Guiding ATM to Broken DNA

Robert T. Abraham and Randal S. Tibbetts

Science **308**, 510 (2005);
DOI: 10.1126/science.1112069

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

thermal flip rate at operating conditions.

Future generations of MRAM will use smaller tunnel junctions and will thus have to readdress the above challenges. Going toward smaller dimensions must not introduce more bit-to-bit variations or jeopardize data retention. The switching current will not substantially increase with reduced bit size (provided that other dimensions, such as the proximity to the write lines and their width, also decrease). But the current density will scale inversely with the conductor area, and electromigration may therefore become an issue. At that point, spin momentum transfer (*10*)—switching by a spin-polarized current through the bit—might become a viable alternative to 2D write selection.

This year, Cypress Semiconductor became the second company (after Freescale Semiconductor) to announce that it has shipped fully functional MRAM samples to potential customers. Many other companies have demonstrated multi-Mb MRAM prototypes. It is now only a matter of time before the first volume shipments of MRAM devices take place.

References

1. F. Masuoka, M. Asano, H. Iwahashi, T. Komuro, *IEEE IEDM Tech. Digest*, 464 (1984).
2. M. Johnson, Ed., *Magnetoelectronics* (Elsevier, Oxford, 2004).
3. L. Savtchenko, B. N. Engel, N. D. Rizzo, M. F. Deherrera, J. A. Janesky, U.S. Patent 6,545,906 B1, 8 April 2003.
4. B. N. Engel *et al.*, *IEEE Trans. Magn.* **41**, 132 (2005).
5. For the Freescale Semiconductor data sheet for MR2A16A, see www.freescale.com and enter Part Number MR2A16A.
6. For the Cypress Semiconductor datasheet for CY9C62256, see www.chipcatalog.com/Cypress/CY9C62256-70SC.htm.
7. S. S. P. Parkin *et al.*, *Nat. Mater.* **3**, 862 (2004).
8. S. Yuasa *et al.*, *Nat. Mater.* **3**, 868 (2004).
9. J. Åkerman *et al.*, *IEEE Trans. Dev. Mat. Rel.* **4**, 428 (2004).
10. J. A. Katine *et al.*, *Phys. Rev. Lett.* **84**, 3149 (2000).

10.1126/science.1110549

CELL BIOLOGY

Guiding ATM to Broken DNA

Robert T. Abraham and Randal S. Tibbetts

DNA damage poses a continuous threat to genomic integrity in mammalian cells. To cope with this problem, these cells have evolved an elaborate network of sensor, transducer, and effector proteins that coordinate cell-cycle progression with the repair of the initiating DNA lesion. A particularly lethal form of DNA damage is the DNA double-strand break (DSB). The cellular response to DSBs must be swift and decisive—requirements that are capably fulfilled by a serine-threonine kinase in the nucleus called ATM (ataxia-telangiectasia mutated). This nuclear protein serves as a key signal transducer in the DSB response pathway. ATM is a member of the phosphoinositide 3-kinase related kinase (PIKK) family, which includes several important proteins required for genome surveillance (*1*). Humans that lack functional ATM suffer from a devastating syndrome called ataxia telangiectasia (AT), characterized by cerebellar neurodegeneration, premature aging, immunodeficiency, extreme sensitivity to radiation, and heightened susceptibility to developing cancer. The severe pathologies associated with AT are attributable largely, if not entirely, to defective DSB recognition and repair. Exposure to ionizing radiation or other DSB-inducing agents triggers a prompt increase in ATM kinase activity, suggesting that ATM is a proximal transducer of DNA damage signals (*1*). On page 551 of this issue, Lee and Paull (*2*) offer new insights into the molecular mechanism that relays damage signals from DNA to ATM.

Enhanced online at www.sciencemag.org/cgi/content/full/308/5721/510

R. T. Abraham is in the Signal Transduction Program, The Burnham Institute, La Jolla, CA 92037, USA. E-mail: abraham@burnham.org R. S. Tibbetts is in the Department of Pharmacology, University of Wisconsin-Madison, Madison, WI 53706, USA. E-mail: rstibbetts@wisc.edu

Seminal studies 2 years ago by Bakkenist and Kastan (*3*) revealed that, in undamaged cells, ATM resides as a catalytically inactive dimer or higher order multimer. DNA damage induced by ionizing radiation triggers the auto- or trans-phosphorylation of the serine amino acid residue at position 1981 (Ser^{1981}) in the ATM polypeptide. This leads, in turn, to the dissociation of inactive ATM complexes into catalytically active ATM monomers. The authors made the striking observation that nearly the entire nuclear pool of ATM molecules was phosphorylated on Ser^{1981} within minutes of cellular exposure to low doses of ionizing radiation that induced only a few DSBs. To explain this highly efficient amplification mechanism, the authors proposed that even a single DSB causes a far broader alteration in chromatin structure that encompasses megabase regions of genomic DNA. This creates a suitable platform for the prompt activation of hundreds of ATM dimers after DSB induction. Consistent with this epigenetic model for ATM activation, the authors demonstrated that treatment of cells with chromatin-disrupting agents provoked widespread phosphorylation of ATM under conditions that did not produce detectable DSBs.

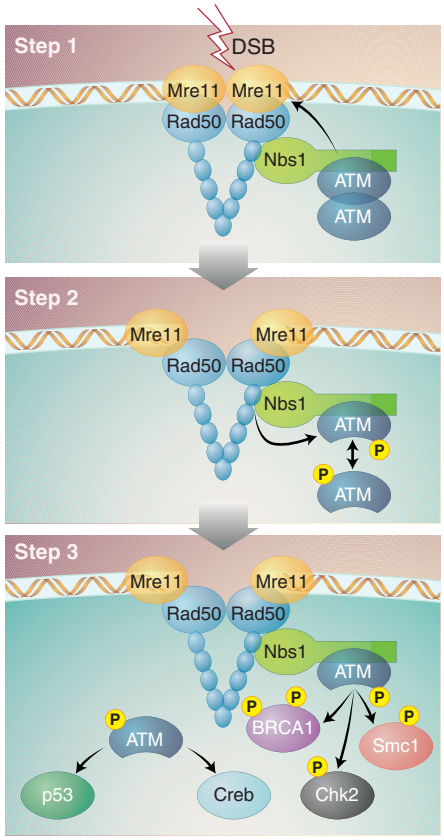
The new study by Lee and Paull (*2*) highlights the Mre11-Rad50-Nbs1 (MRN) complex as an essential mediator of ATM recruitment to and activation by DSBs. The MRN complex has a long history of association with the ATM-dependent checkpoint pathway (*4*). Hypomorphic mutations in the *NBS1* and *MRE11* genes give rise to Nijmegen breakage syndrome (NBS) and an AT-like disorder (ATLD), respectively. The clinical features of ATLD are indistinguishable from those of AT, whereas NBS patients (and cells from these patients) display a somewhat attenuated version of the AT phenotype. Mre11 is a DNA binding protein that possesses 3′,5′-exonuclease activity, as well as an endonuclease activity that cleaves DNA hairpins. Rad50 is a member of the structural maintenance of chromosomes (SMC) family. It forms homodimers that associate with two Mre11 molecules to yield tetrameric Mre11-Rad50 (MR) complexes (see the figure). The two arms of the MR complex allow this structure to form bridges between free DNA ends or between sister chromatids. The contribution of the Nbs1 subunit to the MRN complex is not well understood, although numerous studies show that Nbs1 expression is required for optimal phosphorylation of ATM substrates in cells damaged by ionizing radiation. Bakkenist and Kastan (*5*) recently argued that the partial ATM signaling defects observed in cells from NBS patients indicate that Nbs1 positively influences, but is not essential for, activation of ATM. However, as these authors point out, many NBS cells express a truncated form of Nbs1 that contains an intact carboxyl terminus. It turns out that this region of Nbs1 binds directly to ATM and is important for recruitment of ATM to sites of DNA damage (*6*). Thus, the hypomorphic *NBS1* allele expressed in NBS cells may mask an obligate role for Nbs1 in ATM activation. Earlier findings indicated that the Nbs1 partner protein, Mre11, is equally indispensable for ATM activation after DNA damage (*7*, *8*). Lee and Paull (*2*) now offer compelling biochemical evidence to support the conclusion that the MRN heterotrimer both recruits and activates ATM at DNA damage sites.

Lee and Paull (*9*) earlier demonstrated that the protein kinase activity of purified

human ATM was stimulated by recombinant MR or MRN complexes in the absence of DNA. In these biochemical assays, ATM activation was monitored by phosphorylation of an exogenously added substrate, such as p53. The authors speculated that the binding of MR or MRN to ATM enhanced access of substrate to the ATM kinase domain. However, the lack of a requirement for DNA in these assays hinted that the purification procedure may have favored the isolation of "preactivated" ATM monomers. In their latest study, the authors carefully isolated dimeric (or oligomeric) and monomeric populations of ATM molecules (*2*). With ATM dimers as the starting point, both DNA and the complete MRN complex were required for stimulation of ATM kinase activity. The MR subcomplex was responsible for recognition of the free ends of DNA duplexes, and for Rad50-mediated unwinding of the DNA ends to generate single-stranded DNA (ssDNA), an essential step leading to the recruitment and dissociation of ATM dimers. Interestingly, the ATM-related kinase, ATR, also recognizes ssDNA as a marker for DNA lesions that interfere with progression of the replication fork (*10*). Thus, the accumulation of ssDNA appears to provide a common signal for the recruitment of ATM and ATR to different types of DNA damage. The conservation of recruitment strategies for these two protein kinases apparently extends beyond the configuration of the DNA target. Jackson *et al*. (*6*) recently showed that an evolutionarily conserved motif at the carboxyl terminus of Nbs1 and ATR-interacting protein (ATRIP) mediates direct interactions with the respective partner kinases of these proteins, ATM and ATR. This interaction is required for efficient recruitment of the PIKKs to sites of DNA damage.

The most surprising outcome of the experiments by Lee and Paull (*2*) is that mutation of Ser^{1981} to an alanine (Ala) residue that cannot be phosphorylated has no effect on either the ATM dimer-to-monomer transition, or the stimulation of ATM kinase activity by MRN and DNA. This result is sure to engender considerable debate in the field, given that the Bakkenist and Kastan model (*3*) highlights Ser^{1981} phosphorylation as the pivotal event leading to ATM activation. The discrepant findings reported by Lee and Paull (*2*) may simply reflect the fact that their reconstitution assay offers only a partial view of the mechanism of ATM activation in intact cells. In the biochemical assay, the concentrations of purified DNA and MRN may be sufficiently high to drive the ATM dimer-monomer equilibrium toward the monomeric state, even in the absence of Ser^{1981} phosphorylation. In the intact cell, however, ATM phosphorylation may be critical to prevent the rapid reassociation of ATM monomers at DSB sites. A more trivial explanation is that the recombinant ATM (Ser^{1981}→Ala) "dimers" prepared by Lee and Paull are oligomeric complexes containing cell-derived ATM molecules bearing the Ser^{1981} phosphorylation site. Phosphorylation of one such site in an oligomeric complex may be sufficient to induce complex disassembly and monomer formation in the biochemical assay.

The new studies by Lee and Paull (*2*) focus attention on the MRN complex as both a sensor and effector of ATM activation and signaling in response to DSBs (see the figure). Complementary results from Jackson and co-workers (*6*) indicate that the Nbs1 subunit serves as a bridge between ATM and the DNA-bound MR heterodimer. Where do these new findings leave us with respect to the Bakkenist and Kastan model for ATM activation (*3*)? The results of Lee and Paull are consistent with the idea that the conversion of ATM dimers to monomers is a key event during ATM activation. However, the importance of Ser^{1981} phosphorylation for ATM activation is now open for debate and further experimentation. The generation of mice bearing Ser^{1981}→Ala mutations in both *ATM* alleles should provide definitive evidence for or against the original proposal (*3*) that Ser^{1981} phosphorylation serves as the trigger for ATM activation. Perhaps the most substantive challenge to the Bakkenist and Kastan model relates to the mechanism by which ATM senses DSBs. The original model posited that the DNA damage signal is transmitted to ATM through structural changes in chromatin. The new results indicate that the MRN complex forms a bridge between ATM and the DSB site, and delivers an undefined signal (perhaps a conformational alteration) that triggers ATM autophosphorylation and monomer formation. In their studies, Bakkenist and Kastan found that treatment of cells with chromatin-disrupting agents, such as chloroquine or histone deacetylase inhibitors (HDACs), stimulated ATM in the absence of detectable DNA damage (*3*). However, these drugs may well induce abnormal DNA structures, such as hairpins, that can be recognized and processed by MRN (*11*). It will be interesting to learn whether ATM activation induced by chloroquine or HDACs is also dependent on the MRN complex. Finally, a more detailed understanding of the structural basis of the interaction between ATM and MRN is sure to provide new insights into the mechanism of ATM activation. Considering the pace of research in this field, the ATM activation model may prove to be as dynamic as the process of DNA damage signaling itself.



The MRN complex and ATM activation. (Step 1) The induction of DSBs in DNA leads to prompt recruitment of MR or MRN complexes. These complexes form a bridge between free DNA ends via the coiled-coil arms of Rad50 dimers. Inactive ATM dimers are recruited to the DSBs through interaction with the carboxyl terminus of Nbs1, and possibly a less stable interaction with Rad50. **(Step 2)** The 3′,5′-exonuclease activity of MRE11 catalyzes resection of free DNA ends, creating ssDNA. Activating signals are delivered to ATM dimers, possibly through a conformational change in Nbs1. ATM undergoes phosphorylation at Ser^{1981} accompanied by its conversion from a dimer to a monomer. The MR complex may also trigger a conformational change in ATM that stimulates substrate recruitment. **(Step 3)** Activated ATM monomers either remain in the vicinity of the DSB, where they phosphorylate colocalized substrates, or diffuse away from the DSB sites to phosphorylate nuclear substrates, such as p53 and Creb.

CREDIT: PRESTON HUEY/*SCIENCE*

References

1. Y. Shiloh, *Nat. Rev. Cancer* **3**, 155 (2003).
2. J.-H. Lee, T. T. Paull, *Science* **308**, 551 (2005); published online 24 March 2005 (10.1126/science.1108297).
3. C. J. Bakkenist, M. B. Kastan, *Nature* **421**, 499 (2003).
4. J. H. Petrini, *Curr. Opin. Cell Biol.* **12**, 293 (2000).
5. C. J. Bakkenist, M. B. Kastan, *Cell* **118**, 9 (2004).
6. J. Falck, J. Coates, S. P. Jackson, *Nature* **434**, 605 (2005).
7. V. Costanzo, T. Paull, M. Gottesman, J. Gautier, *PLoS Biol.* **2**, E110 (2004).
8. C. T. Carson *et al.*, *EMBO J.* **22**, 6610 (2003).
9. J. H. Lee, T. T. Paull, *Science* **304**, 93 (2004).
10. L. Zou, S. J. Elledge, *Science* **300**, 1542 (2003).
11. D. D'Amours, S. P. Jackson, *Nat. Rev. Mol. Cell Biol.* **3**, 317 (2002).

10.1126/science.1112069