ATM and the Molecular Pathogenesis of Ataxia Telangiectasia

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Abstract
Ataxia telangiectasia (A-T) results from inactivation of the ATM protein kinase. DNA-damage signaling is a prime function of this kinase, although other roles have been ascribed to ATM. Identifying the primary ATM function(s) for tissue homeostasis is key to understanding how these functions contribute to the prevention of A-T-related pathology. In this regard, because A-T is primarily a neurodegenerative disease, it is essential to understand how ATM loss results in degenerative effects on the nervous system. In addition to delineating the biochemistry and cell biology of ATM, important insights into the molecular basis for neurodegeneration in A-T come from a spectrum of phenotypically related neurodegenerative diseases that directly result from DNA-repair deficiency. Together with A-T, these syndromes indicate that neurodegeneration can be caused by the failure to appropriately respond to DNA damage. This review focuses on defective DNA-damage signaling as the underlying cause of A-T.
ATAXIA TELANGIECTASIA: CLINICAL PRESENTATION

Ataxia telangiectasia (A-T) is an autosomal recessive neurodegenerative disease that results from inactivation of the ataxia telangiectasia mutated (ATM) serine/threonine protein kinase. Individuals in whom this syndrome is fully manifest present with pronounced ataxia early in childhood. The neurodegenerative events that underpin this ataxia are progressive and ultimately cause severe neurological decline. The hallmark neurology is also generally associated with telangiectasia (dilated blood vessels). Although neurodegeneration defines A-T, this disease is multisystemic and also features immune dysfunction, sterility, radiosensitivity, and a pronounced predisposition to cancer (1–4). Elevated α-fetoprotein serum levels are also characteristic of A-T and are a reliable clinical biomarker for the disease.

Immunodeficiency in A-T is variable, but it usually involves decreased or absent immunoglobulin (Ig)A, IgE, and IgG2. However, the immune deficiency is not considered progressive, and frequent infections in A-T are uncommon (5). Cancer occurs in A-T with a lifetime risk of approximately 30%, and such cases are mostly lymphoreticular diseases such as leukemia and lymphoma (6). Children affected with A-T can develop acute lymphocytic leukemia of T cell origin, and leukemia in older A-T patients is usually an aggressive T cell cancer similar to a chronic lymphoblastic leukemia (4, 6). However, B cell disease is also prevalent in A-T (7, 8). ATM mutations in heterozygote carriers are also considered cancer predisposing, and these mutations have been strongly linked to breast and other cancers (9–11). The causes of death in A-T are often diseases of the respiratory system, such as chronic lung disease, or are caused by cancer (5, 12, 13).

Understanding A-T from a molecular pathogenesis perspective remains a central challenge and is required for the rational development of potential therapeutic strategies. There are multiple diseases related to A-T for which the specific biochemical consequence of gene mutations is clear, more so than for A-T. Understanding the etiology of these diseases will provide important additional insights into the ATM functions that prevent A-T.

ATM AND RELATED PROTEINS

ATM is a large (~350-kDa), evolutionarily conserved serine/threonine protein kinase related to a family of proteins with sequence similarity to the phosphatidylinositol 3-kinases (PI3Ks), termed PI3K-like protein kinases (PIKKs). In this family, two other proteins have substantial sequence similarity and related functions to ATM in the DNA-damage response (DDR), namely the ATM- and Rad3-related kinase (ATR) and the catalytic subunit of the DNA-dependent protein kinase (DNA-PKCS) (Figure 1). The primary amino acid sequence of ATM reveals motifs common to the PIKK family, including multiple regions of shared homology (14, 15). ATM is also similar to the mammalian target of rapamycin (mTOR), an important nutrient sensor and regulator of cell growth (16). Although DNA-damage signaling is not a primary function of mTOR, an in vitro functional interaction between this key growth regulator and ATM and oxidative stress has been reported (17, 18).

In many cases, the protein substrates targeted by ATM, ATR, or DNA-PKCS following DNA damage are overlapping, and the modes of activation of all three kinases are also similar (19). However, the essential biological roles of ATM, ATR, and DNA-PKCS are quite different, as demonstrated by the different human diseases resulting from their dysfunction (20–22). Disruption of ATR leads to ATR-Seckel syndrome, which is characterized by neurodevelopmental abnormalities and pronounced microcephaly, whereas disruption of DNA-PKCS function causes an immune system disorder, although without pronounced neural involvement (22, 23). Moreover, inactivation of ATR in the mouse is lethal early in development (24, 25), whereas mice lacking either DNA-PKCS or ATM are viable but have different phenotypic characteristics (26).
In response to DNA damage, ATM undergoes posttranslational modifications involving autophosphorylation at serine 1981, which leads to the dissociation of inactive multimeric ATM to an active monomeric ATM kinase (27). Additionally, ATM is subject to other phosphorylations and acetylation, which may contribute to its functional modulation after DNA damage (28–30). Although these modifications occur in response to DNA damage, mice carrying mutations in either or many of these serine residues fail to show any obvious phenotype that reflects defective ATM signaling, so the biological function of these phosphorylation events is uncertain (31). However, a recent report demonstrated that phosphorylation of ATM at serine 1981 was important for retaining ATM at sites of damage and maintaining DNA-damage signaling (32). It seems likely that posttranslational modification of ATM is a means to fine-tune its activity to modulate the DDR. In response to oxidative stress, ATM forms a disulfide cross-linked dimer via cysteine residue that can activate ATM (see the section titled ATM and Oxidative Stress, below) (33).

Many potential substrates have been identified for the ATM and ATR kinases following DNA damage (many of which are also possible targets for DNA-PKCS), and identifying which of these are central to ATM signaling remains challenging (34). An additional complexity regarding the many substrates for these kinases is the possible tissue specificity associated with ATM signaling.

## ATM SIGNALING AFTER DNA DAMAGE

The ATM kinase functions at the apex of a signaling cascade that responds to DNA double-strand breaks (DSBs) to coordinate cell-cycle arrest, DNA repair, or apoptosis (Figure 2). The outcome of ATM-dependent DNA-damage signaling can be cell and tissue-type dependent. Many ATM substrates are cell-cycle regulators that have essential functions in the response to DNA damage.

![ATM Kinase Diagram](image)

**Figure 1**

ATM (ataxia telangiectasia mutated) is related to other DNA-damage response kinases. ATM is a member of the phosphatidylinositol 3-kinase–like protein kinases (PIKK) family of kinases, which includes ATR (ataxia telangiectasia and Rad3 related) and DNA-PKCS (catalytic subunit of the DNA-dependent protein kinase), all of which are involved in DNA-damage signaling (2). ATM, ATR, and DNA-PKCS are subject to posttranslational modifications that modulate their activity. In the case of ATM, DNA damage induces serine phosphorylation (P) at various residues (most notably serine 1981) and acetylation (AC) of lysine 3016. Common protein motifs in these kinases include FAT (FRAP/ATM/TRAPP), FATC (FAT–C terminal), and a kinase domain. Additionally, each protein has an N-terminal region that recruits a binding partner that facilities activation (19). Other, related proteins exist that have different cellular functions that are not linked to the DNA-damage response. These proteins include SMG1 (suppressor of morphogenetic effect on genitalia), which is involved in nonsense-mediated RNA decay; TRRAP (transformation/transcription domain–associated protein), which is associated with the histone acetyltransferase complex; mTOR (mammalian target of rapamycin), which is involved in cellular nutrient-level sensing; and the lipid kinase PI3K (phosphatidylinositol 3-kinase), which modulates growth factor signaling.

Notably, many of these cell-cycle regulators, including p53, BRCA1, and CHK2, are tumor suppressors, which highlights the important link between ATM signaling, genome stability, and cancer. Many reviews provide a detailed discussion of ATM substrates and the DNA damage–activated signaling pathways that are modulated by this kinase (14, 15, 35–37). Although a broad spectrum of protein substrates involved in multiple cellular pathways have been ascribed to ATM (34), the response to DNA damage appears to be the primary, if not the definitive, in vivo function of this kinase in relation to the phenotypes present in A-T.

DNA can undergo various types of DNA damage (e.g., strand breaks, base modifications,
ATM (ataxia telangiectasia mutated) is activated by DNA damage in an MRN (Mre11-Rad50-Nbs1)-dependent manner. DNA damage promotes MRN localization to the break site and activation of ATM from an inactivated dimer to an active monomer. This activation is associated with multiple posttranslational modifications of ATM, including phosphorylation of serine 1981 (27–32). Various additional factors may modulate ATM activation; they are linked to chromatin modifications or ubiquitination and include mediator of DNA damage and checkpoint control 1 (MDC1), p53-binding protein 1 (53BP1), Krüppel-related factor 1 (KAP-1), the SUMO (small ubiquitin-like modifier) E3 ligase, protein inhibitor of activated STAT (PIAS1/4), ring finger 8 (RNF8), checkpoint protein with FHA and ring domain (Chfr), structural maintenance of chromosomes 1 (SMC1), and Fanconi anemia group D2 (FANCD2). Activated ATM can potentially phosphorylate a large number of different proteins (34). ATM substrates are known to be functionally important for cell-cycle arrest, apoptosis, and DNA repair. In some cases, ATM substrates have multiple cellular roles such as p53 and Chk2, which can invoke cell-cycle arrest or apoptosis, depending upon the cell type and levels of genome damage. Collectively, the signaling pathways controlled by ATM serve the essential biological roles of preventing neurodegeneration, immune deficiency, sterility, and cancer. Abbreviation: TDP1, tyrosyl-DNA-phosphodiesterase 1.

helix-distorting lesions, and strand cross-links), and cells activate specific biochemical pathways depending upon the type of damage; these distinct biochemical pathways reflect the need to address various forms of DNA damage to maintain cellular homeostasis (21, 35, 39, 38). Much has been learned about these different pathways, largely on the basis of work...
investigating a spectrum of human diseases, including A-T and related disorders, that show differential sensitivity to different forms of DNA damage. For example, although A-T cells are particularly sensitive to agents that cause DNA DSBs, such as ionizing radiation, they are not abnormally sensitive to UV irradiation damage. This DNA-repair pathway, nucleotide excision repair, is essentially normal in A-T cells (40). In contrast, individuals with xeroderma pigmentosum, in which the nucleotide excision–repair pathway is affected, are hypersensitive to UV but not to ionizing radiation (41, 42).

DNA damage that induces DNA DSBs is a potent activator of ATM, which depends upon a DNA damage–sensing heterotrimer termed the Mre11-Rad50-Nbs1 (MRN) complex (37, 44, 43). This complex is integral to both the direct activation of ATM signaling following DNA damage via an Nbs1 interaction and DNA DSB repair (19, 37, 45–49). Other components are also required for full ATM activation after DNA damage; these include the E3 ubiquitin ligases, ring finger protein 8, and checkpoint protein with FHA and ring domain (50). In addition to ATM activation, the MRN complex possesses DNA end-resection properties via an endogenous nuclease activity of Mre11 and the modulation of C terminal–binding protein–interacting protein (CtIP) function through interaction with Nbs1 (51). The binding of MRN to DNA ends induces a conformational change that leads to the extension of flexible Rad50 coiled coils and a zinc-hook structure to form an intercomplex tether between separate MRN complexes bound to opposing DNA strands (52–54). Tethering broken DNA ends provides a means for the recruitment of ATM by the MRN complex in a manner dependent upon Nbs1 (47, 55). Localization of ATM to DNA damage depends upon interaction of the ATM C terminus with Nbs1; other PIKKs also use this mode of recruitment to DNA damage. In the case of ATR the binding partner is ATRIP, and in the case of DNA-PKcs it is KU, which highlights conserved modes of DNA-damage activation (19).

The initial response to DNA damage by MRN activation is central to the functions of ATM that prevent A-T. Mutations that partially disable the function of Mre11, Nbs1, or Rad50 can lead to human syndromes that are characterized by marked neuropathology and other features reflective of A-T (56–60). Most notably, hypomorphic mutation of Mre11 can cause a neurodegenerative syndrome with A-T-like features, whereas mutation of either Nbs1 or Rad50 causes marked neuropathology characterized by microcephaly, although the non-neurological features of this disorder are similar to those of A-T (Table 1) (56, 58, 60). Structural analysis of Mre11 indicates that the hypomorphic mutations leading to A-T-like disorder (ATLD) result in impaired Mre11-Nbs1 binding (61). The different neuropathology present in ATLD and Nijmegen breakage syndrome (NBS) has been linked to the relative ability of the mutant MRN complex to activate ATM-dependent apoptosis following DNA damage during neural development (48). Recently, specific Mre11 mutations were found to result in microcephaly rather than neurodegeneration, which underscores the various functional impacts that different hypomorphic mutations can confer toward the MRN complex (62).

Once activated, ATM can phosphorylate multiple cell-cycle regulators to promote cell-cycle arrest at each stage of the cell cycle, which prevents replication of damaged genomic DNA (14, 15). Such arrest allows for DNA repair, a process whose efficiency is controlled by ATM (see the next section). Alternatively, and of particular relevance in the nervous system, DNA damage can result in ATM-dependent apoptosis, although this outcome depends upon the developmental stage and cell type (63–66). Whereas apoptosis eliminates DNA-damaged progenitor cells and ensures the genomic integrity of developing tissue, in ATM-null mice DNA damage fails to activate apoptosis in specific neural regions, which leads to the incorporation of genomically damaged cells into the developing nervous system (63, 64, 66). Thus, by activating either cell-cycle
Table 1  Comparison between human DNA repair–deficiency syndromes

<table>
<thead>
<tr>
<th>Repair syndromea</th>
<th>Defective gene</th>
<th>Nervous system defect</th>
<th>Other phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia (A-T)</td>
<td>ATM</td>
<td>Neurodegeneration</td>
<td>Immune deficiency, telangiectasia, sterility, cancer</td>
</tr>
<tr>
<td>A-T-like disorder</td>
<td>MRE11b</td>
<td>Neurodegeneration (coincident microcephaly can occur with neurodegeneration)</td>
<td>Noneb</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome (NBS)</td>
<td>NBS1b</td>
<td>Microcephaly</td>
<td>Immune deficiency, sterility, cancer</td>
</tr>
<tr>
<td>NBS-like syndrome</td>
<td>RAD50b</td>
<td>Microcephaly</td>
<td>None</td>
</tr>
<tr>
<td>Ataxia with oculomotor apraxia 1</td>
<td>APTX</td>
<td>Neurodegeneration</td>
<td>Noneb</td>
</tr>
<tr>
<td>Spinocerebellar ataxia with axonal neuropathy 1</td>
<td>TDP1</td>
<td>Neurodegeneration</td>
<td>None</td>
</tr>
<tr>
<td>Microcephaly with early onset; seizures and developmental delay</td>
<td>PNKP</td>
<td>Microcephaly</td>
<td>None</td>
</tr>
<tr>
<td>Lig4 syndrome</td>
<td>LIG4b</td>
<td>Microcephaly</td>
<td>Immune deficiency, growth retardation, radiosensitivity</td>
</tr>
<tr>
<td>ATR-Seckel syndrome</td>
<td>ATRb</td>
<td>Neurodevelopment</td>
<td>Growth retardation</td>
</tr>
</tbody>
</table>

*a*Representative (noncomprehensive) inherited syndromes of DNA strand-break repair–deficiency syndromes are compared with A-T.

*b*Mutations in these genes are hypomorphic, as complete inactivation results in developmental lethality.

*c*Lung tumors have been reported in two siblings (57).

*d*Although overt extraneurological phenotypes are absent, hypoalbuminemia and hypercholesterolemia are associated with these syndromes.

arrest or apoptosis, ATM maintains genome integrity, which contributes to the prevention of the spectrum of defects observed in A-T.

ATM AND DNA REPAIR

The profound radiosensitivity present in A-T both in vivo (which affects the gastrointestinal and hematopoietic compartments) and in vitro in A-T-derived cells suggests that ATM is required for DNA repair. This notion is also supported by the propensity of A-T cells to undergo chromosomal instability (1, 4, 15, 40). Notably, other inherited neurodegenerative syndromes, such as Fanconi anemia and xeroderma pigmentosum, which are sensitive to DNA cross-linking agents and UV, respectively, manifest a striking deficiency in DNA repair (42, 67). However, the situation with A-T is less certain, and A-T research has historically been controversial regarding DNA-repair deficiency as a primary cellular defect. Multiple reports during the 1970s and 1980s provided conflicting results as to DNA-repair defects in A-T and as to whether these repair defects contribute to the pathology of the disease (40).

However, recently it has become clear that ATM is important for the repair of a subset of DNA breaks (68, 69). Such repair involves a fraction (∼10%) of breaks observed following ionizing radiation–induced damage, which are repaired with slower kinetics that are specifically associated with heterochromatin (70). ATM is important for the repair of these breaks via its ability to phosphorylate KRAB-associated protein 1 (KAP-1), which causes chromatin relaxation that provides accessibility for repair factors (72). However, Purkinje cells, a target population of the cerebellum that is lost in A-T, contain a mostly euchromatic nucleus and do not have an elaborated heterochromatin composition (73). In contrast, another cerebellar...
population that is also lost in A-T, granule neurons, do have heterochromatin (73), which implies that this aspect of ATM function may be relatively more important in specific cerebellar cell types.

ATM can also directly affect DNA repair through the phosphorylation of specific repair factors. For example, tyrosyl phosphodiesterase 1 (TDP1), which functions to cleave topoisomerase 1–DNA adducts during transcription and DNA repair, can undergo phosphorylation by ATM at serine 1981 to enhance its ability to repair DNA breaks (74). In response to oxidative lesions, ATM can dephosphorylate DNA ligase IIIα, which suggests a potential role in modulating base excision repair (75). Other components involved in DNA repair, such as aprataxin and polynucleotide kinase–like factor, which is implicated in both single-strand break (SSB) repair and nonhomologous end joining, are also targets of ATM kinase activity (76–78).

Homologous recombination (HR) is a principal DNA-repair pathway that deals with DNA DSBs. Many groups have linked ATM to HR, and evidence that ATM can phosphorylate key repair factors such as CtIP further supports a role for ATM during HR (79–83). However, if ATM had a major controlling influence upon this pathway, then deletion of ATM would be lethal, as is the case for inactivation of important key HR factors (e.g., Brca2 and Mre11) (26). More likely is that ATM participates in this pathway via its ability to activate cell-cycle arrest or to phosphorylate substrates associated with nonessential HR repair functions. Alternatively, ATM may function during HR in a restricted tissue-specific manner, such as meiosis in the testis, because loss of ATM leads to sterility due to early defects in meiosis during prophase 1 (84).

In more specialized scenarios, such as V(D)J recombination in the immune system, ATM, together with other factors such as H2AX and XLF, fulfills an important role in the processing and joining of DNA ends (80, 85, 86). In such settings, ATM also ensures that lymphocytes with accumulated DNA damage, which can arise from aberrant gene rearrangements, are effectively eliminated (87). Genetic manipulation of the mouse germ line also strongly supports an important role of ATM during DNA repair. For example, synthetic lethality arises early during embryo development, when coincident genetic inactivation occurs between ATM and various DNA-repair/damage-response factors, including H2AX and DNA-PKcs (88, 89).

Thus, the above evidence indicates that ATM is involved in DNA repair, which suggests that DNA-damage accumulation may contribute to A-T. Importantly, endogenous damage occurs in many target tissues during development; these tissues include the nervous system, the thymus, and the ovary and testis. Efficient DNA repair is required for neurogenesis, as humans with attenuated DNA repair or mice with engineered mutations in DNA-repair factors show striking defects in neurogenesis (21, 26, 39). In the following sections, I discuss the hallmark neurodegenerative feature of A-T and consider how related syndromes caused by direct mutation of DNA-repair enzymes that have similar neuropathology to A-T help us understand how defective ATM signaling leads to neuropathology.

**NEURODEGENERATION IN ATAXIA TELANGIECTASIA**

The neurodegenerative phenotype of A-T is the cardinal aspect of the disease, and the following sections focus on the molecular pathogenesis of this feature. In A-T, the neurodegeneration is progressive and spino cerebellar in nature, and it usually becomes apparent between 6 and 18 months of age. Initial presentation consists of truncal swaying and gait ataxia, which usually results in confinement to a wheelchair by the first decade of life. Patients with A-T manifest hallmarks of cerebellar dysfunction such as dysmetria, muscle hypotonia, truncal swaying while sitting or standing, and sudden falls (3). Also present is oculomotor apraxia, which involves eye-movement defects, strabismus, pursuit abnormalities, hypometric saccades, and sometimes nystagmus.
As discussed below, many of the neurological features of A-T are also characteristic of other syndromes that arise from DDR defects.

Atrophy of the cerebellum, particularly the vermis, is a key feature of A-T and is evident upon magnetic resonance imaging and computed tomography imaging (91–93). Although Purkinje cell loss is a hallmark feature of A-T, granule cell loss is also very extensive and, on the basis of relative cell numbers, is an important underlying reason for the cerebellar atrophy (1, 94). Because granule neurons represent the most abundant neuronal type in the nervous system and contribute substantially to the bulk of the cerebellum, their loss has a profound effect on cerebellar function. Granule cells also influence Purkinje cell dendrites, and loss of parallel fiber synapses can lead to less-branched Purkinje dendrites; similarly, Purkinje cell loss can also affect granule cell neurogenesis (95, 96). In A-T, Purkinje cells have less complex arborizations and are often localized ectopically in the molecular layer of the cerebellum (97). The abnormal localization also suggests that an early developmental defect is present in A-T, as spatiotemporal localization of Purkinje neurons occurs relatively early during genesis of the nervous system (98). Widespread neurodegeneration also occurs in other regions of the nervous system, although these instances are more variable and can occur at later stages (3). For example, peripheral nervous system defects may contribute to muscle hypotonia and swaying, and peripheral neuropathy is present in A-T (3, 26, 99). Consistent with peripheral nervous system defects in A-T, high levels of ATM are also present within postmitotic sensory neurons in the dorsal root ganglia (100).

ATAXIA TELANGIECTASIA VARIANTS

There is variation in the clinical spectrum of A-T; individuals with certain ATM mutations have a less debilitating version of A-T. Such variants are important for our understanding of the severity and progressive nature of the disease, and how the phenotypic spectrum relates to specific mutations in ATM. Initial studies examined A-T individuals in whom the usual clinical phenotype varied; for instance, some patients had no immunodeficiency or had milder neurological presentation or radiosensitivity (4, 101, 102). In these individuals, mild ATM mutations, such as missense rather than truncating mutations, were present, which led to the production of reduced amounts (between 1% and 17%) of functional ATM protein (101). In cases of classic A-T, there is usually no protein made, and kinase activity is absent. Therefore, A-T variants provide a genotype-phenotype relationship for understanding how specific ATM mutations contribute to A-T.

Recently, a comprehensive analysis examined the clinical history and progression of the disease in 13 A-T variants (103). In this study, all A-T variants at initial diagnosis presented with a milder spectrum of disease, showing choreoathetosis or resting tremor; in stark contrast to classic A-T, many of these patients were not confined to a wheelchair as adults. Importantly, in these A-T variants, the causative ATM mutations were missense mutations or splice-site mutations, rather than protein truncations that result in full ATM inactivation typical of classical A-T. These milder mutations resulted in attenuated functional readouts rather than full abrogation of ATM signaling. Whereas truncating mutations can occur in A-T variants, in these situations truncations involve the last few C-terminal amino acids (101, 103). Overall, although the A-T variants were still cancer prone, ataxic, and radiosensitive and had elevated AFP levels, the telangiectasias, oculomotor apraxia, and endocrine issues were markedly reduced, so these systems may be relatively more resistant to ATM loss. However, with age the A-T variants generally developed cerebellar ataxia, indicating that ATM function continues to be important for the maintenance of the mature brain and other tissues. Thus, these findings indicate that A-T is a primary developmental defect that is exacerbated by ongoing neurological decline, which reflects an important postdevelopmental role for ATM function.
Table 2  DNA repair–deficiency syndromes with similar neuropathology to ataxia telangiectasia (A-T)

<table>
<thead>
<tr>
<th></th>
<th>A-T</th>
<th>Ataxia with oculomotor apraxia 1 (AOA1)</th>
<th>Ataxia with oculomotor apraxia 2 (AOA2)</th>
<th>Spinocerebellar ataxia with axonal neuropathy 1 (SCAN1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene function</td>
<td>ATM, a serine/threonine protein kinase that is activated following DNA damage</td>
<td>Aprataxin, a nucleotide hydrolase that processes 5′ DNA adenylation products during DNA strand-break repair</td>
<td>Senataxin, a helicase that participates in DNA repair¹</td>
<td>Tyrosyl-DNA phosphodiesterase 1 participates in DNA repair by removal of 3′ topoisomerase adducts or oxidative DNA end modifications</td>
</tr>
<tr>
<td>Average age of onset (years)</td>
<td>&lt;2</td>
<td>2</td>
<td>&gt;10⁰</td>
<td>&gt;10⁰</td>
</tr>
<tr>
<td>Ataxia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Oculomotor apraxia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes⁰</td>
<td>No</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cancer</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Extraneurological features</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

¹The exact function of this helicase during DNA repair remains to be determined.
²The diseases have a later onset than do A-T and AOA1.
³This feature is variable in AOA2.

DNA SINGLE-STRAND BREAK REPAIR AND ATAXIA TELANGIECTASIA–RELATED DISEASES

There is a spectrum of syndromes with phenotypic similarity to A-T that provide key clues to the causative defect in A-T. These diseases arise from mutations of specific enzymes involved in DNA SSB repair that function to modify the termini of damaged DNA strands for successful repair (Table 2). Importantly, in SSB-repair deficiency diseases, the only functions ascribed to the defective protein are DNA-damage processing and DNA repair, which provides compelling evidence that DNA damage is a probable etiologic agent in A-T. Together with the identification of mutations in the MRN complex, which cause attenuated ATM function that leads to NBS and ATLD, these syndromes collectively indicate that a failure to maintain genome stability accounts for the neuropathology found in classic A-T.

Defects in SSB repair are associated with ataxia with oculomotor apraxia 1 (AOA1) and spinocerebellar ataxia with axonal neuropathy (SCAN1), neurodegenerative syndromes that are similar to A-T (Table 2) (104–109). In fact, the neurological presentation of AOA1 is almost identical to that of A-T and caused AOA1 to be initially classified as an A-T variant (110). Although the phenotypes associated with AOA1 and SCAN1 are similar, oculomotor apraxia is absent from SCAN1 (107, 111). Notably, SSB-repair defects lead almost exclusively to neuropathology without the extraneurological phenotypes associated with A-T or other DSB-repair deficiency syndromes. This nervous system selectivity is probably due to the availability of backup DNA-repair pathways for SSB repair in proliferating and other tissues (112).

DNA SSBs are very common DNA lesions and can occur as a result of direct effects of reactive oxygen species or indirectly as...
an intermediate during repair of damaged bases (38, 113). The repair of a damaged base involves DNA glycosylases, which are endonucleases that initiate base excision, followed by recruitment of repair factors via XRCC1 and poly-ADP-ribose polymerase, end modification by specific repair enzymes, and gap-filling by DNA polymerase and DNA ligation (38, 113, 114). The genes mutated in AOA1 and SCAN1 are aprataxin (APTX) and tyrosyl–DNA phosphodiesterase 1 (TDPI), respectively. TDPI repairs altered 3′ DNA ends arising from oxidative damage and topoisomerase 1–DNA covalent complexes (38, 112, 115–119). APTX is a member of the histidine triad superfamily of nucleotide hydrolases and possesses an AMP–lysine hydrolase activity, which is required for the repair of 5′ AMP intermediates that arise from failed DNA-ligation reactions (108, 120, 121). Additionally, congenital microcephaly can result from DNA-repair deficiency associated with defective DNA SSB repair, or other repair pathways that utilize polynucleotide kinase phosphatase, which implies that in these cases cell loss occurs during development rather than postnatally (39, 122). This finding suggests that some DNA-repair factors within the SSB-repair pathway are relatively more important in proliferating neural progenitors than are APTX or TDPI, which provide important functions to nonreplicating neural cells. These SSB-repair deficiency syndromes underscore the unique susceptibility of the nervous system to DNA damage and reinforce how defective DDRs can cause an A-T-like phenotype (110).

ATM AND OXIDATIVE STRESS

A-T has long been linked to altered oxidative stress, and this feature is often noted as both a cause and a consequence of the disease. Early reports showed that markers for oxidative stress such as protein nitrosylation, increased thiol conjugates, and lipid peroxidation were present in A-T cells and tissues (123–127). Speculation regarding how increased oxidative stress occurs in A-T has considered various perturbations such as changes in nicotine adenosine dinucleotide levels following ATM loss (128). Although the basis for this phenomenon remains unclear, recent data have provided some possible clues. For example, a potential role for ATM in the control of an antioxidant response via the pentose phosphate pathway has been reported and may be relevant in ATM null tissues showing increased oxidative stress (129). ATM loss also increases oxidative levels in hematopoietic stem cells, which prevents these cells’ reconstitutive capacity via increased \( p16^{INK4a} \) expression (130).

It was recently proposed that ATM functions as a redox sensor and, as such, may regulate global cellular responses to oxidative stress. In specific ATM activation by oxidative stress, a disulfide bridge is formed between cysteine (C2991) residues in an ATM dimer (33). Generation of an active ATM dimer by oxidative stress contrasts with the mechanism of DNA-damage activation, in which an active monomer forms from an inactive dimer (27). Importantly, this mode of ATM activation can occur independently of the MRN complex, suggesting a role for ATM in signaling other than direct DNA damage.

The potential importance of oxidative stress–induced activation of ATM was highlighted by a mutant allele of ATM (R3047X) that occurred in an A-T patient. This allele led to ATM with a C-terminal truncation characterized by an in vitro biochemical phenotype similar to that of the C2991L mutant (33); because the C2991 residue is still present in the variant form of ATM, the proximity of the deletion to this key cysteine probably disturbs the required ATM architecture for sensing oxygen stress. This result suggests functional separation in ATM, whereby oxidative stress (\( \text{H}_2\text{O}_2 \) treatment) failed to activate the R3047X ATM, whereas activation by DNA damage still occurred. Overall, these data suggest that oxidative stress is a key etiological agent for A-T, as individuals homozygous for the R3047X mutation develop A-T. However, other reports indicate that A-T individuals with the R3047X mutation have defective DDRs...
and are sensitive to DNA-damaging agents (101, 131).

In considering DNA damage as an etiological agent, it is noteworthy, as discussed above, that specific defects in DNA end-processing during repair can lead to a neural phenotype that is nearly identical to that of A-T. This finding indicates that defective DNA-damage processing is sufficient to account for neurodegeneration in A-T. Oxidative stress–induced activation of ATM may be an additional means of monitoring DNA-damage signaling; clearly, oxidative stress gives rise to increased oxygen radicals that could promote DNA damage. Thus, ATM dimers may become active independent of MRN to provide an additional rapid DDR. Various pools of ATM may also be apportioned throughout the nucleus, with some available for oxidative activation, a feature that may be important at specific developmental stages or in certain cell types. Clearly, further analysis of the importance of oxidative stress–induced activation of ATM will illuminate the possible contribution of this feature toward specific aspects of the A-T phenotype.

NONNUCLEAR FUNCTIONS OF ATM

Although ATM is predominantly nuclear, data suggest that there may be functions for ATM outside the nucleus, and the localization of ATM in the cytoplasm has long held investigators’ attention. Various functions have been ascribed to this cytoplasmic pool of ATM, including peroxisome function, vesicular functions, and synaptic signaling roles (132–134). ATM’s presence in the cytoplasm suggests a function outside of DNA repair. However, conclusive functions for the cytoplasmic pool are elusive. Given the clear links between DNA damage–signaling defects and A-T, it seems unnecessary to invoke cytoplasmic signaling (independent of DNA damage) as an important feature of A-T. A potential issue concerning the findings of cytoplasmic functions of ATM is that most biochemical data derive from cell lines. Analysis of these findings in an in vivo setting will be important for our understanding of the contribution of cytoplasmic ATM in tissue homeostasis.

ATM relocalization to the cytoplasm is a feature of nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) activation following DNA damage (135). NF-κB plays an important role in the modulation of apoptotic responses following genotoxic stress, at least in some settings, such as the immune system (135–138). In response to genotoxic stress, ATM phosphorylates NF-κB essential modulator (NEMO), resulting in nuclear export of an ATM-NEMO complex that interacts with IκB kinase in the cytoplasm to activate NF-κB signaling (138). This scenario involves ubiquitination of another IκB kinase activator, ELKS (protein rich in glutamate, leucine, lysine, and serine), which is critical for TAK-1 (TGF-β-activated kinase)-mediated activation of IKK (IκB kinase) and subsequent NF-κB signaling (139, 140). Also, ATM is central to the modulation of NF-κB-dependent transcription during in vivo lymphocyte development, when antigen receptor gene assembly generates endogenous DNA breaks (136). Additional studies will determine how broadly ATM-dependent activation of NF-κB features in the biology of ATM signaling.

A-T is associated with insulin resistance and metabolic syndrome, which are often present in this disease. ATM has been reported to participate in insulin signaling via the ability to phosphorylate eIF-4E-binding protein 1 (4E-BP1), and ATM-deficient cells are defective in the ability to dissociate 4E-BP1 from eIF-4E following insulin treatment (141). More recently, studies using ATM-deficient mouse models showed that ATM loss worsens the features of the metabolic syndrome, increases insulin resistance, and accelerates atherosclerosis in ApoE<sup>−/−</sup> mice. These results suggest that ATM may mediate susceptibility to the metabolic syndrome and modulate insulin resistance. Although ApoE is generally considered a protein targeted for secretion with a nonnuclear localization, a nuclear role was recently suggested in some cell types, particularly...
after stress (142). Therefore, the involvement of ATM in metabolic syndrome may nonetheless reflect its nuclear function. Along these lines, a nonnuclear/nonrepair role for ATM may be considered in the context of the related protein DNA-PKcs, which has been ascribed a key role in fat metabolism (143); this hypothesis suggests that, by analogy, ATM could perform similar nonrepair functions related to its role in metabolic syndrome in A-T.

A cytoplasmic function, separate from the nuclear DNA damage–response function, was recently reported for ATM in the response to reactive oxygen species to repress mTOR complex 1 (mTORC1) and autophagy via activation of the tuberous sclerosis complex 2 tumor suppressor (15). In this study, cytoplasmic ATM activated tuberous sclerosis complex 2 via LKB1 and AMPK to inhibit mTORC1. Additionally, ATM was recently shown to downregulate mTORC1 signaling under hypoxic conditions by phosphorylation of hypoxia-induced factor (16).

ATM deficiency has also been linked to defects in mitochondrial function (144, 145). Defective mitochondrial function has been identified in ATM-deficient cells and tissue associated with ribonucleotide reductase dysregulation, which resulted in perturbed mitochondria homeostasis (145). This possibility is intriguing because the mitochondria have their own genome, and ATM may also monitor the genomic status of this organelle. Moreover, defects associated with mitochondrial DNA can cause a spectrum of human syndromes, many of which are characterized by neurological and many other defects (146).

**HOW DOES ATM LOSS RESULT IN ATAXIA TELANGIECTASIA?**

ATM is a protein kinase that is activated by specific DNA damage, such as DNA DSBs. Activated ATM phosphorylates substrates that are important in orchestrating the DDR (14, 15). Mutations in ATM lead to the childhood neurodegenerative syndrome A-T, which is also characterized by cancer proneness, telangiectasia, immunodeficiency, sterility, and extreme radiosensitivity (1, 2, 4). Cells isolated from individuals with A-T show defective DNA-damage signaling, are radiosensitive, and manifest genomic instability. Nonetheless, controversy about the etiology of A-T remains, at least in part because of the multitude of apparently diverse functions ascribed to ATM. However, DNA damage–signaling defects probably account for the characteristic phenotype of A-T. This phenomenon is illustrated by ATLD and NBS, syndromes that are similar to A-T and that occur when the MRN DNA damage–sensing complex and upstream ATM activator are mutated (37, 59). The phenotypes of these syndromes overlap with that of A-T and arise from defective DNA-damage signaling due to attenuated ATM activation. Moreover, in syndromes such as AOA1, near-identical neuropathology to A-T is present and results from the inactivation of specific DNA strand break–repair factors that are important for DNA SSB repair (21, 38). More generally, a multitude of other DNA repair–deficiency syndromes show broad neurological involvement, underscoring the profound sensitivity of the nervous system to DNA damage (21). On the basis of data from mouse A-T models, neurodegeneration in A-T may result from accumulated DNA damage during development that becomes fixed in the nervous system, and ongoing DNA damage may require ATM to ensure efficient DNA repair (48, 64, 66). Cell loss leading to neurodegeneration may result from excessive unrepaired DNA damage that directly results in apoptosis, or such damage may activate the cell cycle (147).

Other phenotypic features of A-T can also be attributed to abnormal events after DNA DSBs. The immune dysfunction reflects the need for ATM during the physiological DNA breaks that facilitate rearrangement during V(DJ) recombination, and known meiotic chromosomal recombination defects account for sterility. Furthermore, the cancers that occur in A-T, primarily leukemia and lymphoma, often involve aberrantly rearranged T cell receptor loci, which arise during normal T cell...
maturation, coupled with the failure to eliminate lymphocytes containing proto-oncogenic rearrangements (87).

The intense interest in deciphering ATM biochemistry has unraveled other, various ATM functions. This ever-increasing list of potential cellular functions suggests possibilities other than DNA-damage signaling that may contribute to the phenotypic spectrum of A-T. Although a non–DNA damage role in the nervous system seems highly improbable on the basis of the above argument, some phenotypic aspects of A-T may arise independently from faulty DNA-damage signaling. These features may be diabetes associated with A-T or, in the vasculature, anomalies linked to telangiectasia. However, even in this context, ATM signaling may be involved in the genomic maintenance of stem cells, as DNA repair and DNA-damage signaling are probably very important for these cells to maintain their capacity to generate tissues (148–150).

**CONCLUSIONS**

Although the full scope of the biochemical signaling repertoire of ATM needs to be understood, it is also imperative, from a disease perspective, to focus on ATM functions that are important for preventing A-T. Doing so will be critical for the development of therapeutic approaches to ameliorate the neurological issues that define this disease. Perhaps the use of Occam’s razor would help us understand how loss of ATM function results in A-T—that is, defective DNA-damage signaling ultimately accounts for the spectrum of phenotypes observed in A-T.

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The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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