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meeting report

ATM and the DNA damage response

Workshop on Ataxia-Telangiectasia and Related Syndromes

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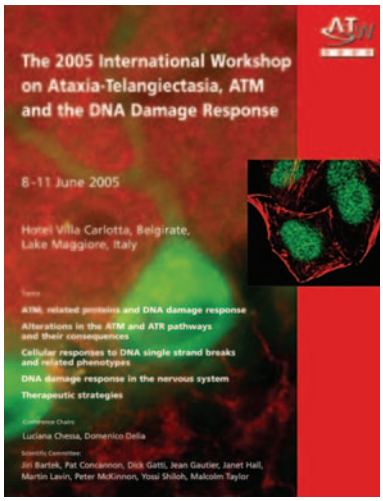

The 2005 International Workshop on Ataxia-Telangiectasia, ATM and the DNA Damage Response took place between 8 and 11 June 2005 on the banks of Lake Maggiore, Italy. The workshop was organized by L. Chessa and D. Delia.

Keywords: ataxia-telangiectasia; ATM; DNA damage signalling; DNA repair; cell-cycle control

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Introduction

This workshop on ataxia-telangiectasia marked the tenth anniversary of the discovery of the gene that is defective in this syndrome—that is, ataxia-telangiectasia mutated (*ATM*; Savitsky *et al*, 1995). At the meeting, several important developments were reported, including: an expansion of the substrate repertoire of the ATM kinase; the use of animal models to analyse the signalling pathways controlled by *ATM* and the functional consequences of disrupting these pathways; new insights into cell-cycle control and the maintenance of genome stability; the influence of modifier genes on *ATM* function; and approaches for correcting the progressive neurodegeneration that is a part of this syndrome.

Ataxia-telangiectasia is an autosomal recessive disorder characterized by neurodegeneration, immunodeficiency, hypogonadism and susceptibility to cancer. At the cellular level, it is marked by genomic instability, which is due to a defective response to double-stranded breaks (DSBs) in DNA. This is manifested by hypersensitivity to ionizing radiation (IR) and radiomimetic compounds, and by a decreased ability to activate the DNA-damage-response network, which includes the cell-cycle checkpoints (Lavin & Shiloh, 1997; Chun & Gatti, 2004). The protein product of the *ATM* gene is present in the nucleus as an inactive dimer or oligomer, and is activated in response to DSBs in a process that involves autophosphorylation on serine (Ser) 1,981. This causes a dissociation of the dimer to form active monomeric forms, which are able to initiate the phosphorylation of many intermediates, such as p53 and the checkpoint kinase Chk2, which are involved in DNA repair and cell-cycle control (Bakkenist & Kastan, 2003). However, ATM is not solely responsible for initiating this cascade of signalling events. The ataxia-telangiectasia and Rad3-related (ATR) protein has a similar role in responding to agents that stress the DNA replication process. Both ATM and ATR are assisted by a host of sensor, transducer and effector molecules that ensure the genome is adequately protected from DNA damage.

Sensing double-stranded breaks in DNA

Two distinct pathways exist for the repair of DNA DSBs: non-homologous end joining (NHEJ), which joins free ends, and homologous recombination (HR), which requires homologous pairing of DNA sequences. Although it is not yet clear how DSBs are detected, signalled to the cell cycle and repaired, some of the presentations at this workshop increased our understanding of these processes. The recruitment to, and activation of, proteins at DSBs is a complex process that involves several levels of control. The MRE11 complex, MRE11–RAD50–NBS1 (MRN) has a central role in sensing DNA DSBs. T. Paull (Austin, TX, USA) used recombinant MRN complex members and purified ATM to show that DNA ends are able to stimulate activation of the inactive ATM dimer, as shown

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by phosphorylation of p53 and Chk2 (Lee & Paull, 2005). ATM was only associated with DNA in the presence of the MRN complex, activation was dependent on the presence of single-stranded DNA (ssDNA) and NBS1 was required for the unwinding of duplex DNA as part of the processing mechanism. By contrast, ssDNA was not required in *Xenopus* extracts incubated with double-stranded oligonucleotides that have blunt ends or overhangs that trigger ATM activation (J. Gautier, New York, NY, USA). Gautier also showed that the association of MRN is the rate-limiting step in the assembly of the ATM, DNA and MRN complex. In addition, although autophosphorylation of ATM on Ser 1,981 is important for ATM activation *in vivo*, it seems to be less important *in vitro*. Paull revealed only weak Ser 1,981 phosphorylation during activation and, in *Xenopus* extracts, neither the presence of the MRN complex nor autophosphorylation were required for ATM monomerization.

M. Weitzman (San Diego, CA, USA) highlighted how viruses can be used to study cellular responses to DNA damage. Infection of cells with adenovirus resulted in inactivation of the DNA-damage-response machinery in the host by mislocalization and degradation of the MRN complex. This is because the MRN complex seems to be important in activation of both ATM and ATR signalling. Conversely, herpes simplex virus (HSV-1) is able to activate and exploit the cellular DNA-damage response for its own replication. Although the response to both adenovirus and HSV-1 is MRN-dependent, Weitzman showed that the complex is not required for ATM activation in adeno-associated virus (AAV)-infected cells.

ATM recognizes and signals to DSBs, whereas ATR responds to damage by ultraviolet (UV) and stalled DNA replication forks. S. Jackson (Cambridge, UK) described related, conserved carboxy-terminal motifs in NBS1, ATR-interacting protein (ATRIP) and Ku80, which are required for interaction with ATM, ATR and the catalytic subunit of DNA-dependent protein kinase, DNA-PK_{cs}, respectively. As DSBs can be resected to single strands and as MRN has such activity, Jackson suggested that they could be considered as two single lesions with different temporal orders of appearance. This raises the possibility that there is co-operation between ATM and ATR. In support of this idea, no DNA-damage-induced ATR foci were observed in ataxia-telangiectasia cells, and replication protein A (RPA) foci, which localize with ATR, were shown to be dependent on both ATM and NBS1. This cross-talk in the DNA-damage response is not confined to ATM and ATR but also exists with DNA-PK. D. Chen (Dallas, TX, USA) showed that DNA-PK_{cs} fused to yellow fluorescent protein (YFP) is recruited rapidly to sites of DSBs and is accompanied by phosphorylation at six clustered sites, including Ser 2,056. Previous data from his and other laboratories have identified these as autophosphorylation sites that, when mutated, result in radiosensitivity and the accumulation of DSBs. Chen showed that some of these phosphorylations (threonine (Thr) 2,609 and Thr 2,447) are ATM-dependent and indicate a direct role for ATM in controlling DNA-PK_{cs} activity. To add to the complexity of recognition of DSBs, T. Halazonetis (Philadelphia, PA, USA) showed that the p53-binding protein, 53BP1, localizes rapidly to DSBs and contributes to ATM activation as an additional sensor. This localization involves the recognition of histone H3, methylated on lysine (Lys) 79, rather than the DNA break *per se*. Suppression of Dot1L, the enzyme that is responsible for histone H3 Lys 79 methylation, prevented the recruitment of 53BP1 to the break. As this lysine residue is constitutively methylated, Halazonetis proposed that this mechanism does not 'sense' the break as such, but rather that it responds to conformational changes in the higher-order chromatin structure. A histone acetyltransferase gene, *MOF*, the human orthologue of the *Drosophila Mof* (males absent on the first) gene also influences the function of ATM. T. Pandita (St Louis, MO, USA) showed that radiation enhances MOF-dependent acetylation of histone H4 on Lys 16. Abrogation of this acetylation decreased ATM phosphorylation and reduced its capacity to phosphorylate downstream targets. Decreased MOF activity also sensitized cells to radiation and led to a loss of cell-cycle checkpoint activation, which is characteristic of the ataxia-telangiectasia cellular phenotype.

Activation of ATM

Previous data have provided evidence of a central role for autophosphorylation (Bakkenist & Kastan, 2003; Kozlov *et al*, 2003) and dissociation of an inactive dimer for ATM activation. However, how these activation steps are controlled is not yet known. S. Lees-Miller (Calgary, AB, Canada) was able to detect protein phosphatase 2A (PP2A) in ATM immunoprecipitates. This enzyme dissociated from ATM in response to IR exposure, and this coincided with activation of ATM kinase. These data suggest that the association of PP2A with ATM suppresses any inherent ability of ATM molecules from undergoing trans-phosphorylation on Ser 1,981. Evidence for two additional autophosphorylation sites on ATM, Ser 367 and Ser 1,983, in response to IR damage was provided by M. Lavin (Brisbane, Qld, Australia). Mutants in these sites were defective in ATM activation and also failed to correct radiosensitivity or the G2 checkpoint defect in ataxia-telangiectasia cells. It is evident from these reports that the process of ATM activation is complex and may involve other modifications and/or interactions.

ATM-dependent substrate phosphorylation

It is known that, once activated, ATM directly or indirectly phosphorylates approximately 30 substrates, and this number was expanded at the meeting. Efficient DNA-damage-induced degradation of human HDMX, the p53 inhibitor, and its dependence on ATM was described by Y. Shiloh (Tel Aviv, Israel). This involves the direct phosphorylation of HDMX on Ser 403 by ATM and additional phosphorylation that might be mediated by other protein kinases activated by ATM (for example, Chk2). This is an excellent example of the fine-tuning carried out by ATM to achieve optimal activation of cell-cycle checkpoints. Shiloh also added another three proteins to the list of ATM substrates: the co-repressor protein KAP1, the ATM-dependent phosphorylation of which mediates chromatin decondensation; CSN3, a subunit of the COP9 signalosome complex, the phosphorylation of which is associated with damage-induced apoptosis; and the transcription factor SP1, the ATM-dependent phosphorylation of which on several sites enhances its specific binding to DNA. Chk2 was also revisited as a substrate by D. Delia (Milan, Italy). It is well-established that phosphorylation on Thr 68 by ATM is an essential step for Chk2 activation, but it is also clear that other Chk2 phosphorylations are involved. Delia identified three new sites in the amino-terminal Ser/Thr-Gln-rich region of Chk2. These phosphorylations were observed at doses greater than 1 Gy of radiation, occurred only during G1 phase and were dependent on ATM and, to a lesser extent, on NBS1. The heterogeneous nuclear nucleoprotein K (hnRNPK), an RNA- and ssDNA-binding protein involved in mRNA processing and translation, was also identified by Jackson as a new target of the ATM pathway. He showed ATM-dependent stabilization of hnRNPK2 by dissociation from mouse double minute (MDM2) after DNA damage similar to that

observed for p53. The loss of hnRNPK2 prevented effective targeting of p53 to its consensus promoter sequences, representing another level of control of p53 by ATM. K.K. Khanna (Brisbane, Qld, Australia) described two types of ATM-interacting protein: transcriptional repressors, which are stabilized rapidly in response to radiation in an ATM-dependent manner; and components of the translation initiation complex, which undergo subcellular redistribution after radiation exposure. Seven new potential targets of ATM, three of which are novel proteins, were identified using the yeast two-hybrid system and Flag-tagged ATM. One of these proteins is the DNA-damage responsive protein 1 (DRP1), which is rapidly induced in response to IR, UV and methyl methanesulphonate treatments. DRP1 binds to ssDNA and MRE11 and localizes to foci after DNA damage. In addition, depletion of DRP1 enhanced cellular sensitivity to DNA-damaging agents. The identification of MRE11 as another ATM substrate by Lavin was not a great surprise as there have been reports that high doses of IR lead to a partial mobility shift in MRE11 that is due to phosphorylation. However, Lavin also identified the ATM phosphorylation sites on MRE11 and described their functional importance.

ATM signalling pathways

ATM controls a complex signalling network in response to DSBs. Given the diversity of events involved, Shiloh and his colleagues have developed a bioinformatics tool, SHARP (showcase for ATM-related pathways), to integrate, visualize and interpret existing and new information on ATM-regulated networks. This tool was introduced in more depth at the meeting. In essence, it has three components: a database for biological interactions that allows input from many users at several sites; a visualization package that allows interactive graphic representations of the biological interactions stored in the database; and an algorithmic inference engine that analyses the data and establishes links between the components to create new regulatory circuits. A graphic representation of the existing network appears in Fig 1 and further details of this system can be obtained at http://www.cs.tau.ac.il/~sharp.

Several substrates phosphorylated by ATM in response to DSBs occur in pathways that are important for cell-cycle checkpoint activation. Two of these checkpoint mediators—mediator of DNA damage checkpoint 1 (MDC1) and 53BP1—were the subject of a spatiotemporal redistribution investigation by J. Lukas (Copenhagen, Denmark). He showed that MDC1 first associates with histone γH2AX, and then MDC1 triggers higher-order chromatin rearrangements to enhance binding of 53BP1 to histone sites. This process localizes ATM-activated proteins to sites of breaks and minimizes the risk of these proteins associating with undamaged DNA. It also ensures that there are local changes in chromatin structure that are required for the productive assembly of downstream signalling components. R. Rothstein (New York, NY, USA) used imaging to show the co-ordinated recruitment of checkpoint proteins and recombination proteins for repairing centres in budding yeast. The repair machinery is able to distinguish between breaks amenable to NHEJ and those that require further processing in the G1 phase of the cell cycle.

ATM maintains genomic integrity

Targeting genes that are involved in DNA-damage recognition and repair has proven to be a useful approach in delineating the role of ATM as a tumour suppressor. The keynote speaker at this meeting, F. Alt (Boston, MA, USA), described the propensity of mice that are deficient in both NHEJ and p53 to form pro-B-cell lymphomas with non-reciprocal translocations. These fuse the IgH locus to chromosome 15 downstream of c-Myc and thus cause its amplification. Conversely, conditional inactivation of NHEJ in *p53*$^{-/-}$ mature B cells gave rise to mature B-cell lymphomas with translocations directly into c-Myc, similar to human tumours. As V(D)J recombination is essentially normal in ataxia-telangiectasia cells, and as the loss of ATM does not lead to defective DSBs, repair to the extent seen in DNA-PK$_{cs}$-deficient or Ku-deficient cells, ATM was not considered significant in NHEJ. However, this and other presentations reveal a more important role for ATM in NHEJ than previously thought. In NHEJ, there is a strong preference for joining DSBs back together, which suggests a significant role for the chromatin proteins localized to the break. Alt showed that class switch recombination was defective in Mdc1, H2ax and 53Bp1 mutant mice, and that these mutants were also characterized by genome instability. He proposed that these downstream targets of Atm formed an intramolecular complex that prevented premature separation of the free ends. In addition, targeting H2ax in an *Atm*-deficient background caused marked genome instability in fibroblasts. In support of such a role for this intramolecular complex, P. Jeggo (Sussex, UK) provided evidence that proteins localizing to γH2AX foci are required for end-processing before rejoining of breaks by the NHEJ. She showed that Artemis nuclease activity is required for end-processing and is dependent on ATM for its activity (Fig 2).

R. Maser (Boston, MA, USA) discussed Atm-independent p53 activation in response to telomere erosion in telomerase (*Terc*)-mutant mice, which, when combined with Atm deficiency, was detrimental to several stem-cell populations. Telomere dysfunction suppressed thymic lymphoma development in *Atm*-mutant mice but, when combined with p53 heterozygosity, the manifestation of tumours was restored. P. McKinnon (Memphis, TN, USA) has previously shown that Atm has a central role in the developing nervous system in differentiating subventricular zone cells. At this meeting, he presented studies with conditional mouse mutants showing that disruption of DNA ligase IV, a component of NHEJ, affects differentiating cells in the nervous system, whereas HR disruption (in this case by mutating the *rad51*-like gene *XRCC2*) affects proliferating cells. Furthermore, DNA-damage signalling requires Atm after the disruption of NHEJ but not HR. These data, together with ATM phosphorylation of DNA-PK$_{cs}$, provide further support for an involvement of ATM in NHEJ. Mutations in *ATM* are associated with an increased risk of breast cancer, and it is notable that the tumour suppressor gene breast cancer 1 (*BRCA1*) is one of the downstream targets of ATM (Gatei *et al*, 2000). However, a defect in this signalling step does not provide a mechanistic explanation for susceptibility to breast cancer. *BRCA1* has an important role in the maintenance of genomic stability by regulating cell-cycle checkpoints and participating in DNA repair. Considerably less is known about its cellular function in the absence of induced DNA damage, a topic addressed by J. Chen (Rochester, MN, USA). He showed that *BRCA1* interacts and localizes with topoisomerase IIa in the S and G2 phases. This enzyme shapes the higher-order chromatin structure by modulating the topological state and chromatin condensation, and by preventing defective chromosome segregation. Cells treated with the topoisomerase inhibitor ICRF-193 had lagging chromosomes during mitosis, which is a phenotype similar to that seen in *BRCA1*-deficient cells and indicates a defect in DNA decatenation.

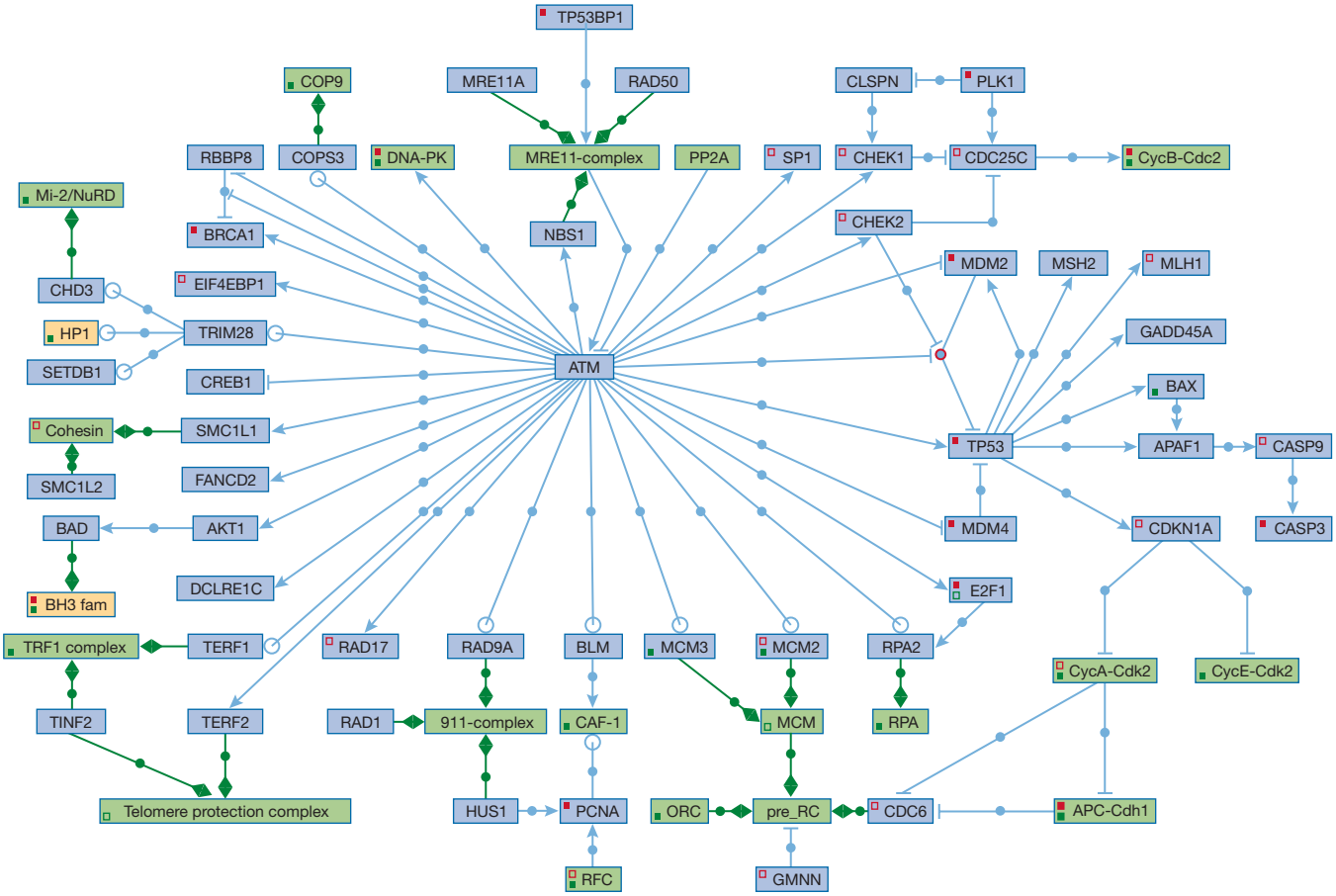

Fig 1 | A comprehensive map of ataxia-telangiectasia-mutated controlled signalling pathways induced in response to DNA double-stranded breaks. This image was created using the SHARP (showcase for ATM-related pathways) tool, which is a highly curated knowledge base for signalling pathways induced by double-stranded breaks. Violet nodes represent single proteins (denoted by the official designation of their encoding genes), yellow nodes represent protein families and green nodes represent protein complexes. Blue lines denote regulatory relations (arrows for activation, blocked lines for inhibition and an open circle for unclear regulation) and green arrows denote containment relations among protein families or protein complexes. Red and green dots within a node indicate that not all of the regulations and associations stored in SHARP for the node are displayed in this map. Researchers can make use of SHARP and help keep it up-to-date at http://www.cs.tau.ac.il/~sharp.

J. Petrini (New York, NY, USA) has also used mouse models to gain an understanding of the molecular transactions that govern chromosome stability and DNA replication. Crossing *Rad50*$^{s/s}$ mice with *Chk2*$^{-/-}$ animals blocked progressive haematopoietic stem-cell failure by apoptosis and increased survival. Introduction of these animals to a *Chk2* heterozygous background also abrogated the lethal phenotype. This was also the case in *Nbs*$^{+/-}$ and *Mre11*$^{+/-}$ backgrounds. Petrini was able to show that the removal of one or both of the *Atm* alleles rescued survival in the *Rad50*$^{s/s}$ background, thus providing further supportive data for MRN signalling through Atm.

Cell-cycle checkpoint activation and DNA repair

It is evident from the network of ATM substrates in Fig 1 that a significant proportion of these control the cell cycle. The role of ATM in DNA repair is less well-understood, although there is evidence that it has a role in HR and, as reported at this meeting by Chen and Jeggo, in NHEJ of DSBs. The introduction of a single DSB into budding yeast DNA triggers HR and the MEC1-dependent DNA-damage checkpoint.

To investigate the importance of γH2AX in HR, S. Takeda (Kyoto, Japan) used chicken DT40 cells with a mutation at the S139A phosphorylation site of H2AX. Higher levels of radiation-induced chromosomal breaks and less efficient formation of RAD51 foci were observed in the mutant. Conditional depletion of wild-type H2AX in a double mutant *H2AX*(S139A)*XRCC3*$^{-/-}$ caused defective RAD51 foci formation, a marked increase in camptothecin-induced chromosomal breaks and a decreased number of sister chromatid exchanges compared with the single mutant *XRCC3*$^{-/-}$. This suggests that γH2AX acts in parallel with XRCC3 to facilitate the assembly of RAD51 foci in HR-mediated repair during DNA replication. Another essential protein in HR in higher vertebrate cells is NBS1. K. Komatsu (Kyoto, Japan) showed that deletion of the C-terminus of NBS1 (the MRE11-binding domain) abolished HR regulation by NBS1, whereas mutations in the Forkhead-associated (FHA) and BRCA1-domains severely inhibited HR. The mutation of ATM phosphorylation sites had only a small effect on HR. Furthermore, HR frequency was not reduced in ataxia-telangiectasia cells, suggesting that ATM is not involved in this process. This contradicts some previous data from *ATM* mutants in

chicken DT40 cells, which suggested a controlling role for ATM in HR-mediated repair.

Syndromes overlapping with ataxia-telangiectasia

Mutations in NBS1 and MRE11 give rise to Nijmegen breakage syndrome (NBS) and ataxia-telangiectasia-like disorder (ATLD), respectively, the clinical and cellular phenotypes of which considerably overlap with ataxia-telangiectasia (Carney *et al*, 1998; Stewart *et al*, 1999). ATM is dependent on NBS1 as part of the MRE11 complex for sensing DSBs, and in turn phosphorylates this protein to enhance its activity in the DNA-damage response. NBS1 also acts as an adaptor molecule for phosphorylation of other downstream substrates of ATM. Notwithstanding this interdependence, NBS1 also has functions that are independent of ATM. The most marked example of this is the early lethality shown by *Nbs*$^{-/-}$ mice, whereas *Atm*$^{-/-}$ mice develop relatively normally. A. Nussenzweig (Bethesda, MD, USA) generated transgenic mice that express human *NBS1* in an *Nbs1*$^{-/-}$ background and showed that this protein interacted with the mouse Rad50 protein, that the DNA-damage response was normal in these mice and that there was no evidence of immunodeficiency. Introduction of the human 5-bp-deletion mutant rescued the lethality of *Nbs1*$^{-/-}$ mice, but they still had the hallmarks of NBS, including genome instability, cancer predisposition and female sterility. The mice exhibited T-cell developmental defects but normal class switch recombination. Cell-cycle checkpoint activation was intermediate in keeping with a partial reduction in Atm kinase activity. Nbs1 was specifically phosphorylated in response to DNA damage but Mre11 and Nbs1 were not as efficiently recruited to γH2ax foci as those in wild-type animals.

Z.-Q. Wang (Lyon, France) described the conditional disruption of the *Nbs1* gene in mice by flanking exon 6 with *loxP* sites. Homozygote mice were crossed with nestin-C transgenic mice to disrupt the *Nbs1* gene in the central nervous system. These mice developed the neurological abnormalities seen in NBS, ataxia-telangiectasia and ATLD, including cerebellar defects and ataxia, growth retardation and microcephaly. Proliferation arrest was seen in granule cell progenitors and apoptosis in post-mitotic cerebellar neurons. Perhaps surprisingly, depletion of *Nbs1* in lens cells caused early postnatal cataractogenesis in these mice. Light and electron microscopy revealed the disruption of lens fibre cell architecture and incomplete denucleation, suggesting a new role for Nbs1 in cell terminal differentiation.

Neurodegeneration and the DNA-damage response

Progressive neurodegeneration, an important characteristic observed in ataxia-telangiectasia, has the greatest impact on patients, as it reduces their mobility and their quality of life (Boder, 1985). Post-mitotic cells are particularly vulnerable in ataxia-telangiectasia patients, and it has been suggested that a checkpoint defect in these cells is responsible for neurodegeneration (Yang & Herrup, 2005). Evidence in support of this was provided previously by Takagi *et al* (1998), who revealed a mitotic spindle defect in ataxia-telangiectasia cells after irradiation. E. Cundari (Rome, Italy) showed that active ATM (that is, phosphorylated on Ser 1,981) localizes to centrosomes. He also showed that p53 is phosphorylated by ATM on Ser 15 at the onset of mitosis, and that p53 then also localizes to centrosomes and is subsequently dephosphorylated. In ataxia-telangiectasia cells, this co-localization is disrupted. These results suggest that ATM is activated 'by default' at the onset of mitosis and pre-emptively phosphorylates p53 to keep it inactive on the centrosome when the spindle is in its correct position. In

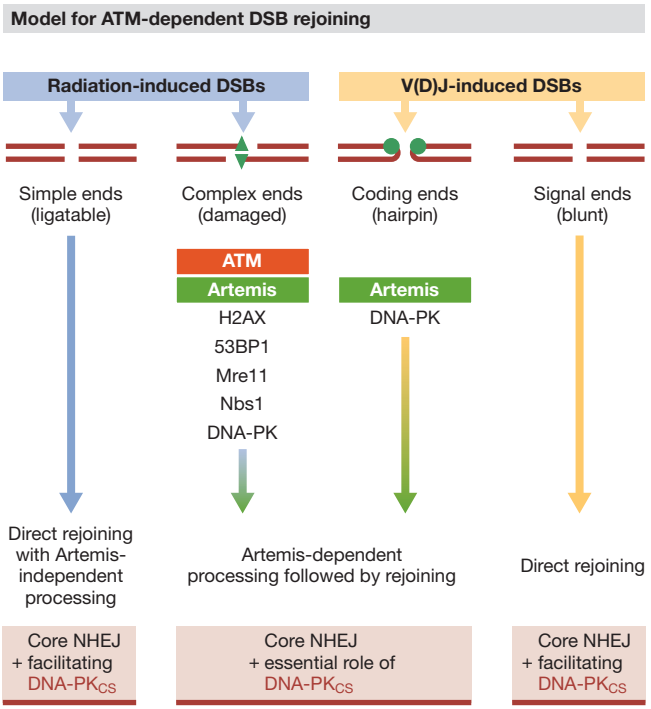

Fig 2 | Ataxia-telangiectasia-mutated activates checkpoint arrest to facilitate the repair of double-stranded breaks regulated by Artemis. A model is provided showing the different pathways for the rejoining of different forms of double-stranded breaks (DSBs). A direct break creating 3′OH and 5′P ends in response to radiation damage is readily repaired by ligation (on the left). This is also the case for signal ends generated during V(D)J recombination (on the right). More complex breaks or ends not amenable to direct ligation require ataxia-telangiectasia mutated (ATM)-dependent processing and the involvement of a series of other proteins including Artemis. NHEJ, non-homologous end joining. Figure provided by P. Jeggo, modified from Löbrich & Jeggo (2005).

ataxia-telangiectasia patients, post-mitotic cells might attempt abortively to re-enter a mitotic cycle, but then die.

The neurological phenotypes of several other autosomal recessive neurodegenerative disorders overlap with ataxia-telangiectasia. It is now evident that several of these are also characterized by defects in the recognition and/or repair of damage to DNA. However, the manifestation of these defects seems to be confined to the brain in these disorders as they do not show the extraneurological phenotype of ataxia-telangiectasia. K. Caldecott (Brighton, UK) presented evidence for a defect in single-stranded break repair in spinocerebellar ataxia with axonal neuropathy 1 (SCAN1). This is a neurodegenerative disease that results from mutations in tyrosyl phosphodiesterase 1 (*TDP1*), which removes topoisomerase 1 peptides from DNA termini during repair. In contrast to what is observed in normal cells, Caldecott showed that camptothecin-induced breaks continue to accumulate in SCAN1 cells even when the cells are incubated in drug-free medium. He showed that *TDP1* is present in an active single-stranded break repair complex in normal cells but that this complex is inactive in SCAN1 cells. Single-stranded breaks that arise from oxidative stress were also shown to accumulate in SCAN1 cells. The persistent DNA breaks in SCAN1 cells resulted in a failure to recover blocked transcription.

Ataxia with oculomotor apraxia type 1 (AOA1) cells have also been shown to be sensitive to agents that cause single-stranded breaks in DNA. L. Chessa (Rome, Italy) described a patient who was homozygous for a mutation (T739C) in *APTX*, the gene that encodes aprataxin and which is defective in AOA1. She described a marked increase in chromosomal aberrations in AOA1 cells compared with controls in response to camptothecin and used an alkaline comet assay to confirm a defect in the repair of DNA single-stranded breaks. Several other autosomal recessive spinocerebellar ataxias exist that overlap with ataxia-telangiectasia. It will be of interest to determine whether any or all of these also have abnormalities in DNA-damage-repair pathways, which might contribute to the neurodegenerative phenotype.

Cross-talk between laboratory and clinic

In his opening address to the meeting, Shiloh emphasized that understanding the pathological and molecular basis of ataxia-telangiectasia was a prerequisite to the search for new treatment modalities for the disease. The use of classical techniques of localization in neurology points to cerebellar brainstem oculomotor and extrapyramidal circuits as the main areas of dysfunction in ataxia-telangiectasia. T. Crawford (Baltimore, MD, USA) described a longitudinal neurological follow-up of ataxia-telangiectasia patients. This analysis was based on the relative impairment of ten measurable neurological traits and showed significant independence of individual traits compared with the overall combined score. He revealed a significant role for the *ATM* genotype in the expression of the various neurological traits. In short, greater homogeneity was observed when comparing ataxia-telangiectasia patients within families than between families. The conclusion from this work is that ataxia-telangiectasia is not an ingravescent (increasing in severity) neurological disorder, as there was a decline with advancing age in the ten measured scale items for ataxia-telangiectasia. This decline stabilized by the mid-teens. This is clearly good news for patients, but it makes determination of the clinical impact of therapy difficult in older patients. Regarding therapy, H. Lederman (Baltimore, MD, USA) described a small-scale clinical trial in which patients were treated with a combination of α-lipoic acid (antioxidant) and the poly ADP-ribose polymerase 1 (PARP1) inhibitor, nicotinamide. The rationale for this was the evidence for oxidative stress in ataxia-telangiectasia and the presence of breaks in DNA that would be expected to activate PARP1 inappropriately. Progress is being monitored by the presence of 8-hydroxyguanine in the urine, lipid peroxidation, the absorbing capacity of serum for oxygen radicals and changes in muscle tone. This is an ongoing trial, but Lederman agreed that antioxidants such as Euk-189 and other nitroxides might be better candidates for therapy in the future because of their broader activity.

Relying on the observation that a certain level of ATM protein was detected in ataxia-telangiectasia patients with a milder phenotype, R. Gatti (Los Angeles, CA, USA) used several aminoglycosides that have the potential to read through premature termination codons in an attempt to restore some ATM function to ataxia-telangiectasia cells. Using an *in vitro* cDNA-coupled transcription/translation protein truncation test, he showed that several aminoglycosides induced read-through expression of ATM fragments in 13 ataxia-telangiectasia cell lines that carry mutations at premature termination codons in the *ATM* gene (Lai *et al*, 2004). He also showed that this read-through gave rise to a functional ATM protein in ataxia-telangiectasia cell lines as shown by radiation-induced autophosphorylation of ATM on Ser 1,981, correction of radiosensitivity and radioresistant DNA synthesis. Gatti and his colleagues are addressing toxicity problems by screening a library of 13,500 compounds and have already identified eight with read-through capacity, four of which are not related to aminoglycosides.

Modifier genes

Before the use of genetic markers, the number of loci predicting disease susceptibility was based on an analysis of phenotypic variation, largely in animal models. The introduction of single-nucleotide polymorphisms (SNPs) allowed for whole-genome scanning to identify SNPs associated with susceptibility to specific diseases. For in-bred animal strains, demonstrating an effect of genetic background on expression is sufficient to implicate modifier genes, which modulate the penetrance, dominance, pleiotropy or expressivity of genes that contribute to specific traits (Nadeau, 2003). However, for segregating human populations, direct evidence of linkage is required.

No data are available at the molecular level on the modification of ATM activity by other signalling proteins, apart from defective ATM activation when MRE11 and NBS1 are mutated. However, a couple of examples of an altered ataxia-telangiectasia phenotype were provided that point to the influence of modifier genes in this disease. Shiloh described two siblings with a mild clinical form of ataxia-telangiectasia even though they did not express *ATM* and had a severely defective DNA-damage response and marked cerebellar atrophy as in the classical forms of ataxia-telangiectasia. T. Dork (Hannover, Germany) described a series of ataxia-telangiectasia patients from the ages of 9 to 66 years old who also displayed milder clinical phenotypes. A patient with an *ATM* missense mutation (V2,716A), and an insertion (9,021 insA) leading to a premature termination, had progressive ataxia but was still gainfully employed at 66 years of age. A 9-year-old patient with V2,716A and 3,883 del4 mutations was initially diagnosed with ataxia-telangiectasia, but this diagnosis was subsequently reversed. This patient had mild dystaxia but no ataxia or telangiectasia. This variability in clinical symptoms suggests that there are polymorphisms in genetic loci other than the *ATM* gene that influence the onset and severity of neurodegeneration and other aspects of the ataxia-telangiectasia phenotype. B. Margus (Boca Raton, FL, USA) described an approach by his company, Perlegen Sciences (San Francisco, CA, USA), that uses high-throughput sample preparation methods and ultra-high-density oligonucleotide arrays to rapidly genotype millions of unique SNPs across the human genome, accurately and at moderate cost. He pointed out that collection of DNA samples for a study that would have sufficient statistical power required a network of clinicians around the world with access to ataxia-telangiectasia patients. The clinicians would need the expertise for assessment of patient clinical condition and the consent and co-operation of patients and their families. Another approach may be to generate crosses between *Atm* mutant mice and other mutants that might be expected to attenuate this condition.

Building a bridge between the laboratory and the clinic has always been a strength of ataxia-telangiectasia workshops, and this interaction has been enhanced by the active participation of patients and their families at these meetings. This meeting was no exception to that tradition, incorporating new insights from the clinic, approaches to therapy, facilitation of reagent collection, new patient accrual and a meeting involving ataxia-telangiectasia family associations.

Conclusions

We have now had a decade of ATM research, and it is evident that its activity permeates many aspects of cellular regulation in response to DSBs. How ATM is activated and how it regulates signalling pathways is under intense scrutiny to further explain the overall DNA-damage response, to determine the relationship of ataxia-telangiectasia with other syndromes that have overlapping features and to develop compounds that influence these pathways. It is hoped that these will ultimately have therapeutic benefit in the treatment of cancer and neurodegeneration.

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