MECHANISMS OF NON-CANONICAL ATM ACTIVATION

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I. INTRODUCTION[1]

ATM (Ataxia Telangiectasia mutated, EC 2.7.11.1) is a serine/threonine protein kinase that belongs to a family of phosphatidylinositol 3-kinase related kinases (PIKK). The *ATM* gene encodes a 350-kDa protein consisting of 3056 amino acids. The domain structure of ATM includes HEAT repeats, FAT (FRAM/ATM/TRRAP), C-terminal FATC and kinase domains as described elswhere [2]. ATR (ATM- and Rad3-related kinase) and DNA-PKcs (catalytic subunit of DNA-dependent protein kinase) display significant homology with ATM. ATM, ATR and DNA-PKcs are all important for the cellular response to DNA damage, although specific functions of these kinases differ significantly [3].

The primary function of ATM is the coordination of the cellular response to DNA damage caused by ionizing radiation. The mechanism of ATM kinase activation in response to ionizing radiation, initially proposed by Kastan's laboratory, involves intramolecular self-phosphorylation of ATM at serine 1981 followed by its monomerization [4]. However, subsequent studies showed that many other amino acid residues undergo post-translational modification, and these include the self-phosphorylation of serines 367, 1893, 2996 and tyrosine 1885 [5-7]. In addition, acetylation of lysine 3016 by the Tip60 histone acetyltransferase is required for kinase activity following self-phosphorylation [8, 9]. These modifications are functionally important in human cell lines, but their relevance in mouse models and *Xenopus* extracts remains controversial [10, 11].

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[1] *Abbreviations:* ATM - Ataxia Telangiectasia mutated; ATR - ATM- and Rad3-related kinase; DNA-PKcs - DNA-dependent protein kinase catalytic subunit; DSB – DNA double-strand break; SSB – DNA single-strand break; MRN - Mre11-Rad50-Nbs1 complex; Top1 – DNA topoisomerase I; Top1cc - Top1-DNA intermediate; A-T - Ataxia Telangiectasia; R-loop – RNA-DNA hybrid

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The canonical model for activation invokes a specific role for DNA double-strand breaks (DSBs), which are highly mutagenic DNA lesions induced by ionizing radiation. ATM activation requires the presence of the MRN (Mre11-Rad50-Nbs1) complex that ensures the initial localization of ATM in a complex with Tip60 at sites of DSB formation [8, 9, 12-15]. In response to ATM activation a cascade of kinase activities leads to the phosphorylation of over a thousand substrates that are required for the coordination of various cellular processes such as chromatin remodeling, transcription and splicing, cell cycle progression, DNA repair, and apoptosis (for review see [16-18]).

It has also been proposed that ATM is activated following oxidative stress by a fundamentally different mechanism [19]. In this case, an active ATM dimer contains a disulfide bond formed upon oxidation of two cysteine 2991 residues. In contrast to DSB-dependent activation of ATM in the nucleus, this oxidative-dependent ATM activation mechanism occurs in the cytoplasm and is important for the coordination of insulin signalling, and mitochondrial and peroxisome functions. [20-22]. Consistent with these proposals, a number of cytoplasmic substrates of ATM have been identified by quantitative proteomics [23]. The mechanisms of ATM activation in response to DSBs and oxidative stress are discussed in detail in a number of comprehensive reviews [18, 24-27].

Given the functional importance of ATM in the cellular landscape, it is not surprising that inactivation of its function underpins the disease Ataxia Telangiectasia (A-T), also referred to as Louis-Bar syndrome [28-30]. A-T is a rare autosomal recessive multisystemic disorder (1 case per 40,000-100,000) that develops in early childhood [31, 32]. A-T is characterised by immunodeficiency, progressive neurodegeneration and increased predisposition to cancer. There are currently no treatments for A-T [33].

In recent years, the exclusivity of DNA double-strand breaks and oxidative stress as inducers of ATM activation has been questioned. In this review, new experimental data are discussed which indicate a wider cellular role for ATM as a sensor and regulator of the cellular response to DNA single-strand breaks, RNA-DNA hybrids, as well as changes to the structure of chromatin and the cytoskeleton.

II. NON-CANONICAL ACTIVATORS OF ATM

DNA LESIONS (EXCEPT FOR DNA DOUBLE-STRAND BREAKS)

DNA single-strand breaks

More than 20 years ago, Tomas Lindahl (2015 Nobel Laureate in Chemistry) proposed that the number of DNA double-strand breaks arising due to the inherent instability of DNA is significantly lower (10-20 lesions per cell per day) than that of endogenous DNA single-strand breaks (15,000-20,000 lesions/cell/day) [34]. In addition, DNA single-strand breaks (SSBs) form as intermediates during the repair of damaged DNA bases (the so-called base excision repair pathway) [35]. The repair of SSBs is crucial for a cell as the replication of SSB-containing DNA leads to the formation of highly mutagenic DSBs [36]. Moreover, the transcription of SSB-containing DNA is inefficient and can be blocked [37, 38]. Defects in SSB repair have been linked to various diseases, including neurodegeneration and cancer [39-43].

Unrepaired SSBs induce activation of ATM in the absence of DSBs [1]. This activation is important for promoting a G_1 cell cycle delay, thus providing time for the controlled repair of SSBs prior to the replication of DNA and therefore prevents the formation of replication-associated DSBs. In addition, ATM-dependent signalling is important for regulating the capacity of DNA base damage and SSB repair [44, 45]. Consequently, inadequate signalling of unrepaired SSBs in the absence of ATM leads to replication of the damaged DNA followed by accumulation of mutagenic DSBs, thus contributing to the genetic instability phenotype characteristic of A-T (Fig. 1).

A role for ATM in SSB signalling is consistent with the sensitivity of A-T cells to DNA damaging agents that cause DNA base damage and SSB formation [46, 47]. However, the mechanism of ATM activation in response to SSBs – its dependence on the presence of MRN complex components or the oxidation of disulfide bond/s, or the existence of an independent mechanism – is presently unknown. It also remains unclear whether SSB-dependent ATM activation contributes to ATM activation in response to ionizing radiation, a treatment that induces significant number of SSBs and DNA base damage in addition to DSBs [48].

Covalent DNA topoisomerase I-DNA adducts

DNA topoisomerase I (Top1) catalyses the relaxation of DNA supercoiling that is produced during DNA replication and transcription. The mechanism of relaxation involves formation of a Top1-DNA intermediate (Top1cc), cleavage of one DNA strand of DNA, followed by ligation [49]. Top1 inhibitors, such as camptothecin, stabilise Top1cc adducts and thus prevent DNA ligation, inducing transcription defects [50].

ATM is activated in response to treatment of quiescent (non-replicating) human cells and post-mitotic mouse cortical neurons with camptothecin [51]. SSBs with a covalent link between the 3'-phosphoryl end and a tyrosine residue of the Top1 active site peptide have been suggested as possible inducers of ATM activity. Such unconventional SSBs form during partial proteasomal degradation of Top1 within camptothecin-stabilised Top1cc adducts. Consequently, the accumulation of Top1cc adducts and ATM self-phosphorylation can be rescued upon inhibition of transcription and proteasomal degradation using DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) and MG-132, respectively. The formation of DSBs, that could contribute to ATM activation, was excluded using DNA Comet assays.

The mechanism of activation and signalling for activation will require further investigation, but it is possible that activation results from local changes to chromatin structure at sites of arrested transcription complexes [51]. It is also possible that ATM activation involves DNA single-strand breaks with a 3'-modification. Interestingly, endogenous Top1cc adducts aberrantly accumulate in the brain cells of *Atm*$^{-/-}$ mice and in human A-T cells [52]. This effect is independent of ATM kinase activity, and is related to abrogated proteasomal degradation of Top1 adducts in the absence of ATM.

R-loops

More recently, R-loops, or RNA-DNA heteroduplexes [53], that form upon inhibition of Top1 activity during transcription [54], have been implicated in ATM activation. However, it remains controversial whether R-loops can activate ATM directly.

ATM is activated in non-replicating cells (primary lymphocytes, rat cortical neurons and synchronized human primary fibroblasts) treated with camptothecin [55, 56]. However, in contrast to some observations [51], DSBs were detected by the accumulation of γH2AX and 53BP1 foci, which serve as DSB markers, and neutral Comet assays. Both ATM activation and DSB formation were rescued upon inhibition of transcription or

expression of RNAse H1 that cleaves RNA within R-loops, indicating an indirect role for R-loops in this activation. It has been proposed that transcription-blocking Top1cc lesions lead to R-loop formation, which are then processed into DSBs that activate ATM [55, 57]. The mechanism by which R-loops can be converted into DSBs is unknown. It is, however, thought that the trancription-coupled nucleotide excision repair endonucleases XPG and XPF-ERCC1 can cleave both DNA strands during resolution of R-loops, resulting in replication-independent DSBs [58]. Alternatively, SSBs with partially cleaved Top1 peptide at the 3'-end might act as precursors of replication-independent DSBs. These DSBs could result from a proximal SSB formed by repair of another Top1cc adduct, an endogenous DNA lesion or an R-loop (cleavage of a single DNA strand during classical transcription coupled nucleotide excision repair) [56]. It therefore appears that R-loops can mediate ATM activation indirectly, through the formation of replication-independent DSBs.

More recently, R-loops were proposed to be the primary inducers of ATM activity, which regulates alternative pre-mRNA splicing [59]. R-loop formation and ATM activation have been observed in UV-treated non-replicating human skin fibroblasts, and ATM activation can be rescued by the treatment of cells with inhibitors of transcription elongation and overexpression of RNAse H1. Importantly and in contrast to [55, 56], ATM activation occurs in the absence of DSBs, as determined by the absence of γH2AX and 53BP1 focus formation. The role for R-loops in ATM activation and its mechanism require further investigation [59].

STRUCTURAL CHANGES

Changes in chromatin structure

DSBs are known to initiate significant changes to the structure of chromatin [60], and it is possible that chromatin remodeling is a direct inducer of ATM activation in response to ionising radiation [4]. Indeed, the importance of chromatin remodeling has been demonstrated by the activation of ATM in the absence of DSBs. This was shown by treatment of human cells in hypotonic solution with chloroquine, using inhibitors of histone deacetylases that promote chromatin decompaction and an siRNA-mediated knockdown of the heterochromatin protein 1α [4, 61]. MRN-independent activation of ATM under hypotonic stress conditions was found to be dependent on the interaction of

ATM with the ATMIN protein, whereas ATM activation in response to irradiation is ATMIN-independent [62]. Further details of a role for chromatin remodeling in ATM activation is discussed elsewhere [63].

Mechanical stress

The activity of ATR kinase, a close homologue of ATM, is canonically induced in the presence of nucleofilaments of single-stranded DNA and the replicative protein A [64]. induced in response to mechanical stress [65]. The localization of ATR to the nuclear envelope, together with the induction of its kinase activity, was observed in human cells under hypertonic conditions that induce osmotic shock and mechanical (membrane) stress in the absence of DSBs. Similar results were obtained upon mechanical stretching of cells and cell compression, within the physiological range of mechanical forces, using a compressive-load system. The mechanism of ATR activation in response to mechanical stress requires further investigation, although it appears to be distinct from the canonical mode of activation. The activation of ATR is important for regulation of the plasticity of the nuclear envelope and the association of chromatin with the nuclear envelope [65]. It remains to be established whether mechanical stress plays a role in ATM activation.

ACTIVATION OF ATM IN THE ABSENCE OF DSB SENSORS

Two independent protein complexes are important for the detection of DSBs in eukaryotes [66]; DSB recognition by the MRN-complex results in the activation of ATM, whereas the Ku (Ku70-Ku80) complex promotes the induction of DNA-PKcs activity, leading to the repair of DSBs via non-homologous end-joining (NHEJ) [67]. In recent elegant work [68], activated DNA-PKcs was shown to functionally substitute for ATM in the absence of MRN in mouse embryonic fibroblasts, whereas MRN-dependent ATM activation was observed in the absence of Ku. Unexpectedly, ATM-dependent phosphorylation of histone H2AX, as well as a G_2M cell cycle delay in response to ionizing radiation, were observed in cells deficient for both DSB sensors. The mechanism of such MRN-independent ATM activation currently remains unclear [68].

III. AETIOLOGY OF A-T

The clinical symptoms of A-T that relate to the functions of ATM in the coordination of the cellular response to DSBs include immunodeficiency, sensitivity to ionizing radiation (radiosensitivity) and other DNA damaging agents, and an increased risk of tumourigenesis [69, 70]. In addition, A-T patients present with progressive neurodegeneration, including the atrophy of spinal cord, cerebellum, and the brain stem, coupled with the loss of Purkinje cells, as well as granular neurons and cells of the molecular layer [71-73], and ataxia.

The neurodegenerative phenotype of A-T is likely to be multifactorial in accordance with the variety of ATM's cellular functions, and the reasons for neurodegeneration are yet to be established. Our progress in understanding the molecular basis underlying A-T has been limited due to the absence of a good animal model for the disease – the progressive neurodegeneration phenotype that is observed in *Atm*$^{-/-}$ mice is rather mild compared to that in humans [74-76]. Interestingly, this mild phenotype is partly rescued by the use of antioxidants, indicating a role for oxidative stress [77, 78]. Increased levels of oxidative stress are also detected in A-T patient cell lines [79, 80].

It is worth noting that no increase in the levels of R-loops has been observed in brain tissues of *Atm*$^{-/-}$ mice, rejecting a role for RNA-DNA heteroduplexes in the aetiology of the disease [81]. The neurodegenerative pathology of A-T can also be linked to defects in the elimination of cells containing unrepaired DSBs during development of the neural system [82]. Moreover, the novel role for ATM in coordination of the repair of SSBs indicates a possible contribution of transcription inhibiting SSBs to the aetiology of ataxia telangiectasia [1].

IV. CONCLUSIONS

The experimental data demonstrate that ATM can be activated by a number of non-canonical inducers (i.e. distinct from DNA double-strand breaks). These include DNA single-strand breaks, RNA-DNA hybrids and changes in chromatin structure (Fig. 2). However, despite our progress in understanding of the functions of ATM kinase, it has yet to be established whether there exists a universal inducer of ATM activation, such as, for example, DNA lesions or chromatin remodeling. In addition, whether or not ATM gets activated in response to various discrete types of stress, and the mechanisms of its activation is yet to be studied.

REFERENCES

1. Khoronenkova, S.V., Dianov, G.L. (2015) ATM prevents DSB formation by coordinating SSB repair and cell cycle progression. *Proc Natl Acad Sci U S A*, **112**, 3997-4002.
2. Lavin, M.F., Scott, S., Gueven, N., Kozlov, S., Peng, C., Chen, P. (2004) Functional consequences of sequence alterations in the ATM gene. *DNA Repair (Amst)*, **3**, 1197-1205.
3. Falck, J., Coates, J., Jackson, S.P. (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*, **434**, 605-611.
4. Bakkenist, C.J., Kastan, M.B. (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature*, **421**, 499-506.
5. Kozlov, S., Gueven, N., Keating, K., Ramsay, J., Lavin, M.F. (2003) ATP activates ataxia-telangiectasia mutated (ATM) in vitro. Importance of autophosphorylation. *J Biol Chem*, **278**, 9309-9317.
6. Kozlov, S.V., Graham, M.E., Peng, C., Chen, P., Robinson, P.J., Lavin, M.F. (2006) Involvement of novel autophosphorylation sites in ATM activation. *EMBO J*, **25**, 3504-3514.
7. Kozlov, S.V., Graham, M.E., Jakob, B., Tobias, F., Kijas, A.W., Tanuji, M., Chen, P., Robinson, P.J., Taucher-Scholz, G., Suzuki, K., So, S., Chen, D., Lavin, M.F. (2011) Autophosphorylation and ATM activation: additional sites add to the complexity. *J Biol Chem*, **286**, 9107-9119.
8. Sun, Y., Jiang, X., Chen, S., Fernandes, N., Price, B.D. (2005) A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci U S A*, **102**, 13182-13187.
9. Sun, Y., Xu, Y., Roy, K., Price, B.D. (2007) DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol Cell Biol*, **27**, 8502-8509.
10. Pellegrini, M., Celeste, A., Difilippantonio, S., Guo, R., Wang, W., Feigenbaum, L., Nussenzweig, A. (2006) Autophosphorylation at serine 1987 is dispensable for murine Atm activation in vivo. *Nature*, **443**, 222-225.
11. Daniel, J.A., Pellegrini, M., Lee, J.H., Paull, T.T., Feigenbaum, L., Nussenzweig, A. (2008) Multiple autophosphorylation sites are dispensable for murine ATM activation in vivo. *J Cell Biol*, **183**, 777-783.
12. Uziel, T., Lerenthal, Y., Moyal, L., Andegeko, Y., Mittelman, L., Shiloh, Y. (2003) Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J*, **22**, 5612-5621.
13. Carson, C.T., Schwartz, R.A., Stracker, T.H., Lilley, C.E., Lee, D.V., Weitzman, M.D. (2003) The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J*, **22**, 6610-6620.
14. Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M.K., Moreau, L.A., Whetstine, J.R., Price, B.D. (2009) Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat Cell Biol*, **11**, 1376-1382.
15. Deshpande, R.A., Williams, G.J., Limbo, O., Williams, R.S., Kuhnlein, J., Lee, J.H., Classen, S., Guenther, G., Russell, P., Tainer, J.A., Paull, T.T. (2014) ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. *EMBO J*, **33**, 482-500.
16. Bhatti, S., Kozlov, S., Farooqi, A.A., Naqi, A., Lavin, M., Khanna, K.K. (2011) ATM protein kinase: the linchpin of cellular defenses to stress. *Cell Mol Life Sci*, **68**, 2977-3006.
17. Shiloh, Y., Ziv, Y. (2013) The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat Rev Mol Cell Biol*, **14**, 197-210.
18. Paull, T.T. (2015) Mechanisms of ATM Activation. *Annu Rev Biochem*, **84**, 711-738.

19. Guo, Z., Kozlov, S., Lavin, M.F., Person, M.D., Paull, T.T. (2010) ATM activation by oxidative stress. *Science*, **330**, 517-521.
20. Yang, D.Q., Kastan, M.B. (2000) Participation of ATM in insulin signalling through phosphorylation of eIF-4E-binding protein 1. *Nat Cell Biol*, **2**, 893-898.
21. Valentin-Vega, Y.A., Maclean, K.H., Tait-Mulder, J., Milasta, S., Steeves, M., Dorsey, F.C., Cleveland, J.L., Green, D.R., Kastan, M.B. (2012) Mitochondrial dysfunction in ataxia-telangiectasia. *Blood*, **119**, 1490-1500.
22. Zhang, J., Tripathi, D.N., Jing, J., Alexander, A., Kim, J., Powell, R.T., Dere, R., Tait-Mulder, J., Lee, J.H., Paull, T.T., Pandita, R.K., Charaka, V.K., Pandita, T.K., Kastan, M.B., Walker, C.L. (2015) ATM functions at the peroxisome to induce pexophagy in response to ROS. *Nat Cell Biol*, **17**, 1259-1269.
23. Kozlov, S.V., Waardenberg, A.J., Engholm-Keller, K., Arthur, J.W., Graham, M.E., Lavin, M.F. (2015) ROS-activated ATM-dependent phosphorylation of cytoplasmic substrates identified by large scale phosphoproteomics screen. *Mol Cell Proteomics*.
24. McKinnon, P.J. (2012) ATM and the molecular pathogenesis of ataxia telangiectasia. *Annu Rev Pathol*, **7**, 303-321.
25. Di Domenico, E.G., Romano, E., Del Porto, P., Ascenzioni, F. (2014) Multifunctional role of ATM/Tel1 kinase in genome stability: from the DNA damage response to telomere maintenance. *Biomed Res Int*, **2014**, 787404.
26. Shiloh, Y. (2014) ATM: expanding roles as a chief guardian of genome stability. *Exp Cell Res*, **329**, 154-161.
27. Lavin, M.F., Kozlov, S., Gatei, M., Kijas, A.W. (2015) ATM-Dependent Phosphorylation of All Three Members of the MRN Complex: From Sensor to Adaptor. *Biomolecules*, **5**, 2877-2902.
28. Syllaba, L., Henner, K. (1926) Contribution à l'e´tude de l'inde´pendance de l'athe´tose double idiopathique et conge´nitale. Atteinte familiale, syndrome dystrophique, signe du re´sau vasculaire conjonctival, inte´grite´ psychique. *Rev Neurol (Paris)*, **1**, 541-560.
29. Louis-Bar, D. (1941) Sur un syndrome progressif cormprenant des télangiectasies capillaires cutanées et conjonctivales symétriques, à disposition naevoïde et des troubles cérébelleux. *Confin Neurol*, **4**, 32-42.
30. Boder, E., Sedgwick, R.P. (1958) Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. *Pediatrics*, **21**, 526-554.
31. Su, Y., Swift, M. (2000) Mortality rates among carriers of ataxia-telangiectasia mutant alleles. *Ann Intern Med*, **133**, 770-778.
32. Swift, M., Morrell, D., Cromartie, E., Chamberlin, A.R., Skolnick, M.H., Bishop, D.T. (1986) The incidence and gene frequency of ataxia-telangiectasia in the United States. *Am J Hum Genet* **39**, 573-583.
33. Teive, H.A., Moro, A., Moscovich, M., Arruda, W.O., Munhoz, R.P., Raskin, S., Ashizawa, T. (2015) Ataxia-telangiectasia - A historical review and a proposal for a new designation: ATM syndrome. *J Neurol Sci*, **355**, 3-6.
34. Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709-715.
35. Dianov, G., Price, A., Lindahl, T. (1992) Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Mol Cell Biol*, **12**, 1605-1612.
36. Kuzminov, A. (2001) Single-strand interruptions in replicating chromosomes cause double-strand breaks. *Proc Natl Acad Sci U S A*, **98**, 8241-8246.

37. Zhou, W., Doetsch, P.W. (1993) Effects of abasic sites and DNA single-strand breaks on prokaryotic RNA polymerases. *Proc Natl Acad Sci U S A*, **90**, 6601-6605.
38. Kathe, S.D., Shen, G.P., Wallace, S.S. (2004) Single-stranded breaks in DNA but not oxidative DNA base damages block transcriptional elongation by RNA polymerase II in HeLa cell nuclear extracts. *J Biol Chem*, **279**, 18511-18520.
39. Date, H., Onodera, O., Tanaka, H., Iwabuchi, K., Uekawa, K., Igarashi, S., Koike, R., Hiroi, T., Yuasa, T., Awaya, Y., Sakai, T., Takahashi, T., Nagatomo, H., Sekijima, Y., Kawachi, I., Takiyama, Y., Nishizawa, M., Fukuhara, N., Saito, K., Sugano, S., Tsuji, S. (2001) Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. *Nat Genet*, **29**, 184-188.
40. Moreira, M.C., Barbot, C., Tachi, N., Kozuka, N., Uchida, E., Gibson, T., Mendonca, P., Costa, M., Barros, J., Yanagisawa, T., Watanabe, M., Ikeda, Y., Aoki, M., Nagata, T., Coutinho, P., Sequeiros, J., Koenig, M. (2001) The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. *Nat Genet*, **29**, 189-193.
41. Takashima, H., Boerkoel, C.F., John, J., Saifi, G.M., Salih, M.A., Armstrong, D., Mao, Y., Quiocho, F.A., Roa, B.B., Nakagawa, M., Stockton, D.W., Lupski, J.R. (2002) Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat Genet*, **32**, 267-272.
42. Shen, J., Gilmore, E.C., Marshall, C.A., Haddadin, M., Reynolds, J.J., Eyaid, W., Bodell, A., Barry, B., Gleason, D., Allen, K., Ganesh, V.S., Chang, B.S., Grix, A., Hill, R.S., Topcu, M., Caldecott, K.W., Barkovich, A.J., Walsh, C.A. (2010) Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. *Nat Genet*, **42**, 245-249.
43. Markkanen, E., Fischer, R., Ledentcova, M., Kessler, B.M., Dianov, G.L. (2015) Cells deficient in base-excision repair reveal cancer hallmarks originating from adjustments to genetic instability. *Nucleic Acids Res*, **43**, 3667-3679.
44. Khoronenkova, S.V., Dianova, II, Ternette, N., Kessler, B.M., Parsons, J.L., Dianov, G.L. (2012) ATM-dependent downregulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. *Mol Cell*, **45**, 801-813.
45. Khoronenkova, S.V., Dianov, G.L. (2013) USP7S-dependent inactivation of Mule regulates DNA damage signalling and repair. *Nucleic Acids Res*, **41**, 1750-1756.
46. Hoar, D.I., Sargent, P. (1976) Chemical mutagen hypersensitivity in ataxia telangiectasia. *Nature*, **261**, 590-592.
47. Yi, M., Rosin, M.P., Anderson, C.K. (1990) Response of fibroblast cultures from ataxia-telangiectasia patients to oxidative stress. *Cancer Lett*, **54**, 43-50.
48. Roots, R., Kraft, G., Gosschalk, E. (1985) The formation of radiation-induced DNA breaks: the ratio of double-strand breaks to single-strand breaks. *Int J Radiat Oncol Biol Phys*, **11**, 259-265.
49. Champoux, J.J., Dulbecco, R. (1972) An activity from mammalian cells that untwists superhelical DNA--a possible swivel for DNA replication (polyoma-ethidium bromide-mouse-embryo cells-dye binding assay). *Proc Natl Acad Sci U S A*, **69**, 143-146.
50. Hsiang, Y.H., Hertzberg, R., Hecht, S., Liu, L.F. (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem*, **260**, 14873-14878.
51. Lin, C.P., Ban, Y., Lyu, Y.L., Desai, S.D., Liu, L.F. (2008) A ubiquitin-proteasome pathway for the repair of topoisomerase I-DNA covalent complexes. *J Biol Chem*, **283**, 21074-21083.
52. Katyal, S., Lee, Y., Nitiss, K.C., Downing, S.M., Li, Y., Shimada, M., Zhao, J., Russell, H.R., Petrini, J.H., Nitiss, J.L., McKinnon, P.J. (2014) Aberrant topoisomerase-1 DNA lesions are pathogenic in neurodegenerative genome instability syndromes. *Nat Neurosci*, **17**, 813-821.

53. Huertas, P., Aguilera, A. (2003) Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell*, **12**, 711-721.
54. Tuduri, S., Crabbe, L., Conti, C., Tourriere, H., Holtgreve-Grez, H., Jauch, A., Pantesco, V., De Vos, J., Thomas, A., Theillet, C., Pommier, Y., Tazi, J., Coquelle, A., Pasero, P. (2009) Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription. *Nat Cell Biol*, **11**, 1315-1324.
55. Sordet, O., Redon, C.E., Guirouilh-Barbat, J., Smith, S., Solier, S., Douarre, C., Conti, C., Nakamura, A.J., Das, B.B., Nicolas, E., Kohn, K.W., Bonner, W.M., Pommier, Y. (2009) Ataxia telangiectasia mutated activation by transcription- and topoisomerase I-induced DNA double-strand breaks. *EMBO Rep*, **10**, 887-893.
56. Cristini, A., Park, J.H., Capranico, G., Legube, G., Favre, G., Sordet, O. (2015) DNA-PK triggers histone ubiquitination and signaling in response to DNA double-strand breaks produced during the repair of transcription-blocking topoisomerase I lesions. *Nucleic Acids Res*.
57. Sordet, O., Nakamura, A.J., Redon, C.E., Pommier, Y. (2010) DNA double-strand breaks and ATM activation by transcription-blocking DNA lesions. *Cell Cycle*, **9**, 274-278.
58. Sollier, J., Stork, C.T., Garcia-Rubio, M.L., Paulsen, R.D., Aguilera, A., Cimprich, K.A. (2014) Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability. *Mol Cell*, **56**, 777-785.
59. Tresini, M., Warmerdam, D.O., Kolovos, P., Snijder, L., Vrouwe, M.G., Demmers, J.A., van, I.W.F., Grosveld, F.G., Medema, R.H., Hoeijmakers, J.H., Mullenders, L.H., Vermeulen, W., Marteijn, J.A. (2015) The core spliceosome as target and effector of non-canonical ATM signalling. *Nature*, **523**, 53-58.
60. Price, B.D., D'Andrea, A.D. (2013) Chromatin remodeling at DNA double-strand breaks. *Cell*, **152**, 1344-1354.
61. Kaidi, A., Jackson, S.P. (2013) KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. *Nature*, **498**, 70-74.
62. Kanu, N., Behrens, A. (2007) ATMIN defines an NBS1-independent pathway of ATM signalling. *EMBO J*, **26**, 2933-2941.
63. Bakkenist, C.J., Kastan, M.B. (2015) Chromatin perturbations during the DNA damage response in higher eukaryotes. *DNA Repair (Amst)*, **36**, 8-12.
64. Zou, L., Elledge, S.J. (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science*, **300**, 1542-1548.
65. Kumar, A., Mazzanti, M., Mistrik, M., Kosar, M., Beznoussenko, G.V., Mironov, A.A., Garre, M., Parazzoli, D., Shivashankar, G.V., Scita, G., Bartek, J., Foiani, M. (2014) ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress. *Cell*, **158**, 633-646.
66. Goodarzi, A.A., Jeggo, P.A. (2013) The repair and signaling responses to DNA double-strand breaks. *Adv Genet*, **82**, 1-45.
67. Taccioli, G.E., Gottlieb, T.M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A.R., Alt, F.W., Jackson, S.P., Jeggo, P.A. (1994) Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. *Science*, **265**, 1442-1445.
68. Hartlerode, A.J., Morgan, M.J., Wu, Y., Buis, J., Ferguson, D.O. (2015) Recruitment and activation of the ATM kinase in the absence of DNA-damage sensors. *Nat Struct Mol Biol*, **22**, 736-743.
69. Epstein, W.L., Fudenberg, H.H., Reed, W.B., Boder, E., Sedgwick, R.P. (1966) Immunologic studies in ataxia-telangiectasia. I. Delayed hypersensitivity and serum immune globulin levels in probands and first-degree relatives. *Int Arch Allergy Appl Immunol*, **30**, 15-29.

70. Boder, E., Sedgwick, R.P. (1970) Ataxia-telangiectasia. (Clinical and immunological aspects). *Psychiatr Neurol Med Psychol Beih*, **13-14**, 8-16.
71. Aguilar, M.J., Kamoshita, S., Landing, B.H., Boder, E., Sedgwick, R.P. (1968) Pathological observations in ataxia-telangiectasia. A report of five cases. *J Neuropathol Exp Neurol*, **27**, 659-676.
72. Paula-Barbosa, M.M., Ruela, C., Tavares, M.A., Pontes, C., Saraiva, A., Cruz, C. (1983) Cerebellar cortex ultrastructure in ataxia-telangiectasia. *Ann Neurol*, **13**, 297-302.
73. Vinters, H.V., Gatti, R.A., Rakic, P. (1985) Sequence of cellular events in cerebellar ontogeny relevant to expression of neuronal abnormalities in ataxia-telangiectasia. *Kroc Found Ser*, **19**, 233-255.
74. Barlow, C., Hirotsune, S., Paylor, R., Liyanage, M., Eckhaus, M., Collins, F., Shiloh, Y., Crawley, J.N., Ried, T., Tagle, D., Wynshaw-Boris, A. (1996) Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell*, **86**, 159-171.
75. Barlow, C., Ribaut-Barassin, C., Zwingman, T.A., Pope, A.J., Brown, K.D., Owens, J.W., Larson, D., Harrington, E.A., Haeberle, A.M., Mariani, J., Eckhaus, M., Herrup, K., Bailly, Y., Wynshaw-Boris, A. (2000) ATM is a cytoplasmic protein in mouse brain required to prevent lysosomal accumulation. *Proc Natl Acad Sci U S A*, **97**, 871-876.
76. Borghesani, P.R., Alt, F.W., Bottaro, A., Davidson, L., Aksoy, S., Rathbun, G.A., Roberts, T.M., Swat, W., Segal, R.A., Gu, Y. (2000) Abnormal development of Purkinje cells and lymphocytes in Atm mutant mice. *Proc Natl Acad Sci U S A*, **97**, 3336-3341.
77. Chen, P., Peng, C., Luff, J., Spring, K., Watters, D., Bottle, S., Furuya, S., Lavin, M.F. (2003) Oxidative stress is responsible for deficient survival and dendritogenesis in purkinje neurons from ataxia-telangiectasia mutated mutant mice. *J Neurosci*, **23**, 11453-11460.
78. Reliene, R., Schiestl, R.H. (2007) Antioxidants suppress lymphoma and increase longevity in Atm-deficient mice. *J Nutr*, **137**, 229S-232S.
79. Rybczynska, M., Pawlak, A.L., Sikorska, E., Ignatowicz, R. (1996) Ataxia telangiectasia heterozygotes and patients display increased fluidity and decrease in contents of sulfhydryl groups in red blood cell membranes. *Biochim Biophys Acta*, **1302**, 231-235.
80. Reichenbach, J., Schubert, R., Schindler, D., Muller, K., Bohles, H., Zielen, S. (2002) Elevated oxidative stress in patients with ataxia telangiectasia. *Antioxid Redox Signal*, **4**, 465-469.
81. Yeo, A.J., Becherel, O.J., Luff, J.E., Cullen, J.K., Wongsurawat, T., Jenjaroenpun, P., Kuznetsov, V.A., McKinnon, P.J., Lavin, M.F. (2014) R-loops in proliferating cells but not in the brain: implications for AOA2 and other autosomal recessive ataxias. *PLoS One*, **9**, e90219.
82. Orii, K.E., Lee, Y., Kondo, N., McKinnon, P.J. (2006) Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development. *Proc Natl Acad Sci U S A*, **103**, 10017-10022.

FIGURE LEGENDS

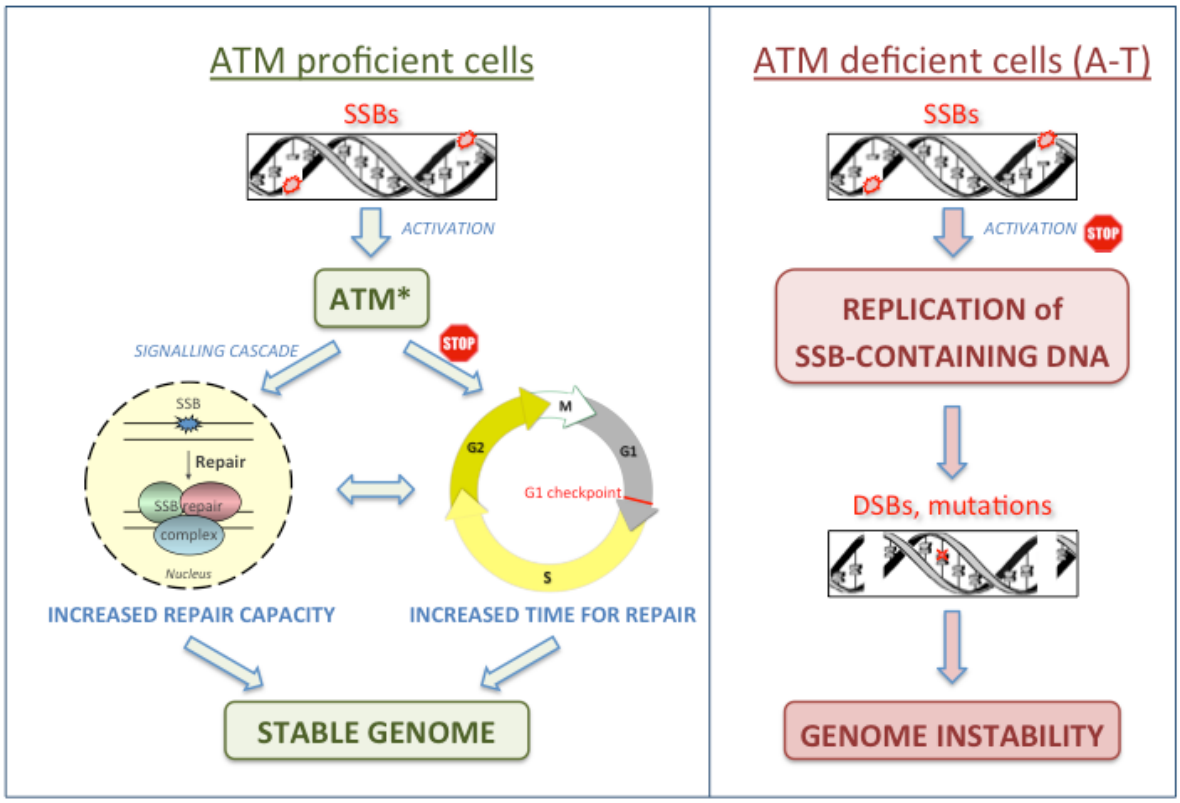

Fig. 1. ATM-dependent coordination of SSB repair. Activation of ATM in response to unrepaired SSBs coordinates their repair by increasing the efficiency of DNA repair and promoting a G_1 cell cycle delay that provides additional time for multiple rounds of repair. This results in coordinated and timely repair of SSBs prior to DNA replication, thus supporting the stability of genome (left panel). In the absence of ATM (A-T), the detection of SSBs is abrogated, leading to replication of the damaged DNA followed by the formation of DSBs and accumulation of mutations (right panel).

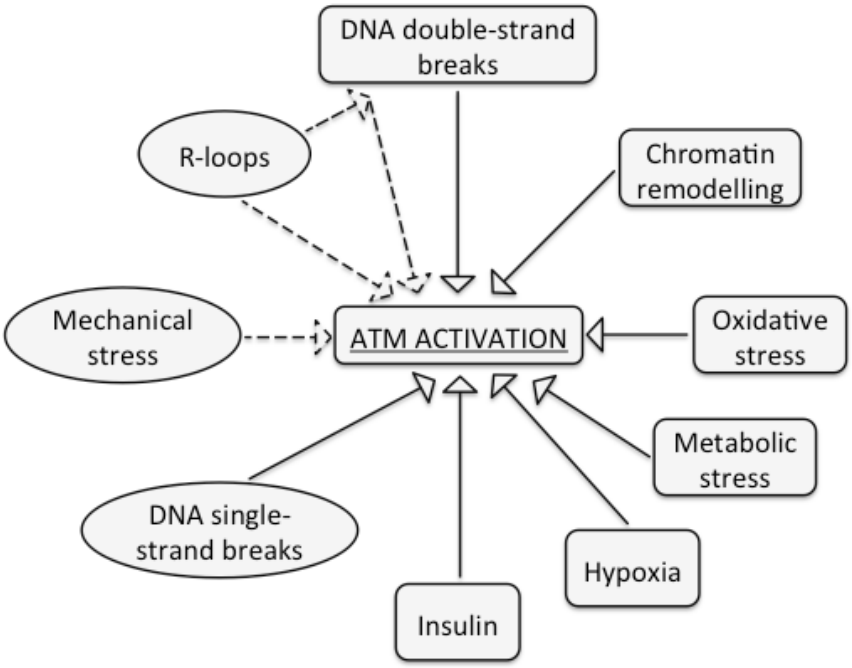

Fig. 2. Inducers of ATM activity. Non-canonical activators that are discussed in this review are designated with ovals.

MECHANISMS OF NON-CANONICAL ATM ACTIVATION

ABSTRACT

ATM is a master regulator of the cellular response to DNA damage. The classical mechanism of ATM activation involves its monomerization in response to DNA double-strand breaks, resulting in the ATM-dependent phosphorylation of more than a thousand substrates required for cell cycle progression, DNA repair and apoptosis. Here, new experimental evidence for non-canonical mechanisms of ATM activation in response to stimuli distinct from DNA double-strand breaks is discussed; these include cytoskeletal changes, chromatin modifications, RNA-DNA hybrids and DNA single-strand breaks. Non-canonical ATM activation may be important for the pathology of the multisystemic disease Ataxia Telangiectasia.

Key words: ATM, Ataxia Telangiectasia, DNA damage, DNA single-strand breaks, DNA double-strand breaks, R-loops

Running title: Non-canonical ATM activation