

## Invited review

**Molecular basis of ataxia telangiectasia and related diseases**Lindsay G BALL, Wei XIAO<sup>1</sup>*Department of Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK Canada, S7N 5E5.***Key words**

ataxia telangiectasia; DNA damage; DNA repair; cdc genes; phosphotransferases

<sup>1</sup> Correspondance to Dr Wei XIAO.

Phn 1-306-966-4308.

Fax 1-306-966-4311.

E-mail wei.xiao@usask.ca

Received 2005-04-08

Accepted 2005-05-05

doi: 10.1111/j.1745-7254.2005.00165.x

**Abstract**

Ataxia telangiectasia (AT) is a rare human disease characterized by extreme cellular sensitivity to radiation and a predisposition to cancer, with a hallmark of onset in early childhood. Several human diseases also share similar symptoms with AT albeit with different degrees of severity and different associated disorders. While all AT patients contain mutations in the AT-mutated gene (ATM), most other AT-like disorders are defective in genes encoding an MRN protein complex consisting of Mre11, Rad50 and Nbs1. Both ATM and MRN function as cellular sensors to DNA double-strand breaks, which lead to the recruitment and phosphorylation of an array of substrate proteins involved in DNA repair, apoptosis and cell-cycle checkpoints, as well as gene regulation, translation initiation and telomere maintenance. ATM is a member of the family of phosphatidylinositol 3-kinase-like protein kinases (PIKK), and the discovery of many ATM substrates provides the underlying mechanisms of heterologous symptoms among AT patients. This review article focuses on recent findings related to the initial recognition of double-strand breaks by ATM and MRN, as well as a DNA-dependent protein kinase complex consisting of the heterodimer Ku70/Ku80 and its catalytic subunit DNA-PKcs, another member of PIKK. This possible interaction suggests that a much greater complex is involved in sensing, transducing and co-ordinating cellular events in response to genome instability.

**Ataxia telangiectasia clinical presentation**

Ataxia telangiectasia (AT) is a rare human disease characterized by extreme sensitivity to radiation<sup>[1–5]</sup>. AT is a progressive neurodegenerative disorder causing a predisposition to cancer, with a hallmark of onset in early childhood<sup>[6–9]</sup>. AT is seen in approximately 1 in every 40 000 live births in the USA, although the frequency varies from country to country<sup>[10]</sup>. At birth, infants appear normal and begin walking at a normal age (approximately age 1 year); however, by age 2–3 ataxia (loss of muscle co-ordination) becomes visible and generally by age 10 patients are confined to a wheelchair<sup>[10]</sup>. Ataxia generally precedes telangiectasia, which is described as the chronic dilation of a group of capillaries causing elevated, dark red blotches on the skin or eyes<sup>[11]</sup>. This disease is additionally characterized by cerebellar degeneration, and immune system defects<sup>[12–14]</sup>. In younger children, diagnosis of AT is somewhat obscure as the cerebellum appears

to be of normal size for several years although onset of ataxia is prevalent. However, by age 10 magnetic resonance imaging (MRI) generally shows an abnormal cerebellum that has decreased in volume<sup>[15]</sup>. Studies have shown a gradual decrease in granular and Purkinje cells, which are large branching cells of the nervous system and are located in the middle layer of the lower part of the brain, or the cerebellum<sup>[14–16]</sup>. Unfortunately, there is currently no treatment for AT except for supportive therapy of secondary symptoms<sup>[17]</sup>.

In addition to an up to several hundredfold increase in developing certain types of cancers (eg lymphoma) in AT patients, epidemiological studies suggest that heterozygote AT mutation carriers are also at increased risk for cancer, particularly breast carcinoma<sup>[18,19]</sup>. This is of great significance as it is estimated that approximately 1% of the population are AT carriers. However, it is noted that a recent study showed that heterozygous AT mutations did not confer genetic predisposition to early onset of breast cancer<sup>[20]</sup>.

Serum alpha-fetoprotein (AFP) levels are elevated in more than 95% of AT patients, which is therefore used to diagnose AT and to distinguish from AT variants<sup>[4,5,10,21]</sup>. When dealing with AFP it is important to note that AFP levels are normally higher at birth than those at age 2, thus diagnostics for AT using AFP cannot be conducted until after the age of 2. There are known malignancies associated with AFP<sup>[10,22]</sup>. For example, AFP is known to be elevated in case of spina bifida and anencephaly, and this elevation is the hallmark test for early detection of both conditions. Spina bifida is part of a group of birth defects called neural tube defects, which affect embryonic structures that eventually develop into the brain, spinal cord and tissues that enclose them. Anencephaly is a congenital condition in which portions of the brain fail to develop. Because AFP is such a diagnostic hallmark for AT, it is surprising that the mechanism of AFP upregulation in normal and AT cells has not been reported. Nevertheless, given the commonality of AFP elevation in all of the above 3 diseases, it is conceivable that the increased AFP level is responsible for the Purkinje cell degeneration in AT patients.

### Ataxia telangiectasia-related diseases

There are several human deficiencies and diseases that are closely related to AT. For example, molecular cloning has now allowed for the distinction between AT and other autosomal recessive cerebellar ataxias (ARCA) such as Friedreich ataxia or A-T<sub>Fresno</sub>, an old-fashioned term for an AT variant<sup>[23–25]</sup>, oculomotor apraxias 1 (aprataxin deficiency), oculomotor apraxias 2 (senataxin deficiency), aicardi syndrome and AT-like disorder (ATLD)<sup>[26–29]</sup>.

AT-like disorder is a very rare disorder with clinical features similar to those of AT, with the most prominent similarity being progressive cerebellar ataxia. However, ATLD patients that do not present with telangiectasia<sup>[30,31]</sup>, have a later onset of neurological features, slower progression, and thus milder symptoms compared to AT patients<sup>[27]</sup>.

Closely related to AT and ATLD is the Nijmegen breakage syndrome (NBS)<sup>[23–25]</sup>. NBS and ATLD have some overlapping features such as hypersensitivity to ionizing radiation and genome instability, and both are characterized by neurological deficits<sup>[32,33]</sup>. Thus, NBS was long considered as a clinical variant of AT. Clinical distinction has been made as NBS patients also present with a characteristic facial appearance and microcephaly as well as growth retardation<sup>[34]</sup>. Clearly the above AT-related human conditions suggest deficiency at common cellular function(s) and genetic pathway(s) with AT.

### Laboratory findings in ataxia telangiectasia and ataxia telangiectasia-related patients

Laboratory findings in patients with AT show: immunodeficiencies emerging as decreased IgA, IgE and IgG2 levels<sup>[5,21,35–38]</sup>, characteristic chromosomal aberrations and an increased rate of telomeric shortening<sup>[39]</sup>, radiosensitivity<sup>[1–5]</sup>, as well as sensitivity to other DNA damaging agents and non-DNA damaging agents<sup>[40]</sup>. Chromosome instability, cell-cycle checkpoint defects<sup>[3]</sup> and elevated serum levels of AFP<sup>[21,22]</sup> are prevalent in most AT patient cells. In addition, approximately one-third of AT patients develop cancer, which is usually lymphoid<sup>[10]</sup>.

Both NBS and ATLD cells share a number of cellular phenotypes with AT cells, with the most prominent being the increased sensitivity to ionizing radiation, abnormal cell-cycle checkpoints, chromosome instability, immunodeficiency and accelerated shortening of telomeres<sup>[41,42]</sup>. In particular, all 3 disorders show an increased level of chromosome translocation in the peripheral blood between the loci of the immunoglobulin and T-cell receptor genes on chromosomes 7 and 14<sup>[43,44]</sup>. Hence, laboratory findings are most consistent with all 3 diseases belonging to a group of disorders referred to chromosome instability syndromes.

### Molecular basis of ataxia telangiectasia and related diseases

AT is the result of mutations in the AT-mutated (*ATM*) gene, which was discovered in 1995<sup>[45,46]</sup>. AT patients suffer as a result of over 400 distinct *ATM* mutations, of which 85% are accounted for by null mutations in the *ATM* gene<sup>[21,47,48]</sup>. Thus, approximately 85% of AT sufferers have no detectable *ATM* protein<sup>[21,48]</sup>. There are a few reported genuine AT cases with normal *ATM* protein levels; however, in these cases the protein is defective in *ATM* enzyme activity<sup>[49,50]</sup>. The establishment of AT as a monogenetic disease assisted in efficient diagnosis and reevaluation of “AT variants”. The fact that some AT patients carry hypomorphic *ATM* mutations (ie mutations with partial functions) provides an underlying explanation for atypical AT patients with minimal signs of symptoms, such as very mild or late-onset of syndromes or a subset of disorders<sup>[51]</sup>.

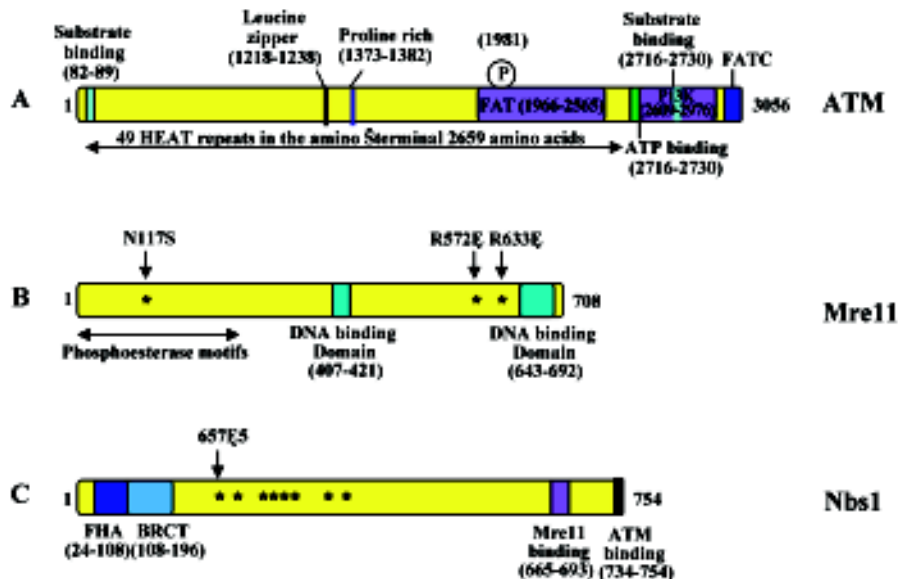
The *ATM* protein is a member of the phosphatidylinositol 3-kinase-like family of serine/threonine protein kinases (PIKK)<sup>[13,52]</sup>. It is thus grouped because all PIKK contain a conserved kinase domain initially reported in phosphatidylinositol 3-kinase. This family represents an atypical subclass of protein kinases responsible for phosphorylation of its substrates on serine or threonine followed by glutamine

(SQ or TQ)<sup>[53-55]</sup>. The mammalian PIKK known to be involved in the DNA damage response are: DNA-PKcs (the catalytic subunit of DNA-dependent protein kinase), ATM (ataxia telangiectasia gene product), ATR (ATM and Rad3-related), mTOR/FRAP (mammalian target of rapamycin/FKBP-rapamycin-associated protein) and ATX/SMG1<sup>[13]</sup>.

The ATM protein is a fairly large (approximately 350 kDa) protein that can be divided into several structural and functional domains (Figure 1A). The first is an FAT domain, which is conserved among the PIKK family of proteins FRAP, ATR and TRRAP; the second is a phosphoinositide 3,4-kinase (PI3K) domain, which ATM has in common with DNA-PKcs; and the third is an FAT carboxy-terminal domain (FATC)<sup>[56]</sup>. In addition, the amino terminus of the ATM protein contains multiple HEAT (huntingtin, elongation factor 3, A subunit of protein phosphatase 2A and TOR1) repeats<sup>[57]</sup>. A recent solution structure analysis of the FATC domain<sup>[58]</sup> revealed an  $\alpha$ -helix and a disulfide-bonded loop that undergoes conformational changes. Hence, the FATC domain may regulate protein activity and stability. The precise function of HEAT repeats is currently unknown, although it is speculated to be involved in the interaction with other proteins, as these repeats are anti-parallel  $\alpha$ -helices linked by a flexible loop<sup>[40,57]</sup>.

The overall shape of ATM is very similar to DNA-PKcs and is comprised of a head and a long arm that is thought to wrap around double-stranded DNA after a conformational change<sup>[59-63]</sup>. One of the hallmarks of the ATM protein is its rapid increase in kinase activity immediately following exposure to ionizing radiation (IR), or in the presence of double-strand breaks (DSB)<sup>[64,65]</sup>.

The gene mutated in NBS was identified as *NBS1*<sup>[25,66,67]</sup> whereas ATLD is caused by mutations in the *MRE11* gene<sup>[29]</sup>. Unlike AT, which can result from complete inactivation of the coding gene, all NBS and ATLD patients carry hypomorphic mutations that express some level of corresponding protein, either truncated or full length with amino acid substitutions<sup>[27,68]</sup>. Indeed, attempts to create null *Mre11* mutations in mouse embryonic stem cells has failed and conditional knockout experiments demonstrated that this gene is required for normal cell proliferation<sup>[69]</sup>, suggesting that *MRE11* is an essential gene in mammals. Similarly, complete *Nbs1* knockout mice are embryonic lethal<sup>[70,71]</sup>. This assertion is further confirmed by a report that disruption of mouse *Rad50*, encoding the third component of the MRN (MRX in yeast) complex consisting of Mre11, Rad50, and Nbs1 (aka nibrin and p95; Xrs2 in yeast), also causes embryonic stem



**Figure 1.** Schematic representation of the ATM, Mre11 and Nbs1 proteins. (A) ATM and its functional domains. Most domains have been discussed in the text. The letter “P” indicates the phosphorylation site required for its activation. (B) Mre11 and its functional domains. The asterisks represent the known mutations that cause ataxia telangiectasia-like disorder (ATLD). Note that only two families of ATLD with *MRE11* mutations were reported. One is homozygous for the mutation C1897T, resulting in R633 $\Delta$  (stop codon), whereas the second family is heterozygous for A350G (N117S) and C1714T (R572 $\Delta$ ). (C) Nbs1 and its functional domains. The asterisks indicate the known *NBS1* mutations that cause Nijmegen breakage syndrome (NBS), among which the homozygous 657 $\Delta$ 5 mutation accounts for over 90% of NBS patients. 657 $\Delta$ 5 contains a 5-bp deletion, resulting in a truncated 26 kDa protein with only forkhead-associated (FHA) and BRCA1 C-terminus (BRCT) domains. Other *NBS1* mutations cause various degrees of symptoms.

cell lethality<sup>[72]</sup>.

The human *MRE11* gene was initially isolated by its possible protein interaction with DNA ligase I<sup>[73]</sup> and was predicted to encode a 708-amino acid protein. However, subsequent reports appear to favor an Mre11B sequence (Figure 1B), which is different from Mre11 primarily at the C-terminal region and significantly strengthens its similarity with Mre11 from other species<sup>[74]</sup>. Mre11 contains a phosphoesterase motif at the N-terminal region that is highly conserved among all eukaryotic Mre11 and a similar motif is also found in *Escherichia coli* SbcD<sup>[75]</sup>. It also contains DNA-binding domains at the C-terminal region and region(s) for homodimerization<sup>[76,77]</sup>. Structural analyses<sup>[78–80]</sup> indicate that the MRN complex consists of two molecules of Mre11 and Rad50 (an *E. coli* SbcC homolog) forming a rope-and-hook structure capable of bridging two DSB ends. *In vitro* characterization of the Rad50–Mre11 complex<sup>[76,81–84]</sup> demonstrates that it possesses 3′ to 5′ exonuclease and single-strand endonuclease activities, as well as ATP-dependent DNA binding and limited unwinding activity.

The phenotypic similarity between NBS and ATLD patients is attributed to the fact that both corresponding proteins are components of the MRN complex. Mre11 interacts with both Rad50 and Nbs1/Xrs2, and its homodimerization or self-interaction appears to be important for the Mre11–Rad50 and Mre11–Nbs1/Xrs2 interactions<sup>[76,77]</sup>. Interestingly, deficiency of the Mre11 protein results in decreased cellular levels of Nbs1 and Rad50, suggesting that the MRN complex is required for protein stability. The primary cellular function of MRN is a sensor for DNA strand breaks and to activate signaling pathways leading to cell-cycle checkpoint and recombination repair<sup>[32,85,86]</sup>. While Mre11 and Rad50 are highly conserved in eukaryotes, from unicellular yeasts to human cells<sup>[87,88]</sup>, the third component, Nbs1, is less conserved structurally among eukaryotes, although the mammalian Nbs1 and yeast Xrs2 play similar roles within their respective MRN/MRX complex. Nbs1 has been suggested to be a regulatory subunit of MRN that is essential for Mre11 phosphorylation upon DNA damage<sup>[89]</sup> and its biochemical activities, such as ATP-dependent DNA unwinding and nuclease activity<sup>[90]</sup>. Although Nbs1 or Xrs2 is not required for the Mre11–Rad50 enzymatic activity *in vitro*, its activity appears to be absolutely required for MRX activity *in vivo*, as inactivation of any 1 of the 3 genes in yeast cells leads to complete loss of the MRX activity and indistinguishable cellular phenotypes<sup>[91–93]</sup>. This essential function of Nbs1/Xrs2 is probably due to its role in recruiting ATM/Te11<sup>[94]</sup>. The Nbs1 protein has 4 known functional regions: the N-terminal forkhead-associated (FHA) domain followed by a

BRCA1 C-terminus (BRCT) domain, a C-terminal Mre11-binding domain and an ATM-binding domain at the extreme C-terminus (Figure 1C). Interestingly, over 90% of all NBS patients analyzed to date contain a homozygous 5 bp truncating mutation, 657Δ5<sup>[25]</sup>, resulting in the production of a 26-kDa protein with FHA and BRCT domains but lacking the Mre11-binding domain (Figure 1C). Knockout mice producing similar truncated Nbs1 proteins are viable and develop symptoms characteristic of the human disease<sup>[95,96]</sup>, thus providing an animal model suitable for NBS research.

### Phenotypic manifestation of genetic defects in ataxia telangiectasia and related diseases

One of the hallmarks of the ATM protein is its rapid increase in kinase activity immediately following DSB formation<sup>[64,65]</sup>, and its kinase activity remains its only known function to date. The phenotypic manifestation of AT is due to the broad range of substrates for the ATM kinase, as compiled recently<sup>[40]</sup>, involving DNA repair, apoptosis, G<sub>1</sub>/S, intra-S checkpoint and G<sub>2</sub>/M checkpoints, gene regulation, translation initiation, and telomere maintenance. Protein phosphorylation and de-phosphorylation is an important cellular regulatory mechanism that governs protein activity, stability, subcellular location and complex formation. We propose that although the primary ATM kinase substrates appear to be related to cellular response to DNA damage, novel ATM substrates and subsequent effects may explain all observed syndromes of AT patients, including elevated AFP level. We also propose that the multiple HEAT repeats in ATM may serve as a platform to interact with various regulatory and substrate proteins and to control its kinase activity in the aftermath of DNA damage. For example, the increased risk for breast cancer in AT patients has been implicated by the involvement of ATM in the interaction and phosphorylation of BRCA1 and its associated proteins following DNA damage<sup>[97,98]</sup>.

The ATM protein also has the ability to interact with the ends of double-stranded DNA<sup>[99]</sup>. A recent model suggests that ATM is in an inactive dimeric or polymeric configuration, in which the kinase domain of each molecule is blocked by the FAT domain of the other<sup>[100]</sup>. Following DNA damage, each ATM molecule phosphorylates the other on a serine residue at position 1981 within the FAT domain and thus converts into fully active monomers. It was shown that protein serine-threonine phosphatase 5 (PP5) is required for the activation of ATM and its subsequent kinase activity<sup>[101]</sup>.

The symptom similarity among AT, ATLD and NBS is apparently due to physical and genetic interactions between

ATM and MRN. Experimental results suggest that the ATM-MRN interaction in response to IR is complicated. First, the histone H2A isoform H2AX is rapidly phosphorylated (within 5 min) after irradiation in an ATM-dependent manner<sup>[102,103]</sup>, and the phosphorylated gH2AX interacts with MRN via the FHA/BRCT region of Nbs1<sup>[103]</sup>. This interaction has been deemed indispensable for the recruitment of MRN<sup>[41]</sup>. Second, in response to IR, ATM phosphorylates Nbs1 *in vivo*<sup>[104]</sup>, which is required for the subsequent phosphorylation of Mre11. Finally, phosphorylation and activation of the S-phase checkpoint effector Smc1 by ATM requires phosphorylated Nbs1<sup>[105,106]</sup>. The above observations suggest that ATM acts upstream of MRN and is required to recruit MRN to the damage sites observed as distinct nuclear foci. On the other hand, MRN/MRX is known to bind DSB ends and a careful choreographic analysis in yeast<sup>[107]</sup> indicates that MRX localization to nuclear DSB foci precedes and is required for that of Tel1, a yeast ATM ortholog<sup>[108,109]</sup>. In mammalian cells, Mre11 appears to be required for ATM activation as well<sup>[110]</sup>, and this interaction was further reinforced very recently by an *in vitro* experiment showing that the MRN complex acts as a DSB sensor for ATM and recruits ATM to broken DNA molecules<sup>[111]</sup>. Interestingly, the unwinding of DNA ends by MRN appears to be essential for ATM stimulation<sup>[111]</sup>, indicating that the MRN enzymatic activity plays a role in this process. In addition, a recent study<sup>[112]</sup> shows that NFB1/MDC1, which interacts with MRN, is required for ATM activation under certain conditions. Alternatively, NFB1/MDC1 may help to recruit substrates, including Nbs1<sup>[113,114]</sup>, to ATM<sup>[113–123]</sup>; suppression of NFB1/MDC1 leads to decreased ATM activation and decreased phosphorylation of ATM substrates<sup>[112]</sup>. These observations support an assertion that MRN plays a crucial role in ATM phosphorylation of other substrates in response to DSB. The argument that MRN functions upstream of ATM is further strengthened by a very recent report that the extreme C-terminal 21 amino acid peptide of Nbs1 is required and sufficient for the interaction with ATM *in vivo* and *in vitro*, and that this interaction mediates ATM deposition to the sites of DNA damage and its checkpoint functions<sup>[94]</sup>. The above genetic and physical interactions between MRN and ATM underlie the molecular basis for the shared cellular functions in cell-cycle checkpoints and recombination repair of DSB, as well as common syndromes among AT, ATLD and NBS.

### DNA-PK: a missing link?

Despite the great advances made in the past decade re-

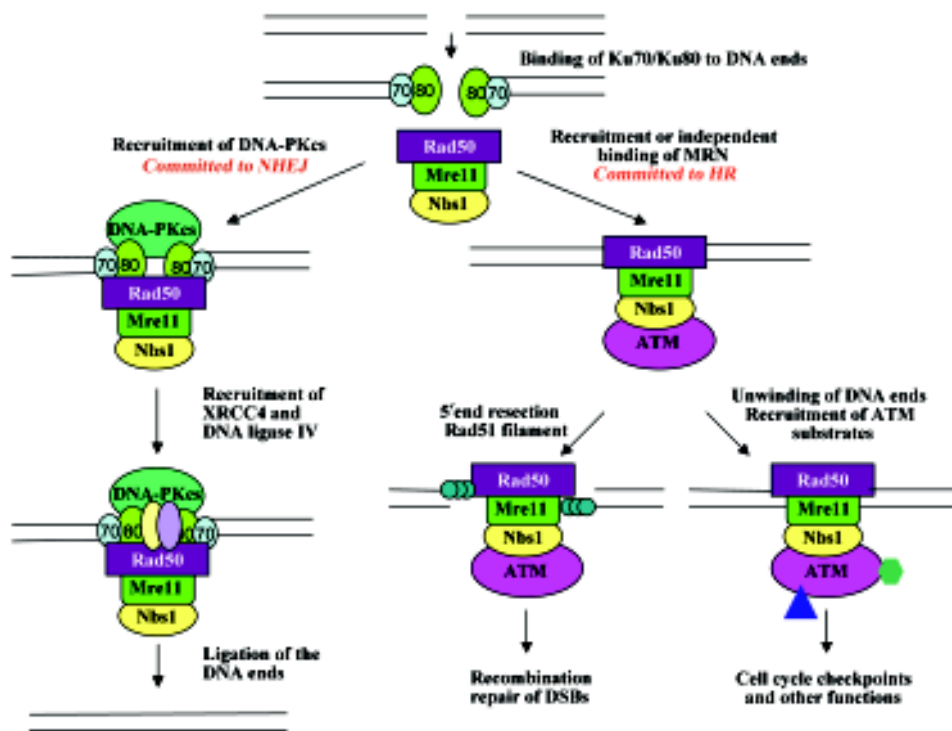
garding AT research, many questions remain to be answered. One question that remains unsolved is the involvement of Ku70/Ku80 in ATM-mediated cellular events in response to DSB. Ku70 and Ku80 form a heterodimer as DNA end-binding subunits of DNA-PK<sup>[59–62]</sup>. It would be plausible to suggest that Ku70/Ku80 may also assist ATM's translocation to DSB, as Ku70/Ku80 is known to play such a role for DNA-PKcs, another member of PIKK with overall structural similarity to ATM<sup>[124]</sup>. Like ATM, DNA-PKcs recognizes the same consensus phosphorylation sequence SQ/TQ, and also undergoes DSB-induced autophosphorylation, which is required for DSB repair<sup>[125]</sup>. In addition, inactivation of either DNA-PK or ATM results in immunodeficiency; the former results in defective V(D)J recombination that leads to *scid* (severe combined immune deficiency) mutations<sup>[126,127]</sup>, whereas the latter results in the disruption of immune gene rearrangement<sup>[128]</sup>. We propose that in the presence of Ku70/Ku80, DNA-PKcs and ATM may undergo conformational changes to be suitable for the suggested role of further wrapping around DNA<sup>[59–63]</sup>. Indeed, recent electron microscopy-based structural analyses of both DNA-PKcs<sup>[129–131]</sup> and ATM<sup>[63]</sup> suggest a similar architecture consisting of a "head" and a "palm" domain connected by an "arm". The palm domain binds DNA, which induces conformational changes and triggers an interaction between initially distant palm and head regions<sup>[130]</sup>. These DNA-induced conformational changes alter the catalytic core and regulate the kinase activity<sup>[131]</sup>. The difference between DNA-PKcs and ATM is that DNA-PKcs is activated in G<sub>1</sub> and is required for non-homologous end joining (NHEJ) and apoptosis if DNA damage is excessive<sup>[132]</sup>, while ATM is activated in S and G<sub>2</sub> phases and is required for S-phase checkpoint and homologous recombination (HR)<sup>[40]</sup>.

Another connection between ATM and Ku70/Ku80 is that yeast MRX is not only required for DSB signaling and HR, but also works with Ku70/Ku80 for NHEJ<sup>[133–136]</sup> and telomere maintenance<sup>[77,93,137,138]</sup>. The budding yeast *Saccharomyces cerevisiae* predominantly employs HR to repair DSB over NHEJ. On some occasions when DSB repair can be processed by either process, NHEJ was shown to precede HR<sup>[139]</sup>. A compiled model can be proposed in which the telomere acts as a repository for Ku proteins, which relocate to DSB after DNA damage<sup>[140]</sup> and recruit MRX<sup>[141]</sup>. MRX can assist to recruit and activate Lif1 and Dnl4<sup>[142]</sup>, resulting in ligation of the broken ends. However, in budding yeast, the MRX complex most likely initiates 5' to 3' end resection<sup>[76]</sup>, hence committing to repair by HR. This commitment is probably due to the broken DSB ends that are unsuitable for direct ligation, or to the recruitment of Tel1 by MRX that

activates cell-cycle checkpoints and subsequent recruitment of other HR proteins<sup>[107]</sup>. A similar regulatory cascade may also exist in higher eukaryotes; however, because mammalian cells have an extended G<sub>1</sub> phase, the initial DSB binding by Ku70/Ku80 may stimulate NHEJ more frequently than in yeast. In addition, mammalian cells contain 3 major PIKK family proteins, namely ATM, ATR and DNA-PKcs, involved in different modes of DNA repair and overlapping signal transduction in response to DNA damage. The mechanism of their recruitment to sites of DNA damage is conserved via a newly discovered motif<sup>[94]</sup>. For example, ATR is recruited to a single-stranded DNA by ATRIP (ATR-interacting protein)<sup>[143,144]</sup>, whereas DNA-PKcs and ATM are recruited to DSB by Ku70/K80 and MRN, respectively. Based on the above observations, we have proposed a working model of cellular response to DSB in mammals, as presented in Figure 2.

Finally, the physical and functional interactions among Ku, MRN/MRX and ATM/Tel1 can also be found in telomeres. Both yeast<sup>[145]</sup> and human<sup>[146]</sup> Ku is bound to

telomeric DNA and protects its 3' overhang. The MRX complex in budding yeast is also associated with telomeres, and mutants deficient in Ku or MRX activity exhibit telomere shortening<sup>[137,147]</sup>. Similar phenotypes are also observed in their homologous mutants in fission yeast and plants<sup>[148-150]</sup>. Tel1 works together with MRX by either regulating the access of telomerase to the DNA end<sup>[151]</sup>, and/or preventing telomere end-to-end fusion<sup>[152]</sup>. Tel1 is activated by the MRX complex and in turn phosphorylates MRX<sup>[153]</sup>, suggesting functional conservation between yeast and mammals. However, unlike Tel1, ATM has not been implicated in telomerase access to the telomere. Nevertheless, AT patient cells were shown to have shortened telomeres<sup>[39,154]</sup>, as well as other telomere abnormalities<sup>[155,156]</sup>. Furthermore, transgenic mice defective in both ATM and telomerase dramatically increase telomere dysfunction compared to single mutants<sup>[157]</sup>, suggesting roles of ATM in telomere maintenance. The telomeric functions of ATM may be related to some symptoms of AT patients.



**Figure 2.** A proposed model for cellular response to DNA double-stranded breaks (DSB) in mammalian cells. The Ku70/Ku80 heterodimer binds to the broken DNA ends first, followed by the recruitment or the independent binding of the MRN complex (consisting of Mre11, Rad50 and Nbs1). If DNA-PKcs is recruited by interacting with the C-terminus of Ku80, cells are committed to non-homologous end joining. On the other hand, if ATM is recruited by interacting with the C-terminus of Nbs1, Ku70/Ku80 may be released and cells are probably committed to homologous recombination repair. Recruitment of ATM also leads to substrate phosphorylation and cell-cycle checkpoints. It should be noted that MRN may also bind to DNA ends without the assistance of Ku70/Ku80. Recent literature suggests that unwinding of DNA ends by MRN activates ATM and cell-cycle checkpoints, whereas DNA end resection by MRN initiates homologous recombination. The 2 processes may be co-regulated.

## Conclusions

Since the initial discovery of the AT mutated gene 10 years ago, knowledge regarding the molecular mechanisms of AT and its related diseases has accumulated rapidly. It is now clear that the primary function of ATM and MRN, which is related to NBS and ATLD, is to respond to DNA damage, especially DSB. Two primary activities are cell-cycle checkpoint and DNA repair through HR, and these are achieved mainly via protein phosphorylation of various substrates, including ATM and MRN themselves. ATM and MRN are also involved in telomere maintenance and protection from chromosomal translocation, as well as efficient immune gene recombination. Although these cellular phenotypes are able to explain most phenotypes observed in AT and AT-related disease, some other associated symptoms may be due to diverse ATM substrates, unique MRN functions not shared by ATM, as well as the hypomorphic nature of the disease. Despite great advances in research, many questions remain to be answered especially with regard to treatment. Future efforts shall be directed to translational research for the cure and prevention of AT its and related diseases.

## Acknowledgements

The authors wish to thank Michelle HANNA for proof-reading the manuscript and other laboratory members for their helpful discussion. This work is supported by a Canadian Institutes of Health Research operating grant (MOP-53240) to WX.

## References

- 1 Gotoff SP, Amirmokri E, Liebner EJ. Ataxia telangiectasia. Neoplasia, untoward response to x-irradiation, and tuberous sclerosis. *Am J Dis Child* 1967; 114: 617–25.
- 2 Taylor AM, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S, *et al*. Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature* 1975; 258: 427–29.
- 3 Young BR, Painter RB. Radioresistant DNA synthesis and human genetic diseases. *Hum Genet* 1989; 82: 113–7.
- 4 Huo YK, Wang Z, Hong JH, Chessa L, McBride WH, Perlman SL, *et al*. Radiosensitivity of ataxia-telangiectasia, X-linked agammaglobulinemia, and related syndromes using a modified colony survival assay. *Cancer Res* 1994; 54: 2544–7.
- 5 Sun X, Becker-Catania SG, Chun HH, Hwang MJ, Huo Y, Wang Z, *et al*. Early diagnosis of ataxia-telangiectasia using radiosensitivity testing. *J Pediatr* 2002; 140: 724–31.
- 6 Boder E, Sedgwick RP. Ataxia-telangiectasia: a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. *Pediatrics* 1958; 21: 526–54.
- 7 Boder E. Ataxia-telangiectasia: an overview. *Kroc Found Ser* 1985; 19: 1–63.
- 8 Gatti RA, Boder E, Vinters HV, Sparkes RS, Norman A, Lange K. Ataxia-telangiectasia: an interdisciplinary approach to pathogenesis. *Medicine (Baltimore)* 1991; 70: 99–117.
- 9 Gatti RA. Ataxia-telangiectasia. In: Vogelstein B, Kinzler KW, editors. *The Genetic Basis of Human Cancer*. New York: Mc Graw-Hill; 2002. p 239–65.
- 10 Chun HH, Gatti RA. Ataxia-telangiectasia, an evolving phenotype. *DNA Repair (Amst)* 2004; 3: 1187–96.
- 11 Cabana MD, Crawford TO, Winkelstein JA, Christensen JR, Lederman HM. Consequences of the delayed diagnosis of ataxia-telangiectasia. *Pediatrics* 1998; 102: 98–100.
- 12 Shiloh Y. ATM: ready, set, go. *Cell Cycle* 2003; 2: 116–7.
- 13 Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003; 3: 155–68.
- 14 Lavin MF, Shiloh Y. The genetic defect in ataxia-telangiectasia. *Annu Rev Immunol* 1997; 15: 177–202.
- 15 Tavani F, Zimmerman RA, Berry GT, Sullivan K, Gatti R, Bingham P. Ataxia-telangiectasia: the pattern of cerebellar atrophy on MRI. *Neuroradiology* 2003; 45: 315–9.
- 16 Nowak R. Discovery of AT gene sparks biomedical research bonanza. *Science* 1995; 268: 1700–1.
- 17 Perlman S, Becker-Catania S, Gatti RA. Ataxia-telangiectasia: diagnosis and treatment. *Semin Pediatr Neurol* 2003; 10: 173–82.
- 18 Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 1991; 325: 1831–6.
- 19 Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987; 316: 1289–94.
- 20 FitzGerald MG, Bean JM, Hegde SR, Unsal H, MacDonald DJ, Harkin DP, *et al*. Heterozygous ATM mutations do not contribute to early onset of breast cancer. *Nat Genet* 1997; 15: 307–10.
- 21 Chun HH, Sun X, Nahas SA, Teraoka S, Lai CH, Concannon P, *et al*. Improved diagnostic testing for ataxia-telangiectasia by immunoblotting of nuclear lysates for ATM protein expression. *Mol Genet Metab* 2003; 80: 437–43.
- 22 McVey JH, Michaelides K, Hansen LP, Ferguson-Smith M, Tilghman S, Krumlauf R, *et al*. A G→A substitution in an HNF I binding site in the human alpha-fetoprotein gene is associated with hereditary persistence of alpha-fetoprotein (HPAFP). *Hum Mol Genet* 1993; 2: 379–84.
- 23 Weemaes CM, Hustinx TW, Scheres JM, van Munster PJ, Bakkeren JA, Taalman RD. A new chromosomal instability disorder: the Nijmegen breakage syndrome. *Acta Paediatr Scand* 1981; 70: 557–64.
- 24 Heil JA, Weemaes CM, van den Heuvel LP, van Engelen BG, Gabreels FJ, Smeets DF, *et al*. International Nijmegen Breakage Syndrome Study Group: Nijmegen breakage syndrome. *Arch Dis Child* 2000; 82: 400–406.
- 25 Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K, *et al*. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 1998; 93: 467–76.
- 26 Moreira MC, Klur S, Watanabe M, Nemeth AH, Le Ber I, Moniz JC, *et al*. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat Genet* 2004; 36: 225–7.
- 27 Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD) – its clinical presentation and molecular basis. *DNA*

- Repair (Amst) 2004; 3: 1219–25.
- 28 Date H, Onodera O, Tanaka H, Iwabuchi K, Uekawa K, Igarashi S, *et al*. Early-onset ataxia with ocular motor apraxia and hypalbuminemia is caused by mutations in a new HIT superfamily gene. *Nat Genet* 2001; 29: 184–8.
- 29 Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, *et al*. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 1999; 99: 577–87.
- 30 Klein C, Wenning GK, Quinn NP, Marsden CD. Ataxia without telangiectasia masquerading as benign hereditary chorea. *Mov Disord* 1996; 11: 217–20.
- 31 Hernandez D, McConville CM, Stacey M, Woods CG, Brown MM, Shutt P, *et al*. A family showing no evidence of linkage between the ataxia telangiectasia gene and chromosome 11q22-23. *J Med Genet* 1993; 30: 135–40.
- 32 D'Amours D, Jackson SP. The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. *Nat Rev Mol Cell Biol* 2002; 3: 317–27.
- 33 Petrini JH. The Mre11 complex and ATM: collaborating to navigate S phase. *Curr Opin Cell Biol* 2000; 12: 293–6.
- 34 Tauchi H, Kobayashi J, Morishima K, van Gent DC, Shiraishi T, Verkaik NS, *et al*. Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature* 2002; 420: 93–8.
- 35 Sanal O, Ersoy F, Yel L, Tezcan I, Metin A, Ozyurek H, *et al*. Impaired IgG antibody production to pneumococcal polysaccharides in patients with ataxia-telangiectasia. *J Clin Immunol* 1999; 19: 326–34.
- 36 Gatti RA. The inherited basis of human radiosensitivity. *Acta Oncol* 2001; 40: 702–11.
- 37 Riballo E, Critchlow SE, Teo SH, Doherty AJ, Priestley A, Broughton B, *et al*. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol* 1999; 9: 699–702.
- 38 Bakhshi S, Cerosaletti KM, Concannon P, Bawle EV, Fontanesi J, Gatti RA, *et al*. Medulloblastoma with adverse reaction to radiation therapy in Nijmegen breakage syndrome. *J Pediatr Hematol Oncol* 2003; 25: 248–51.
- 39 Metcalfe JA, Parkhill J, Campbell L, Stacey M, Biggs P, Byrd PJ, *et al*. Accelerated telomere shortening in ataxia telangiectasia. *Nat Genet* 1996; 13: 350–3.
- 40 Kurz EU, Lees-Miller SP. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. *DNA Repair (Amst)* 2004; 3: 889–900.
- 41 Kobayashi J, Antoccia A, Tauchi H, Matsuura S, Komatsu K. NBS1 and its functional role in the DNA damage response. *DNA Repair (Amst)* 2004; 3: 855–61.
- 42 Tauchi H, Matsuura S, Kobayashi J, Sakamoto S, Komatsu K. Nijmegen breakage syndrome gene, *NBS1*, and molecular links to factors for genome stability. *Oncogene* 2002; 21: 8967–80.
- 43 Chrzanowska KH, Kleijer WJ, Krajewska-Walasek M, Bialecka M, Gutkowska A, Goryluk-Kozakiewicz B, *et al*. Eleven Polish patients with microcephaly, immunodeficiency, and chromosomal instability: the Nijmegen breakage syndrome. *Am J Med Genet* 1995; 57: 462–71.
- 44 van der Burgt I, Chrzanowska KH, Smeets D, Weemaes C. Nijmegen breakage syndrome. *J Med Genet* 1996; 33: 153–6.
- 45 Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, *et al*. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995; 268: 1749–53.
- 46 Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiell A, Collins FS, *et al*. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum Mol Genet* 1995; 4: 2025–32.
- 47 Gilad S, Chessa L, Khosravi R, Russell P, Galanty Y, Piane M, *et al*. Genotype–phenotype relationships in ataxia-telangiectasia and variants. *Am J Hum Genet* 1998; 62: 551–61.
- 48 Becker-Catania SG, Chen G, Hwang MJ, Wang Z, Sun X, Sanal O, *et al*. Ataxia-telangiectasia: phenotype/genotype studies of ATM protein expression, mutations, and radiosensitivity. *Mol Genet Metab* 2000; 70: 122–33.
- 49 Stankovic T, Kidd AM, Sutcliffe A, McGuire GM, Robinson P, Weber P, *et al*. ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. *Am J Hum Genet* 1998; 62: 334–45.
- 50 Stewart GS, Last JI, Stankovic T, Haites N, Kidd AM, Byrd PJ, *et al*. Residual ataxia telangiectasia mutated protein function in cells from ataxia telangiectasia patients, with 5762ins137 and 7271T→G mutations, showing a less severe phenotype. *J Biol Chem* 2001; 276: 30 133–41.
- 51 Lavin MF, Scott S, Gueven N, Kozlov S, Peng C, Chen P. Functional consequences of sequence alterations in the ATM gene. *DNA Repair (Amst)* 2004; 3: 1197–205.
- 52 Goodarzi AA, Block WD, Lees-Miller SP. The role of ATM and ATR in DNA damage-induced cell cycle control. *Prog Cell Cycle Res* 2003; 5: 393–411.
- 53 Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science* 2002; 298: 1912–34.
- 54 Kim ST, Lim DS, Canman CE, Kastan MB. Substrate specificities and identification of putative substrates of ATM kinase family members. *J Biol Chem* 1999; 274: 37 538–43.
- 55 O'Neill T, Dwyer AJ, Ziv Y, Chan DW, Lees-Miller SP, Abraham RH, *et al*. Utilization of oriented peptide libraries to identify substrate motifs selected by ATM. *J Biol Chem* 2000; 275: 22 719–27.
- 56 Bosotti R, Isacchi A, Sonnhammer EL. FAT: a novel domain in PIK-related kinases. *Trends Biochem Sci* 2000; 25: 225–7.
- 57 Perry J, Kleckner N. The ATRs, ATMs, and TORs are giant HEAT repeat proteins. *Cell* 2003; 112: 151–5.
- 58 Dames SA, Mulet JM, Rathgeb-Szabo K, Hall MN, Grzesiek S. The solution structure of the FATC domain of the protein kinase TOR suggests a role for redox-dependent structural and cellular stability. *J Biol Chem* 2005; 280: 20558–64.
- 59 Cary RB, Peterson SR, Wang J, Bear DG, Bradbury EM, Chen DJ. DNA looping by Ku and the DNA-dependent protein kinase. *Proc Natl Acad Sci USA* 1997; 94: 4267–72.
- 60 Smith GC, Jackson SP. The DNA-dependent protein kinase. *Genes Dev* 1999; 13: 916–34.
- 61 Merkle D, Douglas P, Moorhead GB, Leonenko Z, Yu Y, Cramb D, *et al*. The DNA-dependent protein kinase interacts with DNA to form a protein-DNA complex that is disrupted by phosphorylation. *Biochemistry* 2002; 41: 12 706–14.
- 62 Yaneva M, Kowalewski T, Lieber MR. Interaction of DNA-dependent protein kinase with DNA and with Ku: biochemical



- and atomic-force microscopy studies. *EMBO J* 1997; 16: 5098–112.
- 63 Llorca O, Rivera-Calzada A, Grantham J, Willison KR. Electron microscopy and 3D reconstructions reveal that human ATM kinase uses an ATM-like domain to clamp around double-stranded DNA. *Oncogene* 2003; 22: 3867–74.
- 64 Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 1998; 281: 1677–9.
- 65 Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, Chessa L, et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 1998; 281: 1674–7.
- 66 Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR, 3rd, et al. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 1998; 93: 477–86.
- 67 Matsuura S, Tauchi H, Nakamura A, Kondo N, Sakamoto S, Endo S, et al. Positional cloning of the gene for Nijmegen breakage syndrome. *Nat Genet* 1998; 19: 179–81.
- 68 Digweed M, Sperling K. Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks. *DNA Repair (Amst)* 2004; 3: 1207–17.
- 69 Xiao Y, Weaver DT. Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res* 1997; 25: 2985–91.
- 70 Dumon-Jones V, Frappart PO, Tong WM, Sajithlal G, Hulla W, Schmid G, et al. Nbn heterozygosity renders mice susceptible to tumor formation and ionizing radiation-induced tumorigenesis. *Cancer Res* 2003; 63: 7263–9.
- 71 Zhu J, Petersen S, Tessarollo L, Nussenzweig A. Targeted disruption of the Nijmegen breakage syndrome gene *NBS1* leads to early embryonic lethality in mice. *Curr Biol* 2001; 11: 105–9.
- 72 Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, et al. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* 1999; 96: 7376–81.
- 73 Petrini JH, Walsh ME, DiMare C, Chen XN, Korenberg JR, Weaver DT. Isolation and characterization of the human *MRE11* homologue. *Genomics* 1995; 29: 80–6.
- 74 Chamankhah M, Wei YF, Xiao W. Isolation of *hMRE11B*: failure to complement yeast *mre11* defects due to species-specific protein interactions. *Gene* 1998; 225: 107–16.
- 75 Sharples GJ, Leach DR. Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Mol Microbiol* 1995; 17: 1215–7.
- 76 Usui T, Ohta T, Oshiumi H, Tomizawa J, Ogawa H, Ogawa T. Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell* 1998; 95: 705–16.
- 77 Chamankhah M, Xiao W. Formation of the yeast Mre11-Rad50-Xrs2 complex is correlated with DNA repair and telomere maintenance. *Nucleic Acids Res* 1999; 27: 2072–9.
- 78 Anderson DE, Trujillo KM, Sung P, Erickson HP. Structure of the Rad50 x Mre11 DNA repair complex from *Saccharomyces cerevisiae* by electron microscopy. *J Biol Chem* 2001; 276: 37 027–33.
- 79 Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA. Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 2001; 105: 473–85.
- 80 de Jager M, van Noort J, van Gent DC, Dekker C, Kanaar R, Wyman C. Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Mol Cell* 2001; 8: 1129–35.
- 81 Trujillo KM, Yuan SS, Lee EY, Sung P. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. *J Biol Chem* 1998; 273: 21447–50.
- 82 Paull TT, Gellert M. The 3' to 5' exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol Cell* 1998; 1: 969–79.
- 83 Paull TT, Gellert M. Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev* 1999; 13: 1276–88.
- 84 Moreau S, Ferguson JR, Symington LS. The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol Cell Biol* 1999; 19: 556–66.
- 85 Haber JE. The many interfaces of Mre11. *Cell* 1998; 95: 583–6.
- 86 Symington LS. Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. *Microbiol Mol Biol Rev* 2002; 66: 630–70.
- 87 Anderson RA, Boronenkov IV, Doughman SD, Kunz J, Loijens JC. Phosphatidylinositol phosphate kinases, a multifaceted family of signaling enzymes. *J Biol Chem* 1999; 274: 9907–10.
- 88 Kunz J, Wilson MP, Kisseleva M, Hurley JH, Majerus PW, Anderson RA. The activation loop of phosphatidylinositol phosphate kinases determines signaling specificity. *Mol Cell* 2000; 5: 1–11.
- 89 Dong Z, Zhong Q, Chen PL. The Nijmegen breakage syndrome protein is essential for Mre11 phosphorylation upon DNA damage. *J Biol Chem* 1999; 274: 19513–6.
- 90 Steimle PA, Hoffert JD, Adey NB, Craig SW. Polyphosphoinositides inhibit the interaction of vinculin with actin filaments. *J Biol Chem* 1999; 274: 18414–20.
- 91 Ivanov EL, Sugawara N, White CI, Fabre F, Haber JE. Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1994; 14: 3414–25.
- 92 Johzuka K, Ogawa H. Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. *Genetics* 1995; 139: 1521–32.
- 93 Chamankhah M, Fontanie T, Xiao W. The *Saccharomyces cerevisiae mre11(ts)* allele confers a separation of DNA repair and telomere maintenance functions. *Genetics* 2000; 155: 569–76.
- 94 Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* 2005; 434: 605–11.
- 95 Williams BR, Mirzoeva OK, Morgan WF, Lin J, Dunnick W, Petrini JH. A murine model of Nijmegen breakage syndrome. *Curr Biol* 2002; 12: 648–53.
- 96 Kang J, Bronson RT, Xu Y. Targeted disruption of *NBS1* reveals its roles in mouse development and DNA repair. *EMBO J* 2002; 21: 1447–55.

- 97 Chen J. Ataxia telangiectasia-related protein is involved in the phosphorylation of BRCA1 following deoxyribonucleic acid damage. *Cancer Res* 2000; 60: 5037–9.
- 98 Li S, Ting NS, Zheng L, Chen PL, Ziv Y, Shiloh Y, *et al*. Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. *Nature* 2000; 406: 210–5.
- 99 Smith GC, Cary RB, Lakin ND, Hann BC, Teo SH, Chen DJ, *et al*. Purification and DNA binding properties of the ataxia-telangiectasia gene product ATM. *Proc Natl Acad Sci USA* 1999; 96: 11 134–9.
- 100 Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003; 421: 499–506.
- 101 Ali A, Zhang J, Bao S, Liu I, Otterness D, Dean NM, *et al*. Requirement of protein phosphatase 5 in DNA-damage-induced ATM activation. *Genes Dev* 2004; 18: 249–54.
- 102 Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks *in vivo*. *J Cell Biol* 1999; 146: 905–16.
- 103 Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, *et al*. NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol* 2002; 12: 1846–51.
- 104 Zhao S, Weng YC, Yuan SS, Lin YT, Hsu HC, Lin SC, *et al*. Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. *Nature* 2000; 405: 473–7.
- 105 Kim ST, Xu B, Kastan MB. Involvement of the cohesin protein, Smc1, in ATM-dependent and independent responses to DNA damage. *Genes Dev* 2002; 16: 560–70.
- 106 Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev* 2002; 16: 571–82.
- 107 Lisby M, Barlow JH, Burgess RC, Rothstein R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* 2004; 118: 699–713.
- 108 Greenwell PW, Kronmal SL, Porter SE, Gassenhuber J, Obermaier B, Petes TD. *TELI*, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. *Cell* 1995; 82: 823–9.
- 109 Morrow DM, Tagle DA, Shiloh Y, Collins FS, Hieter P. *TELI*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene MEC1. *Cell* 1995; 82: 831–40.
- 110 Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 2003; 22: 5612–21.
- 111 Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* 2005; 308: 551–4.
- 112 Mochan TA, Venere M, DiTullio RA, Jr, Halazonetis TD. 53BP1 and NFB1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. *Cancer Res* 2003; 63: 8586–91.
- 113 Goldberg M, Stucki M, Falck J, D'Amours D, Rahman D, Pappin D, *et al*. MDC1 is required for the intra-S-phase DNA damage checkpoint. *Nature* 2003; 421: 952–6.
- 114 Stewart GS, Wang B, Bignell CR, Taylor AM, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 2003; 421: 961–6.
- 115 Wang B, Matsuoka S, Carpenter PB, Elledge SJ. 53BP1, a mediator of the DNA damage checkpoint. *Science* 2002; 298: 1435–8.
- 116 Lou Z, Minter-Dykhouse K, Wu X, Chen J. MDC1 is coupled to activated CHK2 in mammalian DNA damage response pathways. *Nature* 2003; 421: 957–61.
- 117 Shang YL, Boderer AJ, Chen PL. NFB1, a novel nuclear protein with signature motifs of FHA and BRCT, and an internal 41-amino acid repeat sequence, is an early participant in DNA damage response. *J Biol Chem* 2003; 278: 6323–9.
- 118 Xu X, Stern DF. NFB1/KIAA0170 is a chromatin-associated protein involved in DNA damage signaling pathways. *J Biol Chem* 2003; 278: 8795–803.
- 119 Peng A, Chen PL. NFB1, like 53BP1, is an early and redundant transducer mediating Chk2 phosphorylation in response to DNA damage. *J Biol Chem* 2003; 278: 8873–6.
- 120 Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, *et al*. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol* 2002; 4: 993–7.
- 121 DiTullio RA, Jr, Mochan TA, Venere M, Bartkova J, Sehested M, Bartek J, *et al*. 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat Cell Biol* 2002; 4: 998–1002.
- 122 Ward IM, Minn K, van Deursen J, Chen J. p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice. *Mol Cell Biol* 2003; 23: 2556–63.
- 123 Morales JC, Xia Z, Lu T, Aldrich MB, Wang B, Rosales C, *et al*. Role for the BRCA1 C-terminal repeats (BRCT) protein 53BP1 in maintaining genomic stability. *J Biol Chem* 2003; 278: 14 971–7.
- 124 Hartley KO, Gell D, Smith GC, Zhang H, Divecha N, Connelly MA, *et al*. DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell* 1995; 82: 849–56.
- 125 Chan DW, Chen BP, Prithivirajasingh S, Kurimasa A, Story MD, Qin J, *et al*. Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. *Genes Dev* 2002; 16: 2333–8.
- 126 Hendrickson EA, Qin XQ, Bump EA, Schatz DG, Oettinger M, Weaver DT. A link between double-strand break-related repair and V(D)J recombination: the scid mutation. *Proc Natl Acad Sci USA* 1991; 88: 4061–5.
- 127 Blunt T, Finnie NJ, Taccioli GE, Smith GC, Demengeot J, Gottlieb TM, *et al*. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell* 1995; 80: 813–23.
- 128 Cohen MM, Levy HP. Chromosome instability syndromes. *Adv Hum Genet* 1989; 18: 43–149, 365–171.
- 129 Leuther KK, Hammarsten O, Kornberg RD, Chu G. Structure of DNA-dependent protein kinase: implications for its regulation by DNA. *EMBO J* 1999; 18: 1114–23.
- 130 Boskovic J, Rivera-Calzada A, Maman JD, Chacon P, Willison KR, Pearl LH, *et al*. Visualization of DNA-induced conformational changes in the DNA repair kinase DNA-PKcs. *EMBO J* 2003; 22: 5875–82.
- 131 Rivera-Calzada A, Maman JP, Spagnolo L, Pearl LH, Llorca O.

- Three-dimensional structure and regulation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). *Structure (Camb)* 2005; 13: 243–55.
- 132 Burma S, Chen DJ. Role of DNA-PK in the cellular response to DNA double-strand breaks. *DNA Repair (Amst)* 2004; 3: 909–18.
- 133 Yu J, Marshall K, Yamaguchi M, Haber JE, Weil CF. Microhomology-dependent end joining and repair of transposon-induced DNA hairpins by host factors in *Saccharomyces cerevisiae*. *Mol Cell Biol* 2004; 24: 1351–64.
- 134 Kramer KM, Brock JA, Bloom K, Moore JK, Haber JE. Two different types of double-strand breaks in *Saccharomyces cerevisiae* are repaired by similar RAD52-independent, nonhomologous recombination events. *Mol Cell Biol* 1994; 14: 1293–301.
- 135 Lewis LK, Resnick MA. Tying up loose ends: nonhomologous end-joining in *Saccharomyces cerevisiae*. *Mutat Res* 2000; 451: 71–89.
- 136 Moore JK, Haber JE. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1996; 16: 2164–73.
- 137 Boulton SJ, Jackson SP. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J* 1998; 17: 1819–28.
- 138 Nugent CI, Bosco G, Ross LO, Evans SK, Salinger AP, Moore JK, *et al*. Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr Biol* 1998; 8: 657–60.
- 139 Frank-Vaillant M, Marcand S. Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol Cell* 2002; 10: 1189–99.
- 140 Martin SG, Laroche T, Suka N, Grunstein M, Gasser SM. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell* 1999; 97: 621–33.
- 141 Chen L, Trujillo K, Ramos W, Sung P, Tomkinson AE. Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. *Mol Cell* 2001; 8: 1105–15.
- 142 Teo SH, Jackson SP. Lif1p targets the DNA ligase Lig4p to sites of DNA double-strand breaks. *Curr Biol* 2000; 10: 165–8.
- 143 Cortez D, Guntuku S, Qin J, Elledge SJ. ATR and ATRIP: partners in checkpoint signaling. *Science* 2001; 294: 1713–6.
- 144 Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003; 300: 1542–8.
- 145 Gravel S, Larrivee M, Labrecque P, Wellinger RJ. Yeast Ku as a regulator of chromosomal DNA end structure. *Science* 1998; 280: 741–4.
- 146 Hsu HL, Gilley D, Blackburn EH, Chen DJ. Ku is associated with the telomere in mammals. *Proc Natl Acad Sci USA* 1999; 96: 12 454–8.
- 147 Kironmai KM, Muniyappa K. Alteration of telomeric sequences and senescence caused by mutations in *RAD50* of *Saccharomyces cerevisiae*. *Genes Cells* 1997; 2: 443–55.
- 148 Gallego ME, White CI. RAD50 function is essential for telomere maintenance in Arabidopsis. *Proc Natl Acad Sci USA* 2001; 98: 1711–6.
- 149 Wilson S, Warr N, Taylor DL, Watts FZ. The role of *Schizosaccharomyces pombe* Rad32, the Mre11 homologue, and other DNA damage response proteins in non-homologous end joining and telomere length maintenance. *Nucleic Acids Res* 1999; 27: 2655–61.
- 150 Ueno M, Nakazaki T, Akamatsu Y, Watanabe K, Tomita K, Lindsay HD, *et al*. Molecular characterization of the *Schizosaccharomyces pombe nbs1+* gene involved in DNA repair and telomere maintenance. *Mol Cell Biol* 2003; 23: 6553–63.
- 151 Ritchie KB, Petes TD. The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. *Genetics* 2000; 155: 475–9.
- 152 Chan SW, Blackburn EH. Telomerase and ATM/Tel1p protect telomeres from nonhomologous end joining. *Mol Cell* 2003; 11: 1379–87.
- 153 Usui T, Ogawa H, Petrini JH. A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol Cell* 2001; 7: 1255–66.
- 154 Xia SJ, Shammas MA, Shmookler Reis RJ. Reduced telomere length in ataxia-telangiectasia fibroblasts. *Mutat Res* 1996; 364: 1–11.
- 155 Hande MP, Balajee AS, Tchirkov A, Wynshaw-Boris A, Lansdorp PM. Extra-chromosomal telomeric DNA in cells from *Atm(-/-)* mice and patients with ataxia-telangiectasia. *Hum Mol Genet* 2001; 10: 519–28.
- 156 Pandita TK, Dhar S. Influence of ATM function on interactions between telomeres and nuclear matrix. *Radiat Res* 2000; 154: 133–9.
- 157 Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, Gu Y, *et al*. Telomere dysfunction and *Atm* deficiency compromises organ homeostasis and accelerates ageing. *Nature* 2003; 421: 643–8.