ATMachine

Cells are continuously exposed to DNA-damaging agents, such as ionizing radiation (IR). When such damage goes unnoticed, it rapidly manifests itself as heritable genetic alteration, ultimately leading to cell death or transformation. Given the significant environmental challenges met by cells, it is not surprising that all evolutionary strata have evolved elaborate means to detect such damage, and to react appropriately by allowing sufficient time to repair the damage or by cellular suicide when all is lost. Defective damage responses often result in cellular transformation.

The protein kinase Ataxia-telangiectasia mutated (ATM) is at the heart of the cellular response to double-stranded break DNA damage. *ATM* was first cloned as the gene responsible for a rare but severe human disease, ataxia telangiectasia (AT), characterized by progressive sensitivity to radiation, premature ageing and increased risk of cancers, as well as a host of other complications. It has since become clear that ATM is activated rapidly by IR and is critical for cell cycle checkpoint arrest after DNA damage. Multiple substrates involved in the checkpoint functions of ATM have been described, including several other proteins with well-established roles in cell cycle and cancer, such as BRCA1, p53 and Mdm2, Chk2, Nbs1, SMC1 and hRad17. The mechanisms of how DSBs are detected remain ill-defined, although ATM is thought to be involved at early stages on account of its rapid activation.

Now, in a study published recently in *Nature*, Christopher Bakkenist and Michael Kastan (DOI: 10.1038/nature01368) have made significant headway in our understanding of the ATM activation mechanism. They find that after exposure to IR, ATM is rapidly phosphorylated at a single residue, Ser 1981, in an amino-terminal domain conserved among phosphatidylinositol-3-OH kinases (PI(3)Ks). Low doses of IR that are only sufficient to allow generation of a few DSBs still resulted in stoichometric ATM phosphorylation. Phosphorylation depends on ATM kinase activity, occurs *in vitro* and is sensitive to ATM inhibitors, consistent with auto-phosphorylation. The involvement of other PI(3)Ks is excluded and the auto-phosphorylation is shown to occur *in trans*. Interestingly, substitution with alanine at position 1981 results in a failure to complement AT cells and to target substrates such as p53 *in vivo*. Indeed, the mutant exhibited dominant-negative activity; *in vitro*, however, the mutant retained kinase activity. These results are explained by the observation of inactivating multimerization of ATM involving the region centred around Ser 1981 and the kinase domain. Phosphorylation of this site results in disruption of oligomerization and hence activation of ATM kinase activity. In this model, ATM exists as a homodimer in unperturbed cells, sterically precluding access of substrates. DNA-damage-induced activation results in autophosphorylation, dissociation of the complex and hence accessibility of the kinase domain to substrates.

Figure 1 **Activation of ATM by IR. Primary fibroblasts were irradiated with 1 Gy for 5 min and immunofluorescence staining for phosphoSer 1981 (left) and H2AXγ (middle) was assessed. A merged image is also shown (right).**

It remains unclear exactly how a double-stranded break results in rapid phosphorylation of ATM with high penetrance. The authors hypothesize that this probably involves "action at a distance", through structural changes in the chromatin induced by the break. In support of this, chromatin-modifying drugs and conditions induce phosphorylation of ATM and p53 in the absence of histone H2AX phosphorylation and ATM focus formation, which are indicative of double-stranded breaks. Furthermore, phosphorylation of ATM was observed to occur diffusely across the nucleus and later to congregate into foci, presumably around break sites, to target substrates involved in break repair.

These data begin to uncover a mechanistic understanding of the earliest events of the DNA damage response. It is recommended that readers contemplate this elegant mechanism of ATM activation over a cup of the ATM *trans*-phosphorylation inhibitor caffeine.

BERND PULVERER

intracellular calcium transients within such functional units integrate to yield a flagellar motor response? Fourth, how does enhanced flagellar waveform asymmetry allow sperm to re-orientate towards the resact gradient? Do sperm exhibit the same responses when swimming through a gradual gradient, as compared with the responses seen here by applying acute resact doses in a rapid mixing paradigm? Finally, does sperm chemotaxis in other species also utilize early intracellular calcium transients to initiate primary responses? We know that the receptor guanylyl cyclase family in mammals controls blood volume and blood pressure in response to atrial-, brain- and type C-natriuretic peptides, and also form the major receptor for heat stable enterotoxin[13]. However, these gene products are not expressed in mammalian sperm. Therefore, is the chemotactic response in mammalian sperm then controlled by as yet unidentified receptors that operate through a similar downstream mechanism? In this regard, intracellular calcium levels similarly control the asymmetry of mammalian sperm flagellar beating[14]. Do mammalian sperm chemotactic factors also initiate primary motor responses by activating calcium entry channels? □

Harvey M. Florman, Jackson C. Kirkman-Brown and Keith A. Sutton are in the Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA
e-mail: harvey.florman@umassmed.edu

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