

Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer

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Abstract | First described over 80 years ago, ataxia-telangiectasia (A-T) was defined as a clinical entity 50 years ago. Although not encountered by most clinicians, it is a paradigm for cancer predisposition and neurodegenerative disorders and has a central role in our understanding of the DNA-damage response, signal transduction and cell-cycle control. The discovery of the protein A-T mutated (ATM) that is deficient in A-T paved the way for rapid progress on understanding how ATM functions with a host of other proteins to protect against genome instability and reduce the risk of cancer and other pathologies.

Checkpoint

In this example, a cell-cycle checkpoint is a control mechanism to ensure that chromosomes are intact for cell division.

Adaptor

A protein that assists in the process of downstream signalling. In this case, NBS1 has an adaptor role in ATM signalling.

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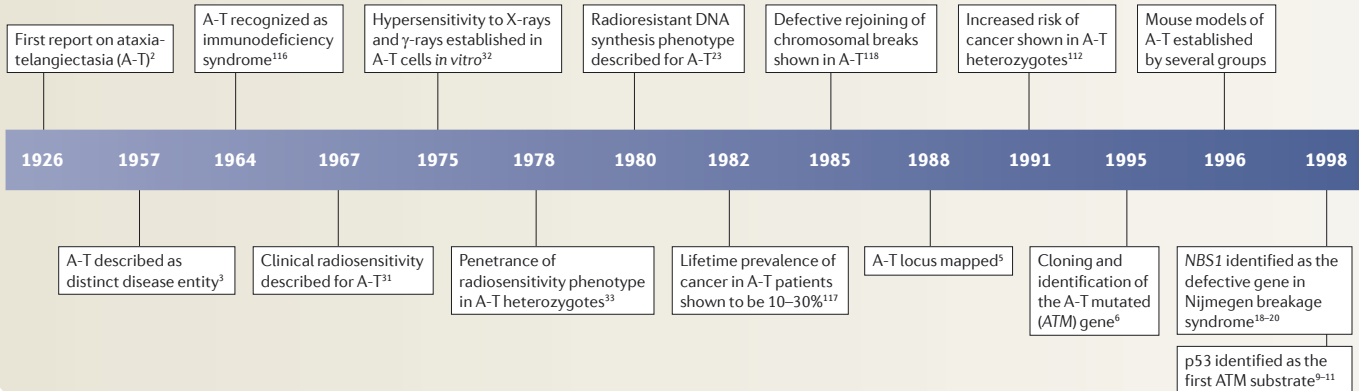
Elena Boder, a pioneer in the field of ataxia-telangiectasia (A-T) research, described A-T as “mysterious from the start — an entity easily diagnosed on purely clinical grounds, often by inspection — but elusive for a long time” (REF. 1). A-T was first reported by Syllaba and Henner² and finally described, with the aid of autopsy, as a separate entity by Boder and Sedgwick³. A-T is an autosomal recessive disorder that is characterized by early onset progressive cerebellar ataxia, oculocutaneous telangiectasia, susceptibility to bronchopulmonary disease, and lymphoid tumours¹. Various other abnormalities are also associated with this disorder, including the absence or the rudimentary appearance of a thymus, immunodeficiency, progressive apraxia of eye movements, insulin-resistant diabetes, clinical and cellular radiosensitivity, cell-cycle checkpoint defects and chromosomal instability⁴. Some of the landmarks of this era of A-T research appear in the [TIMELINE](#).

The gene that is defective in A-T, A-T mutated (*ATM*), was localized to chromosome 11q22–23 (REF. 5) and cloned by positional cloning⁶. ATM is a Ser/Thr protein kinase and is a member of the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family, which also includes ATM and Rad3-related protein (*ATR*), the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and SMG1, a protein kinase that is involved in the DNA-damage response⁷ but is also required for nonsense-mediated decay of mRNA that contains premature terminator codons⁸. The kinase domain is located close to the C terminus in all of these proteins except SMG1, in which the kinase domain is more central. This domain has

protein kinase activity, and p53 was the first substrate of ATM to be identified *in vitro* and *in vivo*^{9–11}. This was not surprising, because the stabilization and activation of p53 was defective in A-T cells, and these cells were also characterized by a defective G1–S checkpoint, in which p53 has a central role^{12,13}. ATM has multiple substrates — indeed, recent proteomic analysis of phosphoproteins induced by DNA damage suggest that the ATM and ATR network might have as many as 700 substrates^{14,15}. ATM also has a number of other domains, including a FAT domain — which is common to ATM, mammalian target of rapamycin (mTOR) and transformation/transcription domain-associated protein (TRRAP)¹⁶ — and an extreme C-terminal FATC domain that is found in combination with FAT in this subfamily of proteins (BOX 1). Other domains include an N-terminal substrate-binding domain, a Leu zipper, a Pro-rich region that enables it to bind to ABL kinase¹⁷ and a peroxisomal targeting signal sequence (PTSI).

ATM associates with the *MRE11–RAD50–NBS1* (MRN) complex as part of its activation process, and this complex subsequently acts as an adaptor for ATM-dependent phosphorylation of at least some downstream substrates. Disorders have also been described for hypomorphic mutants in two members of this complex: Nijmegen breakage syndrome (NBS1 mutants) and A-T-like disorder (MRE11 mutants)^{18–21}. Although all three syndromes are distinct, they share some clinical symptoms and cellular characteristics, A-T-like disorder (ATLD) being closer in phenotype to A-T (BOX 2).

Timeline | Ataxia-telangiectasia (prior to gene discovery) and identification of the ATM gene and its functional role



Radioresistant DNA synthesis

The absence of a steep component of inhibition of DNA synthesis in a dose-response curve when the rate of DNA synthesis is plotted against radiation doses.

Complementation group

This refers to previous studies on A-T cells in which cell fusion was used to determine whether more than one protein was involved in the defect.

The most striking clinical signs in A-T are progressive neurodegeneration and the high incidence (30%) of lymphoid tumours. It is evident that ATM recognizes and responds to DNA double-strand breaks (DSBs) to maintain the integrity of the genome and to minimize the risk of cancer and neurodegeneration. This review is designed to understand how ATM is activated and how it subsequently signals to cell-cycle checkpoints and the DNA-repair machinery to protect against genome instability.

Discovering the gene

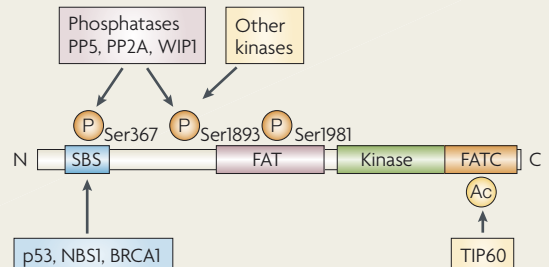
In the early 1980s, much of the A-T research focused on the characterization of A-T cells and on preliminary evidence for a defect in cell-cycle control. Reduced inhibition of DNA synthesis or radioresistant DNA synthesis was reported in A-T cells by the Lavin and Painter laboratories^{22,23}, whose method was adopted as the standard assay to measure the intra-S-phase checkpoint. Indeed it is still used for that purpose. It was over a decade before other defects in cell-cycle checkpoints were recognized in A-T cells. The most notable of these was a failure to activate the G1-S checkpoint, which was linked to a defective p53 response to radiation in A-T cells^{12,13}. It was

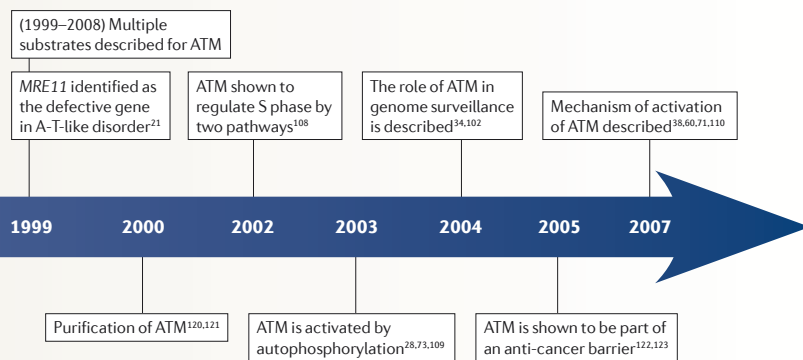
subsequently shown that cell-cycle defects also extended to the G2-M checkpoint²⁴. In retrospect, however, abnormal passage of cells through the cycle was documented for A-T cells considerably earlier, but this behaviour was not linked to cell-cycle checkpoints *per se*²⁵.

Progress towards the identification of the defective gene in A-T was hampered by the description of as many as five complementation groups in these cellular studies²⁶. This was shown by fusion between normal and A-T cells and by assaying for the correction of radioresistant DNA synthesis and radiation-induced chromosomal aberrations. Gatti and colleagues localized the A-T gene(s) to chromosomal region 11q22-23 using genetic linkage analysis of 31 A-T families⁵. This was the forerunner for extensive positional cloning work from several laboratories, which was initially slow because of the apparent heterogeneity of A-T and the shortage of genetic markers in this region of chromosome 11. Exhaustive cloning and physical mapping and the generation of a high-density map narrowed the region of the A-T gene(s) to less than 500 kilobases (kb)²⁷. Several candidate genes were identified and one of these, *ATM*, was found to be mutated in all complementation groups by Yosef Shiloh

Box 1 | Schematic representation of ATM

Members of the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family contain a FAT domain, a protein kinase domain and a FATC domain. Three autophosphorylation sites, Ser367, Ser1893 and Ser1981, have been identified on ataxia-telangiectasia mutated (ATM). Several other Ser and Thr phosphorylation sites (P) are also present on activated ATM, some of which are PIKK Ser/Thr-Gln consensus sites, whereas others are not and might be phosphorylated by other protein kinases. There is also evidence for Tyr phosphorylation on ATM, but the significance of this is unknown. Three phosphatases, protein phosphatase-2A (PP2A), PP5 and WIP1, have been implicated in the control of ATM activation. In addition to binding to the kinase domain, several known ATM substrates bind to a region near the N terminus of the protein (the substrate binding site, SBS). This is a crucial region of ATM because its deletion inactivates the protein. The ATM FATC domain functions as a binding site for the acetyltransferase TIP60, which acetylates (Ac) ATM on Lys3016; this post-translational modification is important for its activation. BRCA1, breast cancer susceptibility protein-1.





and colleagues^{6,28}. The identification of a single gene dispelled the existence of several complementation groups, but did not provide an explanation for their detection in earlier studies. The *ATM* gene was shown to occupy 160 kb of genomic DNA, encoding a 13 kb transcript of 66 exons without evidence of different splice forms²⁹. Four hundred and thirty two unique mutations have been reported for *ATM* that extend across the full length of the gene (see the Leiden Open Variation Database; further information). Most mutations in A-T patients are truncating or splice-site mutations that give rise to shorter, unstable ATM proteins. Most ATM mutations are unique to single families, are compound heterozygous, and founder effects have been observed among many populations, including Moroccan Jews, Costa Ricans, Norwegians, Turkish, Russians and Polish³⁰.

ATM and DNA DSB repair

Hypersensitivity to ionizing radiation (X-rays, γ -rays) was reported in A-T patients after radiotherapy for cancer in the 1960s³¹ and in A-T cells in culture in the 1970s^{32,33}. ATM is activated by DNA DSBs and signals to the cell-cycle checkpoint to slow the passage of cells through the cycle to facilitate DNA repair⁴. It seems likely that ATM also signals to the DNA-repair machinery to assist in the repair of DNA DSBs. Although there is no gross defect in DNA repair of DSBs in A-T cells, pulse-field gel electrophoresis and loss of γ H2AX foci indicated defective repair for a small fraction of breaks^{34,35}. These foci show an accumulation of DNA-damage-response proteins at sites of DNA damage. Cells that are deficient in non-homologous end-joining (NHEJ) repair can repair DNA DSBs slowly over several days, but in A-T cells a small proportion of breaks persist even over long periods of time³⁶. Up to 90% of DSBs are repaired by NHEJ in G1 phase of the cell cycle by an ATM-independent mechanism.

However, a subset of DNA breaks requires processing by the nuclease Artemis, a processing step that is dependent on ATM that phosphorylates Artemis in response to DNA damage³⁴. It was initially suggested that this subset of breaks are those that arise from more complex DNA damage and are refractory to DNA repair³⁴.

However, more recent results suggest that the complexity might relate more to the chromatin environment rather than to the nature of the lesion³⁷. These results suggest that, in the absence of ATM, persistent DNA DSBs are localized to heterochromatin. Thus, ATM seems to facilitate entry of the DNA-repair machinery into heterochromatin by phosphorylating the transcriptional corepressor Kruppel-associated box (KRAB)-associated protein-1 (KAP1) (REF. 37). Overall lack of ATM would be expected to result in a fraction of unrepaired DNA DSBs. Persistence of DNA DSBs that are induced by the eukaryotic homing endonuclease I-PpoI in ATM-null cells and after ATM depletion also support a repair defect in A-T³⁸. A reduced capacity to repair these residual breaks could account for the increased radiosensitivity that is observed in A-T³⁶. The components of the ATM-signalling pathway(s) that contribute to the protection of cells against DNA DSBs are unclear. Yazdi *et al.*³⁹ have shown that overexpression of phosphorylation site mutants of SMC1 (structural maintenance of chromosomes protein-1; a substrate for ATM) sensitizes these cells to radiation, suggesting that signalling through SMC1 has an important role in protecting cells against DNA damage. This was further strengthened by the observations that fibroblasts from SMC1-Ser957Ala/Ser966Ala-mutant mice showed increased radiosensitivity and a decreased rate of disappearance of chromosomal breaks after irradiation⁴⁰.

Lymphocyte development. ATM also responds to physiological breaks in DNA during the development and differentiation of B and T cells⁴¹. Bredmeyer *et al.*⁴² showed that ATM functions in the resolution of DNA DSBs that are generated during V(D)J recombination. Consistent with this, a role for ATM in supporting efficient V α -J α -coding end joining has been shown in double-positive thymocytes⁴³. In addition, increased accumulation of unrepaired coding ends during different steps of antigen receptor-gene assembly, for both immunoglobulin and T-cell receptor loci, has been reported in ATM-deficient B and T lymphocytes⁴².

The thymic lymphocytes that arise in ATM-mutant mice do not have translocations that involve the T-cell receptor, and therefore it is likely that ATM controls other processes in these cells. Constitutive activation of JUN N-terminal kinase in macrophages from ATM-deficient mice, together with other possible abnormalities, might contribute to the defects that are observed. ATM localizes to V(D)J-recombination-induced DNA DSBs⁴⁴, and has been shown to have a key role in maintaining T-cell receptor- α (TCR α) expression during recombination⁴⁵. Impaired TCR α expression and defective maturation of double-positive T cells reduces the number of mature thymocytes in ATM-mutant mice. ATM deficiency also impairs class-switch recombination of immunoglobulin genes⁴¹, and T- and B-cell precursors in *Atm*^{-/-} mice have a reduction in normal coding joins and a high frequency of abnormal hybrid joins between signal ends and coding ends during inversion rearrangements⁴². These data provide further evidence for an increased half-life of DNA breaks at these loci in immature T cells and B cells.

Non-homologous end joining (NHEJ) repair

The repair of DNA double-strand breaks (free ends) by DNA-PK and other cofactors without the requirement for DNA recombination.

Heterochromatin

A cytologically distinct, tightly packed form of chromatin in which transcription is repressed.

V(D)J recombination

A mechanism that involves gene rearrangement in the maturation of immunoglobulin and T-cell receptor genes.

Box 2 | Characteristics of ataxia-telangiectasia

Characteristic	Ataxia-telangiectasia	ATLD	Nijmegen breakage syndrome
Defective protein	ATM	MRE11	NBS1
Appearance of symptoms	Infancy	First decade	Infancy
Progression	Rapid	Slow	Rapid
Neurological Defect	Cerebellar atrophy	Cerebellar atrophy	Microencephaly
Oculomotor apraxia	+	+	-
Immunodeficiency	+	-	+
Telangiectasia	+	-	-
Alpha-fetoprotein	High	Normal	Normal
Growth defect	-	-	+
Radiosensitivity	++	+	+
Cell-cycle checkpoint defect	+	+	+
DNA-repair defect	+	?	+
Chromosomal instability	+	+	+
Cancer predisposition	+	-	+

Ataxia, the presenting symptom in ataxia-telangiectasia (A-T), is manifested when a child becomes ambulatory. This ataxia is of gait and truncal movements — it is progressive and affects the extremities; involuntary movements are evident and it leads to immobilization by the end of the first decade¹. Although the cerebellum is predominantly affected, degenerative changes are widespread in the CNS. Telangiectasia has a later onset, appearing between 2 and 8 years of age. The telangiectasia might represent progressive changes or might be linked to the radiosensitivity that is characteristic of this disease. A-T is a primary immunodeficiency disease that involves both cellular and humoral immunity. This defect can be explained by the role of A-T mutated (ATM) in responding to DNA double-strand breaks, and the importance of the rearrangement of immunoglobulin and T-cell receptor genes during T-cell and B-cell ontogeny⁴. Radiosensitivity is also a hallmark of A-T patients^{4,31}. This is a universal characteristic and in most cases is severe. It was initially assumed that, as in yeast, this enhanced sensitivity to radiation was due to defects in cell-cycle checkpoints. It is more probable that the radiosensitivity is due to defective signalling to specific substrates, in the absence of functional ATM, which results in a compromised cellular defence against DNA damage. A-T shares similar clinical symptoms and cellular characteristics with two other syndromes: A-T-like disorder (ATLD) and Nijmegen-breakage syndrome (see table).

Even though A-T is an autosomal recessive disorder, it is evident that aspects of the A-T phenotype show penetrance in A-T carriers. What is intriguing is the observation that cells from A-T heterozygotes are intermediate between controls and A-T homozygotes in their sensitivity to radiation³³. These results are borne out in a series of subsequent reports using different assays for radiosensitivity. This is the case, not only for cellular radiosensitivity, but also for a heightened risk of cancer, especially breast cancer¹¹². A series of epidemiological studies support a two to fourfold risk of breast cancer in A-T heterozygotes. In a more recent study of individuals with familial breast cancer, 12 ATM sequence variants were identified compared with 2 in controls ($p < 0.005$)¹¹³. It is possible that the penetrance of some aspects of the phenotype in A-T carriers might be due to interference with normal function by the expression of mutant ATM.

These data also provide a mechanistic explanation for the increased number of chromosomal abnormalities in T cells and B cells from A-T patients, and for the propensity to develop lymphoid neoplasia⁴³. Up to 30% of A-T patients develop lymphoid tumours.

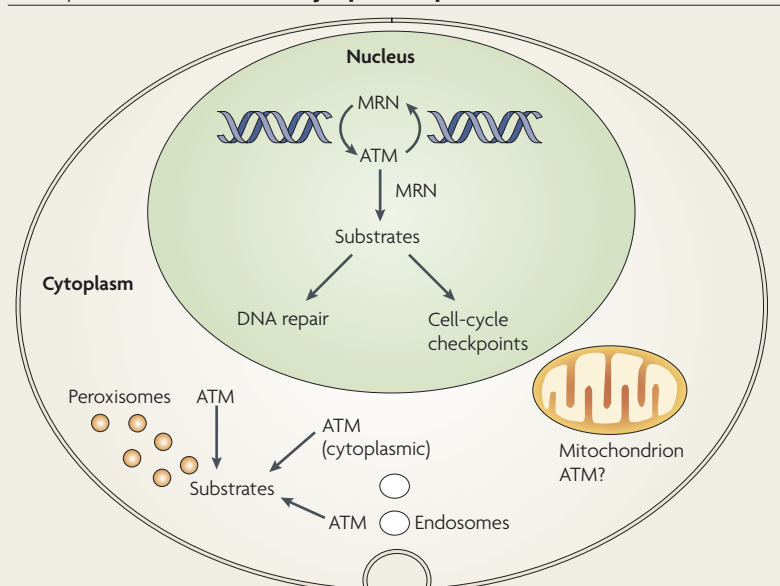
Peroxisomes
Subcellular organelles that metabolize fatty acids and clear the cell of toxic peroxides.

Does ATM have a role outside the nucleus? Although ATM is predominantly a nuclear protein that responds to DNA DSBs and signals to the cell-cycle checkpoints and DNA-repair pathways, there is also evidence for a cytoplasmic form, which has been found to be associated with both peroxisomes and endosomes^{46,47} (BOX 3). There is increasing support for a more general signalling role for ATM that does not involve direct DNA damage. A series of abnormalities in ATM-deficient cells provide indirect evidence for an extranuclear role for ATM⁴⁸. These abnormalities include reduced internalization of phytohaemagglutinin (PHA), defective Ca²⁺ mobilization, depolarization in response to extracellular K⁺, a decrease in the duration of Ca²⁺ and Na⁺ firing, greater growth factor demand and defective signalling through the epidermal growth factor (EGF) receptor. More direct evidence demonstrates the ability of insulin to activate ATM, as shown by the phosphorylation of 4EBP1 and the subsequent dissociation of eukaryotic translation-initiation factor (eIF)4E from 4EBP1, thereby making it available for initiation of mRNA translation⁴⁹. Dissociation of this complex was defective in A-T cells.

Insulin resistance and metabolic syndrome. Pertinent to these results is the observation that some A-T patients develop insulin-resistant diabetes^{50,51}. This abnormality is also seen in metabolic syndrome. Loss of one or both alleles of *Atm* accentuated the features of metabolic syndrome in *ApoE*^{-/-} mice fed on a high-fat diet⁵². Chloroquine treatment decreased atherosclerosis and blood pressure, and improved glucose tolerance in an ATM-dependent manner in these mutant mice. These results suggest that chloroquine enhances ATM activity to protect against metabolic syndrome and glucose resistance. Indeed, chloroquine and other agents that alter chromatin structure can cause partial activation of ATM in the absence of DNA DSBs⁵³. Although these data point to a role for cytoplasmic ATM in this process, they do not exclude shuttling of ATM from the nucleus.

Neurodegeneration. Progressive neurodegeneration due to Purkinje-cell loss has the most marked effect on A-T patients¹. Whether this neuronal effect is due to a defective response to DNA DSBs was difficult to analyse as early observations showed that ATM is predominantly cytoplasmic in neuronal cells⁵⁴. More recently, Biton *et al.*⁵⁵ showed that ATM is predominantly nuclear in human neuronal-like cells, and that the ATM-mediated response is as robust as in proliferating cells⁵⁵. Knockdown of ATM abolished that response. Similar results came from murine cerebellar neurons, in which ATM was essentially nuclear and ATM activation measured by autophosphorylation and downstream signalling was comparable with that in other cell lines⁵⁶. This is supported by genetic evidence showing that MRE11 facilitates the activation of ATM at DNA DSBs, and that patients that are hypomorphic for mutations in *MRE11* (patients with ATLD) have a neuronal phenotype that is similar to that in A-T⁵⁷.

Box 3 | ATM is a nuclear and cytoplasmic protein



Ataxia-telangiectasia mutated (ATM) is predominantly present in the nucleus but a variable amount (~10–20%) has been reported in the cytoplasm. The actual proportions of nuclear and cytoplasmic ATM in neuronal cells are disputed. The major known role of nuclear ATM is to participate in the response to DNA double-strand breaks for DNA repair and cell-cycle checkpoint activation. ATM is present in the cytoplasm in peroxisomes, a subcellular organelle that is involved in β -oxidation of fatty acids and detoxification, and in endosomes, which are responsible for endocytosis and intracellular routing of receptors and other molecules. ATM might also exist as a soluble protein in the cytoplasm. Although direct evidence for activation of cytoplasmic ATM has not been provided, it has been shown that ATM phosphorylates the cytoplasmic protein 4EBP1 on Ser111 *in vitro*, and that insulin induces phosphorylation at this site in the cell⁴⁹. Other possible ATM targets include proteins that are involved in oxidative metabolism or proteins that are designed to protect against the symptoms of metabolic disorder. MRN, MRE11–RAD50–NBS1 complex.

Activation of ATM

Rapid recruitment of DNA-damage recognition and repair proteins to distinct foci is observed in response to agents that damage DNA or cause the arrest or collapse of DNA-replication forks^{58,59}. Although this recruitment is rapid, the order in which these proteins localize to sites of DNA damage is gradually being resolved. DNA DSBs lead to the accumulation of the MRN complex, the retention of which on chromatin is dependent on the mediator of DNA-damage checkpoint protein-1 (MDC1) adaptor protein (FIG. 1). This retention by MDC1 increases the local concentration of the MRN complex at the sites of DNA DSBs. ATM also arrives early at the damaged site, initially associating with DNA regions that flank the break, before associating with the MRN complex at the break site through the C terminus of NBS1 (REFS 38,60).

Interaction of MDC1 through its FHA domain with ATM regulates the accumulation of ATM at damaged sites. MDC1 also mediates the interaction between ATM and γ H2AX, which presumably contributes to the long-range phosphorylation of H2AX and the maintenance of the response⁵⁹. Given the key role of ATM in mediating signalling from DNA DSBs, it is not

surprising that its activation is tightly controlled. It seems likely that ATM is at least partially activated adjacent to DNA DSBs³⁸, probably owing to the initial relaxation of chromatin structure by the break. The activation of ATM by chloroquine, histone deacetylase inhibitors or hypotonic buffer supports this hypothesis⁵³. However, ATM that is activated by these factors does not localize to nuclear foci and fails to phosphorylate H2AX, but it is capable of phosphorylating p53, which suggests that ATM needs to be localized to the break for complete activation. Full activation of ATM and localization to DNA DSBs is facilitated by the MRN complex³⁸.

The role of the MRN complex. The MRE11–RAD50 complex binds to DNA as a heterotetramer, tethering the broken ends of a DSB⁶¹. This binding is achieved through the two DNA-binding motifs of MRE11, which are arranged as a globular domain with RAD50 Walker A and Walker B nucleotide-binding motifs (ATPase domains)⁶². The bridging of DNA molecules is achieved through Cys-X-X-Cys sequences in the hook region of RAD50 (FIG. 2). The Cys-X-X-Cys sequences are arranged at the ends of coiled-coil regions and are thought to dimerize by the coordination of a Zn²⁺ ion⁶³. The dynamic architecture of the MRN complex is altered by DNA binding to generate a parallel orientation of the coiled-coils of RAD50, thereby preventing intracomplex interaction and favouring intercomplex association⁶⁴. Association with RAD50 stimulates both the exonuclease and endonuclease activities of MRE11 (REF. 65), and NBS1 stimulates its endonuclease activity⁶⁶. However, other nucleases, such as Artemis, also seem to have a role in DSB processing, at least in a subgroup of breaks with damaged termini³⁴.

The MRN complex functions as a sensor of DNA DSBs, and there is no requirement for ATM for MRN association with sites of DNA damage⁶⁷. Evidence for a role for MRN upstream of ATM comes from studies with NBS and ATLD cells (which are hypomorphic for members of the complex), from cells in which the MRN complex was depleted during viral infection, from *in vitro* investigations using recombinant proteins, and from *Xenopus laevis* extracts that have been reconstituted for DNA-damage signalling. ATM activation is retarded in both NBS and ATLD cells in response to DNA DSBs⁶⁸. An NBS1 construct, NbFR5, which retained the MRE11-binding site, stimulated ATM activation⁶⁹. The most common NBS mutation (657 Δ 5, a 5-base-pair deletion that gives rise to a hypomorphic allele) generates two fragments of the NBS1 protein⁷⁰, an N-terminal fragment containing the MRE11-binding site, and a C-terminal fragment containing a conserved domain that is responsible for recruiting ATM⁷¹. NBS cells that express NbFR5 Δ ATM, which lacks the ATM-binding site, had dramatically reduced levels of ATM activation⁷².

Post-translational modifications. As indicated above, ATM undergoes autophosphorylation on at least three sites (Ser367, Ser1893, Ser1981), at least one of which seems to be instrumental in the monomerization and

Endosomes

Vesicles derived from the plasma membrane to transport proteins and other substances into the cell.

Purkinje cell

Neuronal cells found on the cerebellum between the molecular and granular layers.

Autophosphorylation

The process by which a protein kinase uses itself as a substrate for phosphorylation in many cases for self-activation.

Downstream signalling

Part of a signal transduction pathway in which a protein, such as ATM, phosphorylates a series of substrates that assist in controlling various cellular processes.

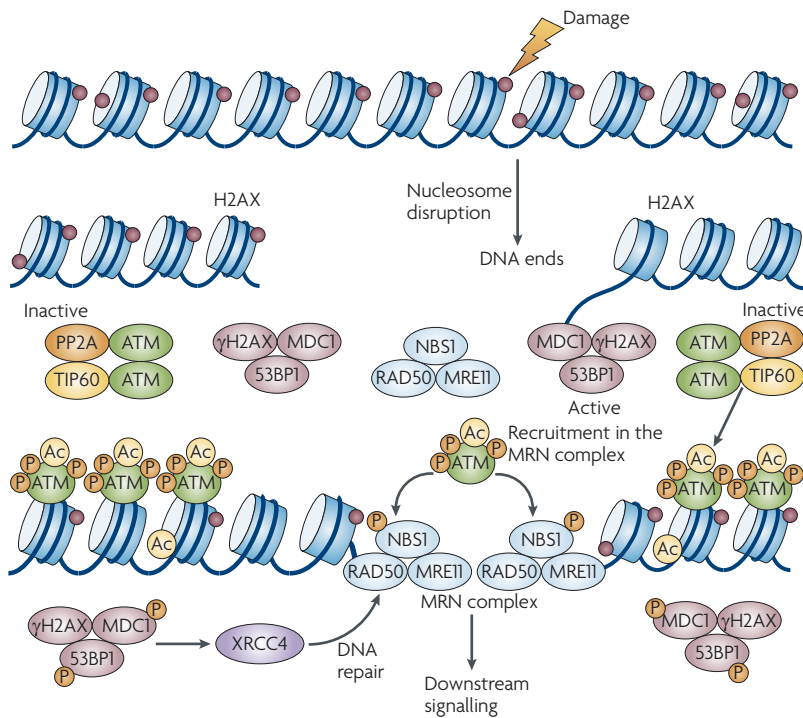


Figure 1 | Activation of ATM. Activation of ataxia-telangiectasia mutated (ATM) is a complex process that involves the relaxation of chromatin as a consequence of a DNA double-strand break (DSB), involves the recruitment of the MRE11–RAD50–NBS1 (MRN) complex to the break and also the recruitment of ATM to regions that flank the break. In these flanking regions, ATM is partially activated and phosphorylates p53 and possibly other substrates. ATM is then recruited to the site of the break by the MRN complex and phosphorylates members of the complex and other downstream substrates. The MRN complex is not essential for this signalling, but in the presence of hypomorphic mutations in members of the complex, signalling is delayed and/or reduced. An inactive ATM dimer is monomerized in response to DNA DSBs, and concomitantly transphosphorylation (autophosphorylation) occurs on at least three sites: Ser367, Ser1893 and Ser1981. Phosphatases also regulate ATM activation. Protein phosphatase-2A (PP2A) is constitutively associated with ATM, presumably to ensure that it is not inappropriately activated by autophosphorylation. In the presence of DNA DSBs, PP2A dissociates from ATM and loses its activity, therefore minimizing the risk of competition between phosphorylation and phosphatase activities. The phosphatase WIP1 is also capable of removing phosphates from all three autophosphorylation sites. PP5 removes phosphates from ATM as part of the process of activation. Acetylation (Ac) also contributes to the process of activation. The acetyltransferase TIP60 is constitutively associated with ATM, and in the presence of a DNA DSB it becomes activated and acetylates ATM at Lys3016 within the C-terminal FATC domain. Lys3016 mutants fail to upregulate ATM activity after DNA damage, prevent monomerization of ATM and inhibit downstream signalling through p53 and checkpoint kinase-2 (CHK2). XRCC4, the requisite cofactor of DNA ligase 4 and non-homologous end joining (NHEJ) is detected at the break site after ATM recruitment. 53BP1, p53 binding protein-1; MDC1, mediator of DNA-damage checkpoint protein-1.

FHA domain
(Forkhead-associated domain). A short sequence of amino acids that binds to phosphothreonine residues on various proteins.

ATPase domain
The region of a protein that is responsible for the hydrolysis of ATP and is coupled to protein phosphorylation.

activation of ATM^{53,73}. Activation of ATM by okadaic acid suggested that phosphatase activity was important in maintaining ATM in a basal state⁷⁴. ATM co-immunoprecipitates with protein phosphatase-2A (PP2A) in undamaged cells, but this interaction was abrogated in response to DNA DSBs, which would then allow ATM autophosphorylation. A second phosphatase, WIP1, can dephosphorylate ATM *in vitro* at both the Ser367 and Ser1981 sites, and deficiency of this enzyme causes upregulation of ATM⁷⁵. A third phosphatase, PP5, co-immunoprecipitates with ATM in DNA-damaged

cells and a dominant-interfering form of PP5 inhibited both ATM activation and the phosphorylation of ATM downstream substrates⁷⁶.

Phosphorylation is not the only post-translational modification that alters ATM activity. Sun *et al.*⁷⁷ showed that DNA DSBs induce the acetylation of ATM in parallel to Ser1981 autophosphorylation. Overexpression of a dominant-negative form of TIP60 acetyltransferase reduced levels of both acetylation and autophosphorylation of ATM, reduced ATM-kinase activity and sensitized cells to radiation. A single acetylation site was identified as Lys3016, within the C-terminal FATC domain and adjacent to the kinase domain of ATM⁷⁸. Mutation of Lys3016 prevented the upregulation of ATM activity by DNA damage, inhibited monomerization of the inactive ATM dimer and prevented ATM-dependent phosphorylation of p53 and checkpoint kinase-2 (CHK2).

What is the role of ATM autophosphorylation? There is evidence that autophosphorylation is a consequence rather than a cause of ATM activation in murine cells⁷⁹. This group used a bacterial artificial chromosome (BAC) reconstitution system to generate an ATM-Ser1987Ala (the mouse equivalent of Ser1981Ala) mutant expressed on an *Atm*^{-/-} background. Cells from these mice showed normal levels of DNA-damaged-induced phosphorylation of several ATM-dependent substrates, including SMC1, CHK2 and p53. They also showed that these cells were not hypersensitive to radiation and that activation of both the intra S phase and G2–M-phase checkpoints was normal. Pellegrini *et al.*⁷⁹ also revealed that enhanced retention of ATM-Ser1987Ala occurred at sites of DNA damage. This seems to be inconsistent with binding to I-PpoI-induced DNA DSBs, in which neither ATM kinase-dead nor ATM-Ser1981Ala were capable of binding³⁸. Another report showed that ATM activation is dispensable for initial recruitment to DNA breaks⁶⁰. This study postulates that although wild-type and mutant ATM can be recruited along damaged chromatin, ATM mutants that cannot be activated might subsequently dissociate from chromatin⁶⁰.

Notwithstanding the different observations on recruitment of ATM, there seem to be substantial differences between the importance of ATM autophosphorylation and downstream signalling in determining the radiosensitive status of cell-cycle control in humans and in mice. It is suggested that increased pool size of ATM in the region of the break is sufficient for ATM activation, without a need for autophosphorylation⁷⁹. These data suggest that either there is a fundamental difference in the mechanism of ATM activation in the two species, or the ATM-mutant mice that are generated through BAC reconstitution are different to those produced by more conventional methodology. Furthermore, as in the case of DNA-PKcs, the level of ATM protein is significantly lower in the mouse (M. Kastan, personal communication). However, there are examples from human cells in which ATM can be activated without phosphorylation on Ser1981 (REFS 80,81). This seems to be due to the nature of the stimulus involved.

The ATM-dependent signalling cascade

Assembly of DNA-damage-response proteins. The activation of ATM leads to the rapid phosphorylation of a multitude of proteins that are involved in DNA repair, cell-cycle checkpoint activation and transcription^{82,83}. A series of large-scale proteomic studies have identified extensive phosphoprotein networks that are responsive to DNA damage^{14,15,84}. In these studies, as many as 900 induced phosphorylations on ATM and ATR consensus sites of over 700 proteins have been identified. These proteins were found to be arranged in interconnecting modules that are involved in DNA repair, DNA replication and cell-cycle control¹⁴. It is probably unlikely that all of these phosphorylations are direct substrates for ATM and might be dependent on other PIKKs. We focus on ATM substrates that are involved in DNA repair and cell-cycle checkpoint activation.

Although the series of events that occur after ATM activation are rapid, it is possible to sub-categorize substrate phosphorylations by their position in the cascade, their role in signal amplification and on their requirement for MRN. As described above, it seems that ATM associates with DNA/chromatin in the vicinity of a DSB, where it is at least partially activated^{138,60}. In this case, ATM does not need to be recruited to the MRN complex at the break. At low doses of radiation (1–3 Gy), ATM autophosphorylation and Ser15 p53 phosphorylation are maximal, which indicates a lack of correlation with dosage⁴⁶. Agents that alter chromatin structure activate ATM and cause phosphorylation of p53, but fail to induce phosphorylation of SMC1, NBS1 and other substrates. Diffuse nuclear staining of phosphorylated Ser15 p53 is consistent with these data^{40,53}. In addition, in cells from NBS patients, ATM-dependent phosphorylation of CHK2 and other substrates is defective, but diffuse nuclear staining for p53 phosphorylation is observed^{40,53}.

Although p53 and ATM itself are the only defined substrates of ATM during break-independent activation of ATM, it is possible that other substrates exist. Some of these might have a role in the prevention of metabolic syndrome. Administration of chloroquine to *Atm*^{+/-} mice on an APOE-null background lowers blood pressure, improves glucose tolerance, suppresses JNK activity and decreases diet-induced atherosclerosis⁵². As ATM haploinsufficiency exacerbates the APOE phenotype, it seems likely that ATM activation is responsible for alleviating these symptoms. Thus, the proteins that undergo ATM-dependent phosphorylation under these conditions are substrates that are capable of being phosphorylated after minimal activation of ATM.

The order of assembly of DNA-damage-response proteins at DNA DSBs, and the structural requirements for their interaction, has been facilitated by the use of microlasers and charged particle tracks to generate DSBs^{85,86} (FIG. 3). ATM-dependent phosphorylation of the histone variant H2AX to produce γ H2AX seems to be the initial signal for subsequent accumulation of DNA-damage-response proteins^{87,88}. A second substrate of ATM, MDC1, binds to γ H2AX via its breast cancer susceptibility protein-1 (BRCA1) C-terminal (BRCT) domain and has a key role as a 'master regulator' of the

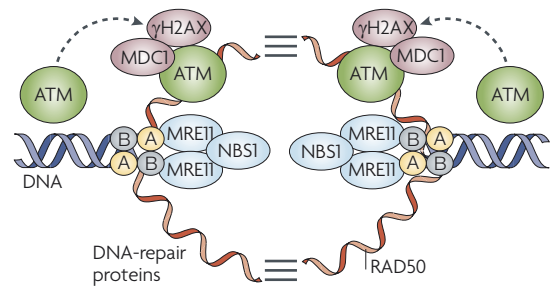


Figure 2 | The MRN complex tethers DNA broken ends.

The MRE11–RAD50–NBS1 (MRN) complex acts as a sensor of DNA double-strand breaks, binding as an MR heterotetramer. MRE11 binds the DNA ends together with the ATPase-domain region of RAD50, whereas the coiled-coil regions of RAD50 bring the two ends together in a bridge-like structure, aided by the hook regions (Cys-X-X-Cys)⁶¹. Ataxia-telangiectasia mutated (ATM) and other DNA-damage response proteins, such as mediator of DNA-damage checkpoint protein-1 (MDC1) and γ H2AX, are subsequently recruited to the break site. A and B represent Walker A and Walker B domains.

recognition and repair of DNA DSBs⁸⁹. NBS1, as part of MRN, arrives at the break with the same kinetics as MDC1, and it is also phosphorylated by ATM⁸⁵. However, its retention on chromatin is not dependent on ATM phosphorylation but rather on casein kinase-2 phosphorylation of Ser-Asp-Thr (SDT) repeats in the N terminus of MDC1 (REFS 90,91). The RING-finger ubiquitin ligase, RNF8, also assembles at the DSB through interaction of its FHA domains with phosphorylated MDC1 (consensus sites for ATM phosphorylation)^{92–94}. RNF8 thus ubiquitylates H2AX and facilitates the accumulation of p53-binding protein-1 and BRCA1 at the site of damage. It is of interest that both of these proteins are also substrates for ATM in response to DNA DSBs^{79,80}. Thus, ATM has a key role in phosphorylating various proteins that constitute the core components of the DNA-damage recognition machinery and those responsible for amplification of the signal (FIG. 4).

MRN as an adaptor in downstream signalling. The MRN complex is not only involved in the activation of ATM but is also a target for ATM kinase in downstream signalling to the DNA-repair machinery and the cell-cycle checkpoints⁹⁵. Having an intact complex is important for downstream signalling, and there is also evidence that ATM-dependent phosphorylation of NBS1 has a role as an adaptor for downstream signalling^{96–98}. It is now well established that NBS1 is rapidly recruited to a DSB, where it remains stably bound^{38,99,100}. By contrast, ATM substrates such as CHK2 move rapidly throughout the nucleus after DNA damage¹⁰¹. In addition, SMC1, which has a prominent role in the DNA-damage response, cannot be localized to sites of damage, except after exposure of cells to high doses of radiation¹⁰².

In establishing a downstream role for MRN in ATM signalling, conditions must be established that allow normal ATM activation. In cells from NBS patients, activated ATM does not appear in DNA-damaged-induced foci

Exonuclease

The enzyme that degrades nucleic acids from 3' to 5' free ends.

Endonuclease

An enzyme that is capable of hydrolysing phosphodiester bonds in nucleic acids that are away from the free ends.

Ubiquitylation

Post-translational modification of a protein by the covalent attachment of a ubiquitin protein to enable degradation or other forms of regulation.

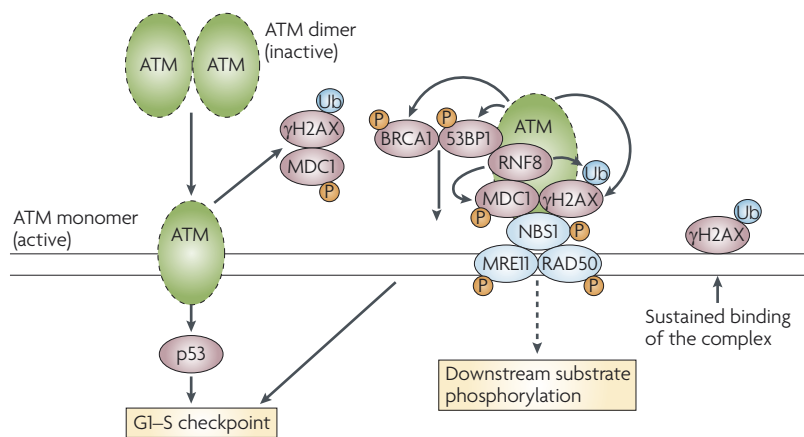


Figure 3 | Assembly of DNA-damage response proteins at a double-strand break.

The order of the assembly of DNA-damage response proteins at a DNA double-strand break has been established using cleavage of DNA at specific break sites, high linear energy transfer (LET) radiation tracks in nuclei and by the use of microlasers in nuclei. An early event during the DNA-damage response is the monomerization of ataxia-telangiectasia mutated (ATM) adjacent to the break by some form of relaxation of chromatin, followed by the phosphorylation of histone H2AX. A second substrate for ATM, mediator of DNA-damage checkpoint protein-1 (MDC1), binds to γ H2AX and acts as a scaffold or platform to ensure the retention of the DNA-damage recognition/repair complex on chromatin. The MRE11–RAD50–NBS1 (MRN) complex binds to MDC1 through Nijmegen breakage syndrome-1 (NBS1) for retention on chromatin. The ubiquitin ligase RING-finger protein-8 (RNF8) is also recruited to MDC1 and it is then capable of ubiquitylating H2AX to facilitate the accumulation of breast cancer susceptibility protein-1 (BRCA1) and p53 binding protein-1 (53BP1) at the break site. Most of these proteins are phosphorylated by ATM. The minimum essential consensus sequence for phosphorylation by ATM is Ser/Thr-Gln. Phosphorylation is also influenced by the amino-acid sequences on the N-terminal and C-terminal sides of this consensus sequence^{114,115}. P, phosphorylation; Ub, ubiquitylation.

but is present in a diffuse form throughout the nucleus, and phosphorylation of downstream substrates is defective⁴⁰. The observation that ATM is activated normally in NBS1-deficient cells in response to radiation doses >1 Gy (and yet SMC1 phosphorylation is defective) supports a role for the NBS1 protein in downstream events. The appearance of phosphorylated Ser957 SMC1 in ATM and NBS1 foci in cells that contain wild-type NBS1, but not in NBS1-deficient cells, is consistent with an adaptor role for NBS1 in signalling⁴⁰. The stable binding of MRN to the break site and its co-localization with ATM makes it an ideal candidate to facilitate downstream substrate phosphorylation³⁸. Radiation-induced CHK2 phosphorylation was impaired in NBS1-deficient cells 30 minutes after irradiation. This defect was also evident at the level of CHK2 activation. ATM-dependent phosphorylation of the Fanconi anaemia protein FANCD2 was also shown to be dependent on both NBS1 and MRE11 (REF. 97). Radiation-induced phosphorylation of CHK1 (REF. 98) and SMC1 (REF. 39,40) also requires NBS1. All of these observations are compatible with an adaptor role for the MRN complex in determining phosphorylation of ATM substrates. It might be that many more of the ATM substrates also depend on the presence of MRN.

There is also evidence that the phosphorylation status of NBS1 might influence downstream phosphorylation of ATM substrates. In response to radiation damage, NBS1 is phosphorylated on two sites (Ser278 and Ser343);

these phosphorylation events are dependent on ATM^{103–106}. The functional importance of these phosphorylations for downstream events is somewhat controversial. There is general agreement that NBS1-phosphorylated Ser343 is required for activation of the S-phase checkpoint^{39,40,103,104,106}. SMC1 has been identified as a downstream effector of the ATM/NBS1-controlled S-phase checkpoint, and ATM-dependent phosphorylation of SMC1 on Ser957 and Ser966 is required for activation of the checkpoint, cell survival and maintaining the integrity of the genome^{39,40}. Furthermore, phosphorylation of NBS1 is required for SMC1 phosphorylation. This led to the proposal of a separate branch of the S-phase pathway that contains ATM, NBS1 and SMC1 (REF. 39). The importance of SMC1 phosphorylation, not only for this S-phase checkpoint but also in protection against radiation-induced cell death, suggests that ATM-dependent phosphorylation of NBS1 is also important for cell survival post-irradiation. This is supported by several reports^{103–105}.

NBS1 is the key member of the MRN complex that influences the DNA binding, unwinding and nuclease activities of the MR complex¹⁰⁷. By binding through its C terminus to the N terminus of MRE11, it ensures that MR is localized to the nucleus⁶⁹. MR is unable to activate dimeric ATM, which supports a key role for NBS1 at the break. As outlined above, NBS1 and the ATM-dependent phosphorylated form of this protein have an adaptor role in phosphorylation of downstream substrates. However, it is now evident that both MRE11 and RAD50 are also phosphorylated in response to DNA DSBs. Several recent proteomic studies have identified RAD50 as an ATM-dependent substrate^{14,15,84}. In addition, MRE11 is phosphorylated by agents that cause breaks in DNA^{108,109}. At present, it is unclear as to the functional consequences of the phosphorylations of MRE11 and RAD50, but it is tempting to suggest that they might also have important roles as adaptor proteins in regulating the phosphorylation of the multitude of substrates described for ATM. This in turn would be expected to influence DNA repair and cell-cycle control.

Conclusions and future perspectives

Much progress has been made on how ATM responds to DNA DSBs. It is evident that recruitment to chromatin is an important part of the ATM activation mechanism and its interaction with the MRN complex, the sensor of DNA DSBs, enhances the process of activation. Although there is growing agreement that the initial stimulus for ATM activation is a relaxation of chromatin structure, the series of events leading to the translocation of ATM to the MRN complex at the break site and the subsequent repair of the DSB await further elucidation. It is also becoming increasingly obvious that the retention of ATM and other DNA-damage response proteins on chromatin is a crucial part of the overall response. Stable binding of individual components of the DNA-damage-response machinery is sufficient to induce the DNA-damage response in the absence of DNA DSBs¹¹⁰. Immobilization of NBS1, MRE11 or MDC1 leads to the phosphorylation of H2AX, NBS1 and ATM, which is indicative of an activated DNA-damage response.

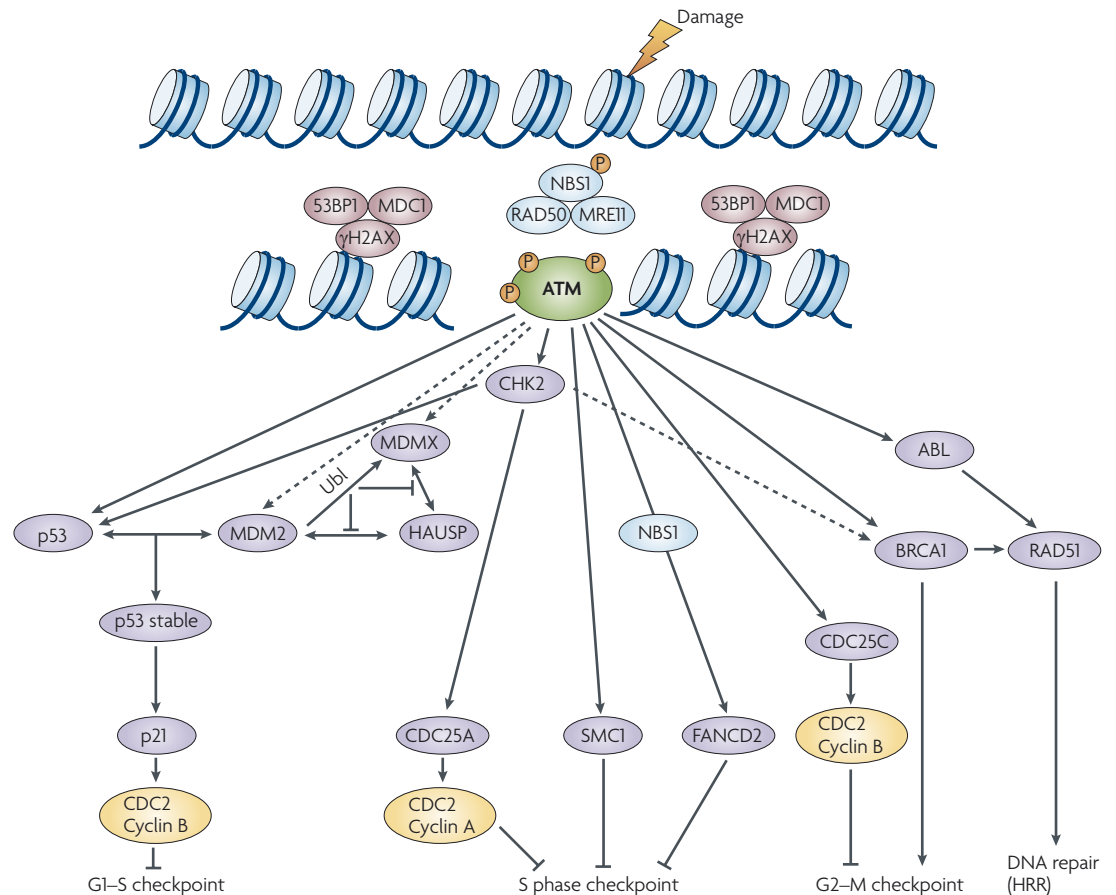


Figure 4 | ATM activation and signalling to downstream substrates in response to DNA double-strand breaks. Signalling in response to DNA double-strand breaks (DSBs) controls a number of processes, including cell-cycle checkpoint activation, DNA repair, transcriptional and translation events, and apoptosis. Ataxia-telangiectasia mutated (ATM) is activated by a complex series of events (see FIG. 1). This activation is complete when it interacts with the DNA DSB sensor, the MRE11–RAD50–NBS1 (MRN) complex. It then phosphorylates a series of downstream substrates, including NBS1. Phosphorylated NBS1 acts as an adaptor for the ATM-dependent phosphorylation of other substrates, including structural maintenance of chromosomes protein-1 (SMC1). It is noteworthy that in controlling a specific process, for example, the G1–S checkpoint, ATM phosphorylates or mediates the phosphorylation of several different proteins to ensure tight regulation of that checkpoint. In the G1–S checkpoint, ATM phosphorylates p53 on Ser15 and other sites; although this modification does not stabilize and activate p53, it is a marker for that process. Stabilization of p53 is also influenced by ATM phosphorylation of the checkpoint kinase CHK2, murine double minute-2 (MDM2; HDM2 in humans) and MDMX. Phosphorylation of MDM2 decreases its affinity for p53, thereby preventing ubiquitylation (Ubl) and proteasomal degradation of p53 and contributing to its stabilization. The regulation of MDMX is even more complex. In response to DNA damage, ATM phosphorylates MDMX on Ser403 and mediates CHK2 phosphorylation of MDMX on Ser342 and Ser367. These phosphorylation events, together with ubiquitylation by MDM2 (stimulated by CHK2), leads to MDMX degradation and to p53 stabilization. The picture that emerges is that p53 is stabilized by ATM in a series of modifications. The same applies for both intra-S phase and G2–M phase checkpoint control. Many downstream players are involved in parallel pathways to achieve control. 53BP1, p53 binding protein-1; BRCA1, breast cancer susceptibility protein-1; CDC2, cell division control-2; FANCD2, Fanconi anaemia, complementation group D2; HAUSP, herpes virus-associated ubiquitin-specific protease; HRR, homologous recombination repair; MDC1, mediator of DNA-damage checkpoint protein-1.

Although the system used is somewhat artificial, it does reflect what is seen in the process initiated by DNA DSBs. When H2AX is phosphorylated by ATM in response to DNA damage, MDC1 binds to it and then acts as a scaffold for the binding of other proteins and therefore also the amplification of the response.

Autophosphorylation is an inherent part of ATM activation. It occurs at several sites and is responsible for the dissociation of the inactive dimeric ATM to an active monomeric form^{53,73}. It is also likely that autophosphorylation

of ATM alters the interaction with other proteins that alter the DNA-damage response. This is certainly the case in human cells, although evidence in some mouse studies suggests that ATM autophosphorylation and phosphorylation of downstream substrates is a consequence of ATM activation, rather than contributing to it. It is suggested that increased concentration of ATM in the region of the break is sufficient for activation. Clearly these differences between human and mouse is an area for further investigation.

Agents that damage DNA are mutagenic and in many cases also carcinogenic. The response to DNA DSBs is complex and is designed to minimize genome instability that arises from unrepaired breaks. The presence of unrepaired breaks in DNA increases the risk of genome rearrangements or translocations, and consequently the risk of inappropriate regulation of expression of specific genes, including oncogenes, is also increased. DNA breaks *per se* can also be beneficial in that they activate a DNA-damage response in pre-cancerous cells, thereby establishing a barrier to tumour progression¹¹¹. However, that barrier can be readily circumvented by mutation in

genes that impair the DNA-damage response and allow the development of cancer. Not unexpectedly, syndromes such as A-T and NBS that are characterized by a defective response to DNA DSBs are prone to chromosomal instability and cancer predisposition. Unrepaired breaks can also contribute to other pathologies such as neurodegeneration, immune-system defects, infertility and endocrine dysfunction. These abnormalities might arise as a direct consequence of the presence of unrepaired breaks, leading to cell death, or indirectly owing to the build-up of oxidative stress as a consequence of the persistence of breaks in DNA.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

ATM

UniProtKB: <http://www.uniprot.org>

ATR | MRE11 | NBS1 | RAD50

FURTHER INFORMATION

Leiden Open Variation Database:

<http://chromium.liacs.nl/LOVD2/home.php>

Martin Lavin's homepage: <http://www.qimr.edu.au/research/labs/martin/index.html>

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CORRIGENDUM

Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer

Martin F. Lavin

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In the timeline of the above article, reference 28 in the box with the text “ATM is activated by autophosphorylation” should be replaced with reference 53. Reference 22 should appear in the box “Radioresistant DNA synthesis phenotype described for A-T”, also in the timeline.