DNA DAMAGE

The ATM protein kinase: regulating the cellular response to genotoxic stress, and more

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Abstract | The protein kinase ataxia-telangiectasia mutated (ATM) is best known for its role as an apical activator of the DNA damage response in the face of DNA double-strand breaks (DSBs). Following induction of DSBs, ATM mobilizes one of the most extensive signalling networks that responds to specific stimuli and modifies directly or indirectly a broad range of targets. Although most ATM research has focused on this function, evidence suggests that ATM-mediated phosphorylation has a role in the response to other types of genotoxic stress. Moreover, it has become apparent that ATM is active in other cell signalling pathways involved in maintaining cellular homeostasis.

This Review is dedicated with deep indebtedness and appreciation to the memory of Maimon M. Cohen (1935–2007), a pioneer in human genetics and the research of ataxia-telangiectasia.

Protein phosphorylation is a central post-translational modification (PTM) in cellular signalling. Phosphorylation can modulate substrate activity, stability, interaction with other proteins and subcellular localization and often primes it for additional PTMs. Phosphorylation–dephosphorylation cycles can dynamically fine-tune the function and turnover of a protein. Cascades of protein phosphorylation are often the backbones of signalling pathways. Such cascades can be initiated by the activation of an apical protein kinase that alone is sufficient to mobilize an extensive signalling network.

The Ser/Thr protein kinase ataxia-telangiectasia mutated (ATM) is a prime example of this principle. The gene encoding ATM is mutated in the human genomic instability syndrome ataxia-telangiectasia (BOX 1). This disorder involves a marked defect in responding to a severe DNA lesion, the double-strand break (DSB)[1,2]. Not surprisingly, ATM is best known for its role as a chief mobilizer of the cellular response to this DNA lesion. DNA DSBs can be generated by DNA damaging agents, following the collapse of stalled replication forks[3] or in response to uncapped telomeres[4]. DSBs are also obligate intermediates in meiotic recombination and in the assembly of the genes encoding antigen receptors during lymphocyte maturation through V(D)J and class switch recombination (not discussed in this Review). Unrepaired DSBs can severely disrupt DNA replication in proliferating cells, usually leading to cell death, or leave chromosomal aberrations that may initiate a vicious cycle of further genomic alterations, setting the stage for cancer formation.

The vigorous cellular response to DSBs is a highly ordered cascade of events divided into several major stages, which partly overlap in time (BOX 2; FIG. 1a). Its early phase is characterized by the recruitment to DSB sites of a large, heterogeneous group of proteins collectively dubbed 'sensors' or 'mediators', which form large protein complexes that appear as nuclear foci when imaged under a fluorescence microscope. This is accompanied by the activation of 'transducers', which are protein kinases such as ATM that relay a strong, wide-spread signal to numerous downstream effectors. Some of these effectors are sensors, hence a feedback loop is created to maintain the construction and shape of the DSB-associated focus as well as the processes within this structure. This leads to the modulation of pathways and processes throughout the cell in which the various effectors are key players. An important determinant of the broad span of the DSB response is the extensive range of substrates of the main transducer of this response, ATM[5–7].

Recent work suggests that ATM might also be involved in the responses to other types of genotoxic stresses, perhaps in a less predominant position compared with its leading role in the DSB response. Furthermore, evidence is steadily accumulating that the broad capacity of ATM as a protein kinase is exploited in undamaged cells in other signalling pathways that

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Box 1 | **Ataxia-telangiectasia**

Ataxia-telangiectasia (Online Mendelian Inheritance in Man (OMIM) database ID: 208900) is a human genomic instability disorder inherited in an autosomal recessive manner[1,129]. It is caused by mutations in *ATM* (ataxia-telangiectasia mutated), which encodes the ATM protein[187,188]. The disease is characterized by progressive neurodegeneration that affects mainly the cerebellum and develops into severe neuromotor dysfunction, telangiectasia (that is, the dilation of blood vessels observed primarily in the eyes), immunodeficiency that spans the B cell and T cell systems, thymic and gonadal atrophy, marked predisposition to malignancies (primarily lymphoreticular), increased serum levels of α-fetoprotein and carcinoembryonic antigen, acute sensitivity to ionizing radiation, and, occasionally, growth retardation, premature ageing and insulin resistance. The hallmarks of the cellular phenotype of ataxia-telangiectasia are increased chromosomal breakage, premature senescence of cultured primary fibroblasts and sensitivity to DNA-damaging agents, which is most evident when cells from patients with ataxia-telangiectasia are treated with physical or chemical double-strand break (DSB)-inducing agents. This sensitivity represents a marked defect in the activation of the cellular response to DSBs, the chief mobilizer of which is ATM. Notably, however, typical readouts of the DSB response are attenuated in these cells rather than completely abolished, attesting to the activity of redundant protein kinases.

The classic ataxia-telangiectasia phenotype is caused by homozygosity or compound heterozygosity for *ATM*-null alleles, which typically truncate ATM or inactivate it via missense mutations (see Leiden Open Variation Database). Milder forms of the disease, which are characterized by later onset or slower progression of symptoms, are associated with mutations that leave residual amounts of functional ATM protein. Another disorder, ataxia-telangiectasia-like disorder (ATLD), is similar to mild ataxia-telangiectasia, with a later age of onset and slower progression than the classic condition[189]. ATLD is caused by hypomorphic mutations in *MRE11A*, which encodes a component of the MRN (MRE11–RAD50–NBS1) complex[190] (see main text).

Many of the major symptoms of ataxia-telangiectasia are readily explained by the defective response to physiological DSBs and DSBs induced by DNA-damaging agents. However, the debate continues over the cause of the most devastating symptom of the disease — the relentless cerebellar degeneration. This discussion is continuously fertilized by the discoveries of new roles for ATM in maintaining cellular homeostasis.

respond to various stimuli or physiological situations. The role of ATM under these conditions is probably less pronounced than in acute DNA damage and hence less visible than in the ATM-mediated DNA damage response (DDR) — ATM's main claim to fame. Some of these pathways are not nuclear and are involved in various metabolic processes. Is ATM a kinase for all purposes and a willing 'servant' of different, unrelated processes, or is there a common thread to ATM-mediated pathways in apparently different arenas? This Review directs the spotlight on this question.

ATM: a member of the PIKK family

A large, mostly uncharted molecule. ATM is a relatively large protein with a molecular weight of 350 kDa and comprising 3,056 residues (FIG. 1b). The landmark domain of ATM is its carboxy-terminal active site, which contains a PI3K signature despite the associated protein kinase activity of ATM. This domain places ATM within the family of PI3K-like protein kinases (PIKKs), most of which are involved in cellular responses to various stresses[8,9]. The PI3K domain occupies about 10% of this large protein. The rest of it presumably contains regulatory and interaction domains that determine its modes of activation and broad substrate specificity. Very few distinct domains have been defined in this largely uncharted territory, among them several repeats of a HEAT domain (common to huntingtin, elongation factor 3, protein phosphatase 2A (PP2A) and yeast target of rapamycin 1 (TOR1)) and a few interaction sites with other proteins (reviewed in REF. 10). Other notable sites are those that become decorated with PTMs during ATM activation (FIG. 1b) (see below). Clearly, the lack of structural data, which probably stems from technical challenges in ATM crystallization, is currently hindering progress in further understanding the functional significance of ATM domains.

A PIKK trinity? The PI3K domain is a signature of PIKKs, most of which are active in various stress responses[8,9]. Other members of this group are ATR (ATM and RAD3-related), DNA-PKcs (DNA-dependent protein kinase catalytic subunit), SMG1 (suppressor of mutagenesis in genitalia 1), mTOR (mammalian TOR) and TRRAP (transformation/transcription domain-associated protein; the only member of this group that lacks catalytic activity). The PI3K-like domain and two other C-terminal domains, FAT (conserved in FRAP, ATM and TRRAP) and FATC (FAT C-terminal) (FIG. 1b), are shared among PIKKs.

Three of the PIKKs are major players in responses to genotoxic stresses: ATM, DNA-PKcs and ATR. Notably, they preferentially phosphorylate their respective targets on Ser or Thr residues followed by Glu (S/T-Q motif). The diversity of their functions has recently come to light in a similar way: traditionally, each was associated with a specific canonical pathway, but awareness is growing of other signalling pathways that they regulate. DNA-PKcs is the catalytic subunit of the DNA-PK holoenzyme, which also contains the KU70–KU80 heterodimer. It is best known for its central role in the non-homologous end-joining (NHEJ) DSB repair pathway[11,12] (BOX 2), but its involvement in other processes, such as cell proliferation and regulation of oxidative stress, is being noted[13,14]. ATR has a major role in coordinating DNA replication origin firing and guarding replication fork stability, and its canonical pathway activates a DDR network following replication fork stalling, which may lead to subsequent collapse[3,15].

The primary stimuli, modes of activation and interacting proteins of the three PIKKs are different. Furthermore, they have unique physiological functions, evident from the diverse phenotypes caused by their deficiencies in humans and their genetic manipulation in mice[2,8,9,13,16–22]. Nevertheless, there are certain overlaps in their targets, functional crosstalk and even collaboration among them following certain genotoxic stresses, depending on the type and extent of the damage and the time point after damage induction (see REFS 23–26 for recent examples). This delicate balance and cooperation are abrogated by the loss of ATM in patients with ataxia-telangiectasia. Interestingly, cells from patients with ataxia-telangiectasia do show many DDRs that are considered ATM dependent, but these are markedly attenuated compared with wild-type cells[27]. It is possible that the absence of ATM affects the responses of DNA-PK and/or ATR to DSBs, and their collaborative activity is boosted to partially replace the missing member of the trio.

ATM: transducing the DNA damage alarm

ATM bursts into action. Following DSB induction, ATM undergoes spatial relocalization and catalytic activation. Although its total amount does not change[28], a portion of nuclear ATM is rapidly recruited to DNA damage sites, where most of it stays for several hours[29], whereas another portion presumably remains nucleoplasmic. ATM thus becomes an active component of the protein 'turret' that associates with the DSB site and is responsible, directly or indirectly, for many phosphorylation events and other protein PTMs within this structure. This may explain why the presence of catalytically inactive (kinase-dead) ATM in cells is more detrimental than its loss (BOX 3).

ATM activation was first noticed as modest enhancement of its kinase activity in cells treated with DNA damaging agents[30,31]. This moderate response *in vitro* was later found to represent a robust process in cells, which turns quiescent ATM into an avid phosphorylation machine[32–34]. Understanding the mechanisms of ATM activation and pinpointing the responsible stimuli are vital to understanding the physiological functions of ATM. A seminal study[32] showed that, in undamaged cells, quiescent ATM exists as homodimers, which dissociate into active monomers upon activation. This model was supported using *in vitro* purified proteins[34] and *Xenopus laevis* extracts[35]. A mere few DSBs were sufficient to induce massive, quantitative activation of the cellular ATM pool[32]. This study also identified the first PTM associated with ATM activation, autophosphorylation at Ser1981, a hallmark of activated human ATM. Three additional autophosphorylation sites have been identified in activated ATM[7,36,37], as well as TIP60-mediated acetylation at Lys3016 (REFS 38,39) (FIG. 1b). Abrogation of these modifications hampers human ATM function in the DDR[7,32,36,37,39], as expected from modifications that promote the transition from a dormant to an active kinase. Surprisingly, however, abolishing three equivalent autophosphorylation sites in mouse ATM did not affect its DDR functions[40,41]. Why the human and mouse orthologues differ remains unresolved[42]. Notably, *in vitro* ATM autophosphorylation at Ser1981 was not required for monomerization of ATM dimers[34]. Clearly, however, these modifications mark activated ATM in both organisms. It was recently shown that these autophosphorylation sites are required for retention of activated ATM at DSBs, albeit not for the initial recruitment of ATM[7,43]. Importantly, dephosphorylation events might also contribute to ATM activation, as at least two protein phosphatases, PP2A[44] and PP5 (REF. 45), were reported to be required for this process.

What sparks ATM activation following DSB induction is still being debated. It was suggested that the initial trigger of ATM activation is a chromatin conformational change that follows DSB formation rather than direct contact of ATM with broken DNA[32]. Moreover, it has been proposed that the mere tethering of ATM or several other DDR players to undamaged chromatin is sufficient to induce the ATM-dependent DDR[46]. Other studies suggested that direct interaction of ATM with broken DNA is required for its activation[47], specifically a contact with single-stranded stretches at DSBs[48]. Oligonucleotides emanating from DSBs following end resection were also suggested to stimulate ATM activity[49]. The above factors may indeed contribute to the rapid and robust activation of ATM, but the biophysical nature of the activation process and the mechanism of action of its physical trigger are still unclear.

Nevertheless, it has been shown *in vitro* and *in vivo* that the MRE11–RAD50–NBS1 (MRN) complex is required for optimal ATM activation at DSBs (for a review, see REF. 10). MRN is one of the first complexes to be recruited to DSB sites, where it acts as a damage sensor that can also form a physical bridge spanning the DSB ends[50]. It is required for timely repair by both NHEJ and homology-directed repair (HDR) (BOX 2), and by virtue of its nuclease component, MRE11, it takes part in DSB end resection, which is essential for HDR. Notably, the interaction between specific domains of ATM and NBS1 is central to ATM recruitment and retention at DSB sites[34,51–54]. Two major sensor proteins, 53BP1 (p53-binding protein 1) and BRCA1 (breast cancer type 1), assist in optimizing this interaction[55], which also requires Lys63-linked ubiquitylation of NBS1

Box 2 | **The DSB response**

The cellular response to DNA double-strand breaks (DSBs) induced by DNA-damaging agents comprises a vast signalling system, perhaps one of the broadest cellular responses to stimuli. Beginning seconds after DSB induction, it modulates numerous cellular processes in a concerted, structured manner (FIG. 1). It calls repair mechanisms to action, activates special cell cycle checkpoints, affects gene expression on a large scale, moderates protein synthesis, activity and turnover, and affects many aspects of cellular metabolism[89,191]. This system relies on a core of DNA damage response (DDR)-dedicated proteins, but its broad reach is enabled by the temporary recruitment of factors that function in other processes. The early phase of the DSB response includes the massive build-up of the rapidly expanding, multiprotein focus at DSB sites, which is highly structured in space and time[192]. This process is accompanied by extensive induction of post-translational modifications (PTMs; including phosphorylation, ubiquitylation, sumoylation, acetylation, methylation and poly(ADP)-ribosylation) of many of the recruited proteins as well as of the core histones at the vicinity of the breaks, followed by a subsequent change in the epigenetic landscape in that region[88]. One notable example is phosphorylation of the tail of histone H2A.X; phosphorylated H2A.X (γH2A.X) anchors some of the founders of the DSB-associated focus[88,192,193]. Chromatin reorganization, initial processing of the DSB ends and finally their actual repair occur within these protein complexes, which disassemble after DNA integrity is restored.

Much of the feverish activity around the DSB is aimed at setting the scene for the actual repair of the lesion[191,194]. In addition to chromatin reorganization and relaxation, this includes DNA end processing, which reshapes the DNA ends to make them legitimate substrates for DNA metabolizing enzymes, and may entail 5′–3′ resection that leaves variable lengths of single-strand stretches. DSB repair is then mediated by non-homologous end-joining (NHEJ) or homologous recombination repair (HRR; also known as homology-directed repair (HDR)). The lion's share of DSB repair is handled by NHEJ, which results in the ligation of DSB ends at the cost of leaving microdeletions at the junctions. In mammals, this pathway is active throughout the cell cycle. HRR can take place in the late S phase or G2 phase of the cell cycle and involves recombination between the damaged DNA molecule and an intact sister molecule, and is thus error-free. Several specialized variations on these two repair pathways have recently been described[195]. Each pathway is carried out via sequential action of many proteins, and the delicate balance between the two is affected by the degree of end resection at the break and a crosstalk between the cell cycle machinery and the DDR[196].

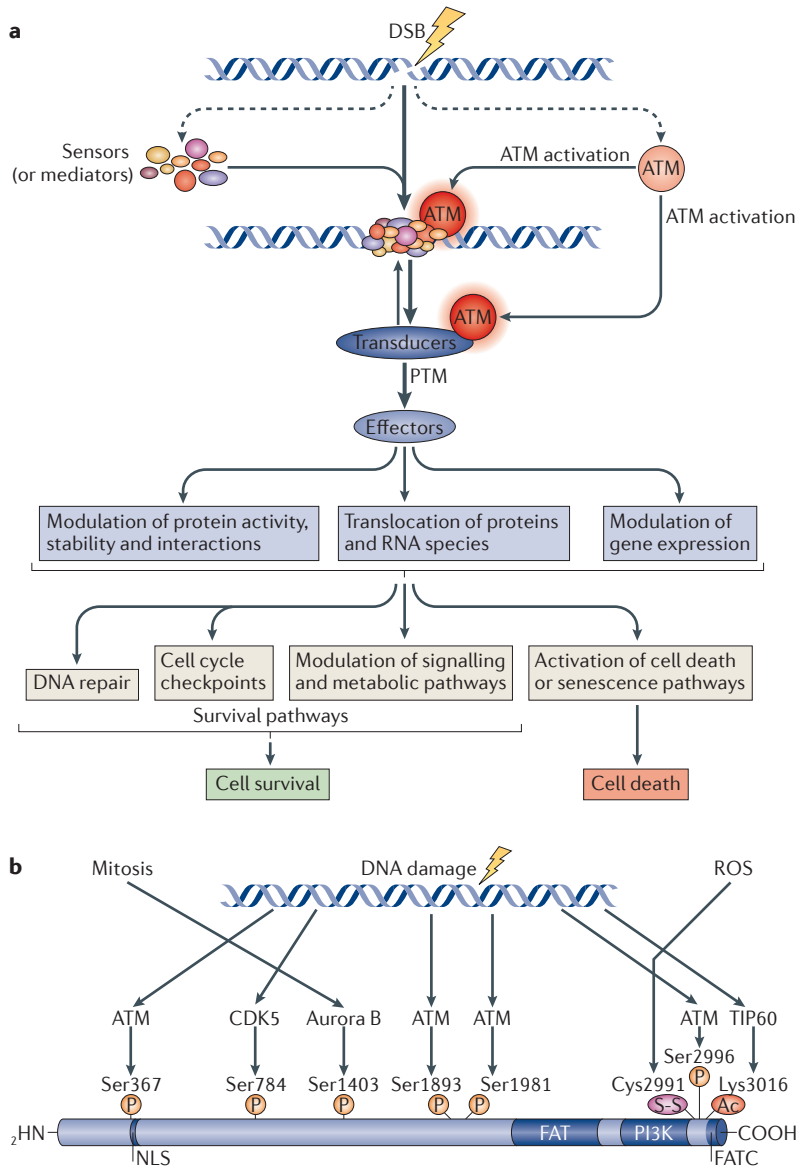

Figure 1 | **The DDR cascade and ATM.** **a** | The broad but incredibly structured and meticulous DNA damage response (DDR) cascade is initiated by the formation of large protein complexes (which include sensors (or mediators)) at double-strand break (DSB) sites, which appear as nuclear foci under the microscope. Extensive activity within these foci (including chromatin reorganization and DNA damage signal amplification), accompanied by many protein post-translational modifications (PTMs), sets the scene for DSB repair and leads to the activation of protein kinases, most notably ataxia-telangiectasia mutated (ATM), which transduce the DSB alarm to numerous downstream effectors. These, in turn, activate both cell survival and cell death pathways, and the final outcome depends on a delicate balance between these opposing processes. **b** | Schematic representation of ATM depicting its major domains. The sites of PTMs associated with ATM activation in various contexts and the proteins responsible for these modifications, including ATM itself (see text for details), are indicated. Ac, acetylation; FATC, FAT carboxy-terminal; NLS, nuclear localization sequence; P, phosphorylation; ROS, reactive oxygen species; S-S, disulphide bridge.

mediated by the E3 ubiquitin ligase SKP2 (S phase kinase-associated protein 2)[56]. Importantly, MRN complex components are themselves phosphorylated by ATM, and these phosphorylation events contribute to the timely activation of various DDR branches[57–61] and may also create a positive feedback loop that maintains ATM activity. The ATM–MRN complex connection is thus one of the central functional links of ATM during its activation.

Another important interaction of ATM is with the sensor protein MDC1 (mediator of DNA damage checkpoint protein 1). MDC1 is also required for timely ATM activation[62]. It is anchored to DSB sites via its interaction with phosphorylated histone H2A.X (γH2A.X)[63] (BOX 2) and in parallel binds ATM. This allows ATM to phosphorylate additional H2A.X moieties, which in turn serve to bind additional MDC1 molecules in a repeated process that accelerates the expansion of the DSB-associated focus[43,64,65]. Similarly to the MRN complex, MDC1 is an ATM substrate, and its ATM-mediated phosphorylation enhances its oligomerization at DSB sites[66–68], demonstrating once again a positive feedback loop between ATM and a protein that is required for its proper positioning at DSB sites. Optimal ATM activation was found to depend on several other proteins, such as the cell death regulator AVEN (an ATM target)[69], FOXO3A (forkhead box O3A)[70], the histone acetyltransferase KAT8 (also known as MOF)[71], the chromatin protein HMGN1 (high mobility group nucleosome-binding domain-containing protein 1)[72] and the ubiquitin ligases RNF8 (RING finger 8) and CHFR (checkpoint with forkhead and RING finger domains), which are involved in histone ubiquitylation in response to DNA damage[73].

Understanding the role of all these proteins in ATM activation awaits the elucidation of the structural nature of this process. Currently, the data point to a combination of DNA damage-induced protein–protein interactions and PTMs on ATM and other proteins that collectively support and streamline ATM activation and relocalization to DSB sites. None of these interactions and PTMs is absolutely and solely required for this process, but together they optimize it in space and time. Such concerted action of many, sometimes partially redundant, players for fine-tuning processes is a recurring theme in the DDR.

Although most of the work concerning the ATM-mediated network revolves around its mobilization following DNA damage induction, less attention is given to the inactivation of this enormous network after successful DNA repair. Presumably, the return of the cell to a normal life cycle should follow a well-organized process in which the DDR winds down in a streamlined, highly structured process. One well-documented protein phosphatase that removes several ATM-dependent phosphorylations, including at least one autophosphorylation event of ATM itself, is the type 2C phosphatase WIP1 (also known as PPM1D)[74–76]. Interestingly, *in vivo* evidence for the functional link between ATM and WIP1 was obtained in mice, in which loss of WIP1 partially rescued the ATM-deficiency phenotype[77]. It is reasonable to assume that there is a lot more to the recovery of the ATM-mediated DDR than the action of a single phosphatase, and this process begs for experimentation.

Box 3 | **Losing ATM or losing its activity: not exactly the same**

Researchers have long suspected that the physiological consequences of loss of ataxia-telangiectasia mutated (ATM) as opposed to harbouring inactive ATM may not be the same[197]. Solid evidence of this notion was obtained recently using mice with different *Atm* mutations. *Atm*-knockout mice have long been known to recapitulate most symptoms that are characteristic for ataxia-telangiectasia with the exception of neurodegeneration and hence present a relatively moderate phenotype[177–180]. Strikingly, mice producing physiological levels of catalytically inactive (kinase-dead) ATM were recently found to die early in embryonic life, and conditional expression of the mutant protein in the immune system caused marked genomic instability in lymphoid cells[198,199]. Mechanistic explanations for this startling observation are still lacking, but as kinase-dead ATM was recruited to DSB sites[198,199], it is possible that the presence of catalytically inactive ATM within the DNA damage response hub that surrounds these sites severely disturbs the damage response network. At any rate, these studies clearly indicate that the presence of the inactive form of ATM affects the cellular response to genotoxic stress more than its absence.

ATM signalling: a robust but finely tuned operation. Although the apical position of ATM in the DDR cascade was clear from the beginning, nothing prepared investigators for the striking wealth of ATM targets that has unfolded in recent years. FIGURE 2a depicts a recent map of the functional links of ATM in the DDR. The map contains ATM effectors that have been documented in detail, but proteomic screens point to hundreds more putative ATM substrates[5–7]. This plethora of downstream effectors is unusual even when compared with those of most diversified protein kinases and raises the inevitable question of whether ATM is a promiscuous or meticulous protein kinase. Several considerations lead us to believe that most of the putative ATM-dependent phosphorylation events are likely to be functionally significant. This notion stems from the structure of the DDR network and the principles that underlie its function: its extremely broad reach to many aspects of cellular metabolism, including profound, large-scale effects on the cellular transcriptome and proteome[78–81] (FIG. 2b); and the DDR-driven regulation of specific processes by simultaneously modulating several pathways that control each one of them, a typical example being the special cell cycle checkpoints that are induced by DNA damage[82]. This redundancy and the commonly observed ATM-dependent phosphorylation of several substrates in the same pathway allow fine-tuning of the corresponding processes (see below).

It should be noted that ATM phosphorylates and thus modulates the activity of several protein kinases, which in turn phosphorylate their own substrate repertoires. Checkpoint kinase 2 (CHK2) is perhaps the best-documented example[83], and DNA-PK[84], AKT[85] and homeodomain-interacting protein kinase 2 (HIPK2)[86] should be noted too. Proteomic experiments and meta-analysis of the literature suggest several other protein kinases[87]; thus, the number of ATM-dependent phosphorylation events in the DDR may considerably exceed that of direct ATM-mediated ones. Indeed, components of the DDR may undergo multiple ATM-dependent phosphorylation events, some carried out by ATM itself and others by protein kinases that depend on ATM for their activation. Often these phosphorylation events prime the substrates for additional PTMs, such as ubiquitylation or sumoylation, and these PTM combinations collectively affect the action and/or fate of the substrate[88–90].

A prototypic example of the multilayered regulation of an ATM-dependent pathway is the activation and stabilization of p53 in response to DNA damage[91,92]. The first ATM-mediated phosphorylation event characterized in detail was that of p53, and this was used to demonstrate ATM activation[30,31,93]. The ATM-dependent activation and stabilization of p53 are central to the modulation of the cellular transcriptome following induction of DSBs[79]. Activated p53 drives the expression of genes that are involved in the activation of cell cycle checkpoints (a cell survival mechanism) but also genes that promote programmed cell death[94,95]. The interplay between these opposing mechanisms presumably determines cell fate and is influenced by the type and extent of the damage. ATM activates and stabilizes p53 via a complex signalling subnetwork within the larger DDR system, one that includes extensive PTM-mediated modulation of a range of proteins that affect p53 activity and stability (FIG. 3).

Although activated p53 drives the expression of many genes that promote programmed cell death, ATM activates the transcription factor nuclear factor-κB (NF-κB), which promotes the expression of several anti-apoptotic genes[78,96]. ATM activates NF-κB mainly by phosphorylating IKKγ, a subunit of IκB kinase (IKK), which is also ubiquitylated and sumoylated in response to DNA damage[97]. IKK is then activated, phosphorylates and thus leads to the degradation of the NF-κB inhibitor IκBα, which sequesters NF-κB in the cytoplasm. This allows NF-κB to translocate to the nucleus, where it binds to target genes. In the nucleus, ATM regulates a delicate cascade that relocates ATM itself outside the nucleus and includes ATM-dependent modifications on several proteins.

There is more to the decision between cell survival and activation of cell death programmes than just gene regulation. Similarly to the regulation of the cell cycle checkpoints, this decision is also mediated by more rapid pathways that are modulated by protein PTMs. ATM was recently shown to phosphorylate PIDD (p53-inducible protein with a death domain) — a molecular switch between cell survival and cell death in response to genotoxic stress — thereby modulating its interactions with specific proteins that lead to these opposite biological processes[98].

Another example of the multipronged approach of ATM to pathway regulation concerns a cardinal facet of ataxia-telangiectasia, the acute radiosensitivity of the patients, which stems from a partial defect in DSB repair. The fraction of DSBs that depends on ATM for timely repair is relatively small and estimated to be in 10–15% of the breaks in commonly used cell lines[99]. However, ATM approaches its apparently modest share of DSB repair from several directions by phosphorylating direct players in DSB repair such as the nuclease Artemis[99–101], CtBP-interacting protein (CtIP)[102,103], DNA-PKcs[84], polynucleotide kinase 3′-phosphatase[104–106], the checkpoint clamp protein RAD9 (REF. 107) and the three components of the MRN complex[61,108,109]. Moreover, ATM mobilizes

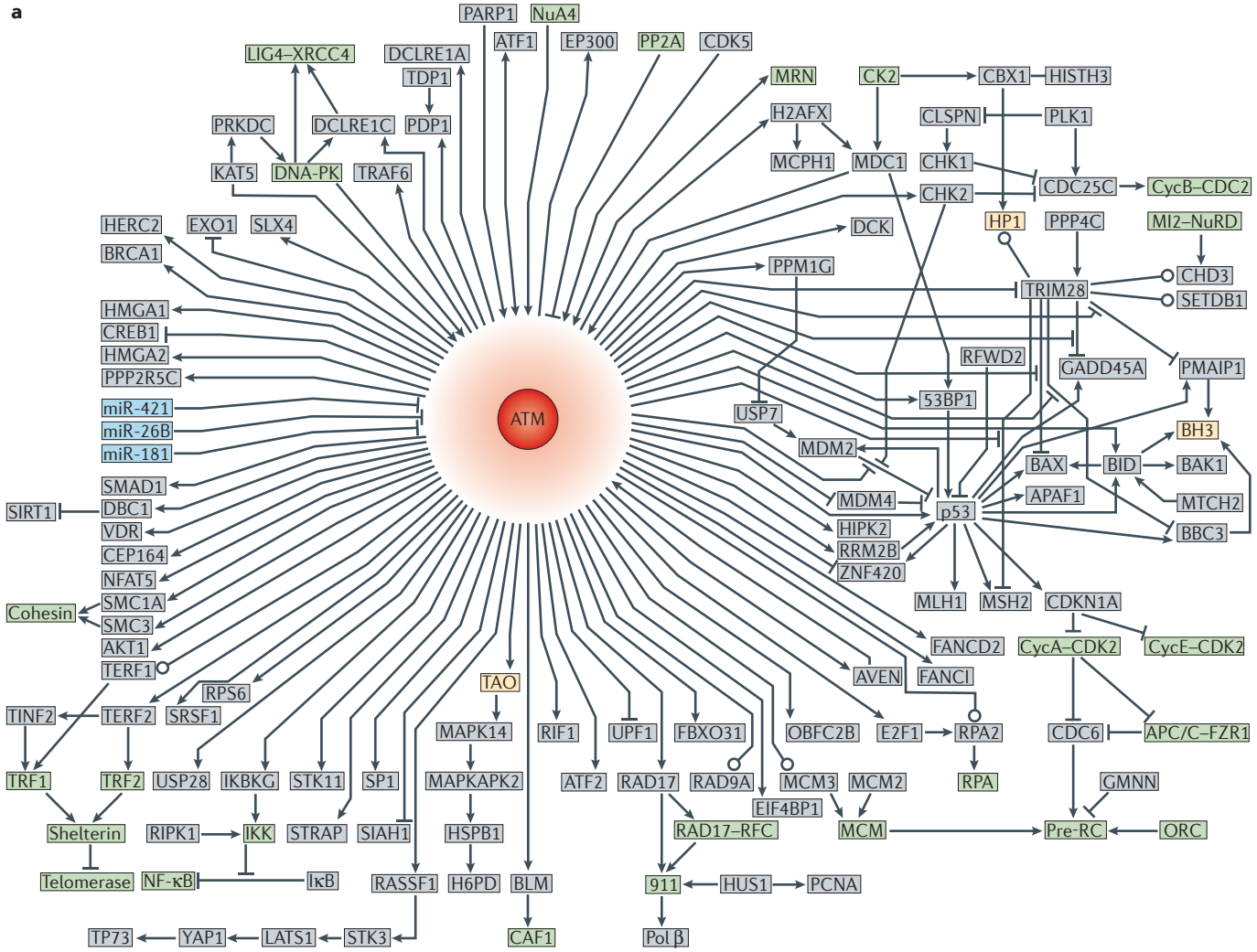

Figure 2 | **The scope of the ATM-mediated DNA damage response.** **a** | Map of ataxia-telangiectasia mutated (ATM) functional interactions, each of which has been thoroughly documented in at least one publication. The map is based on information collated from the SPIKE database of signalling pathways[200]. In most cases, proteins that functionally interact with ATM are shown for each pathway, most of which are ATM substrates. Proteins are depicted in grey, microRNAs (miRNAs) in blue, protein complexes in green and protein families in yellow. Arrows correspond to activation, T-shaped edges to inhibition, and open circles denote regulations the effect of which is still unclear. **b** | Functional classification of 1,077 proteins identified in proteomic screens as putative ATM substrates[5–7]. Initial functional division was made using Gene Ontology annotations, and closely related classes were subsequently assembled into the shown groups. Some of the groups partially overlap.

several pathways that lead to chromatin relaxation, which is necessary for timely repair, by phosphorylating various players in this process, such as KRAB-associated protein 1 (KAP1)[110–113] and the RNF20–RNF40 heterodimer (which ubiquitylates histone H2B)[114,115]. Notably, ATM also phosphorylates CHD4 (chromodomain helicase DNA-binding protein 4), a member of the NuRD (nucleosome remodelling and histone deacetylation) complex, the recruitment of which to DSB sites is important for DSB repair[116–119], and the transcription factor SP1, which is recruited to DSB sites and phosphorylated by ATM to facilitate DSB repair via an unknown mechanism[120].

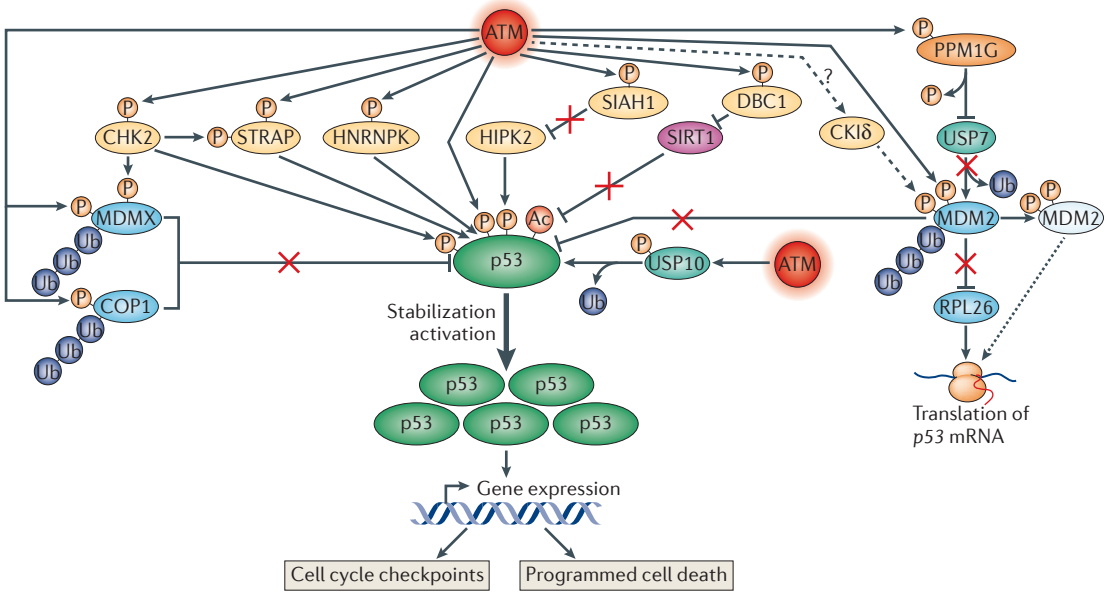

Figure 3 | **ATM-mediated activation and stabilization of p53 in response to DNA damage induction.** Within the broad DNA damage response (DDR) network, a complex but finely concerted subnetwork, governed by many ataxia-telangiectasia mutated (ATM) targets, is devoted to stimulating and increasing the amount of p53 protein, a key player in transcriptome dynamics in the face of DNA damage. For simplicity, most accessory proteins that are not under ATM control are not shown. In addition to ATM, prominent protein kinases in this network are CHK2 (checkpoint kinase 2) and HIPK2 (homeodomain-interacting protein kinase 2). Others include STRAP (Ser/Thr kinase receptor-associated protein) and HNRNPK (heterologous nuclear ribonucleoprotein K). DNA damage-induced post-translational modifications (PTMs) of p53 (which include phosphorylation, dephosphorylation and acetylation (mediated by sirtuin 1 (SIRT1)) contribute mainly to its activation and direct its transcriptional activity towards genes that are involved in cell cycle arrest and programmed cell death. The rapid stabilization and subsequent accumulation of p53 are achieved primarily by modulating the activity, stability and subcellular localization of a range of proteins. A central player in this process is MDM2, which in unprovoked cells functions as an E3 ubiquitin ligase towards p53, keeping low basal p53 expression levels in check. Following ATM- and CKIδ (casein kinase Iδ)-mediated phosphorylation, MDM2 undergoes an allosteric change that interferes with this activity. Concomitantly, ATM-mediated phosphorylation stimulates MDM2 binding to *p53* mRNA, which enhances its translation (dotted arrow). This translation enhancement is also achieved by relieving MDM2-mediated polyubiquitylation of the ribosomal protein RPL26, a positive regulator of p53 translation. It is unclear whether CKIδ is stimulated by ATM (dashed arrow). Another E3 ubiquitin ligase of p53, COP1 (constitutive photomorphogenesis 1), and the p53 inhibitor MDMX undergo enhanced polyubiquitylation and proteasomal degradation following their DNA damage-induced phosphorylation by ATM, and ATM and CHK2, respectively. Two deubiquitylating enzymes (DUBS) have been found to play parts in this network to date: USP7 (ubiquitin-specific protease 7; also known as HAUSP), which alternately modulates the stability of MDM2, MDMX and p53 (not shown), and USP10, which, following its ATM-mediated phosphorylation, undergoes nuclear import and stabilization, deubiquitylates p53 and thus further contributes to its stabilization. Notably, the phosphatase WIP1 (also known as PPM1D) removes ATM-mediated phosphorylation of MDM2 and p53, thereby antagonizing this network (not shown). For further details, see REFS 91,92,201–205. DBC1, deleted in breast cancer 1; SIAH1, seven in absentia homologue 1.

Another ATM-dependent process, rapid recruitment of proteasomes to sites of DNA damage, depends on a newly identified target of ATM, the nuclear proteasome activator PA28γ (also known as PSME3). This recruitment plays a part in maintaining the balance between the two major DSB repair pathways: NHEJ and HDR[121].

It has recently emerged that, in addition to regulating the activity of transcription factors, ATM reshapes the cellular RNA landscape by other means. A study revealed an ATM-dependent pathway that represses transcription in the vicinity of DSBs[122]. This is presumably necessary for streamlining DSB repair and preventing the generation of erroneous transcripts. This pathway is mediated by inhibiting transcription elongation-dependent chromatin decondensation and involves histone H2A ubiquitylation. It should be noted that a later study suggested that this effect is mediated primarily by DNA-PK rather than ATM[123]; the reason for this discrepancy remains unclear. Another way by which the transcriptome can be modulated is by altering mRNA stability through the activity of microRNAs (miRNAs). A recent study showed that a subset of miRNAs is upregulated in an ATM-dependent manner following treatment with a radiomimetic chemical[124]. This pathway depends on ATM-mediated phosphorylation of KSRP (KH-type splicing regulatory protein), a key regulator in miRNA biogenesis. The increase in expressed miRNAs may lead to the rapid downregulation of proteins the levels of which should be reduced in the course of the DDR. Notably, regulators of RNA processing are frequently identified in proteomic screens for potential ATM targets (FIG. 2b).

Altogether, the ATM-mediated network is a prime example of a multilayered signalling network brought to action by a single, powerful protein kinase that sparks a rapid, highly structured cascade of protein PTMs. This endows ATM with a strong grip on the network while allowing it to delicately regulate its various branches and their meticulous coordination with each other. In the laboratory, the size and complexity of this network sets up such a noisy background that it can be a daunting task to ferret out the functional analysis of a single pathway, often a single PTM.

ATM: fine-tuning the responses to other genotoxic stresses. ATM clearly mobilizes and orchestrates the vast DSB response network in response to induction of acute DNA damage. Is this ATM's sole 'raison d'être'? In the large range of DNA lesions that regularly occur in body tissues under normal physiological conditions, the DSB is in stark minority, outnumbered by other endogenous lesions, mainly single-strand breaks (SSBs). Furthermore, the vigorous ATM-mediated DSB response is usually studied in the laboratory under artificial conditions using radiation doses that most living organisms never encounter. This raises the question of whether ATM has roles in other signalling pathways. The 'non-DDR' functions of ATM are addressed below, but evidence suggests that ATM may have roles in genotoxic stress responses in addition to controlling the DSB response; it may continuously be acting in the background of cellular responses to various DNA lesions. ATM may not be an integral player in these responses, but may, when needed, use its capacity as a protein kinase to streamline, enhance and sometimes rescue these processes.

This notion traces its origins to scattered reports on moderate sensitivity of cells from patients with ataxia-telangiectasia to alkylating and crosslinking agents[125,126] or SSB-inducing agents[127], which was strongly overshadowed by the striking hypersensitivity of these cells to DSB-inducing agents or thought to reflect DSBs that are secondary to the primary lesions induced by these agents[128,129]. Furthermore, among the well-documented ATM substrates are proteins with roles in responses to different DNA lesions. These include TDP1 (tyrosyl-DNA phosphodiesterase 1)[130], polynucleotide kinase 3′-phosphatase[104–106], the RecQ helicase-like BLM (Bloom's syndrome helicase)[131–134] and several proteins in the Fanconi anaemia pathway of DNA crosslink repair (reviewed in REF. 135). Moreover, phosphoproteomic screens had shown that many enzymes involved in the repair of various DNA lesions as well as PARP1 (poly(ADP-ribose) polymerase 1) are ATM substrates[5–7]. Collectively, these observations suggest that ATM-mediated phosphorylation events may have supporting roles in various genotoxic stress responses and in resolving aberrant DNA structures that are occasionally formed in the course of DNA transactions. This ongoing, low-profile role of ATM, masked by its highly visible role in the DSB response cascade, may constitute a considerable portion of its daily work. The inherent chromosomal instability observed in cells from patients with ataxia-telangiectasia[1] or in cells in which ATM is chemically inhibited[136] may reflect this ongoing duty of ATM. The loss of this function in these patients may be no less important than that of the timely DSB response in causing the symptoms.

ATM: expanding roles in cell signalling

Most ATM investigations focus on its canonical pathway — the mobilization of the DSB response. These studies, which typically involve treatment of cultured cells with high doses of DNA damaging agents, depict an apparent dichotomy between a 'dormant' ATM in undamaged cells and an explosively active ATM responding to DNA damage. Is the diverse capacity of this powerful protein kinase used only in this critical situation? We suggested above that in maintaining genomic stability, ATM continuously assists and streamlines cellular responses to various genotoxic stresses, most of which are presumably endogenous, and to various faults in DNA metabolism. Evidence is accumulating that the capacity of ATM as a protein kinase is also continuously exploited in signalling processes that are not associated with DNA damage, some of which are cytoplasmic. Such a function would require downstream effectors with key roles in these pathways and ongoing activity of ATM or various modes of ATM activation. ATM may therefore be a versatile protein kinase that is continuously operating in different signalling pathways involved in maintaining cellular homeostasis. Analogous to the temporary recruitment in the DDR of players from various cellular processes when the DNA damage alarm is sounded, ATM may leave its routine duties, go into high gear and take over the vast DDR network.

Activation of ATM (monitored by its autophosphorylation and phosphorylation of its downstream substrates) was observed in cells challenged by hypoxia[137–139], which leads to replication stress followed by activation of ATR- and ATM-mediated responses without apparent DNA damage. In this case, ATM activation was found to be MRN-independent, but ATM-mediated phosphorylation of the downstream substrate KAP1 in this context required MDC1 (REF. 138). Hyperthermia was also found to activate ATM in an MRE11-independent manner[140]. ATM activation by replication stress (which is triggered by hypotonic stress) or by treatment with the chemical chloroquine[32] (which affects chromatin organization) was dependent on the nuclear zinc-finger protein ATMIN (ATM interactor; also known as ASCIZ)[141]. Importantly, DSB formation led to the dissociation of ATM from ATMIN and its interaction with the MRN complex component NBS1 (REF. 141), possibly reflecting a transition from a DSB-independent ATM activation to a DSB-dependent mechanism. However, it was recently reported that antagonism and redundancy of ATMIN and NBS1, which compete for ATM binding, modulate ATM activity also in response to DSBs, and deficiency in both NBS1 and ATMIN severely abrogated ATM signalling and led to radiosensitivity[142]. This change of the guard between ATMIN and NBS1 in ATM activation suggests that ATM may be activated by several pathways in response to different stimuli.

Fanconi anaemia pathway
A DNA damage response pathway that responds predominantly to DNA interstrand crosslinks. Mutations leading to loss of any of the15 proteins in this pathway cause the genomic instability syndrome Fanconi anaemia in humans.

DNA crosslink
Covalent linkage of two positions in the DNA molecule (in the same strand or in opposite strands), caused by endogenous or exogenous bifunctional agents that interact with both positions.

Replication stress
Stress on DNA metabolism imposed by inefficient DNA replication that leads to slowing or stalling of replication forks. It can be caused by DNA damage, depletion of dNTP pools or decreased or extremely increased replicon initiation.

Hypotonic stress
Stress on cellular homeostasis imposed by a hypotonic environment in which the extracellular solute concentration is lower than the intracellular one. Osmosis then causes a flow of water into the cell, compromising its integrity.

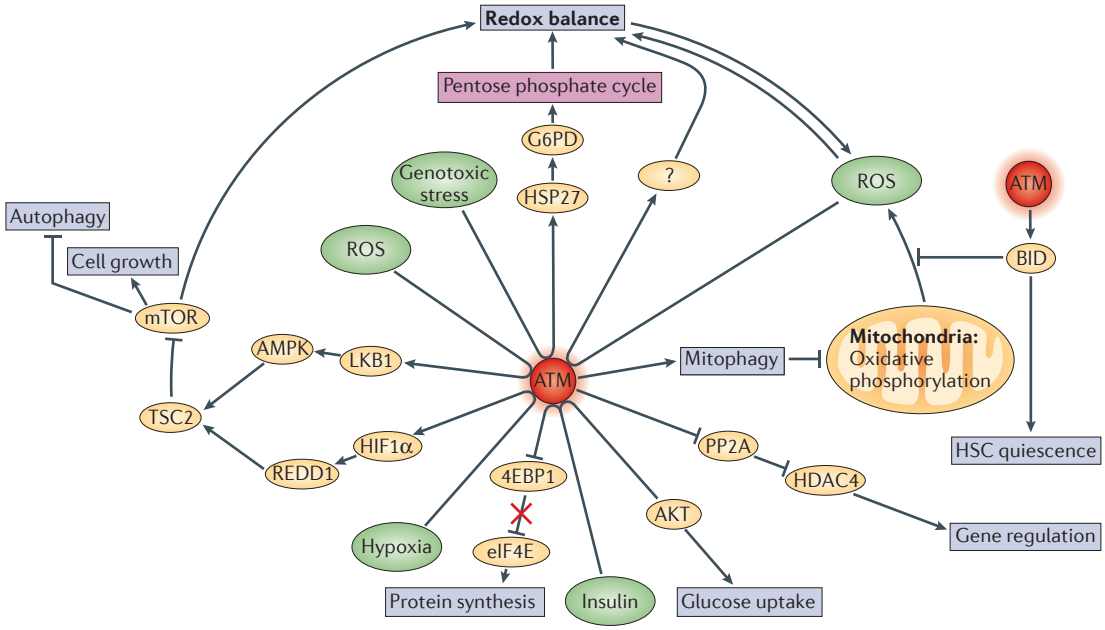

Figure 4 | **Involvement of ATM in cellular homeostasis pathways.** Roles for ataxia-telangiectasia mutated (ATM) that are distinct from its functions in the response to double-strand breaks have been identified. In response to insulin, ATM phosphorylates and thus inhibits the translation repressor 4EBP1 (eIF4E-binding protein 1), thereby promoting protein synthesis. ATM also activates AKT to enhance glucose uptake. In addition, ATM has been shown to regulate histone deacetylase 4 (HDAC4)-mediated gene expression. In response to reactive oxygen species (ROS), ATM activates tuberous sclerosis complex 2 (TSC2), which negatively regulates mammalian target of rapamycin (mTOR), thus blocking autophagy and promoting cell growth. This can also occur in response to hypoxia through phosphorylation of the transcription regulator HIF1α (hypoxia-inducible factor 1α). In response to genotoxic stress, ATM enhances the pentose phosphate cycle, which is a source of the antioxidant NADPH. ATM has also been implicated in mitochondrial homeostasis and the secretion of ROS by modulating mitophagy. This pathway, together with enhanced pentose phosphate cycle and inhibition of mTOR, all contribute to maintaining redox homeostasis. Interestingly, BID (BH3-interacting domain death agonist) is an ATM substrate that has emerged as an important mediator of stress responses, including the regulation of mitochondrial metabolism and of haematopoietic stem cell (HSC) quiescence. AMPK, AMP-activated protein kinase; eIF4E, eukaryotic translation initiation factor 4E; G6PD, glucose-6-phosphate dehydrogenase; HSP27, heat shock protein 27; LKB1, liver kinase B1; PP2A, protein phosphatase 2A; REDD1, regulated in development and DNA damage response 1.

Another interesting example of a non-DDR ATM-mediated pathway is the recently identified role of ATM in the mitotic spindle checkpoint. This pathway involves low-level activation of ATM that is not associated with DNA damage and is MRN independent. Instead, it requires phosphorylation of ATM by Aurora B kinase, which is followed by the canonical ATM autophosphorylation that is observed following DNA damage. This leads to ATM-mediated phosphorylation and activation of the kinetochore protein BUB1, which is essential for the spindle checkpoint[143].

Collectively these findings point to several modes of ATM activation by different physiological stimuli. The search for non-DDR functions of ATM is motivated largely by an ongoing debate on the molecular basis of the neurodegeneration phenotype in ataxia-telangiectasia and other aspects of this disease, such as the premature ageing and insulin-resistant diabetes observed in some patients (BOX 1). Notably, several of the non-DDR functions of ATM converge to regulate redox signalling and the cellular response to oxidative stress[144] (FIG. 4). The loss of some of these functions, particularly the regulation of oxidative stress, may explain the striking premature senescence of primary fibroblasts from patients with ataxia-telangiectasia under ambient oxygen pressure[145].

ATM reaches out of the nucleus. Evidence for a cytoplasmic pool of ATM or extranuclear shuttling of ATM has been steadily accumulating[97,146–150], and some reports indicated that ATM loss affects various cytoplasmic signalling pathways, such as calcium and potassium ion mobilization, in which ATM deficiency leads to defects in calcium and potassium currents in human fibroblasts and mouse neurons[151–153]. Moreover, in response to DNA damage, ATM was reported to regulate *de novo* synthesis of ceramide, which occurs in the endoplasmic reticulum[154]. Furthermore, recent proteomic analyses suggested the involvement of ATM in various cytoplasmic metabolic pathways[80,155–157]. With cytoplasmic ATM-dependent pathways becoming a common notion[158], the pioneering work by Yang and Kastan[159] on the involvement of ATM in insulin signalling should be noted. These investigators showed early on that in response to insulin, ATM phosphorylates the translation repressor 4EBP1 (eIF4E-binding protein 1). 4EBP1 reversibly binds to and

Kinetochore
A multiprotein complex that assembles on centromeric DNA. It mediates the attachment of chromosomes to spindle fibres and their subsequent movement to the mitotic spindle poles.

inhibits eIF4E, a component of the translation initiation complex, and its phosphorylation leads its dissociation from eIF4E and enhancement of protein synthesis. They suggested that the loss of this pathway may contribute to the poor growth and insulin resistance reported in some patients with ataxia-telangiectasia. Interestingly, it was later found that loss of one or both *Atm* alleles in mice lacking mouse apolipoprotein E (APOE) worsened the features of metabolic syndrome in these animals[160], including atherosclerosis and insulin resistance (the latter is commonly associated with metabolic syndrome in humans). ATM responds to insulin also by activating the protein kinase AKT (also known as PKB) in an incompletely charted pathway that enhances glucose uptake in certain cell types (for a review, see REF. 85).

A role in redox homeostasis. ATM has also been implicated in the regulation of oxidative stress, and its effects in this pathway were recently shown to modulate cytoplasmic pathways. Our laboratory suggested early on that ATM could be an upstream sensor that is activated by oxidative damage or stress[161]. Subsequent reports indicated that oxidative stress was not properly controlled in cells from patients with ataxia-telangiectasia and in tissues of ATM-deficient mice and tied this phenomenon to various features of the disease (reviewed in REF. 144). Reactive oxygen species (ROS) can directly affect protein structure by oxidizing Cys residues, which in turn react with other amino acids, for example by forming disulphide bonds with other Cys residues[162]. In a landmark paper it was recently shown that direct oxidation of certain Cys residues in ATM by ROS leads to the formation of active disulphide-crosslinked ATM dimers in a manner independent of DSBs or the MRN complex[163]. Interestingly, oxidation-mediated activation of ATM rules out further activation by the DSB- and MRN-mediated pathway, and it was suggested that it may direct the activity of ATM to different sets of targets[164]. The importance of this discovery is that ATM was shown to respond to different stresses through distinct activation mechanisms. The involvement of ATM in regulating oxidative stress adds to our understanding of the phenotypic outcomes of *ATM* mutations, most notably cerebellar attrition caused by ATM loss in humans[165]. The recent report showing that ATM-regulated oxidative stress has a role in promoting pathological angiogenesis in the mouse retina is of particular interest[166]. This finding could have an impact on the attempts to develop ATM-based cancer therapies and the treatment of neovascular diseases.

Notably, several cytoplasmic ATM-mediated pathways that affect ROS levels were recently identified (FIG. 4). In response to genotoxic stress, ATM was found to enhance the pentose phosphate cycle, a major source of the important antioxidant cofactor NADPH. The pathway involves ATM-mediated phosphorylation of heat shock protein 27 (HSP27), which in turn binds to and stimulates the activity of glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate cycle[167]. Besides attenuating ROS accumulation, increased NADPH levels may promote nucleotide synthesis for DNA repair.

Other ATM-mediated pathways that modulate redox homeostasis enter the territory of another highly influential PIKK, namely mTOR. This protein kinase integrates various environmental signals to modulate protein synthesis and pathways that control cellular growth and homeostasis[168]. In response to increased ROS, ATM activates TSC2 (tuberous sclerosis complex 2), a negative regulator of mTORC1 (mTOR complex 1), and TSC2-mediated mTORC1 repression in turn enhances autophagy[169,170]. ATM-dependent TSC2 activation occurs in the cytoplasm via phosphorylation of the tumour suppressor protein LKB1 (liver kinase B1). Phosphorylated LKB1 activates AMPK (AMP-activated protein kinase), which in turn phosphorylates and activates TSC2. Interestingly, ATM can mediate suppression of mTORC1 signalling also in response to hypoxia, in this case by phosphorylating the transcription regulator HIF1α (hypoxia-inducible factor 1α)[171]. This results in increased levels of HIF1α, leading to upregulation of its target genes, for example the gene encoding the TSC2 activator REDD1 (regulated in development and DNA damage response 1; also known as DDIT4) (FIG. 4). This pathway is probably linked to the above-mentioned activation of ATM by hypoxia[137–139].

An emerging mitochondrial connection. Insights into the role of ATM in regulating the cellular redox balance were also obtained by studies on its impact on mitochondrial physiology. Several laboratories noted increased numbers of mitochondria in ATM-deficient cells, concomitant with mitochondrial dysfunction[172–174], but the data on mitochondrial respiratory activity in these cells were conflicting[172,173]. Recent work in mouse thymocytes showed that a fraction of cellular ATM can be found in mitochondrial preparations, supporting the notion of reduced mitochondrial function in ATM-deficient cells[174]. Importantly, this work provided an explanation for the increased number of mitochondria in the absence of ATM: instead of increased mitochondrial biogenesis, there is defective destruction of abnormal mitochondria (mitophagy) (FIG. 4). Furthermore, partial or complete loss of the autophagy regulator Beclin 1 delayed the appearance of lymphomas in *Atm*-null mice. The work pointed to an important addition to the homeostatic roles of ATM and suggested a link between mitochondrial physiology, ROS metabolism, autophagy and tumour predisposition. A key question is whether ATM affects mitochondrial physiology and turnover by directly phosphorylating mitochondrial proteins and whether it is activated by damage to mitochondrial DNA.

BID: an ATM effector on several fronts. ATM action in these diverse cellular processes requires appropriate effectors. A recent example of an ATM substrate that bridges different ATM-controlled arenas is BID (BH3-interacting domain death agonist), a protein that is best known for its role in triggering the mitochondrial apoptotic programme following death receptor activation (reviewed in REF. 175). This programme starts with profound alterations in mitochondrial metabolism (FIG. 4).

Apolipoprotein E
(APOE). A class of apolipoprotein that is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. APOE transports lipoproteins, fat-soluble vitamins and cholesterol into the lymphatic system and then into the blood.

Reactive oxygen species
(ROS). Important signalling intermediates with special roles in stem cell renewal and apoptosis. They are byproducts of cellular metabolism that also pose a constant threat to cellular constituents. Excess ROS or other oxidants beyond the cellular antioxidant capacity leads to oxidative stress, with broad pathological consequences.

Pentose phosphate cycle
A cytoplasmic chain of reactions that oxidizes glucose, reduces NADP to NADPH and generates pentoses (5-carbon sugars). It is the major source of the NADPH required for anabolic processes.

Autophagy
A tightly regulated catabolic process involving degradation of cellular components through the lysosomal machinery. During steady-state conditions, it maintains homeostasis through the elimination of damaged organelles and proteins. Under stress conditions, such as nutrient starvation, it is highly enhanced, reallocating nutrients from less essential processes to crucial ones and providing the cell with building blocks for survival.

Thymocytes
Haematopoietic progenitor cells that are present in the thymus. They differentiate into mature T cells.

Interestingly, ATM-mediated phosphorylation of BID was recently found to have a role in the canonical DDR, and BID was also identified as a player in the ATR-mediated response to replicative stress. Furthermore, BID is now emerging as an important mediator of various stress responses in the liver and the haematopoietic system. In the haematopoietic system, ATM-mediated phosphorylation of BID plays a major part in maintaining the quiescence and survival of haematopoietic stem cells (HSCs) and myeloid progenitor cells, a process that is crucial for bone marrow homeostasis. ATM controls this in the absence or presence of external stress. Interestingly, in the bone marrow, the ATM–BID pathway acts as a regulator of a mitochondrial rheostat to dictate whether HSCs will remain quiescent, enter the cell cycle or undergo apoptosis[176]. This pathway further exemplifies the emerging role of ATM as a homeostatic protein kinase.

Roles in neuronal cell function and retinal vascularization. The striking feature of the neurological phenotype seen in patients with ataxia-telangiectasia is the cerebellar atrophy (BOX 1). No less striking is the absence of similar cerebellar degeneration in several strains of ATM-deficient mice[177–179] and the very mild cerebellar defect observed in one strain[180]. However, close examination of other aspects of the nervous system in *Atm*$^{-/-}$ mice has indicated malfunction of the nigrostriatal pathway[181–183], age-dependent reduction in dopaminergic neurons and reduced synaptic function in hippocampal neurons[148]. The nature of the responsible developmental defects is unclear. Cultured *Atm*$^{-/-}$ neurons exhibited defective neural network activity following DNA damage. Interestingly, the firing activity of individual neurons was normal, but the function of the culture as a network was impaired[184].

A specific ATM-dependent pathway was recently proposed to contribute to the neurodegeneration associated with ATM loss in humans. ATM was found to indirectly control the nuclear–cytoplasmic shuttling of histone deacetylase 4 (HDAC4)[185]. HDAC4 must be phosphorylated to remain cytoplasmic. This phosphorylation is negatively regulated by PP2A, the activity of which is negatively regulated by ATM-mediated phosphorylation. Thus, lack of ATM leads to enhanced PP2A activity and consequently to the accumulation of HDAC4 in the nucleus, where it alters gene expression via histone deacetylation. Because HDAC4 deficiency had previously been associated with cerebellar atrophy in mice, the authors suggested that the altered balance between cytoplasmic and nuclear HDAC4 may contribute to the cerebellar atrophy observed in patients with ataxia-telangiectasia.

An interesting abnormality identified in ATM-deficient mice was impaired vascularization of the retina, which leads to retinal pathology[186]. Although it is unclear whether these characteristics are reflected in the human ataxia-telangiectasia phenotype and their mechanistic aspects are unknown, this may suggest additional roles for ATM in nervous system homeostasis.

Nigrostriatal pathway
A neural pathway that connects two areas in the brain, the substantia nigra and the striatum. It is one of the four major dopaminergic pathways in the brain and is involved in the production of movement.

Dopaminergic neurons
The main source of dopamine in the mammalian central nervous system. Their loss is associated with Parkinson's disease and various mood disorders.

Hippocampal neurons
Cells of the cerebral cortex that are involved in memory formation, consolidation, indexing and storage, as well as spatial orientation and navigation.

Neural network
Circuits based on groups of neurons and glial cells that are connected or functionally related and together perform a specific physiological function.

Conclusions and future perspectives

This Review reflects what seems to be a transition in our perception of ATM: from a protein kinase identified exclusively with the mobilization of the DSB response to a versatile kinase involved in the response to various genotoxic stresses and in diverse aspects of cellular homeostasis. In the face of a DSB emergency, this versatile kinase can abruptly suspend its routine duties in several cellular compartments and take command over a vast signalling network, to which many other players are similarly recruited from their daily chores. Thus, an in-depth analysis of the effects of ATM loss and ATM inhibition on the cellular metabolome would be a timely experiment.

A key question in determining the role of ATM in cellular metabolism is how it is activated in these different contexts. What mechanistic and structural changes occur in this protein as a result of various stimuli? How do these stimuli bring about those changes? Do these changes affect the substrate preference of ATM? What are the structural determinants of such preferences? Answers to most of these questions are likely to come from protein structure analysis, which is technically challenging in the case of ATM, the protein structure of which is still 'virgin soil'.

Numerous laboratories are searching for the functional significance of individual 'trees' in the vast 'forest' of ATM-mediated phosphorylation events. Particularly interesting, and sometimes surprising, are those events discovered by laboratories working in other cell biological arenas altogether, only to find that their proteins of interest are ATM targets in various contexts. It is expected that the 'fingerprints' of ATM will be found in additional cellular signalling pathways.

No less important than the activation and rise of the ATM-mediated DDR network is its fall after DNA damage repair. This process too may be complex and highly structured in space and time. It may have its own unique players that remove DNA damage-induced PTMs from proteins that are to be recirculated or that degrade others. Another cardinal question concerns the death mechanisms that are activated in cells destined not to return to the cellular life cycle after suffering extensive DNA damage.

Notably, most of the work on the DDR and the various ATM-mediated pathways was carried out with proliferating cell lines, whereas most of the cells in higher organisms are post-mitotic. Revisiting some of the current concepts in these fields using specific types of differentiated cells will probably be inevitable.

The phenotypes associated with ATM mutations will continue to attract considerable interest. Why isn't the complete loss of ATM embryonic lethal when this very phenotype is associated with catalytically inactive ATM? How do the new roles of ATM in cellular metabolism explain the many symptoms of ataxia-telangiectasia and the associated multifaceted cellular phenotype? And how can understanding ataxia-telangiectasia help us to treat this devastating human disorder? Indeed, these are some of the same questions that led to the discovery of ATM in the first place.

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Competing interests statement
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