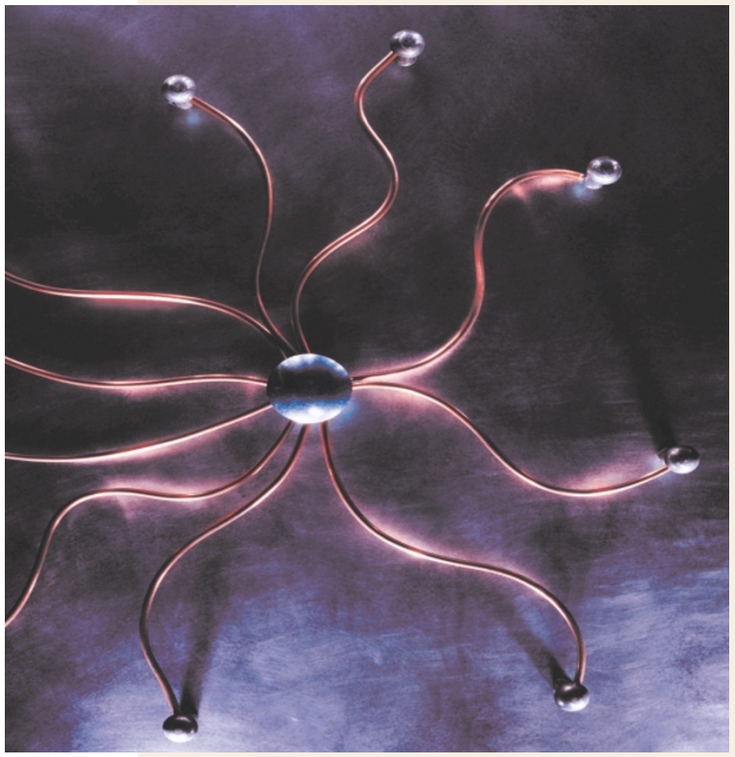
CYTOSKELETON

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

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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)

axonal growth of hippocampal neurons, whereas CRMP-2-Δ323–381 inhibited endogenous microtubule assembly. In addition to inducing axonal growth, CRMP-2 is also required for axonal branching.

The authors propose that CRMP-2 promotes microtubule assembly by binding and delivering tubulin heterodimers, with which it co-polymerizes, to the growing (plus) ends of microtubules. So, microtubule assembly might then affect microtubule-polymer length during axonal growth. But although CRMP-2 is a strong candidate for promoting microtubule assembly, this function alone cannot explain the role(s) of CRMP-2 in axonal differentiation and growth, as the 59-residue microtubule-assembly-promoting fragment couldn't mediate axonal growth on its own. It remains to be seen, then, what CRMP-2's other talents are.

Katrin Bussell

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ORIGINAL RESEARCH PAPER Fukata, Y. *et al.* CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nature Cell Biol.* **4**, 583–591 (2002)

APOPTOSIS

Clean debris

Apoptotic cells are tidy — they organize their funeral while they are dying, so cellular remnants are promptly removed without any spillage of intracellular content or collateral damage. By contrast, necrotic cells become leaky, and this causes damage to neighbouring cells and inflammation. When apoptotic cells escape clearance, however, they undergo secondary necrosis. A report in *Nature* now describes a fail-safe mechanism in apoptotic cells that avoids inflammation after secondary necrosis, in which apoptotic cells change the structure of their chromatin, which allows it to trap a pro-inflammatory protein called high mobility group protein-1 (HMGB1).

HMGB1 functions as an architectural DNA-binding factor that is involved in nucleoprotein complex assembly. It also acts as a mediator of inflammation when it is secreted by activated monocytes and macrophages. Marco Bianchi and colleagues now show that these two functions are linked.

The authors first observed that HMGB1 leaks out of necrotic, but not apoptotic, cells. HMGB1 was indeed found to be tightly associated with the chromatin of apoptotic cells, even when their membrane is permeabilized artificially with detergents. As nucleosomal fragmentation is one of the hallmarks of apoptosis, the authors next tested whether DNA fragmentation is responsible for the affinity of HMGB1 for chromatin in apoptotic cells. But inhibition of DNA fragmentation did not block HMGB1 binding. Acetylation also affects chromatin structure, so they tested whether treatment with a deacetylase inhibitor before the induction of apoptosis would suppress HMGB1 binding to chromatin — and it did. This indicates that chromatin hypoacetylation occurs during apoptosis, and allows HMGB1 binding. Consistent with this histone H4 was found to be hypoacetylated in apoptotic cells.

What does HMGB1 binding to chromatin do in apoptosis? *Hmgb1*-deficient cells are as sensitive as wild-type cells to apoptosis, which indicates that HMGB1 is not required for apoptosis. So, could HMGB1 binding to chromatin avoid its leakage and the subsequent inflammation? To test this, Bianchi and colleagues incubated bone-marrow cells with wild-type or *Hmgb1*-deficient dead fibroblasts. The *Hmgb1*-deficient necrotic cells were much less efficient than wild-type necrotic cells at eliciting the production of tumour-necrosis factor-α (TNF-α) by monocytes. Remnants from cells that had been induced to undergo secondary necrosis after prolonged exposure to an apoptotic stimulus also failed to induce any inflammation. However, remnants from fibroblasts that were treated with both TNF-α and a deacetylase inhibitor triggered inflammation as potently as wild-type necrotic cells.

The role of HMGB1 in inflammation after tissue necrosis was confirmed *in vivo* — mice that were exposed to an overdose of paracetamol undergo massive liver necrosis and subsequent inflammation. And, although anti-HMGB1 antibodies did not block liver damage, they reduced inflammation considerably. So, by changing the structure of their chromatin and tethering the pro-inflammatory protein HMGB1, apoptotic cells altruistically die in silence and spare their neighbours.

Valerie Ferrier, Associate Editor,
Nature Cell Biology

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WEB SITE

Marco Bianchi's laboratory:
http://w3dibit.hsr.it/PhD/people/bianchi.html

IN THE NEWS

Accentuate the negative

At last! Here's a newspaper article that understands the true plight of researchers. "The sad truth about science is that most experiments fail and the hypotheses that seduced researchers turn out not to be true or, at least, the studies provide no evidence that they are true", revealed a story in *The New York Times* (7 July 2002).

Bjorn Olsen at Harvard Medical School is one of many scientists who want to see these negative studies published, as they can still add to our knowledge and they would help prevent other researchers from going down the same, unsuccessful route.

Olsen is setting up an online journal, *The Journal of Negative Results in Biomedicine*, which is expected to publish its first papers this summer. He hopes that this will overcome the difficulty of publishing studies that fail to prove hypotheses in some existing journals.

But other researchers say the attitude of journals is not the only reason for not publishing negative data. "Those [scientists] conducting the studies do not want to share them [negative data] ", Scott Kern told *The New York Times*.

Kern started NOGO (*Negative Observations in Genetic Oncology*), which focuses on negative studies of gene mutations that might predispose to cancer. This, said Kern, received great support, but few submissions. Kern suspects this is because scientists are unwilling to share this information to their peers. "They now know something they're not going to do again and their competitor does not", he said.

Simon Frantz