SIGNALLING

Ready and Abl

As most tyrosine kinases need to be activated to elicit a downstream response, understanding the mechanism of activation is of fundamental importance. Surprisingly, the activation of the oncogenic tyrosine kinase, c-Abl, has remained undiscovered for the past 20 years. In this month's issue of *Nature Cell Biology*, Ann Marie Pendergast and colleagues unravel some of this elusive activation mechanism.

c-Abl is localized to many cellular compartments and seems to be regulated through numerous external stimuli. Whilst the kinase has effects on diverse processes such as cell proliferation, cell death, migration, the cytoskeleton and gene expression, the work by Pendergast and colleagues indicates how the protein is activated during platelet-derived growth factor (PDGF) stimulation, including chemotaxis.

Cell lines expressing mutant forms of the PDGFR (PDGF receptor) lack c-Abl activation in response to PDGF. This activation could be restored after transfection of a receptor subunit that can bind the downstream effector phospholipase C (PLC)γ. Inhibitors of PLCγ prevent an increase in c-Abl activity. Chemotaxis towards a source of PDGF is known to increase PLCγ activity, whilst PLCγ overexpression can potentiate cell movement towards a PDGF source. This effect on chemotaxis is enhanced in cells expressing activated c-Abl and inhibited by kinase-inactive c-Abl.

Following activation by PDGF, PLCγ hydrolyses phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). The authors found that depleting PtdIns(4,5)P_2 also increased c-Abl activity. PtdIns(4,5)P_2 seems to bind to the SH2-SH1 (Src-homology) domains in c-Abl, which presents a direct means for inhibiting enzyme activity. How this inhibition of c-Abl is regulated is not yet known, but the authors propose that, through PDGF signalling, PtdIns(4,5)P_2 binding would be abrogated, allowing c-Abl to be activated.

Other results did surprise the authors, especially the identification of a cellular complex of PLCγ and c-Abl.

50TH ANNIVERSARY OF DNA

More Cinderella than ugly sister

Watson and Crick changed biology forever when they described the right-handed double helical structure of DNA in 1953. Below, Shuguang Zhang gives a personal view on the less well-known story of the equally beautiful and functional left-handed DNA.

When I was an undergraduate in China, in 1979, I asked my biochemistry professor why all biological helices seemed to be right-handed, and whether there might be left-handed ones? My professor did not know. Shortly after, my question was answered when Alexander Rich and colleagues reported the discovery of left-handed DNA.

Left-handed DNA consists of two anti-parallel chains, with bases that still form Watson–Crick base pairs. It was named Z-DNA as a result of its zigzag phosphodiester backbone. Before this unexpected discovery, DNA was viewed as structurally static. This finding made it obvious that the molecule is a dynamic entity: its structure depends on its environment.

The new discovery provoked a worldwide race to study Z-DNA. One key finding was that biologically negative supercoiling stabilized Z-DNA. This clearly indicated that Z-DNA could have a functional role.

To investigate this potential role, Rich's lab used antibodies to Z-DNA to probe nuclear activities. They found that the anti-Z-DNA antibodies localized in transcriptionally active macronuclei in ciliates, and in transcriptionally active polytene chromosomes in *Drosophila*.

Further studies by Rich's group, and others, were consistent with this finding, confirming that Z-DNA was involved in regulating some genes as well as chromatin remodelling. Studying unstable Z-DNA in cells is a technically daunting and unfashionable pursuit that has discouraged many. Undeterred, Rich and co-workers have pressed on alone, accumulating an impressive body of evidence that shows that Z-DNA is not only biologically relevant but is also important.

The latest exciting findings might indicate a link between the structure of Z-DNA and viral pathogenesis. In a series of experiments, Rich and colleagues show that the Z-DNA-binding domain found in vaccinia viruses is required for them to be pathogenic. These results raise the intriguing possibility that smallpox could be treated by blocking Z-DNA binding in variola — the virus that causes it — which has a nearly identical binding domain to vaccinia.

Alexander Rich has a passion for Z-DNA and relentlessly pursues its biological function. His early passions led him to numerous discoveries, including the molecular structure of collagen with Francis Crick in 1955, DNA–RNA hybridization and the mechanism of protein synthesis on polyribosomes. I anticipate that Rich and colleagues will not only elucidate the biological function of Z-DNA, but will also inspire many more discoveries in the coming years.

Shuguang Zhang, Massachusetts Institute of Technology Laboratory of Molecular Self Assembly

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WEB SITE

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http://web.mit.edu/lms/www/index.shtml

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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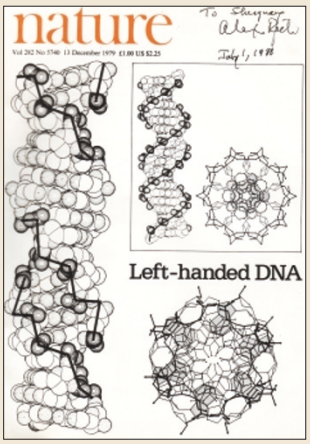

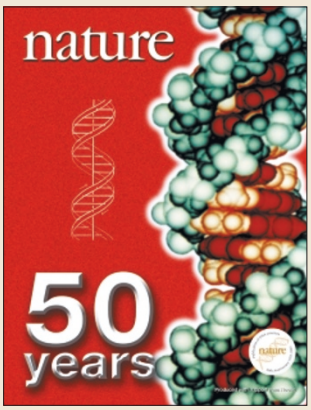

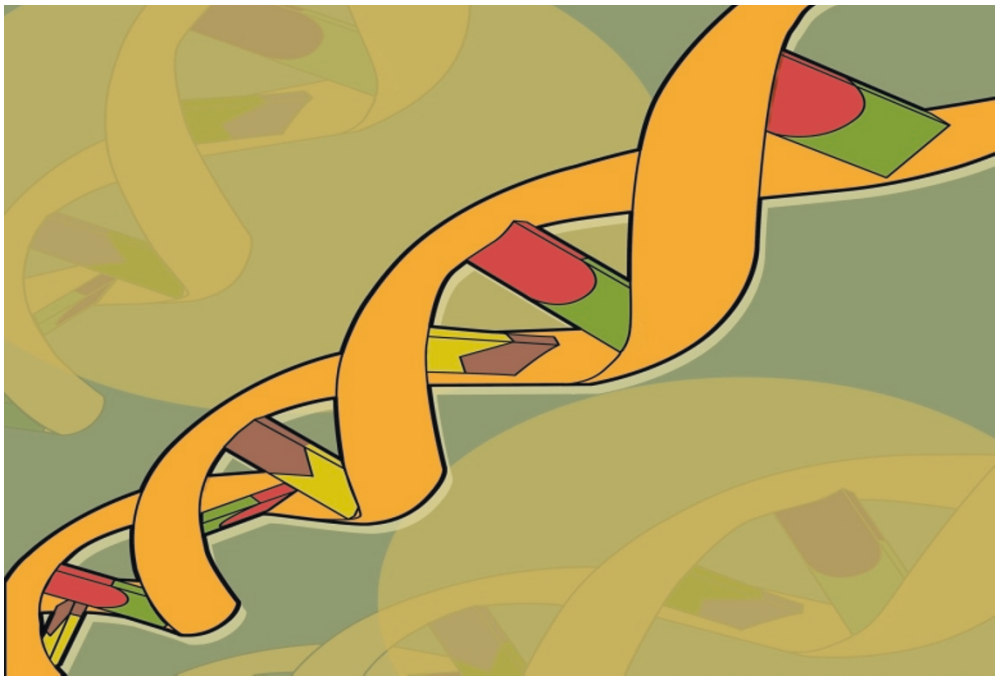

DNA RECOMBINATION

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.

Alison Mitchell

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