Ataxia Telagiectasia
Cell Cycle Checkpoints and Cancer
Apoptosis and Aging

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Concluding remarks about ataxia telangiectasia in the text, DNA Repair and Mutagenesis. Friedberg, E.C., Walker, G.C., Seide, W. 1995.

"Consultation with one of our colleagues in the field, whose opinion we respect highly, led to the suggestion that the AT field "is a mess", and that [our] discussion of it reflects little more insight than that! If so, we hope that enthusiastic young (and old) investigators who are undaunted by messy fields will recognize this as a challenge... The next frontier is surely the isolation and characterization of the AT gene(s), with the hope that the translated structure of this gene will be informative and with the expectation that it will prove to be a powerful probe for exploring interactions between the AT and other cellular proteins whose properties will be at least as informative."

In the future, attention undoubtedly will be centered on the genome, with greater appreciation of its significance as a highly sensitive organ of the cell that monitors genomic activities and corrects common errors, senses unusual and unexpected events, and responds to them, often by restructuring the genome."

- Barbara McClintock, 1984

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Interests:
Abnormalities of cell cycle regulation in cancer;
Development and application of function based assays to detect mutation of oncogenes and tumor suppressor genes in human tumors.

Because of the remarkable level of conservation of the cell cycle control proteins from yeast to humans, we are using a yeast genetic approach to study the mechanism of disruption of cell cycle control by oncogenes. I have developed and used a method to isolate yeast *web* (wants E1A badly) mutants that require the adenovirus E1A oncogene for growth. The *WEB2* gene encodes a DNA helicase required for DNA replication. We have found that Web2 has a second important function in addition to its replication function. It is required for signaling in the S-phase checkpoint pathway, that normally delays entry into mitosis until replication of DNA is complete. This pathway has a counterpart in human cells, and is disrupted in the cancer prone disease, ataxia telangectasia. We have developed a model in which Web2 functions in maturation of Okazaki fragments and in signaling the completion of Okazaki fragment maturation to the S-phase checkpoint pathway. Our current focus is on characterizing the mechanism of Web2 function in replication and in cell cycle checkpoint signaling.

We are adapting a functional assay of the p53 tumor suppressor gene for use in rapid identification of mutations in human tumors. We will optimize the assay to reduce the rate of false positives, and will validate the assay using our large bank of cell lines and tumors with known p53 mutations, and with fresh biopsy specimens. Such assays will be used to identify patients with respect to prognosis and likelihood of response to treatment. For example, it has been shown that p53 status is prognostically important in bladder cancer. A functional test of p53 status may allow identification of patients with more aggressive tumors who would benefit from earlier cystectomy to prevent early metastatic disease.

Ataxia telangiectasia (AT) is a rare disease with an interesting history. The extreme sensitivity to X-rays and the markedly increased incidence of cancer in AT has attracted the attention of many researchers. Although AT is rare (between 1 in 40,000 and 1 in 100,000), AT heterozygotes are relatively common. The finding that AT heterozygotes have an increased incidence of cancer makes mutation of the AT gene am important public health problem.

Progressively increasing understanding of AT has led to a succession of models to explain the mechanism of the disease. Recognition of X-ray sensitivity in AT led to an early "DNA Repair Model" in which it was proposed that the underlying problem was defective repair of DNA breaks. However, repair defects cannot be consistently identified in AT cells.

Further work has shown that AT cells are defective in mobilizing the normal response to the presence of X-ray induced damage, leading to a "Cell Cycle Checkpoint Model" in which the AT gene normally acts to delay cell cycle progression while DNA damage is repaired. However, this model has been shown to be incomplete. While this model may explain the increased incidence of cancer and, possibly, some of the immune deficits of AT, it does not explain the neurologic deficits and the finding that nondividing AT cells are also sensitive to X-rays.

AT cells have recently been shown to have a lowered threshold for apoptosis (programmed cell death), in response to X-rays. This effect has been shown to require function of the p53 tumor suppressor gene. This has led to a model called the "Damage Surveillance Network Model". It incorporates the "Cell Cycle Checkpoint Model" and additionally proposes that the AT gene product acts to block p53 dependent programmed cell death while X-ray induced DNA damage is repaired. This more comprehensive model can explain most of the features of AT.

In 1995, the ATM (**a**taxia **t**elangiectasia **m**utated) gene was cloned. All cases of AT carry mutations in ATM. ATM is a member of a gene family that includes similar members from mammals, flies and yeast with related functions. Study of ATM and its homologs in other organisms is leading rapidly to better understanding of the molecular mechanism of ATM function. As a direct result of the identification of ATM, we now have mouse, *Drosophila* and yeast models of AT. Each of these will be useful for clarifying the nature of ATM function.

I will begin by describing the clinical features of AT. Then, I will give a historical perspective on the progress in understanding the mechanism of AT, describing the evolution of AT diseasemodels. I'll discuss whether AT heterozygosity is clinically important with respect to cancer risk and cancer screening, and whether AT heterozygotes are at high risk from the very X-ray based screening tests used to detect early cancers. I'll conclude with one proposal for exploiting ATM mutations for treatment of human cancer.

A Case of AT from (1)

A seven year old boy presented with four months of anorexia, weight loss and generalized weakness. He was an apparently normal boy until he began to walk, when he was noted to have poor coordination. Although this initially improved, he went on to develop slowly progressive moderate ataxia. He had been hospitalized several times over the preceding two years for recurrent respiratory tract infections. He had a normal older brother, and a sister that died at age five from recurrent pulmonary infections.

He was small for a seven year old at 43 inches, 37 pounds. He had ataxia of all limbs, slurred speech, and oculomotor apraxia (halting, irregular conjugate gaze). Telangiectatic vessels were present in both conjunctiva and ears.

Serum IgA and IgG levels were below normal. Chest X-ray showed a large mass in the right upper chest, encompassing half the chest diameter.

At thoracotomy, a large tumor was found in the right upper lobe that surrounded the mainstem bronchus. A pneumonectomy was performed. Pathology showed a large cell lymphoma.

Pneumonectomy was followed by radiotherapy. After 10 days of treatment (200 rads/day), he developed marked dysphagia and erythema within the irradiated field. These progressed, and treatment was stopped after 15 days of treatment. The dysphagia became very severe. The skin sloughed from the irradiated areas of his chest. He died three weeks after discontinuing radiotherapy with continuing progression of dysphagia and necrosis of the chest wall.

Recognition of AT as a Disease

The first report of patients with an AT-like syndrome was in 1926. Syllaba and Henner (2) reported three siblings with ataxia, choreoathetosis, and conjunctival telangiectasia. They made motion pictures of the patients to record their movement disorder. In retrospect, these patients were found to have the features of AT (3). Another report described a 9 year old boy with an AT-like syndrome in 1941.

AT was not recognized as a disease entity until less than 40 years ago, in 1957, when Boder and Sedgwick described eight cases in the USC Medical Bulletin (4). All had ataxia and oculocutaneous telangiectasia, leading to the naming of the disease. Two other reports of AT patients independently appeared in 1957 (5, 6). Boder (7) speculated that AT was recognized at this time because increasing use of antibiotics allowed more AT patients to survive long enough to develop the telangiectasias that distinguished them from other ataxia syndromes.

From the earliest descriptions, it was clear that AT was inherited, because multiple siblings were often affected (2, 4). AT inheritance was shown to be autosomal recessive in 1965 (8).

The first report by Boder and Sedgwick (4, 9) included description of an autopsy on a ten year old AT patient. This autopsy identified several features often found in AT. There was severe bronchiectasis and pulmonary fibrosis. No vascular abnormalities were seen in the lung. The brain was remarkable mainly for cerebellar degeneration primarily due to loss of Purkinje cells, which had pyknotic changes. There was decreased cerebellar white matter due to loss of Purkinje cell axons. There were no visible telangiectasias in the brain, but histologic examination showed that small veins were enlarged, thin walled and engorged. There was hypertrophic osteoarthropathy of fingers and toes presumed secondary to the pulmonary disease. Arteriosclerotic changes were found in the aorta and pulmonary artery. The thymus and ovaries were absent. These features are typical of AT patients.

Since the recognition of AT, there has been gradually increasing awareness of the disease. The AT Children's Project, was founded in late 1993 by a family in Florida with two young sons who have AT. The A-T Children's Project is active in raising funds to accelerate scientific research aimed at finding a cure and improving the lives of all children with Ataxia Telangiectasia. You can find the A-T Children's project on the world wide web at http://www.med.jhu.edu/ataxia/index.html.

Clinical Features of AT

Neurologic Features

The presenting sign of AT is nearly always ataxia, usually first noted when the child is learning to walk (4, 9). The ataxia may appear to improve transiently because of increasing skills of the child, but it then continues to slowly progress to severe cerebellar ataxia with a broad based, staggering gait. Ataxia of the head and trunk progress until standing is difficult. Many develop choreo-athetosis. Children with AT generally become wheelchair bound by adolescence, even though muscle strength is preserved. Sensation is also preserved. Dysarthric slurring of speech and drooling occur. During or after the second decade, other neurologic deficits develop as well, including signs of spinocerebellar ataxia and spinal muscular trophy.

The primary neuropathologic finding is progressive loss of Purkinje cells in the cerebellum. It is thought that the Purkinje cells are slowly lost due to cell death. The neurologic abnormalities other than cerebellar ataxia are thought to result from a slower, yet progressive loss of other neurons.

Vascular Features

Telangiectasias usually first appear in the conjunctiva beginning at about age 5 (4, 9). Cutaneous telangectasias generally appear shortly after this. These are often located on the ears, face, and the extremities. The cause of these telangiectasias is not known. The autopsy described by Boder and Sedgwick (4, 9) noted significant arteriosclerotic changes in a ten year old AT patient. In AT heterozygotes, mortality from ischemic heart disease is increased 3.8-4 fold in women and 1.5-1.8 fold in men (10, 11).

Immunologic Features

Most AT patients have defects in both humoral and cell mediated immunity. The result of this is recurrent sinobronchial infections, beginning during childhood. This is the leading cause of death in AT patients.

In the autopsy reported by Boder and Sedgwick (4, 9), there was absence of the thymus. Subsequent autopsies have show either thymic absence, or arrest of thymic development at a very early embryonic stage (12). Consistent with this poor thymic development, AT patients have substantial defects in cell mediated immunity. Mild lymphopenia is common, and is due to reduced numbers of T-cells (13). Most patients have defects in generating cytotoxic T-cells in response to mitogens (13). There is considerable variability in the array of immune deficits among AT patients (14).

Many AT patients have decreased levels of IgA, IgE and IgG (13). It is unclear whether this is a primary B-cell defect or is secondary to the T-cell defects.

Hypogonadism

Female AT patients generally have absent or hypoplastic ovaries, as first reported by Boder and Sedgwick, with frequent amenorrhea (4, 9). Male patients do not have absence of the testes, but do have signs of hypogonadism. They have oligospermia or aspermia often with abnormal morphology. I have not found any report of an AT patient contributing to a pregnancy. Although this does not necessarily imply that they are sterile, it is likely that they at least have a severe reduction in fertility. There has been no evidence for an endocrine cause of the hypogonadism in AT.

Endocrine abnormalities

A significant number of AT patients develop mild diabetes mellitus. AT patients in one family developed severe insulin resistant diabetes with acanthosis nigricans and an antibody to the insulin receptor (15).

Growth Retardation

AT patients are typically smaller than normal in both height and weight. The cause of this growth retardation is unknown. It has been speculated that this results from increased cell death, but this remains to be clarified.

Premature aging, Premature Senescence

Median survival in AT patients is about 20 years (7). However, some survive considerably longer. Their relatively short survival due to infection and cancer makes it difficult to evaluate other signs of aging. However, AT patients do seem to exhibit early graying of the hair and early vascular disease. The signs of premature aging are not nearly as marked as in Werner's syndrome.

Primary cultures of AT cells grow to lower than normal cell density, and senesce after fewer divisions than do normal cells. Some have proposed that telomere shortening is associated with cellular senescence. AT cells have abnormally short telomeres.

Elevation of Embryonic Antigens

Elevated levels of several embryonic antigens occur in AT. Elevation of alpha fetoprotein (AFP) is essentially universal among AT patients, with the exception that it may not appear in very young infants. The presence of elevated AFP with ataxia is considered diagnostic of AT (16). The reason for elevation of AFP is unknown. However, some autopsies have shown unexplained cirrhotic changes with regenerating nodules in the liver. It has been proposed that there is excess cell death which is compensated by liver regeneration. CEA is often elevated in AT.

Increased incidence of Cancer

The first report of Boder and Sedgwick (4, 9) included a patient with a fatal malignancy, probably a lymphoma. It quickly became clear that there is a substantial increase in cancer in AT (17), when they found three cases of lymphoma in the first 101 collected AT patients. The incidence

of cancer in AT is estimated at roughly 15% overall, with a risk of lymphoreticular neoplasms increase nearly 1000 fold. Since many AT patients die in childhood from infection, the risk of cancer may be higher than this figure suggests.

During childhood, the majority of cancers are Hodgkin's disease, non-Hodgkin's lymphomas of both T and B-cell type, and acute leukemias , usually of T-cell type (18).

The frequency of chromosomal abnormalities in increased in lymphocytes from AT patients. Although no single abnormality is seen, frequent sites involved are rearrangements involving sites of T-cell receptor genes and sometimes immunoglobulin genes, that normally undergo rearrangements during maturation of the immune system. It is thought that AT cells have a defect that allows or promotes incorrect gene rearrangements. These rearrangements may well be involved in the increased incidence of lymphomas and leukemias.

There is also an increased incidence of carcinomas, the proportion of which increases with age. A wide variety of sites and histologies are found. This shows that the increased potential for malignancy is not limited to the lymphatic system, but appears to be a feature of all cell types. It is interesting that many families have concordance of cancer incidence, site and histology among siblings (18). For example, one family had three daughters with AT who all developed gastric adenocarcinoma. This raises the possibility of modifying genes that affect the penetrance of the predisposition to malignancy.

Sensitivity to Ionizing Radiation - The DNA Repair Model of AT

The sensitivity of AT cells to ionizing radiation was first appreciated because of a clinical observation that AT patients had untoward reactions from radiation therapy. Two cases of severe reaction to radiation therapy for treatment of malignancy were reported in 1967 (19) and 1968 (20). A third case (the case described earlier in this presentation) was reported in 1975 (1). It is now generally recognized that AT patients should not be treated with radiation therapy. The clinicians involved in the third case considered the possibility that AT cells might be X-ray sensitive. Fibroblast cell lines were obtained from this and other AT patients and found to be profoundly sensitive to killing by X-rays (21). AT cells were also found to be extremely sensitive to chemotherapeutic agents that induce DNA strand breaks, such as bleomycin (22). Such agents should be avoided in treating AT patients. AT cells are, however, not sensitive to all forms of DNA damage. For example, they have a normal level of sensitivity to ultraviolet light.

By analogy to the sensitivity to ultraviolet light in xeroderma pigmentosa, this led to a model in which it was proposed that AT cells were defective in repair of X-ray induced DNA damage. Such a model has been shown to be essentially correct for xeroderma pigmentosa with respect to a defect in repair of uv induced DNA damage (23). Many studies were done testing for DNA repair defects in AT cells. However, no specific repair defect could be consistently identified in AT cells. In fact, the very first paper to demonstrate X-ray sensitivity of AT cells reported that AT cells showed no defect in rejoining DNA strand breaks (21). This makes it unlikely that AT is due to a primary defect in DNA repair.

AT cells are Defective in the S-Phase Cell Cycle Checkpoint

Painter and Young (24) at UCSF, did a simple experiment that led to an important advance in understanding AT. The experiment was to measure the effect of X-ray treatment on the rate of DNA replication in normal and AT cells. AT cells were found to have radioresistant DNA synthesis (RDS). This important result indicated that AT cells are defective in recognizing or responding to X-ray induced DNA damage. It has since been recognized that Painter and Young demonstrated that AT cells are defective in a cell cycle regulatory signaling pathway known as the S-phase checkpoint pathway. This led to the Cell Cycle Checkpoint Model of AT.

Cell Cycle Checkpoints, Genomic Integrity and Cancer

Consider how accurate the replication of the genome must be to avoid significant mutation. The average human adult contains about 10^{13} cells and generates over a thousand times that during a normal life span in renewing tissues (25-27). Thus, a typical human generates over 10^{16} cells. The average mutation rate for a single gene is about 10^{-6} per gene per division. Since we are diploid and

have two copies of most genes, the rate of loss of function by recessive mutation is reduced to 10^{-12} per division. These numbers imply that a substantial number of cells acquire mutations during a normal human lifetime. Any event that increases the rate of accumulation of mutations is expected to lead to an unwelcome result-a malignancy, for example.

Cell cycle checkpoints are a key element of the control mechanism that regulates progression through cell division such that the various steps of cell division are correctly coordinated and so that genomic integrity can be maintained. A cell cycle checkpoint pathway is an intracellular signal transduction pathway that senses the completion of a step of cell division, and transmits a signal resulting in blocking cell cycle progression until the sensed step is completed. Several different checkpoint pathways have been identified that regulate progression from G1 to S, through S, and from G2 to M phases. These checkpoint pathways insure that the events of cell division occur in the correct order. These checkpoint pathways have been found in all eukaryotic cells.

The S-phase checkpoint pathway senses the presence of DNA damage and incompletely replicated DNA and transmits a signal that blocks S-phase progression until the DNA damage is repaired. This pathway has been found in humans and in yeast. If the S-phase checkpoint pathway is defective, then S-phase is not slowed in response to DNA damage. The replication machinery is then free to make its best possible copy of the damaged DNA. Replication through mild DNA damage may result in mutations that are propagated to progeny cells. Replication in the presence of double strand breaks, as are induced by X-rays, would be expected to lead to serious consequences including the possibility of chromosomal translocation or cell death. The accumulation of genetic damage resulting from a defective S-phase checkpoint pathway could lead to activation of oncogenes and inactivation of tumor suppressor genes, resulting in cancer. So, the S-phase checkpoint pathway is critical for maintaining genome integrity and for preventing cancer.

When normal cells are treated with X-rays, an abrupt decrease in DNA synthesis occurs. This reduction in DNA synthesis is dose dependent and biphasic, with a steep slope at lower doses and a lesser slope at increased doses. This slowing of DNA replication is a result of activation of the S-phase checkpoint pathway by DNA damage.

The Cell Cycle Checkpoint Model of AT

Painter and Young (24) showed that AT cells have a greatly decreased ability to slow DNA replication in response to X-ray treatment. The defect is not complete, but the initial steep sloped response is lost. Normal cells block both initiation of replication and elongation of initiated strands in response to X-rays. Evaluation of replicative intermediates shows that AT cells have partially lost the ability to block initiation of replication, and have completely lost the ability to block progression of strands already initiated.

This result suggested that the primary defect in AT cells is not in DNA repair, but in recognition or response to X-ray induced DNA damage. This "Cell Cycle Checkpoint Model" of AT is more consistent with current knowledge than is the DNA Repair Model. It explains why no repair defect could be consistently found in AT cells. It also can better explain the increased in cancers seen in AT. For example, p53, a tumor suppressor gene that is the most commonly mutated gene in human cancer, is required for normal checkpoint control. It is thought that this loss of checkpoint control allows accumulation of genetic damage eventually resulting in cancer. Similarly, loss of the S-phase checkpoint pathway due to mutation of the AT gene would be expected to result in genomic instability, thereby increasing the rate of onset of cancer.

This model may also explain why there is such a preponderance of lymphoid malignancies seen during childhood in AT patients. Normal maturation of lymphocytes involves a DNA rearrangement at either the immunoglobulin locus or the T-cell receptor locus. These rearrangements involve double strand DNA breaks. There is evidence that lymphocytes delay progression through cell division while the DNA rearrangements are occurring, using a cell cycle checkpoint pathway. AT cells are defective in the checkpoint pathway that detects DNA strand breaks induced by X-rays. AT lymphocytes may also be defective in sensing the presence of the DNA strand breaks involved in normal gene rearrangements, resulting in loss of coordination of immune gene rearrangement and cell division. This could result in mitotic entry with broken chromosomes, often leading to cell death and, thereby accounting for the hypoplastic, underdeveloped thymus and cell mediated immune deficits seen in AT patients.

AT Cells are Also Defective in the G1/S and G2/M Checkpoint Pathways

Normal human cells respond to X-rays by arresting at the G1/S transition, in S-phase, and at the G2/M transition. AT cells are unable to arrest at any of these cell cycle locations in response to X-rays and, therefore, AT cells are defective in both the G1/S and G2/M checkpoint pathways as well as the S-phase checkpoint pathway described above. Therefore, AT cells have lost several checkpoint pathways that act to maintain genome integrity.

Arrest at G1/S in response to X-rays requires p53. The involvement of p53 in mediating arrest in S or at G2/M is less clear (28). Irradiation of normal cells in G1 increases p53 activity (by postranslational mechanisms) resulting in transcriptional induction of the p21/Cip1/Waf1 gene and the GADD45 gene. These genes act to block entry into S-phase. p21/Cip1/Waf1 is a direct inhibitor of the cyclin dependent kinases required for induction of the G1/S transition, including cyclin E and cyclin A associated Cdk2 (29) and also blocks DNA replication through direct interaction with the proliferating cell nuclear antigen (PCNA).

AT cells are defective at each step of the G1/S checkpoint pathway. AT cells do not increase p53 activity in response to X-rays. They do not induce p21/Cip1/Waf1 or GADD45. In addition, irradiation of AT cells does not inhibit cyclin E or cyclin A-associated Cdk2 (29). Overexpression of p21/Cip1/Waf1 restores the G1/S checkpoint in AT cells (29). This indicates that the AT gene product must act upstream from p53 in detecting X-ray induced DNA damage in G1. There is evidence both for and against involvement of the p53, p21/Cip1/Waf1 pathway in the S-phase and G2/M checkpoint pathways, but p53 does not appear to be required for arrest in S-phase or at G2/M (28).

The Cell Cycle Checkpoint Model Does Not Explain Some Features of AT

Although the Cell Cycle Checkpoint Model can explain many features of AT, there are important features that it cannot explain. In particular, this model cannot explain several features of excessive cell death in AT. First, the presenting sign of AT is ataxia due to progressive loss of Purkinje cells. Purkinje cells are fully differentiated and are post mitotic. Therefore, a defect in cell cycle checkpoint control should not cause death of these nondividing cells. In addition, some AT patients with brain tumors have, unfortunately, undergone radiotherapy to the brain. These patients have had serious neurologic complications from cranial irradiation that results, at least partly, from death of nondividing neurons. X-ray induced death of nondividing cells is not expected from a defect in cell cycle checkpoint pathways.

It is unlikely that the G1/S checkpoint defect leads to excess cell death in AT. If cell cycle checkpoint defects were the cause of X-ray sensitivity of AT cells, then sensitivity to X-rays should be blocked by holding the cells at a particular cell cycle stage long enough for the damage to be repaired. Several attempts to do this kind of experiment have shown no reduction in X-ray sensitivity of AT cells that are kept from cycling. For example, holding AT cells in G0 for 7 days after irradiation does not improve their survival (30).

Another point suggesting that the checkpoint defects of AT cells are not likely to be the cause of excess cell death is that most irradiated AT cells die before their first postirradiation mitosis. AT cells irradiated in G1 or S-phase progress without delay to G2, where they undergo apoptosis (30, 31). AT cells irradiated in G2 complete the following mitosis without delay (because they lack the G2/M checkpoint) and then go on to arrest at the following G2 where they die. So, the majority of irradiated AT cells die before entering mitosis, indicating that "mitotic catastrophe" is not responsible for their death.

Cells with deletion of p53, which also results in a G1/S checkpoint defect, are not sensitive to X-rays (32). Mice with deletion of p53 are not sensitive to X-rays, do not have ataxia, do not have immune defects, and are not sterile, although they do have a high incidence of cancer. Overexpression of the p53 gene in AT cells corrects the G1/S checkpoint defect, but does not correct the X-ray sensitivity (29). This suggests that another cause for cell death is responsible for the major features of AT, although checkpoint defects are likely responsible for increased cancer incidence.

AT Cells Have a Lowered Threshold for Apoptosis

An important finding that may explain this discrepancy is that AT cells have a lowered threshold for induction of programmed cell death in response to low doses of radiation. Meyn (30) showed that the radiation sensitivity of AT fibroblasts and lymphocytes results from apoptotic death. Substantial apoptosis occurs in AT cells in response to low doses of X-rays that do not induce appreciable cell death in normal cells.

The low threshold for apoptosis may explain several cardinal features of AT. For example, the ataxia may result from progressive apoptosis of Purkinje cells. Consistent with this is the observation that Purkinje cells in AT patients often have histologic features suggesting apoptosis (30). It may explain the marked reduction of cell number in the gonads and thymus. It might explain the cirrhotic changes with nodules of regenerating cells and resulting increased AFP seen in many AT patients.

Increased Apoptosis in AT requires p53

The low threshold of X-ray induced apoptosis in AT cells appears to require p53. Blocking p53 function abolishes the increased X-ray sensitivity and apoptosis in AT cells. Expression of a dominant negative p53 allele, and expression of the human papilloma virus E6 gene both block p53 function as shown by loss of the G1/S checkpoint pathway. Both methods of blocking p53 function reverse AT cell X-ray sensitivity (30).

This result suggests that the AT gene product has another function in addition to signaling in cell cycle checkpoint pathways. It suggests that the AT gene product acts to inhibit a p53 dependent pathway to apoptosis in response to X-ray induced damage, thereby promoting cell survival. So, the AT gene product appears to activate p53 for induction of cell cycle checkpoint control, and block p53 activity for induction of apoptosis. It remains to be determined whether the AT gene product actually does affect p53 in two different ways, or whether both effects may somehow result from a single effect on p53.

The Damage Surveillance Model of AT

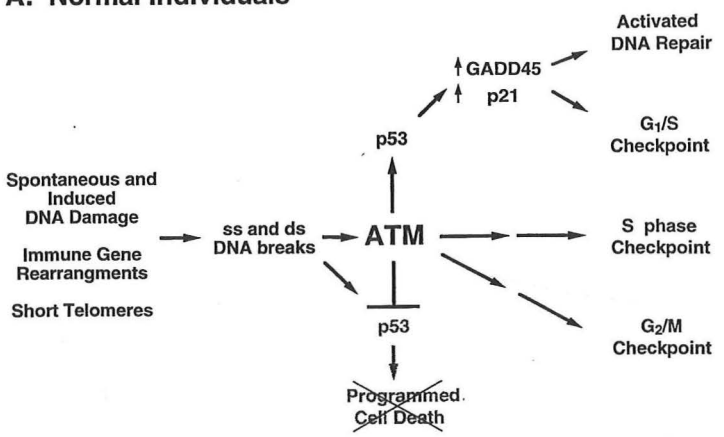
The finding of increased apoptosis in AT has led to a new model called the Damage Surveillance Model (30, 33). This model incorporates all of the Cell Cycle Checkpoint Model and adds the role of the AT gene product in regulating p53 dependent apoptosis. The model proposes that the AT gene product is a central component of a signal transduction network that acts to generate several cellular responses to certain types of damage, including that caused by X-rays. It proposes that ATM acts to sense and/or transmit a signal when such damage is present, thus causing cells to halt in G1, S, or G2 phases, activate repair mechanisms, and suppress apoptotic cell death while repair is being completed. This model is somewhat reminiscent of the SOS system of *E. coli* that mediates response to several types of stress. The model predicts that loss of function of the AT gene product would lead to increased genomic instability because of loss of checkpoint control pathways, and to increased apoptotic cell death in response to spontaneous and ionizing radiation induced damage, and to other inducers of the p53 dependent programmed cell death pathway.

This model explains several features of AT that were not explained by previous models. 1) It explains why both cycling and noncycling cells are sensitive to X-rays. 2) It explains why arresting the cell cycle does not protect AT cells from X-ray induced death. 3) It explains why most irradiated AT cells die before undergoing mitosis.

There is evidence that the rearrangements of the immunoglobulin and T-cell receptor genes in lymphocytes activate cell cycle checkpoint pathways. Presumably, this minimizes the chance of having double strand DNA breaks during S-phase or mitosis, when such breaks would be detrimental. The lack of checkpoint pathways in AT lymphocytes may result in chromosomal rearrangements caused by strand breaks present during replication or mitosis. In addition, the breaks associated with immune gene rearrangement may be enough to activate p53 dependent apoptosis. This excessive death of lymphocytes would explain the marked reduction of the thymus and the defect in immunity in AT.

DNA Damage Surveillance Network Model of AT - from (33)

A. Normal Individuals



B. A-T Homozygotes

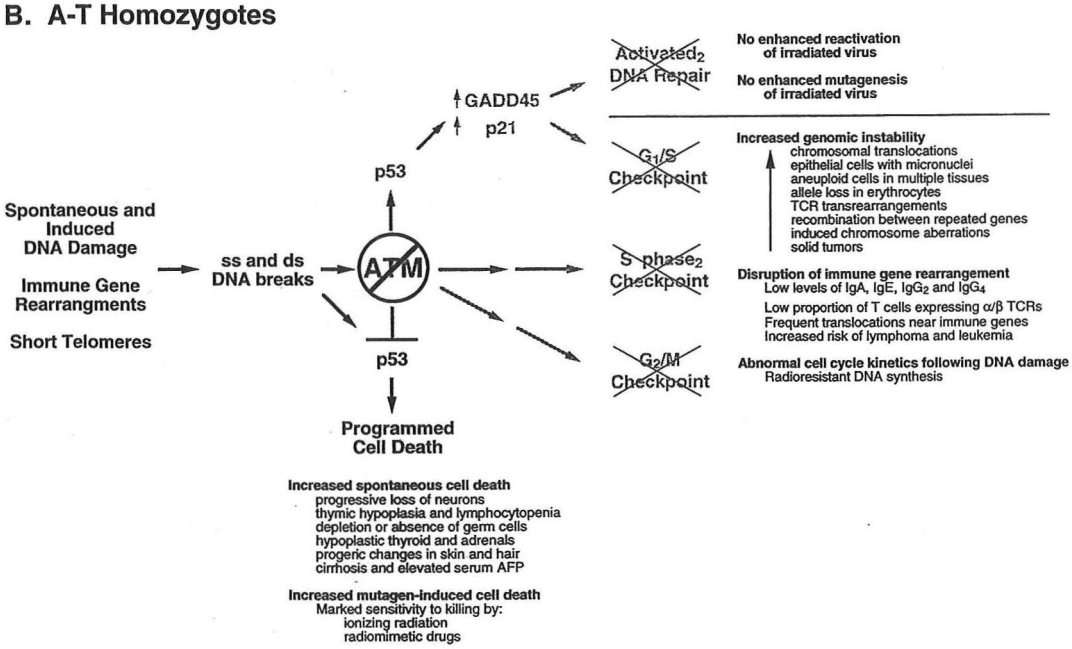


Fig. 1. *A*, a DNA Damage Surveillance Network. As part of this signal transduction network, the ATM protein activates at least five cellular functions in response to the detection of spontaneous or induced DNA damage. In *B*, the DNA damage surveillance network is defective in A-T homozygotes. A-T homozygotes cannot activate ATM-dependent functions in response to DNA damage, resulting in the pleiotropic A-T phenotype.

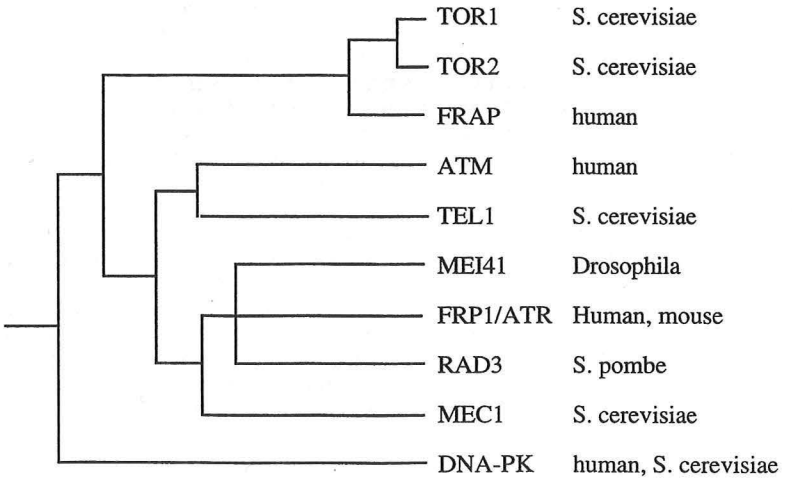
Cloning of the ATM gene

In 1995, a large effort to clone the AT gene by positional cloning identified a gene that was mutated in AT patients (34, 35). Since then, the ATM (**a**taxia **t**elangiectasia **m**utated) gene has been found to be mutated in every AT patient studied. Although cell fusion studies had identified several complementation groups of AT, all patients carry mutations in the same gene. Two patients that had been assigned to different complementation groups carried the exact same mutation. So, for now, there appears to be only one AT gene.

The ATM gene is large, covering 150 kb. Its transcript is spliced to include 66 exons resulting in a 12 kb mRNA encoding a 3056 amino acid, 350 kd protein. The sequence of ATM is very interesting. Its C-terminal region contains sequences conserved in phosphatidyl inositol 3 kinases. ATM is a member of a family of proteins that each contain a PI3-kinase domain. Although it is possible that these proteins do phosphorylate phosphatidyl inositol, such an activity has not been demonstrated. However, several members of this group, including ATM, have been shown to phosphorylate proteins (36). Therefore, it is likely that these proteins are all protein kinases.

All known members of the ATM related family (See below) function in control of cell cycle progression and/or in responding to DNA damage. For example, the DNA dependent protein kinase (DNA-PK) binds to double strand DNA breaks, resulting in activation of its protein kinase activity. DNA-PK is required for immunoglobulin gene rearrangements. Mutation of DNA-PK is responsible for the inability to rearrange immunoglobulin genes in severe combined immunodeficiency (SCID). Such a function is very similar to that predicted by the Damage Surveillance Network Model of AT. DNA-PK has been found in mammals and yeast.

Several members of this family are known to be required for function of cell cycle checkpoints. For example, the S. cerevisiae *MEC1* gene is required for the G1/S, S, and G2/M checkpoints, as is ATM in humans. Similarly, the fission yeast RAD3 is required for checkpoint pathways. These genes appear to act in yeast checkpoint pathways much in the way predicted for ATM in humans. The yeast gene most similar in sequence to ATM is TEL1. Mutations in TEL1 do not disrupt checkpoint pathways, but overexpression of TEL1 can replace the requirement for MEC1 in checkpoint control, so, TEL1 and MEC1 have partially overlapping functions. Mutation of TEL1 leads to shortened telomeres, which have also been described in AT. Phenotypes resulting from mutation of either MEC1 or TEL1 or both are highly similar to the phenotypes of AT cells. Therefore, we have a quite good yeast model of AT.



Phylogenetic Relatedness of Proteins of the ATM Family

Mouse Models of AT

IN the last few months, two groups have reported the results of disruption of the ATM gene in transgenic mice. Barlow et al. (37), reported that mice homozygous for ATM disruption have many features similar to human AT. These mice had growth retardation, hypogonadism and infertility, abnormalities in the development of T-lymphocytes, and extreme sensitivity to ionizing radiation. All of these mice developed highly aggressive thymic lymphomas between 9 weeks and four months of age. The mice had subtle neurologic defects consistent with mild cerebellar dysfunction, although no histologic abnormalities of the brain were seen. All of the cardinal features of AT except for telangiectasia were seen to some degree. It is possible that the telangiectasias and the neurologic abnormalities would have become more evident if the mice were able to survive longer than their lymphomas allowed.

Xu et al. (38) found very similar results of ATM knockout in mice. In addition, they showed that the hypogonadism and infertility of mice homozygous for ATM disruption is due to arrest of meiosis at prophase with abnormal chromosomal synapsis and extensive chromosome fragmentation. Few or no mature germ cells developed in these mice. This indicates that ATM is required for meiosis and its inactivation results in death of germ cells, possibly by an apoptotic mechanism. In addition, these mice have reduced numbers of thymocytes.

Both groups showed that cell lines homozygous for disruption of ATM were extremely radiation sensitive, and that cell cycle checkpoints were defective, as in AT. Xu and Baltimore (39) confirmed that disruption of ATM blocks X-ray induced activation of p53. Xu and Baltimore (39) further showed that irradiation induced apoptosis of thymocytes derived from ATM-disrupted mice.

These mouse models will be extremely valuable for future study of AT. An experiment that will very likely be done soon is to generate a mouse with homozygous deletion of both ATM and p53 by crossing. Prior experiments discussed earlier using different means of blocking p53 function in AT cells showed that blocking p53 function rescued the X-ray sensitivity of AT cells. Thus, transgenic mice with both genes disrupted will probably be much less sensitive to X-rays than AT mice. However, they would be expected to retain a high incidence of cancer.

ATM and ATR Associate with Different Sites in Meiotically Paired Chromosomes

Using antibodies specific for ATM and the related protein, ATR (AT related), Keegan et al (36) studied the location of these proteins in meiotic cells. Their remarkable finding was that both proteins bind to meiotic chromosomes, and that they bind to complementary regions. Meiotic chromosomes undergo synapsis during their recombination in early meiosis. ATM protein was found only at synapsed regions of the chromosomes. ATR was found only at the unsynapsed regions. They propose that both proteins are involved in recognizing and responding to DNA strand breaks that occur during normal meiotic recombination. It remains unclear whether or not these proteins could actually be directly involved in the recombination process, or whether they are solely involved in sensing the presence of the recombination intermediates for signaling in a yet to be characterized meiotic checkpoint control pathway. It has been proposed that p53 acts together in direct association with ATM at the recombination site (40).

The binding of ATM to regions involved in recombination is again reminiscent of the SOS system in E. coli, in which the recA protein is a key element of a damage sensing pathway and also binds to single stranded DNA regions, becoming activated to signal induction of the SOS response.

The cloning of the ATM gene, the recognition of closely related genes in organisms more amenable to genetic analysis than humans, and the generation of mouse models of AT have greatly increased insight into the nature of AT and into the important roles of ATM in maintaining genome integrity, preventing cancer, and preventing apoptosis. It is likely that there will be rapid progress in further understanding of these processes. A lot has changed since last year when it was concluded in Dr. Friedberg's text (23) that the AT field "is a mess".

Increased Cancer Risk in AT heterozygotes

An Example Case (41)

A 27 year old, otherwise healthy woman presented with a breast cancer in the right breast, and was treated with mastectomy. No lymph nodes were involved and she received no adjuvant therapy. Her family history was notable only for breast cancer in her great grandmother. At age 29, she presented with a 3 cm cancer in the left breast. This was removed by lumpectomy and axillary dissection, with no involvement of lymph nodes. Postoperatively, she was treated with radiotherapy to the breast, complicated by moderate erythema within the irradiated field. This was followed by six cycles of cytoxan, methotrexate and 5-fluorouracil, which was tolerated without major complication. Fibroblast and lymphoblastoid cell lines were established from the patient and shown to have moderately increased sensitivity to X-rays, although not nearly to the degree found in AT cell lines. Evaluation of her ATM gene revealed that she was heterozygous for a mutation in which a stop codon caused truncation of the protein within the kinase domain. At the last followup three years after her last treatment, she remained free of disease and without substantial complication from her treatment.

AT Heterozygote Cells have Intermediate Radiosensitivity

Lymphoblastoid cells from AT heterozygotes were first shown to have intermediate sensitivity to ionizing radiation by Chen et al (42), and later by many other groups. However, the radiosensitivity of AT heterozygotes overlapped with normal controls to some extent, making it impossible to use radiosensitivity as a screening test. An assay scoring only cells irradiated in G2 can discriminate AT heterozygotes from normal controls fairly reliably (43). Although AT heterozygotes have increased sensitivity to X-rays, they have not been shown to have abnormalities in cell cycle checkpoint controls. The increased sensitivity to X-rays raised the question of whether AT heterozygotes might be at increased risk for cancer.

Even before it was recognized that AT heterozygotes have increased sensitivity to X-rays, Swift et al (44) showed a significant increase in malignancy among family members of AT patients. This association was highly significant at ages below 45.

A further retrospective family study of 110 families again showed a significantly increased risk for malignancy in AT blood relatives compared to spouses, with an overall increased risk of 2-3 fold (45). The cancer most significantly associated with AT blood relatives was breast cancer, with a relative risk for AT heterozygotes estimated at 7.6. A smaller British study also showed a significant increase in breast cancer incidence among blood relatives of AT patients (46). A small Norwegian study also identified a significant increase in incidence of breast cancer among blood relatives of AT patients (47).

Swift et al (10) went on to do a large prospective study of 1599 blood relatives of AT patients, followed for a mean time of 6.4 years. 821 spouses not related to AT patients were used as controls. 91 new cancers occurred in the AT relatives during the study compared to 19 cancers in the spouses. The overall increased risk of cancer in AT heterozygotes was estimated at nearly 4 fold, and that for breast cancer over 5. Among the AT blood relatives, those with breast cancer were very significantly ($p=0.005$) more likely to have had prior exposure to ionizing radiation compared to those without cancer. The rate of death from ischemic heart disease was also higher in AT relatives, but the difference did not reach statistical significance. Based on these figures and on the estimated frequency of AT heterozygotes (roughly 1% of the population), the authors estimate that nearly 5% of all cancers in people between the ages of 20-79 occur in AT heterozygotes. Because the incidence of breast cancer was higher than other cancers in AT relatives, it was estimated that as many as 7-20% of all cases of breast cancer occur in AT heterozygotes. A further analysis that lumped several of these studies supported an association of increased risk of breast cancer in AT heterozygotes, but suggested that approximately 3.8% (with a range of 1-13 %) of breast cancers occurred in AT heterozygotes (48).

All of the studies described so far were done before the ATM gene was cloned. Now that the gene is available, it will be possible to answer the question of cancer risk in AT heterozygotes

much more accurately. Only one paper has appeared that evaluates breast cancer patients for mutation of ATM. Screening of 38 unselected breast cancers by a PCR-SSCP analysis failed to detect either somatic or germ line mutations of ATM (49). This suggests that neither somatic nor germline mutation contributes greatly to the incidence of breast cancer, and that the lower estimates from the above studies are likely to be more accurate. It is also possible that the assay method was not comprehensive enough to detect all ATM mutations. However, this study is a relatively small sample, and a larger group of patients should be examined before a conclusion is drawn. Hopefully, DNA samples have been kept from the large studies done previously so that the AT relatives can be assigned as normal vs. AT carriers. This would certainly improve the accuracy of information obtained from those studies.

A Function Based Assay for ATM Mutation

Development of an accurate screening method is difficult because of the very large size of the gene. This makes it difficult to detect all mutations. In an analysis of many AT mutations, it has been found that there are a wide variety of different ATM mutations. Thus, there are no common mutant alleles that can be easily identified by screening. In the future, it may be possible to develop simpler function based assays to detect ATM mutations. The strong sequence and functional homology of ATM with yeast TEL1 and MEC1 suggests that it may be possible that ATM can rescue lethality of deletion of the yeast genes. If so, then it would be possible to develop a quick, relatively cheap assay of ATM function in yeast. Because such an assay is based on function, it would likely be more accurate in detecting the presence of significant ATM mutations.

Such an assay would be useful for screening for AT heterozygotes. Studies need to be done to determine whether AT heterozygotes 1) are at risk for developing cancer as a result of diagnostic X-rays, 2) make up a significant proportion of breast cancer patients, 3) have complications from either chemotherapy or radiotherapy, and 4) have different outcomes from other patients when treated for cancer. A rapid function based assay for ATM mutations would greatly aid such studies.

Exploiting ATM Mutations in Treatment of Human Tumors

It is likely that ATM heterozygotes will have a significant incidence of tumors that have lost both ATM alleles, by analogy to other tumor suppressor genes such as p53 and the retinoblastoma gene. Tumors with complete loss of ATM may be extremely sensitive to radiation therapy and chemotherapy. Identification of such AT-like tumors may allow the use of less toxic treatment without compromising results. A function based assay that can be used on human tumor samples would be clinically useful to test this possibility.

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