But more surprising still was the identification of PLCγ as a phosphorylation target for activated c-Abl *in vivo*. c-Abl complexes more tightly with PLCγ when it is active and through phosphorylation can inhibit PLCγ function, so forming an activation feedback loop.

Although this activation mechanism will not be universal, it is the first link between c-Abl and phosphoinositide signalling, and has uncovered one new way in which to control activation of this tyrosine kinase. The work also shows how chemotaxis of cells towards a PDGF source requires active c-Abl. As c-Abl and PLCγ regulate the activity of one another, this work does not simplify the known roles of c-Abl, but further complicates the understanding of this kinase. Nothing in life ever seems easy, and c-Abl seems to need more than one mechanism to ensure it is ready for activation.

Sarah Greaves, Senior Editor,
Nature Cell Biology

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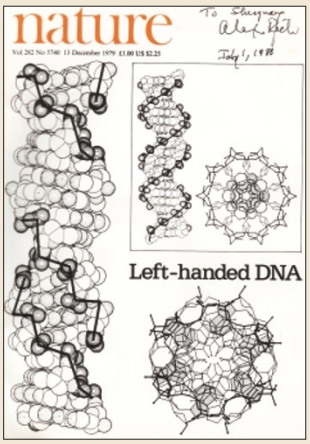

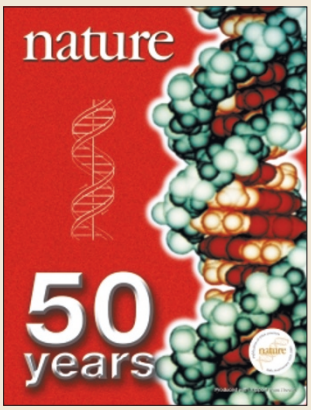

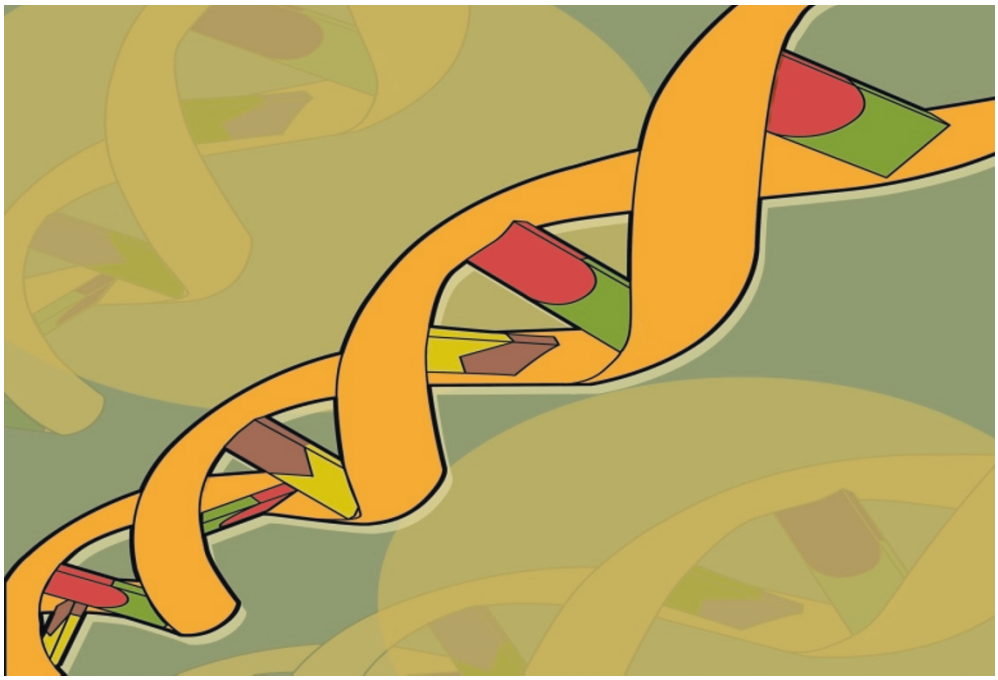

DNA RECOMBINATION

A pushy protein

In vitro **biochemistry can tell us much, but the situation *in vivo* is often more complicated. Take homologous recombination, for example, which has been extensively studied using purified proteins and oligonucleotides. The Rad51 protein can catalyse pairing of homologous sequences and strand exchange *in vitro*, but what happens in chromosomes, where the DNA is wrapped around nucleosomes?**

Stephen Kowalczykowski and colleagues have addressed this question with their study of *Saccharomyces cerevisiae* Rad54, published in *Nature Structural Biology*. The Rad54 protein belongs to the SWI2/SNF2 group of ATP-dependent chromatin-remodelling factors. These complexes allow DNA-binding factors access to the DNA by moving the nucleosomes out of the way.

Rad54 has been shown to interact with Rad51 *in vitro*, where it stimulates Rad51-mediated strand exchange. This stimulatory effect could be explained if the function of Rad54 were to move nucleosomes out of Rad51's path, so Kowalczykowski and colleagues first asked whether Rad54 can indeed redistribute nucleosomes on DNA. To study this, they reconstituted nucleosomes on short fragments of DNA, generating a mixture of nucleosomes at different places along the DNA. They then isolated three nucleosome species (N1, N2 and N3), which could be identified based on electrophoretic mobility — where the nucleosomes were closer to the centre of the DNA fragment (N3), the species migrated more slowly than if the nucleosomes were positioned nearer to the DNA ends (N1).

Kowalczykowski and colleagues incubated the isolated nucleosomes with Rad54/ATP and, in each case, the nucleosomes became redistributed. The nucleosomes in N1 were moved to a more central position, whereas in N2 and N3 they were located closer to the DNA ends. Some free DNA was also generated, suggesting that some of the nucleosomes had been moved off the DNA fragments. The authors favour the idea that the nucleosomes were moved by sliding along — rather than by dissociating from, and then reassociating with — the DNA, as the amount of free DNA generated was greatest with the N1 species, where the nucleosomes had less far to travel to fall off the end.

The authors next wondered where this chromatin-remodelling activity might fit in to the process of Rad51-mediated recombination. Rad51 forms a helical nucleoprotein filament on single-stranded (ss)DNA, which has previously been shown to stimulate Rad54's other activities (ATPase and DNA-unwinding). So the authors examined the effects of incubating N3 and Rad54 with increasing amounts of Rad51/ssDNA. They found that the Rad51 complex enhanced the nucleosome-remodelling activity of Rad54 in a concentration-dependent manner. The optimal stoichiometry was one Rad54 monomer:one Rad51 monomer, suggesting that Rad54 might co-assemble with the Rad51 nucleoprotein filament at an early stage of recombination — before the DNA-pairing and strand-exchange steps.

The authors propose, then, that Rad54's job *in vivo* could be to remodel chromatin and clear the DNA of nucleosomes while the recombinational repair machinery searches for homologous sequences. Interestingly, Rad54's close association with Rad51 could also indicate a role for it after strand exchange, when it might clear Rad51 from the DNA to complete the repair process.

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