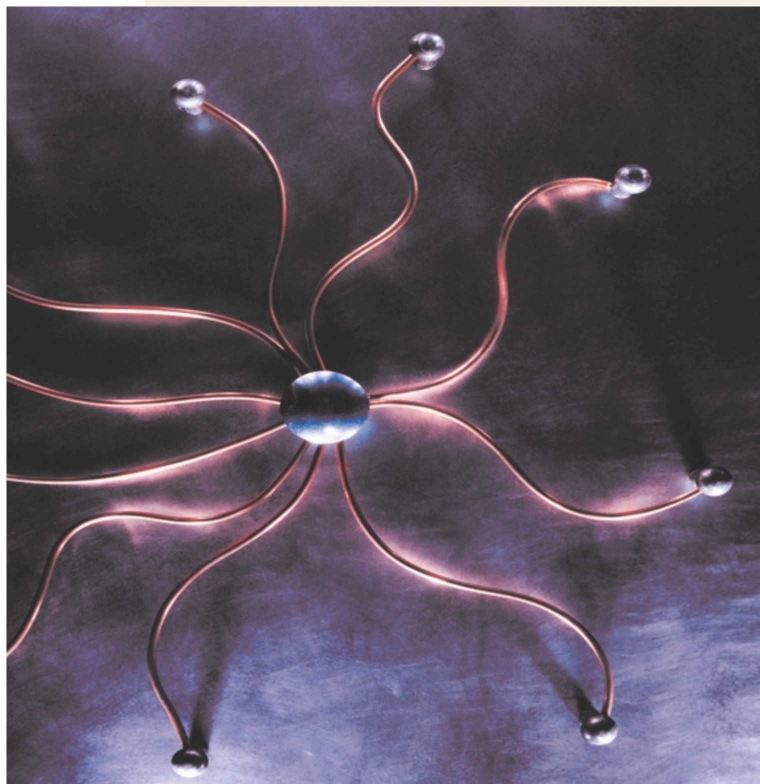
CYTOSKELETON

Meandering microtubules



One phenomenon that intrigues neuronal cell biologists is how immature neurites develop into an axon and mature dendrites. In 2001, Kaibuchi and colleagues showed that collapsin response-mediator protein-2 (CRMP-2) is required for axonal growth and axon–dendrite fate, and the group now reports that CRMP-2 regulates axonal growth and branching by binding to tubulin heterodimers and promoting microtubule assembly.

They began by searching for CRMP-2-binding proteins, and identified α- and β-tubulin from developing rat brain extracts. Under conditions that favoured tubulin-dimer formation, purified tubulin and purified CRMP-2 interacted directly. Further analysis showed that the CRMP-2–tubulin complex comprises one CRMP-2 molecule and one tubulin α/β-heterodimer.

To find out what influence this might have on microtubule dynamics, Kaibuchi and co-workers incubated purified tubulin with wild-type or deletion mutants of CRMP-2. Wild-type CRMP-2 and the carboxy-terminal half (residues 323–572) efficiently assembled microtubules, but the amino-terminal half (1–322) did not. Subsequent studies found that residues 323–381 effectively mediated microtubule assembly. The wild-type construct even increased the growth rate of plus ends of pre-formed microtubules. Surprisingly, though, CRMP-2 bound to pre-formed microtubules about ten times less efficiently than to tubulin heterodimers. The authors then found that CRMP-2 seems to co-polymerize with tubulin dimers into microtubules.

So what happens in a cellular context? In transfected fibroblasts, CRMP-2 associates with cellular microtubules. When it is overexpressed in a neuronal cell line, CRMP-2 induces the cells to differentiate and extend neurites. Deletion of residues 323–381 (CRMP-2-Δ323–381), however, inhibited the neurite-inducing activity. So although this 59-amino-acid fragment could induce microtubule assembly, it couldn't induce neurite formation. This indicates that the microtubule-assembly activity of CRMP-2 is required, but is not sufficient, for neurite outgrowth. Similarly, expression of residues 323–381 had no effect on the

ANTIBODY RESPONSES

Deamination unlocks diversity

It looks like an RNA editor, but it seems that the B-cell-specific activation-induced cytidine deaminase (AID) might, in fact, work directly on DNA. A new study published in *Nature* shows that AID can deaminate deoxycytidine (dC) — an event that could precipitate antibody gene diversification according to the authors.

AID is essential for all three types of antibody gene diversification — somatic hypermutation (SHM), in which single base-pair changes are introduced into the antigen-binding variable (*V*) regions; gene conversion, in which sequence changes are copied from upstream *V* pseudogenes; and class-switch recombination (CSR), in which recombination between 'switch' sequences leads to a change of antibody isotype. But, the function of AID is unknown and its physiological role in these different processes has been the subject of much speculation.

AID is homologous to APOBEC1, a cytidine deaminase that specifically edits APOB messenger RNA. Therefore, it has been suggested that AID might similarly edit the mRNA of an unidentified mutator enzyme. However, Neuberger and colleagues suggest that SHM, gene conversion and CSR could all be initiated by the direct action of AID on DNA to deaminate dC, resulting in a U–G mismatch lesion.

The authors envisage 4–5 ways in which such a lesion could be resolved, assuming that it is not corrected fully by base-excision repair. First, the mismatch is not repaired at all, and DNA replication leads to C→T (and G→A) transitions. Second, base-excision repair is initiated and the uracil is excised; replication over this abasic site will lead to the C (and G) being replaced by any of the other three bases (thereby allowing transversions at C and G). Third, the U–G lesion undergoes mismatch repair, possibly involving error-prone polymerases, which corresponds to the second phase of SHM. Fourth, the lesion undergoes template-mediated repair on an upstream *V* pseudogene, which results in gene conversion. Finally, if the lesion occurs in a switch site, repair that involves another switch region would lead to CSR.

But, can AID act directly on DNA? To test this, human *AID* was expressed in *Escherichia coli*, and the frequency of acquisition of rifamycin resistance — which normally occurs at low levels — was used as a measure of the frequency of mutation. The bacteria that were transformed with *AID* mutated at an increased frequency, and sequencing revealed that there was a strong bias towards C→T and G→A mutations, which is consistent with the deamination of dC by AID. If this is the case, then AID-transformed bacteria that are deficient for uracil-DNA glycosylase, which is involved in the repair of such mutations, should have an increased mutation rate. This was, indeed, found to be the case.

So, this study strongly supports a DNA-deamination mechanism of antibody diversification. Just how AID specifically targets antibody genes remains an important question for future study.

Jennifer Bell, Associate Editor,
Nature Reviews Immunology

References and links

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FURTHER READING Kinoshita, K. & Honjo, T. Linking class-switch recombination with somatic hypermutation. *Nature Rev. Mol. Cell. Biol.* **2**, 493–503 (2001)

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