DNA REPAIR

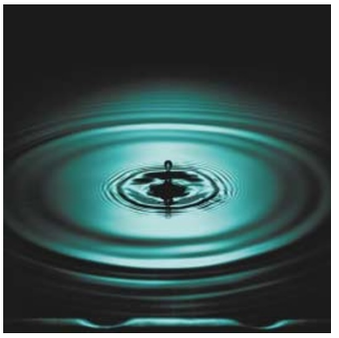
Small cause, large effect

The Mre11 complex is a multisubunit nuclease that contains three components — Mre11, Rad50 and Nbs1 (known as Xrs2 in yeast). It acts as a sensor of double-stranded DNA breaks, but also has roles in checkpoint signalling and DNA replication. Research into the individual components has been hampered by the fact that null mutants are not viable, but John Petrini and colleagues now present their studies of a functionally impaired *Rad50* mutant. And the results underline the importance of the Mre11 complex in the homeostasis of proliferative tissues.

Reporting in *Genes and Development*, Petrini and co-workers describe the generation of a group of hypomorphic *Rad50* mutants, one of which — $Rad50^{K22M}$ — was used to derive so-called $Rad50^{S/S}$ mutant mice. $Rad50^{S/S}$ mouse-embryo fibroblasts contained wild-type levels of Mre11, Nbs1 and $Rad50^{K22M}$, and these components could assemble functional complexes. However, the $Rad50^{S/S}$ mice showed partial embryonic lethality, and those that survived were only 60% of the weight of their wild-type littermates. By 4–8 weeks of age, the mice showed signs of anaemia, and most of them died by 4 months. Of those that survived up to 7 months, 20% died from a variety of tumours.

As the premature death was associated with severe anaemia, the authors first looked more closely at haematopoietic cells in the $Rad50^{S/S}$ mice. At 2 weeks there was no difference between wild-type and $Rad50^{S/S}$ mice, but by 4 weeks of age, lymphocytes, macrophages, red blood cells and platelets were severely depleted in the mutant mice. Further experiments indicated that the progressive depletion was due to the failure of haematopoietic stem cells. Moreover, a similar depletion of cells was observed in the spermatogenic lineages of $Rad50^{S/S}$ mice.

Given the age-dependent cellular depletion in the bone marrow and



testes, and the fact that those mice that survived longest developed tumours, Petrini and co-workers wondered whether the $Rad50^{K22M}$ mutation might cause genotoxic stress. If this were the case, mutation of p53 — which is involved in the response to genotoxic stress — could be expected to lessen the severity of the observed defects. And this is just what the authors saw. For example, macrophages were increased three-fold and T cells 3–20-fold in $p53^{-/-}$ $Rad50^{S/S}$ mice compared with $Rad50^{S/S}$ mice. Moreover, $p53^{-/-}$ $Rad50^{S/S}$ mice developed tumours and died much earlier than $p53^{-/-}$ mice alone, again supporting the idea that the $Rad50^{K22M}$ mutation causes genotoxic stress.

A final confirmation came when the authors analysed various indices of genotoxic stress. Levels of phosphorylated histone H2AX — which correlate with levels of DNA damage — were the same in unirradiated $Rad50^{S/S}$ cells as in irradiated wild-type cells. And karyotype analyses of $Rad50^{S/S}$ thymic lymphoma cells revealed increased chromosome breaks and rearrangements (including telomeric short-arm fusions) compared with wild-type cells. So, as the authors conclude, "the data clearly indicate that the Mre11 complex exerts a profound influence on homeostasis in mammalian tissues, even when its checkpoint and DNA recombination functions are not overtly impaired".

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References and links

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STRUCTURE WATCH

A powerful weak handshake

Integrins are heterodimeric (α, β) cell-surface receptors that mediate interactions between cells, and between cells and the extracellular matrix. The α and β subunits both have a small cytoplasmic tail, a transmembrane domain and a large extracellular domain, and the extracellular domains form sites that bind numerous ligands. Activation of the ligand-binding function, however, only occurs on receipt of an 'inside–out' signal from the cytoplasmic tails — a process that has remained poorly understood. Now, though, two papers in *Cell* provide new insights.

In the first paper, Qin and colleagues present the NMR structure of the cytoplasmic face of the integrin $\alpha_{IIb}\beta_3$, which shows a "weak handshake" between the membrane-proximal helices of the α_{IIb} and β_3 cytoplasmic tails. The residues that mediate this interaction are highly conserved in all integrins, which indicates that this interaction and its functions are probably conserved. When the authors studied cytoplasmic-tail point mutations that are known to produce a constitutively active form of $\alpha_{IIb}\beta_3$, they found that they disrupted the tail interface. In addition, they showed that talin — a cytoskeletal protein that activates $\alpha_{IIb}\beta_3$ *in vivo* by binding to the β_3 tail — seems to compete with the α_{IIb} tail for binding to β_3, which disrupts the tail interface. These results have provided "a structural mechanism by which a handshake between the α and the β cytoplasmic tails restrains the integrin in a resting state and unclasping of this interaction triggers the inside-out conformational signal that leads to receptor activation".

The big picture

In the other *Cell* paper, Springer and colleagues used techniques including electron microscopy to study the integrin-structure rearrangements that are involved in 'outside–in' and 'inside–out' signalling and that control the affinity of integrins for their ligands.

The authors studied the extracellular region of $\alpha_V\beta_3$ in two forms — 'clasped' (α and β subunits were linked through a carboxy-terminal 'clasp' to mimic the interaction of the α/β cytoplasmic tails (see above)) and 'unclasped'. They saw that integrins have, at the least, three conformational states — bent (V-shaped), extended with a closed headpiece and extended with an open headpiece. They showed that the bent conformation, which was previously observed in a crystal structure, is physiologically relevant, and has a low ligand-binding affinity. The clasped form of $\alpha_V\beta_3$ favours this bent conformation, which fits with the observation that α/β cytoplasmic-tail interactions restrain integrins in an inactive form (described above). On addition of either a high-affinity ligand-mimetic peptide or Mn^{2+} (which activates all integrins by binding to the extracellular region) to $\alpha_V\beta_3$, Springer and co-workers observed a "switchblade-like opening" to the high-affinity extended conformer with the open headpiece. Unclasped $\alpha_V\beta_3$ favours this conformation, which again concurs with the results described above. They propose that the extended conformer with the closed headpiece represents an intermediate conformation with an intermediate ligand-binding affinity.

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