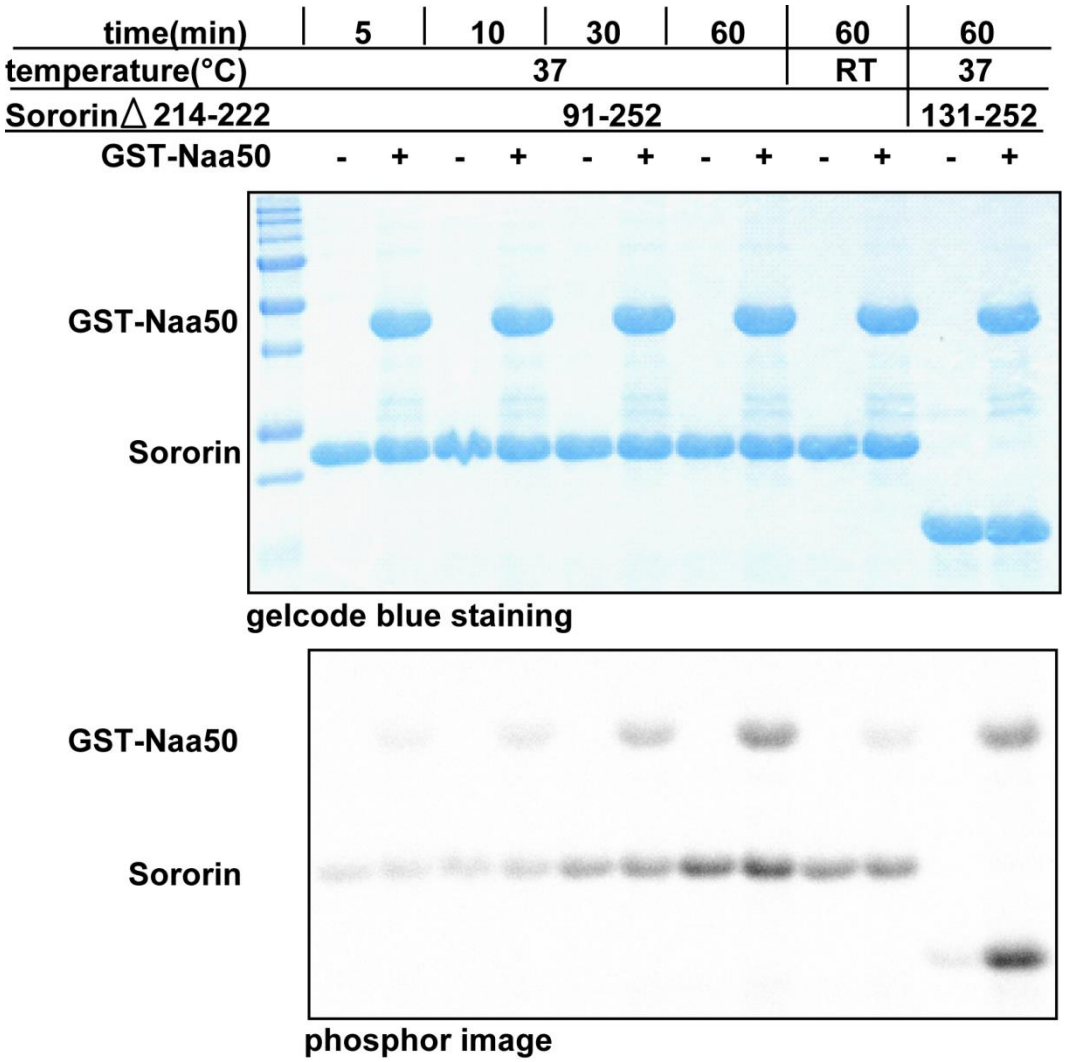


Fig 12: Naa50 acetylates Sororin in vitro. Sororin 91-252(Δ214-222) and Sororin 131-252(Δ214-222) were used to perform in vitro acetylation assay. Different conditions were tested as indicated. ^{14}C-acetyl-CoA was added into all samples. Naa50 was added or not into mixture. Gelcode blue staining of proteins and phosphor image of acetylation signals are shown.

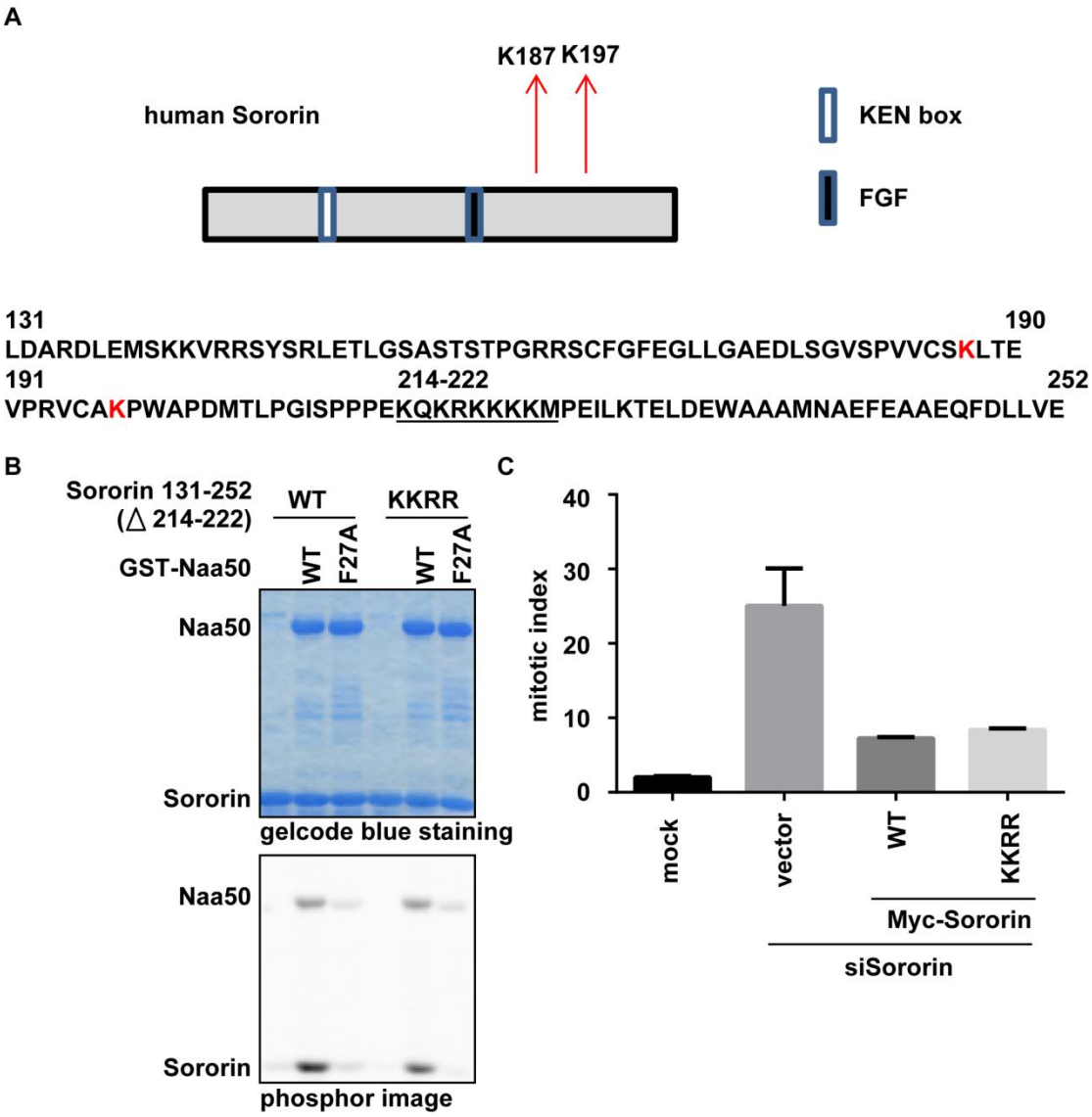


Figure 13: Sororin KKRR mutant has no detectable defects. (A) Schematic and sequence of human Sororin. Red letters mark the lysine acetylation sites observed by mass spectrometry (B) Sororin 131-252(Δ214-222) WT or K187R197R were tested by in vitro acetylation assay. ^{14}C-acetyl-CoA was added into all mixtures. Samples were treated without Naa50 (NA), with Naa50 WT or with Naa50 F27A. Gelcode blue staining of proteins and phosphor image of acetylation signals were shown. (C) FACS analysis and quantification of the mitotic indices (defined as the percentage of MPM2-positive, 4n cells) of cells. The means and the SDs (represented by error bars) of the percentage of the mitotic index were calculated using the data from two independent trials.

5.3 Discussion

By using Naa50 F27A, a mutant with decreased acetyltransferase activity, the requirement of acetyltransferase activity of Naa50 was confirmed for sister chromatid cohesion. The partially rescue of mitotic defects by F27A mutant may be explained by its residual enzymatic activity. Another explanation is that Naa50 may not only mediate cohesion through catalytic activity, but also through other mechanisms like association with some unknown proteins.

Once the importance of the acetyltransferase activity of Naa50 was confirmed, I attempted to identify substrates of Naa50 which is involved in regulating sister chromatid cohesion.

First, Scc1 was hypothesized as a potential substrate of Naa50. Although N-terminus of Scc1 can be acetylated by Naa50 in vitro, the Scc1 Nt acetylation signal was weak in cells and this signal was not affected by Naa50 depletion. These results indicate Scc1 may not be the cohesin substrate of Naa50.

By in vitro acetylation assay, two other potential candidates for Naa50 acetylation were tested: Pds5B and Sororin. Pds5B exhibited increased acetylation signals upon Naa50 treatment. However, when Sororin 91-252(Δ214-222) was used, even the sample without Naa50 exhibited an acetylation signal. One possibility is that some unknown acetyltransferase may be purified with Sororin 91-252(Δ214-222) and facilitates the acetylation of Sororin. It is also possible that Sororin 91-252(Δ214-222) may autoacetylate. Later, Sororin 131-252(Δ214-222) was tested and a much stronger acetylation signal was detected in sample with Naa50 WT and ^{14}C-acetyl-CoA. Thus,

both Pds5B 1-1120 and Sororin 131-252(Δ214-222) are acetylated by Naa50 in vitro.

Through mass spectrometry analysis, some lysine sites were revealed. Mutants of each protein were made but none of them show reduced acetylation upon Naa50 treatment. The failure of reducing acetylation signals by mutants suggests that these mapped sites may not be the major acetylation sites. Other acetylation sites may exit in proteins which have not been identified. Another possibility is that the acetylation signals detected also include Nt-acetylation. Consistent with this observation, Pds5B 7KR had no defects in interaction with Sororin. Also, Sororin K187RK197R was functionally intact in rescuing cohesion defects caused by Sororin depletion.

Taken together, the requirement of Naa50 acetyltransferase activity for sister chromatid cohesion was verified, however, the cohesin substrate is still unclear. Although some proteins have been identified to be acetylated by Naa50 in vitro, there are no evidences showing any of them are acetylated in cells by Naa50. It is possible that the acetylation sites identified by mass spectrometry are false-positive interpretations of the acetylation assays due to the likely existence of acetyl-CoA spontaneously reacting with substrate, the contamination of other acetyltransferase in protein prep, or the pre-existence of acetylation sites in purified protein. Also, since the in vitro acetylation assay cannot discriminate lysine acetylation and Nt-acetylation, the acetylation signals I observed for Pds5B and Sororin may be from the N terminus and not from internal lysines. All of these potential caveats make the identification of cohesion substrates of Naa50 more complicated.

Chapter 6

The role of NatA in Naa50 mediation of cohesion

6.1 Introduction

In eukaryotic cells, N-terminal acetylation is the most wildly found modification for proteins. Among the NAT protein complexes, NatA is one of the important enzymes. It is composed of two main subunits: Naa10 and Naa15. There are also data to show that Naa50 can interact with NatA and form another NAT complex. However, unlike depletion of Naa50, single depletion of NatA subunits causes no mitotic arrest phenotype (Hou et al., 2007). The function of NatA in sister chromatid cohesion is still unclear.

Surprisingly, in my study, a double depletion experiment showed NatA depletion rescued the mitotic defects caused by Naa50 depletion. The opposing roles of Naa50 and NatA in sister chromatid cohesion allow us to propose a hypothesis to interpret this phenotype.

6.2 Results

6.2.1 Depletion of NatA complex subunits can rescue the defects caused by Naa50 depletion.

In my study, Naa50 and NatA (Naa10 or Naa15) were depleted either individually or simultaneously from HeLa Tet-On cells. Unexpectedly, when Naa50

was depleted together with NatA, the increase of mitotic index caused by Naa50 depletion was decreased to nearly the basal level, meaning the mitotic arrest was remarkably suppressed (Fig. 14A). Depletion efficiency of Naa50 siRNA, Naa10 siRNA and Naa15 siRNA was analyzed by immunoblotting (Fig. 14B). I also prepared mitotic chromosome spreads from these cells. Single depletion of Naa50 caused massive premature chromatid separation. This defective phenotype was effectively inhibited when either subunit of NatA was simultaneously depleted with Naa50 (Fig. 14C, 14D). Therefore the cohesion defects induced by Naa50 depletion were rescued upon double depletion of NatA and Naa50.

6.2.2 Depletion of NatB/C complex subunits cannot rescue the defects caused by Naa50 depletion.

NatB and NatC are two other major types of NATs in cells. The function of NatA complex in opposing Naa50 to regulate sister chromatid cohesion led us to wonder if any other NAT complex had similar roles during this process. Double depletion of Naa50 and Naa20 (catalytic subunit of NatB) or Naa50 and Naa30 (catalytic subunit of NatC) was performed. However, as judged by mitotic indices through FACS analysis, the mitotic arrest defect was not rescued as effectively as NatA (Fig 15). In conclusion, NatA depletion, but not NatB or NatC depletion can rescue the defects caused by Naa50 depletion.

6.2.3 Acetyltransferase activity of Naa50 and NatA is not affected after Naa50/NatA complex formation.

To elucidate how Naa50 and NatA play antagonizing roles in regulating sister

chromatid cohesion, some hypothesis were proposed. One of them is that NatA may inhibit the catalytic activity of Naa50 when a complex is formed. Previous reports revealed the existence of Naa50 alone, NatA complex and Naa50/NatA complex in cells (Hou et al., 2007). To investigate this hypothesis, in vitro acetylation assays with single Naa50, NatA complex, or Naa50/NatA complex was performed (Fig. 16B, data from Robert Magin in Ronen Mormenstein's lab in University of Pennsylvania.). MLGP peptide was a known substrate of Naa50, while SASE peptide was considered as a peptide substrate of NatA. Naa50/NatA complex was capable of acetylating both Naa50 and NatA substrates. The lower amount of acetylation of MLGP peptide by Naa50/NatA complex compared with Naa50 was likely to be caused by differences of Naa50 protein amount between Naa50 alone and Naa50/NatA complex (Fig. 16A). Therefore, the formation of Naa50/NatA complex does not disrupt the acetyltransferase activity of either Naa50 or NatA.

6.2.4 Depletion of MetAP1 and MetAP2 cannot rescue the defects caused by Naa50 depletion.

Recently, a study suggested the possible involvement of MetAP in mediating the opposing roles of Naa50 and NatA for cohesion. It revealed that Naa50 acetylated the initiator Methionine (iMet) at the N-terminus of proteins with a small amino acid at the second position (Van Damme P et al., 2015). After their N-terminal Met was removed by MetAPs, such proteins were typical NatA substrates. Moreover, there were data showing that Nt-acetylation of iMet prevented the excision of the N-terminal Met by MetAP (Van Damme P et al., 2015). These findings led to another

hypothesis: in cells, same proteins are acetylated by Naa50 and NatA at different sites through MetAP processing which stabilize and destabilize sister chromatid cohesion respectively. If this hypothesis was correct, we expected to see the rescue of mitotic defects after double depletion of Naa50 and MetAP1&2. FACS analysis was performed, but MetAP1/2 depletion did not rescue the mitotic arrest defect caused by Naa50 depletion (Fig. 17). Therefore, our hypothesis was incorrect.

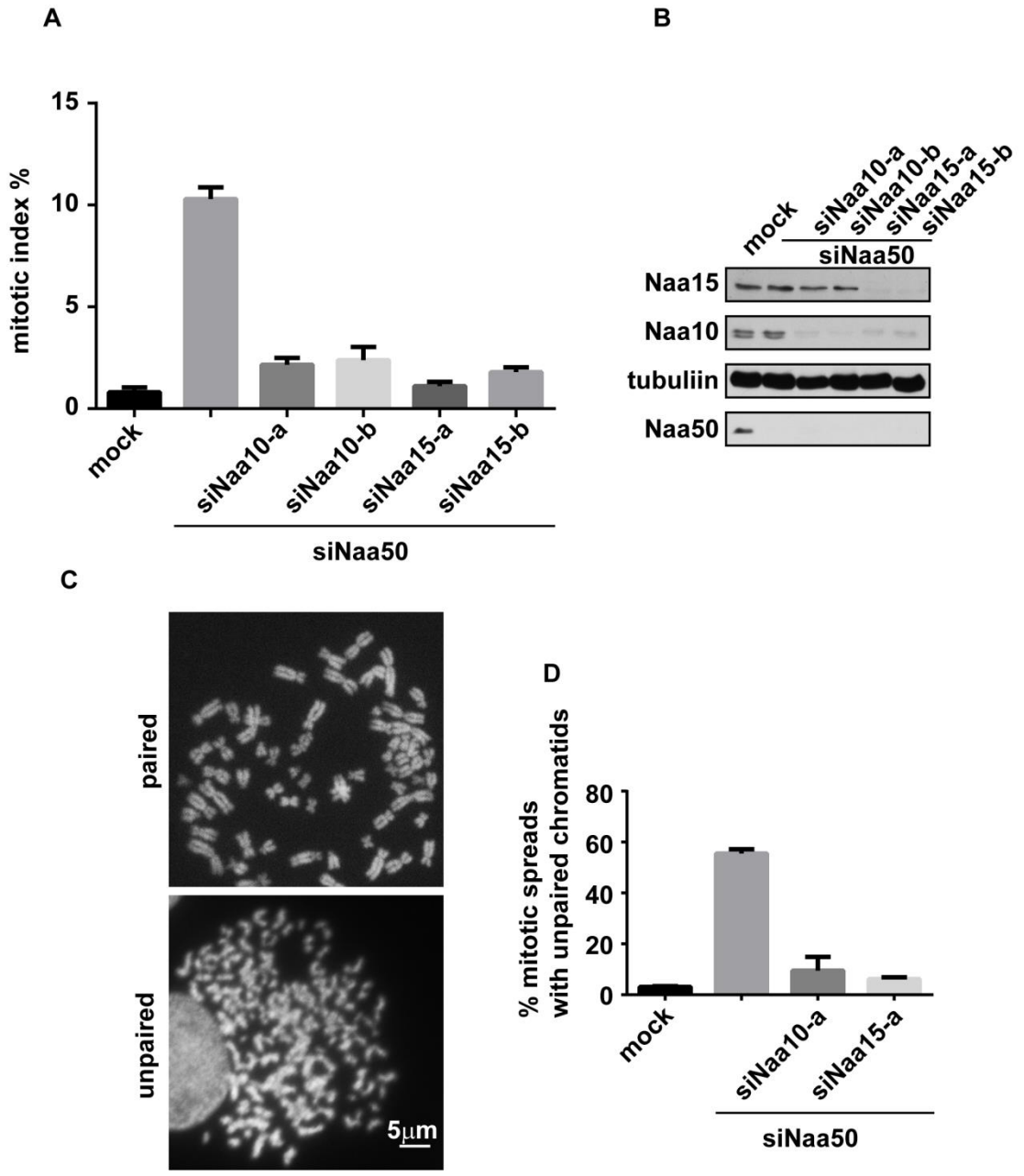


Figure 14: Antagonizing roles of Naa50 and NatA for cohesion. (A) FACS analysis and quantification of the mitotic indices (Mean±SD, n=2 independent experiments). (B) Naa50 and the subunits of the NatA complex were effectively depleted by siRNA in HeLa Tet-ON cells. The levels of Naa50, Naa10, and Naa15 were determined by immunoblot assay in mock and siRNA cells. The level of α-tubulin was blotted as a loading standard. (C) Cells were treated with nocodazole for 3 h and subjected to chromosome spreads. Paired (top) and unpaired (bottom) sister chromatids in mitosis were observed. (D) Quantification of percentage of premature separated chromatids in mitotic spreads. The means and the SDs (represented by error bars) of the percentage of the mitotic index were calculated. Data were collected from two independent trials.

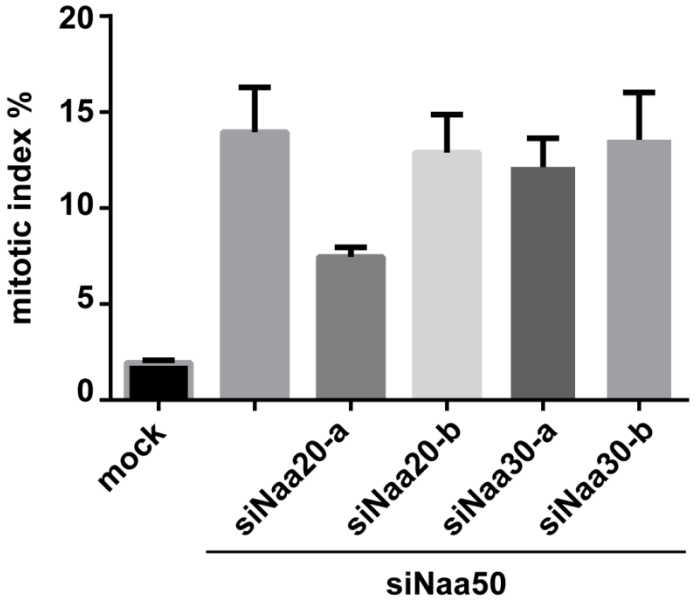


Figure 15: Depletion of NatB or NatC does not rescue the phenotypes caused by Naa50 depletion. Naa50 and NatB/NatC subunit (Naa20 or Naa30) were depleted individually or simultaneously from HeLa Tet-On cells. Quantification of the mitotic indices was analyzed (Mean±SD, n=2 independent experiments).

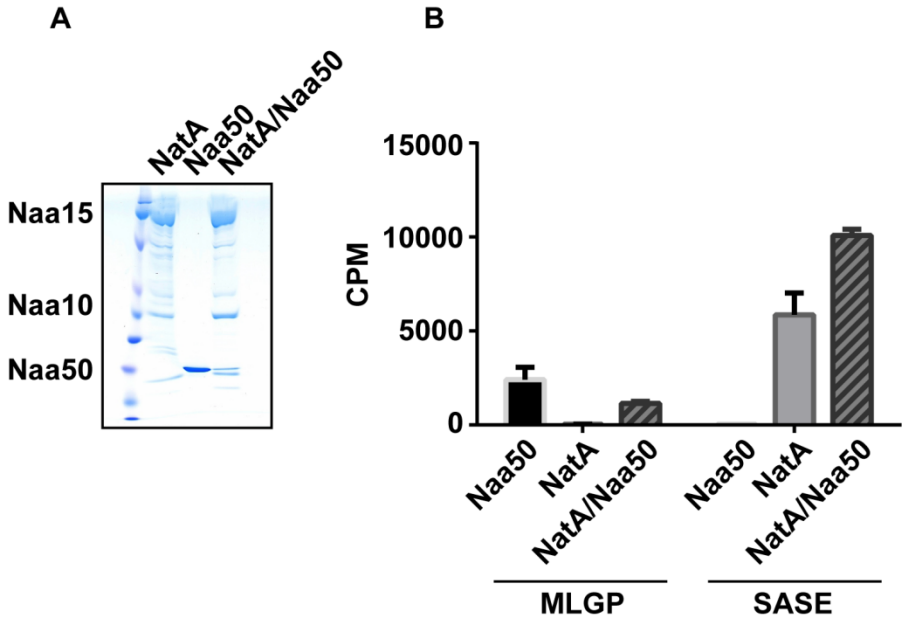


Figure 16: Binding of Naa50 and NatA does not inhibit the acetyltransferase activity of Naa50 or NatA. (A) Purified Naa50, NatA, and Naa50/NatA complex were analyzed by SDS-PAGE. Gel was stained with Coomassie Blue staining reagent. (B) In vitro acetylation assay to test if the formation of Naa50/NatA complex affects substrate specificity. Purified acetyltransferase used in this assay was shown as (A). Different types of peptides were used. MLGP is believed to be a typical substrate of Naa50 while SASE is a substrate of NatA. Acetyltransferase activity of different enzymes to both peptides was calculated.
Original data was made by Robert Magin in Ronen Mormenstein's lab at University of Pennsylvania.

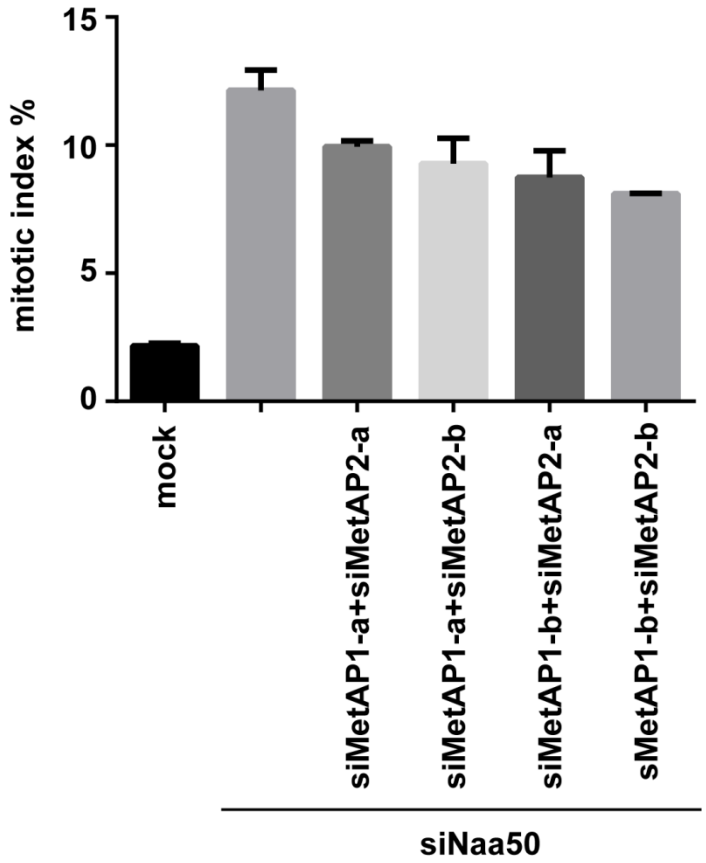


Figure 17: Depletion of MetAP1 and MetAP2 does not rescue the phenotype caused by Naa50 depletion. Naa50 and MetAP1/MetAP2 were depleted by siRNA in HeLa Tet-ON cells. Quantification of the mitotic indices was analyzed (Mean±SD, n=2 independent experiments).

6.3 Discussion

The discovery that NatA depletion rescued the cohesion defects caused by Naa50 depletion suggests Naa50 regulates sister chromatid cohesion by antagonizing NatA. To further understand the underlying mechanism, several models were proposed and experiments were performed to investigate on the hypotheses.

The first hypothesis was the interaction of Naa50 and NatA might directly inhibit acetyltransferase activity of Naa50, and thus the decrease of Naa50 catalytic activity in cells resulted in the cohesion defects. However, results obtained from the in vitro acetylation assay demonstrated the formation of Naa50/NatA complex did not affect acetyltransferase activity of either Naa50 or NatA.

Next, another hypothesis was suggested that Naa50 and NatA may affect the same proteins through mediating acetylation at different sites with the help of MetAPs. Depletion of Naa50 might cause increased removal of the iMet by MetAPs and increased NatA-targeted protein acetylation, which destabilizes sister chromatid cohesion. If this was true, MetAP depletion should have similar effects as NatA depletion. However, the results of the experiments in Figure 17 indicate this is unlikely to be true. Therefore, the mechanism by which Naa50 antagonizes NatA and the reason for this function has yet to be discovered.

Chapter 7

Conclusion

Although the importance of Naa50 for sister chromatid cohesion has been implicated in different species, the detailed mechanism needs to be investigated. *We propose that* Naa50 affects cohesion in a cohesin-related pathway. The observation of simultaneously depletion of Naa50 and Wapl rescued the cohesion defects caused by Naa50 depletion, supporting this view. Our data further indicates that Naa50 is required for the establishment of cohesion but not for the loading process. However, unlike Esco1/Esco2, Naa50 does not acetylate Smc3. Therefore, Naa50 is likely to use a different method in promoting establishment of cohesion.

The effects of Naa50 on cohesin suggested the interaction of cohesin and related regulators may be affected by Naa50. Sororin and Sgo1 are two important proteins for cohesion. It is believed Sororin begins to paly roles starting in S phase during cohesion establishment. It is also important for cohesion maintenance until anaphase. Unlike Sororin, Sgo1 is required for protection of centromeric cohesion during prophase. Previous data revealed that localization of Sgo1 was not changed when Naa50 was depleted. Consistent with these findings, immunoprecipitation results in this study indicate Naa50 affects Sororin/cohesin/Pds5B interaction but not Sgo1/cohesin interaction. Moreover, Naa50 does not affect the dynamic of cohesin as analyzed by FRAP.

As an acetyltransferase, the relevance of enzymatic activity of Naa50 to sister

chromatid cohesion was studied by a phenylalanine-27 to alanine mutant of Naa50. According to the resolved structure of Naa50-CoA-peptide complex, this site is the residue that contacts the N-terminal methionine. Decreased catalytic activity of the mutant was detected by an in vitro acetylation assay. Furthermore, this mutant cannot rescue Naa50 depletion defects as efficiently as WT, although they have the similar protein expression level. These observations indicate the acetyltransferase activity of Naa50 is important for its function in sister chromatid cohesion. However, the partial rescue by the mutant may suggest other non-catalytic mechanisms of Naa50 in regulating sister chromatid cohesion, for example, by affecting cohesion through direct protein binding.

In human cells, more than 80% proteins are Nt-acetylated. Among those NATs, the interaction of NatA and Naa50 in cells implies the possible role of N-terminal acetylation in sister chromatid cohesion. However, single depletion of NatA subunits has no similar phenotype to Naa50 depletion. Surprisingly, co-depletion of NatA subunits and Naa50 rescues the defects in Naa50 depleted cells. Furthermore, co-depletion of other NAT subunits, such as Naa20 or Naa30, cannot rescue the defects as efficiently as NatA subunits. It was therefore proposed that the acetyltransferase activity of Naa50 may be inhibited through interaction with NatA. If this was the case, Naa50/NatA complex should have significantly decreased the catalytic activity of Naa50. However, Naa50/NatA is still able to acetylate both Naa50 and NatA substrates with no major specificity differences, indicating that this hypothesis was not correct.

The interplay of Naa50, NatA and MetAPs leads to another hypothesis: in cells, acetylation of N-terminal methionine of some proteins may stabilize cohesion by protecting proteins from processing by MetAPs, while acetylation of the following residue after cleavage of the initiator Met may destabilize cohesion. If this hypothesis was correct, we expected to see the rescue of defects after co-depletion of Naa50 and MetAP1/2. However, MetAP1/2 depletion does not restore the mitotic arrest defects in Naa50 depleted cells.

So far, cohesin substrates of Naa50 have not yet been identified. Although Naa50 displays both NAT and KAT activity, there are evidences suggesting that Naa50 has preference to be a NAT. The fact that Naa50 is mainly localized in the cytosol in interphase cells also suggests that NAT activity of Naa50 may be more important for cohesion than KAT activity, since NAT is cotranslational while KAT is posttranslational. Finally, a hypothetic model is proposed in which Nt-acetylation of different proteins by Naa50 and NatA has opposing roles in regulating sister chromatid cohesion (Figure 18). For further investigation, large scale identification of Naa50 substrates by mass spectrometry may need to be performed in cells depleted or not with Naa50 siRNA.

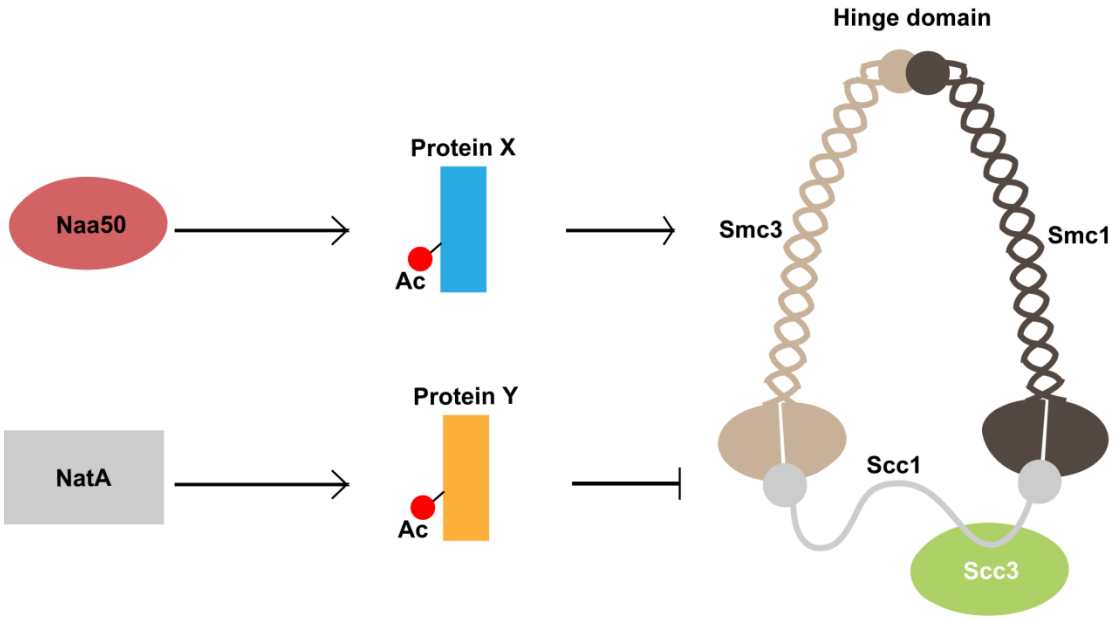


Fig 18: Hypothetic model for Naa50 regulating sister chromatid cohesion by antagonizing NatA. In cells, Naa50 and NatA acetylate different sets of proteins. Nt-acetylation of protein by Naa50 promotes the stabilization of sister chromatid cohesion, while Nt-acetylation of protein by NatA destabilizes sister chromatid cohesion.

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