HIGHLIGHTS

CHROMATIN

A fork load

In a recent study, Peter Adams and colleagues showed that repression of histone synthesis causes S-phase arrest. Adams' group now reports, in *Molecular Cell*, that the assembly of nucleosomes onto newly synthesized DNA near the replication fork — rather than histone synthesis *per se* — is tightly coupled to DNA synthesis in human cells.

Adams and co-workers showed that a dominant-negative mutant of the p150CAF-I subunit of the heterotrimeric chromatin-assembly factor CAF-I inhibits CAF-I-dependent chromatin assembly in an *in vitro* assay. The mutant protein (called HA-p150C) disrupts the endogenous CAF-I complex *in vivo* by preventing the binding of wild-type p150CAF-I to the p60CAF-I subunit. As a result, p60CAF-I can no longer associate with chromatin at replication foci.

To investigate what happens at the site of DNA synthesis, the replication fork, Adams and co-workers transfected cells with a plasmid encoding HA-p150C, and found that DNA synthesis and progression through S phase were blocked. Co-expression of the wild-type p150CAF-I subunit abolished cell-cycle arrest, confirming that perturbation of CAF-I inhibits DNA synthesis. As expected, the cells that were arrested in S phase had an abnormal chromatin structure that was hypersensitive to micrococcal nuclease digestion (that is, the DNA is more accessible to the nuclease, because the chromatin structure is perturbed).

S-phase arrest can be caused by DNA damage. So was this the case for defective chromatin assembly-induced arrest? As it turned out, yes — Adams and colleagues found that HA-p150C-expressing cells contained fragmented DNA, and that histone H2AX was phosphorylated, presumably, in response to double-strand breaks.

What causes the inhibition of DNA synthesis and S-phase arrest? The S-phase checkpoint protects the cell's genome integrity during S phase, and is activated in response to DNA damage. So, Adams and colleagues reckoned that inhibition of chromatin assembly might activate the S-phase checkpoint, which is indeed what happens.

H2AX is a substrate for ATR and/or ATM, both S-phase checkpoint kinases, and the authors showed that ATR nuclear foci, the sites of active ATR, are present preferentially in HA-p150C-expressing cells arrested in S phase. Other substrates of ATR and ATM — p53 and BRCA1 — were also activated. Phosphorylation of p53 was induced, and dispersed BRCA1 foci, which are typical of activated BRCA1, were enriched in HA-p150C-expressing cells that contained ATR foci.

The authors propose that "…defects in S-phase chromatin assembly cause double-strand breaks due to stalling and inappropriate processing of replication forks". This indicates that defects in chromatin assembly, combined with inactivation of the S-phase checkpoint, might promote genome instability, and Adams and colleagues are now testing whether genes encoding chromatin-assembly factors might be mutated in human cancers.

Arianne Heinrichs

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VIRAL TRANSMISSION

Deadly contact

Transmission of the human T-cell lymphotropic virus type I (HTLV-I) seems to require cell–cell contact — the contribution to infection by cell-free HTLV-I virions is minimal. In their report in *Science*, Igakura *et al.* now show that HTLV-I is transmitted across cell–cell junctions after polarizing the cytoskeleton of the infected cell at sites of cell–cell contact.

The authors first looked at the distribution of the viral (Gag) core proteins and the glycoprotein envelope (Env) protein in isolated infected T cells, and in uninfected cells that had conjugated with HTLV-I-infected cells. After 40 minutes, they saw a strong polarization of both proteins from around the cell periphery in infected cells to the area of cell–cell contact in conjugates — a significant finding because the nucleocapsid p15 Gag protein is known to incorporate the retroviral genome into virions. In addition, another Gag protein, p19, was detected in the 'uninfected' cells of the conjugates, which might represent the initial establishment of HTLV-I infection.

Following on from the detection of p15 at the cell–cell contacts, Igakura *et al.* studied the localization of the HTLV-I genome. The HTLV-I nucleic acid was not polarized in single infected T cells, but it accumulated at cell–cell junctions of infected–uninfected conjugated cell pairs, similar to what was seen for the Gag and Env proteins. As was also seen for the Gag p19 protein, viral nucleic acid was later transferred to the 'uninfected' cell.

What is the cause of this asymmetrical localization? The authors noticed that polarized Gag proteins at the cell–cell junctions were frequently closely juxtaposed to a reorientated microtubule-organizing centre (MTOC). As nocodazole, which depolymerizes microtubules, inhibited the cell–cell accumulation and subsequent cell transfer of Gag, this implicates microtubule dynamics in the polarization of Gag. In addition, Igakura *et al.* showed that MTOC

PLANT DEVELOPMENT

Channelling elongation

Everybody knows that to grow, plants need minerals and water from the soil, which they obtain through roots and root hairs. The formation of these structures requires cell expansion — by way of elongation — which, in turn, needs calcium (Ca^{2+}) acquisition. But, until now, what regulated the Ca^{2+} influx wasn't so obvious. Research led by Liam Dolan's group, though, has pinpointed the production of reactive oxygen species (ROS) by an NAPDH oxidase in the activation of Ca^{2+} channels in elongating root cells.

Because *Arabidopsis thaliana rhd2* mutants develop very short root hairs and stunted roots, and are defective in Ca^{2+} uptake, the authors decided to clone the gene encoding RHD2. They found that the gene — *At5g51060* — had previously been defined as *Arabidopsis thaliana* respiratory burst oxidase homologue C (*AtrbohC*). Rather unsurprisingly, as implied by the name, the *At*rbohC protein and other *At*rbohs are homologous to the $gp91^{phox}$ subunit of the mammalian NAPDH oxidase that catalyses ROS production.

What, then, is the connection between RHD2/*At*rbohC and growth? ROS production was reduced by ~50% in root apices from *rhd2* mutants compared with wild-type apices. Normally, ROS are present as the root hair emerges as a bulge and further increase as the elongation rate goes up. Adding an inhibitor of NADPH oxidase to the apices of wild-type plants prevented ROS accumulation, the elongation of root-hair bulges and the extension rate of the primary root, thereby phenocopying the *rhd2* mutant.

The authors then tried the opposite approach. Could ROS applied to *rhd2*-mutant root-hair bulges induce root-hair growth? Indeed it could. Application of the most reactive ROS, hydroxyl radicals ($OH^{\bullet}$), to *rhd2*-mutant root-hair bulges restored root-hair growth, although the growth lacked the polarity found in wild-type hairs. Moreover, this was coincident with a rapid increase in the cytoplasmic levels of Ca^{2+} ($[Ca^{2+}]_c$), which was blocked in the presence of 0.1 mM Gd^{3+}, a Ca^{2+}-channel antagonist.

These data implicated ROS in the increase of $[Ca^{2+}]_c$ by Ca^{2+} influx, so the next step was to see if plasma-membrane Ca^{2+} channels could be activated by ROS. Within a few minutes of $OH^{\bullet}$ treatment, a Ca^{2+}-permeable, inwardly rectifying, hyperpolarization-activated conductance was detected in protoplasts from the elongation zone epidermis. This was again blocked by 0.1 mM Gd^{3+}, which also decreased the root elongation rate, as did a Ca^{2+} chelator. Because *rhd2* mutants and wild-type cells didn't differ significantly in their current amplitudes, the *rhd2* mutation seems not to affect the ROS-mediated channel sensitivity or the number of channel proteins. In root-hair apical spheroplasts, $OH^{\bullet}$ activated a Ca^{2+}-, Ba^{2+}- and TEA^{+}-permeable, inwardly rectifying, hyperpolarization-activated conductance.

So in protoplasts from the elongation zone epidermis and apical spheroplasts, ROS is involved in cell elongation by activating Ca^{2+} channels. The influx of Ca^{2+} is likely to modulate actin dynamics and other growth processes, and this mechanism could well extend to all plant cells. As the mammalian $gp91^{phox}$ is regulated by Rac, the authors propose that RHD2/*At*rbohC could be similarly controlled by Rac-like proteins in plants — ROPs.

Katrin Bussell

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WEB SITE

Liam Dolan's laboratory:
http://www.jic.bbsrc.ac.uk/science/cdb/dolanwebpage.htm

polarization occurred within the infected cell, rather than towards it.

So, when an HTLV-I-infected T cell contacts another cell, microtubule rearrangements in the infected T cells occur, and the viral genome is subsequently transferred to the recipient cell. The identity of the molecules that initiate contact and polarization is unknown; the Env protein is one candidate for fusion, being the only HTLV-I protein that is expressed on the outside of the infected cell, but HTLV-I also upregulates the expression of some adhesion molecules that could favour cell–cell transmission. Other viruses that depend on cell contact for transmission, or that are lymphotropic — such as HIV-1 — might similarly subvert normal T-cell physiology to propagate between cells.

Katrin Bussell

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ORGANELLE TRAFFICKING

Breakdown to arrive

When you're travelling to a particular destination, there's always the risk that mechanical breakdown will thwart your arrival. However, Weisman and colleagues now report in *Nature* that transport-machinery breakdown can actually be essential for cellular organelles to reach their final destination.

For yeast vacuole inheritance to occur correctly, vacuoles must be transported from the mother cell to the growing bud. The yeast class V myosin Myo2 moves organelles along actin to different destinations during the cell cycle, and previous studies indicated that the yeast vacuole membrane protein Vac8 is needed for Myo2's role in vacuole inheritance. However, Vac8 and Myo2 had not been shown to interact directly. Weisman and co-workers therefore began by identifying Vac17 as the vacuole-specific receptor for Myo2. However, Vac17 is not a membrane-bound protein, so how does it link Myo2 to vacuoles?

The authors showed that a Vac17–Vac8 interaction is the missing link and that the regions of Vac17 that interact with Vac8 and Myo2 are distinct. Vac17 can therefore interact with both proteins simultaneously — Vac17 links Myo2 to Vac8 and, as a result, to the vacuole membrane.

Vacuole inheritance was found to be blocked by mutations that disrupted either Myo2–Vac17 or Vac17–Vac8 interactions, and the authors found that this block resulted in Vac17 accumulation. Furthermore, they showed that Vac17 protein levels and the levels of vacuole-associated Vac17 change during normal cell-cycle progression. The levels of vacuole-localized Vac17 increase on formation of the nascent bud, decrease with increasing bud size, and decrease further on vacuole-inheritance completion. So, are these changes in Vac17 levels due to increased synthesis, decreased breakdown, or both? And could changes in Vac17 levels control vacuole inheritance?

Sequence analysis allowed the authors to identify a predicted PEST sequence in Vac17, which is a signal for rapid protein degradation. They found that deletion of PEST resulted in increased Vac17-ΔPEST levels, although Vac17-ΔPEST supported normal vacuole inheritance. These results indicate that, in this case and in vacuole-inheritance mutants, Vac17 accumulation is a result of defective Vac17 degradation, rather than because of a significant increase in Vac17 synthesis.

Using immunofluorescence, Weisman and co-workers showed that, in *VAC17-ΔPEST* mutants, Vac17-ΔPEST accumulates in the bud, whereas, in vacuole-inheritance mutants, Vac17 accumulates in the mother cell. These results support the idea that Vac17 degradation occurs after it has arrived in the bud.

When vacuole inheritance is complete, vacuoles are localized near the centre of the bud, and the authors found that, in the *VAC17-ΔPEST* mutant, several vacuoles were localized to the mother–bud neck. Using time-course experiments, they showed that removal of the PEST sequence stabilizes the Myo2–Vac17 interaction, which causes the vacuoles to move 'backwards' from the bud centre to the mother–bud neck.

Together, these results support a model in which newly synthesized Vac17 binds to Vac8 and Myo2 in the mother cell to form the Myo2–Vac17–Vac8 transport complex, which moves the vacuole along actin to the bud. In the bud, Vac17 is degraded in a PEST-dependent manner, which releases Myo2 from the vacuole and results in the vacuole being deposited near the centre of the bud. So, transport breakdown doesn't always hinder arrival after all!

Rachel Smallridge

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DEVELOPMENT

Jaguar driven

In *Drosophila*, the asymmetric cell division of embryonic neuroblasts requires, among other things, the formation of a basal crescent-shaped complex by cell-fate determinants and their adaptor proteins, one of which is Miranda. Because Miranda is localized to the apical cortex before the basal crescent forms, Yuh Nung Jan and colleagues figured that Miranda might require a motor protein that targets it to the basal site. Now they've found one — myosin VI Jaguar (Jar) — according to a report in *Developmental Cell*.

The Jan laboratory isolated Miranda-containing protein complexes from fly embryos using anti-Miranda antibodies and identified myosin II Zipper (Zip), which negatively regulates basal transport of Miranda, and Jar. Jar binding to Miranda is direct, as shown in glutathione *S*-transferase (GST) pull-down studies.

So what's the functional significance of this interaction? To address this question, Jan and co-workers took three independent approaches to reduce Jar activity in neuroblasts. In all cases, reduced Jar activity resulted in mislocalization of Miranda and misorientation of mitotic spindles — spindle reorientation being another feature of asymmetric neuroblast cell division.

Double-mutant studies indicated that Jar functions synergistically with Lethal giant larvae (Lgl), which is known to be required for basal-crescent formation. The apical complex was not affected, which led the authors to conclude that Jar and Lgl function downstream of, or in parallel to, apical localization.

All the myosins previously implicated in asymmetric cell division (including Zip in fly, as well as the ones in worm and yeast) are barbed-end-directed myosins, which move towards the plus ends of actin filaments. Jar is the first pointed-end-directed myosin. No doubt, future studies will be aimed at uncovering the mechanism by which Jar mediates basal targeting and spindle reorientation.

Arianne Heinrichs

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NUCLEOLUS

Disassembly dissected

In keeping with the physiological nucleolar dynamics in early embryogenesis, the nucleoli of transplanted nuclei disassemble in eggs and early embryos and reassemble at the blastula stage. Now, in *Nature Cell Biology*, Nobuaki Kikyo and colleagues have begun to analyse the molecular mechanisms underlying nucleolar disassembly.

Using dispersal of nucleolar phosphoprotein B23 in the nucleoplasm of embryonic XL2 nuclei as a marker of disassembly, a nucleolar-disassembly activity from *Xenopus laevis* egg extract was purified. The activity contained two germ-cell proteins — FRGY2a and FRGY2b (FRGY2a/b) — that share 83% amino-acid identity and are known to function as transcription factors and maternal messenger RNA-masking proteins in messenger ribonucleoprotein (mRNP) particles in oocytes. The authors then tested the ability of FRGY2a/b, recombinant (r)FRGY2a and rFRGY2b to disassemble nucleoli — in each case, nucleoli were disassembled in a similar and dose-dependent manner, showing that FRGY2a or FRGY2b alone is sufficient for nucleolar disassembly.

By creating FRGY2a mutants, Kikyo and colleagues went on to show that the disassembly property of FRGY2a is restricted to the carboxy-terminal domain (rFRGY2a-C), and that four BA islands (basic/aromatic amino acids) — which provide extensive, non-selective charge interactions with RNA — contribute cumulatively to disassembly.

To examine its *in vivo* effects, XL2 cells were transfected with rFRGY2a or rFRGY2a-C. rFRGY2a-C localized preferentially to the nucleus and induced both dispersal of B23 and nucleolar disappearance. By contrast, rFRGY2a localized mainly in the cytoplasm and didn't disperse B23 or reduce nucleolar size. However, by mutating the RNA-binding cold-shock domain in the amino terminus some rFRGY2a entered the nuclei and triggered disassembly.

Because transcription of rRNA is crucial for maintaining nucleolar integrity, the authors monitored rRNA synthesis during disassembly. Importantly, FRGY2a/b didn't disrupt rRNA synthesis and is, to the authors' knowledge, "…the first protein to be identified that can disassemble nucleoli without disrupting rRNA synthesis". Finally, Kikyo and colleagues showed that disassembly by FRGY2a/b could be reversed, indicating that the essential core structure of these nucleoli is retained.

Nucleolar disassembly by FRGY2a/b will have a considerable impact on the inefficient nuclear-cloning process, because proper nucleolar disassembly and reassembly is presumed to be crucial for the normal development of cloned embryos. In addition, this type of nuclear-remodelling study will "…provide key insights into the structural and functional organization of the nucleus".

Natalie Wilson

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RNA REPAIR

Mending the message

Repair of alkylation damage in DNA is clearly important, as at least three repair mechanisms have been described. On the other hand, little is known about what happens to alkylated RNA. However, Hans Krokan and colleagues now report in *Nature* that damaged RNA can be repaired *in vivo* — by some of the same enzymes used in DNA repair.

One of the DNA-repair systems involves the oxidative demethylation of 1-methyladenine and 3-methylcytosine. This is catalysed in *Escherichia coli* by the enzyme AlkB. Krokan and co-workers identified two human *AlkB* homologues — *ABH2* and *ABH3*. To test the enzymatic activity of these new enzymes, they did high-performance liquid chromatography (HPLC) analysis of [^{3}H]methylated DNA incubated with recombinant ABH2 and ABH3. And indeed, like AlkB, these enzymes were oxidative DNA demethylases capable of removing 1-methyladenine and 3-methylcytosine from DNA by demethylation.

When determining the substrate specificity of ABH2 and ABH3, Krokan and colleagues found that ABH2 was more active on double-stranded (ds)DNA, whereas ABH3 — like AlkB — had a preference for single-stranded (ss)DNA. Remarkably, AlkB and ABH3, but not ABH2, also repaired RNA oligonucleotides efficiently.

Next, the authors tested whether this newly discovered RNA-repair activity was biologically relevant by measuring the survival of chemically methylated (by methyl methanesulphonate; MMS) bacteriophage in the presence of plasmid-expressed AlkB, ABH2 or ABH3 in an AlkB-deficient *E. coli* strain. Consistent with the *in vitro* data, AlkB and ABH3, but not ABH2, were able to reactivate MMS-treated ssRNA phage MS2. Methylated ssDNA phage M13 could be reactivated by ABH2 and AlkB, and only partially by ABH3, whereas AlkB and ABH2, but not ABH3, reactivated dsDNA phage λ.

By studying the subcellular localization of ABH2 and ABH3, Krokan and co-workers noticed that ABH2 colocalizes with replication foci during S phase, which is consistent with its higher activity on dsDNA. By contrast, the dispersed nuclear localization of ABH3 throughout the cell cycle, and its preference for ssDNA and RNA, indicates that it might have a maintenance role in actively transcribing genes.

So, given that about 20 of the 62 known naturally occurring RNA modifications are sequence-specific methylation events, Krokan and colleagues conclude "…it is not surprising that cells have evolved RNA repair mechanisms that operate to prevent dysfunction of RNA as a result of alkylation damage".

Arianne Heinrichs

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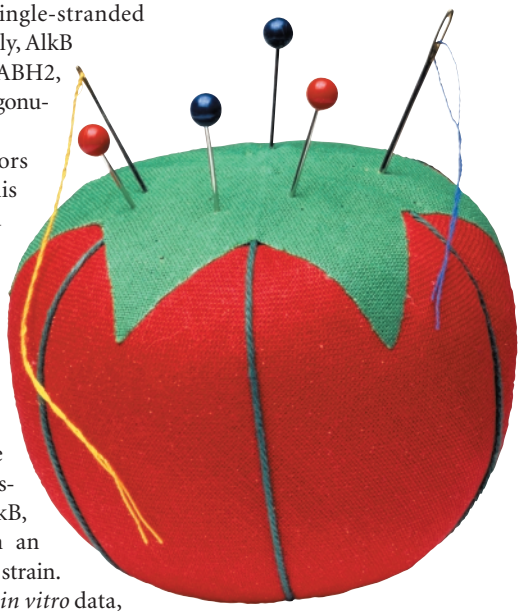

STRUCTURE WATCH

It takes two

Apoptosis is crucial for metazoan development and homeostasis, and it is executed by cascades of caspase activation. The catalytic activity of these caspases can, however, be inhibited by the inhibitor of apoptosis (IAP) proteins. Although we now have a good understanding of effector-caspase inhibition by IAPs, the mechanism of inhibiting initiator caspases — such as caspase-9 — has remained unclear. Now, though, in *Molecular Cell*, Shi and colleagues provide new insights by describing the 2.4-Å-resolution crystal structure of the catalytic domain of caspase-9 in complex with the third baculoviral IAP repeat (BIR3) of X-linked IAP (XIAP).

It has been shown, at least for caspase-3 and -7, that caspases must form homodimers to be catalytically active, because a crucial supporting loop (L2′) for the active site is provided by the adjacent monomer. In their structure, Shi and co-workers found that XIAP-BIR3 heterodimerizes with caspase-9 using the surface of caspase-9 that would otherwise mediate its homodimerization. XIAP-BIR3 therefore sequesters caspase-9 in its monomeric, inactive state and, in addition, it traps the caspase-9 active-site loops in an inactive conformation. The authors further showed that caspase-9 mainly exists as a monomer in solution, and that these monomers are catalytically inactive. This inactivity results primarily from the absence of the supporting L2′ loop, which is needed to stabilize the other active-site loops of caspase-9. These data, in conjunction with other studies, have therefore defined "…a unified mechanism for the activation of all caspases".

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Not a SET pattern?

Methylation is an important type of histone modification, which has been associated with various processes including transcriptional control. Although a large family of histone methyltransferases that all contain a SET domain has been identified, the *Saccharomyces cerevisiae* Dot1 protein was recently found to be the first histone lysine methyltransferase that lacks a SET domain. Dot1 mono-, di- and trimethylates lysine 79 in the core domain of histone H3. So, to further understand lysine methylation of histone core domains, as well as the nucleosome specificity of the unique Dot1 protein family, Xu and colleagues determined the 2.5-Å-resolution crystal structure of the catalytic domain of human Dot1 (hDOT1L) bound to *S*-adenosyl-L-methionine (SAM).

The structure revealed that hDOT1L has a unique organization of a mostly α-helical amino-terminal domain together with an open α/β structure carboxy-terminal domain that resembles several SAM-dependent methyltransferases. The active site consists of SAM, which binds between the loop that connects the two domains and the open α/β structure, and a narrow, potential lysine-binding channel that leads to the SAM methyl group. The narrowest part of this channel is ~4 Å, which could accommodate a mono-, di- or trimethylated lysine. Furthermore, Xu and co-workers showed that a disordered, positively charged region at the carboxyl terminus of the hDOT1L catalytic domain is essential for nucleosome binding and enzymatic activity.

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IN THE NEWS

Tricking the ribosome
Nerve cells can take over the normal cellular protein synthesis machinery in the same way that viruses do when they infect a cell, according to a report published in *Nature Neuroscience*. When a virus infects a cell, it 'kidnaps' the host cell's ribosomes to boost the production of virus particles. The virus achieves this by using an internal ribosome entry site (IRES), which shuts down and bypasses the normal mechanisms that regulate binding of messenger RNAs to ribosomes.

In a study of egg laying in the sea slug *Aplysia*, researchers from McGill University observed that the protein production of the egg-laying hormone (ELH) in neurons increased markedly. They found that the 5′ untranslated region of *ELH* mRNA contained an IRES — which are common in viral mRNAs but not in normal cellular mRNAs.

Wayne Sossin and colleagues also noticed that the stimulus for egg laying caused dephosphorylation of the initiation factor eIF4E. And this single event was sufficient for the cells to switch to IRES-mediated translation. "Egg laying is an important investment for an animal, thus when stimulated to do so, it wants to get it right," explains Sossin (*ScienceDaily*, 20th February 2003).

"The discovery … reveals an unexpected regulatory role of the IRES in nerve cells" according to Nahum Sonenberg, who first discovered the IRES in poliovirus in 1988 (*ScienceDaily*, 20th February 2003). IRES-regulated protein production might be important in other physiological events, and Sossin predicts that "…IRES regulation may be particularly important at synapses where there are limiting numbers of ribosomes" (*Nature Neuroscience*).

Arianne Heinrichs

SIGNALLING

New Polo player

Phosphopeptide-binding domains and phosphorylated serine, threonine or tyrosine residues fit together like molecular lego to mediate protein–protein complexes. The number of domains that specifically recognize phosphorylated motifs is growing, and in their study, published in *Science*, Elia, Cantley and Yaffe used a proteomic approach to identify the 'polo-box domain', a new phosphoserine (pSer)/phosphothreonine (pThr)-binding domain, which they found to be present in the mitotic kinase Polo-like kinase 1 (Plk1).

Because protein kinases and phosphopeptide-binding domains recognize overlapping amino-acid motifs, the authors took the approach of biasing a partially degenerate phosphopeptide library towards the phosphorylation motif of a kinase and then immobilizing this library and using it as bait in a screen for interacting proteins. In this case, they used a pThr-proline library biased towards the motif that is generated by cyclin-dependent kinases (CDKs) and mitogen-activated protein kinases, which is also recognized by the antibody MPM-2, a mitotic phosphoprotein-specific monoclonal antibody. Using a collection of peptides, rather than a single peptide, increases the chances of an interaction. Conversely, to control for phospho-independent binding, an identical unphosphorylated peptide library was used.

One of the clones isolated encoded the carboxy-terminal part of Plk1. It was missing some of its kinase domain, though, implying that phosphopeptide binding occurred independently of catalytic activity. Polo kinase family members have two 'polo boxes' in their carboxy-terminal domain, and a series of deletion constructs showed that both polo boxes and the linker between the kinase domain and polo box 1 constituted what the authors called the 'polo-box domain' (PBD).

By screening several pSer- and pThr-containing orientated peptide libraries and using isothermal titration calorimetry, the authors determined the optimal binding motif for the PBD — a strong preference for serine in the (pThr or pSer) –1 position and proline at (pThr or pSer) +1, leading them to propose a core consensus binding motif of Ser-(pThr/pSer)-(Pro/X).

The conserved (pSer/pThr)-Pro epitope that is recognized by the MPM-2 antibody occurs on ~50 mitotic phosphoproteins. Many of these were specifically bound by the Plk1 PBD, and the interaction was blocked by incubation with its optimal phosphopeptide ligand. One mitotic phosphoprotein, the phosphatase Cdc25C, has a consensus Plk1 PBD-binding motif in its amino terminus. Transfection of Cdc25C point mutants that are expected to have a disrupted PBD-binding motif abrogated their interaction with the Plk1 PBD and interfered with their subsequent mitotic phosphorylation.

In addition, Yaffe and colleagues found the PBD of Plk1 to be responsible for localizing Plk1 to centrosomes during prophase. As the PBD also binds to the kinase domain of Plk1, there seem to be parallels between PBD and phosphotyrosine-binding Src-homology-2 (SH2) domains, in that both domains target their kinases towards phosphorylated substrates on activation, but inhibit phosphotransferase activity in the basal state. Also similar to SH2 domains, PBDs might provide particularly attractive targets for small-molecule mimetics as potential therapeutics.

Katrin Bussell

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GLYCOPROTEIN DEGRADATION

Time for a trim

Quality control is essential for cell viability and, in the endoplasmic reticulum (ER), the calnexin/calreticulin chaperone cycle ensures that only correctly folded glycoproteins reach their final destination. Persistently misfolded glycoproteins undergo ER-associated degradation (ERAD); that is, they are retro-translocated to the cytosol and degraded by the proteasome. Previously, EDEM — a transmembrane α-mannosidase-I-like protein that lacks mannosidase activity — has been shown to accelerate ERAD, and now two *Science* papers clarify its exact role in this process.

Glycoproteins displaying the *N*-linked glycan $Glc_1Man_9GlcNAc_2$ (where Glc is glucose, Man is mannose and GlcNAc is *N*-acetylglucosamine) bind calnexin or calreticulin, which allows these chaperones to facilitate protein folding, and trimming of the terminal glucose disrupts this interaction. However, if folding is incomplete, the free glycoprotein can be either reglucosylated by a glucosyltransferase to allow further interaction with chaperones or further trimmed by ER α-mannosidase I to produce $Man_8GlcNAc_2$, which targets glycoproteins for ERAD.

In the first paper, Nagata and colleagues began by assessing EDEM's interactions. They showed that EDEM interacts with the transmembrane chaperone calnexin, but not with the soluble chaperone calreticulin, and that the transmembrane region of calnexin is required for this interaction.

Next, they used an ERAD substrate called NHK (an α_1-antitrypsin variant) to study the roles of calnexin and EDEM in ERAD. They compared NHK-transfected cells with cells transfected with NHK and EDEM, and found that NHK degradation was accelerated in the latter. When they disrupted the calnexin–NHK interaction, they found that NHK degradation was only mildly inhibited in NHK-expressing cells, but that the accelerated degradation was strongly suppressed in cells expressing NHK and EDEM. Furthermore, they showed that, in both types of transfected cell, calnexin overexpression suppressed NHK degradation. So, both substrate binding to calnexin and substrate release from calnexin are needed for EDEM-mediated ERAD.

Finally, Nagata and co-workers showed that EDEM overexpression accelerates ERAD by promoting the release of persistently misfolded glycoproteins from calnexin. EDEM therefore seems "…to function in the ERAD pathway by accepting substrates from calnexin".

In the second paper, Molinari *et al.* showed that EDEM overexpression accelerated the degradation of a membrane-bound and soluble form of an ERAD substrate, without affecting the same substrate lacking *N*-glycans. They also showed that ERAD-substrate degradation was slower when intracellular EDEM levels were reduced.

When Molinari *et al.* compared wild-type and EDEM-overexpressing cells, they found that glucose persisted for shorter times on the *N*-glycans of ERAD substrates in the latter case, which would be expected to affect ERAD-substrate interactions with calnexin and EDEM. Indeed, they showed that, although initial calnexin–ERAD substrate interactions were the same, ERAD substrates dissociated from calnexin faster, and were degraded quicker, in EDEM-overexpressing cells.

Molinari *et al.* further showed that varying the levels of EDEM did not affect the maturation of correctly folding glycoproteins. EDEM therefore specifically regulates the release of terminally misfolded glycoproteins from the calnexin cycle.

The work of Nagata and colleagues and Molinari *et al.* has therefore shed light on the role of EDEM in ER quality control. EDEM functions in the ERAD pathway by accepting terminally misfolded glycoproteins from calnexin and, as EDEM levels are upregulated when protein folding is perturbed, it might promote cell recovery at times of ER stress.

Rachel Smallridge

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IN BRIEF

ADHESION

TES is a novel focal adhesion protein with a role in cell spreading.

Coutts, A. S. *et al. J. Cell Sci.* **116**, 897–906 (2003)

TES was previously identified as a candidate tumour-suppressor gene. As reported here, Coutts *et al.* carried out yeast two-hybrid analysis and found that TES interacts with several focal adhesion and/or cytoskeletal proteins. Further studies showed TES to localize to regions of cell–substrate and cell–cell contact. Focal-adhesion-associated proteins might mediate cell adhesion, migration or cell signalling, and TES, when overexpressed, was found to confer on fibroblasts an increased ability to spread.

DEVELOPMENT

Retraction of the *Drosophila* germ band requires cell–matrix interaction.

Schöck, F. & Perrimon, N. *Genes Dev.* **17**, 597–602 (2003)

The absence of integrins in *Drosophila* affects germ-band retraction, which involves large-scale epithelial movements. Here, α1, 2 laminin and αPS3βPS integrin were shown to be needed for a small group of cells of the amnioserosa (a squamous epithelium) to spread over the end of the germ band. In the absence of βPS integrin, lamellipodia formation was inhibited and no cell–matrix adhesion between the amnioserosa and the tail end of the germ band occurred.

SIGNALLING

Redox-dependent downregulation of Rho by Rac.

Nimnual, A. S., Taylor, L. & Bar-Sagi, D. *Nature Cell Biol.* **5**, 236–241 (2003)

Balancing the opposing effects of Rac and Rho on the cytoskeleton is crucial for determining cell morphology and migratory behaviour. Here, the authors found that Rac, through its unique insert region, generates reactive oxygen species (ROS). A resultant decrease in Rho activity occurs through ROS-mediated inhibition of the low-molecular-weight protein tyrosine phosphatase (LMW-PTP) and subsequent activation, by increased tyrosine phosphorylation, of p190Rho-GAP, enabling Rac to induce membrane ruffling and integrin-mediated spreading.

CHROMOSOME BIOLOGY

Global chromosome positions are transmitted through mitosis in mammalian cells.

Gerlich, D. *et al. Cell* 2003 March 4 (DOI: 10.1016/S0092867403001892)

How and when are chromosomes positioned non-randomly in mammalian cells? Gerlich *et al.* used non-invasive labelling and 4D imaging to show that no global chromosome rearrangements occur in interphase, G1, S or G2 of the cell cycle, but that global positioning occurs during mitosis and is transmitted from one cell generation to the next. They propose that this occurs by chromosome-specific timing of sister-chromatid separation.

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

Shuguang Zhang's laboratory:
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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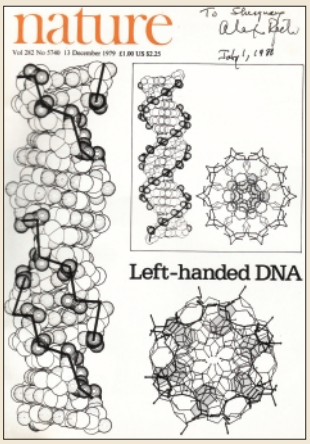

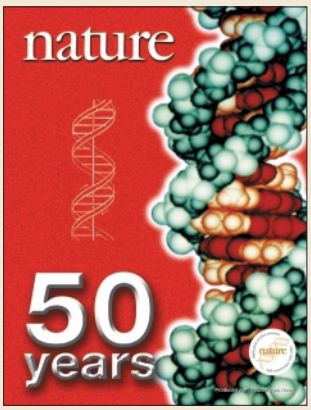

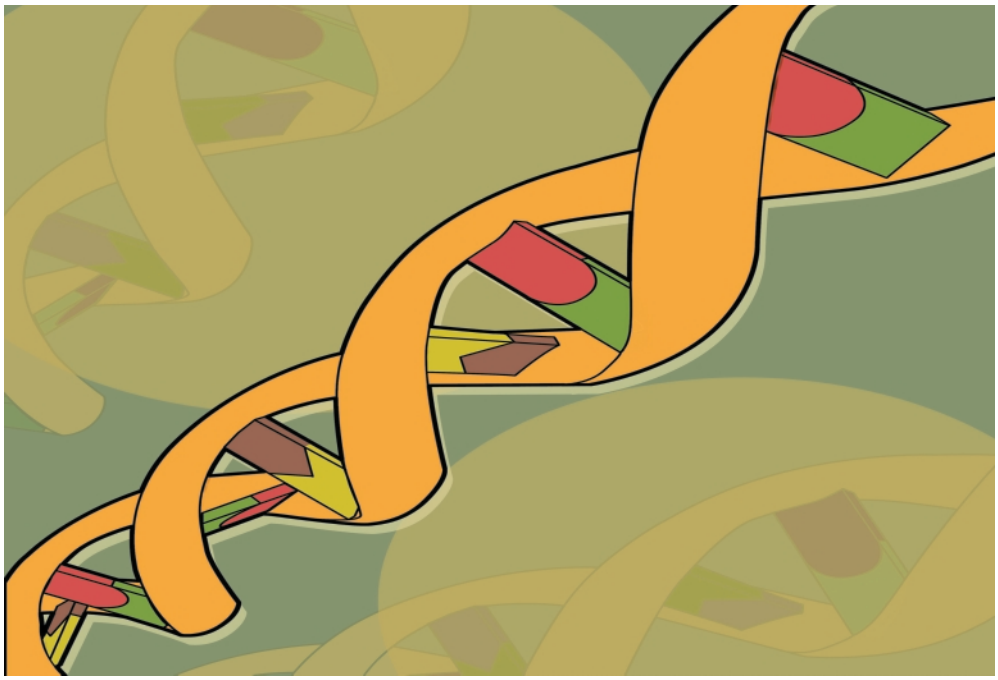

DNA RECOMBINATION

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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CELL CYCLE

A way out

Exit from mitosis is characterized by low Cdk1 kinase activity and activation of the phosphatase Cdc14. This final stage in the cell cycle is triggered by Tem1, a GTPase located on the spindle pole body that, on entering the daughter cell, is activated and signals the mitotic exit network (MEN) pathway. However, cells must be able to reverse the state of mitotic exit to enter the next cell cycle. Stephen Elledge and colleagues now report, in *Cell*, new insight into how cells 'exit' mitotic exit.

Elledge and co-workers identified 'antagonist of MEN' (*AMN1*) in a genetic screen and set out to establish whether Amn1 is a bona fide negative regulator of MEN. They found that Amn1 overexpression was toxic to MEN mutant strains, and wild-type cells were delayed or arrested in mitotic exit. Amn1 was also required for the nuclear orientation and spindle checkpoints, indicating that *AMN1* is a checkpoint gene and might function coordinately with other checkpoints to control mitotic exit.

Next, the authors showed that *AMN1* expression is cell-cycle regulated and peaks in late M/G1. Moreover, intracellular localization studies using a green fluorescent protein (GFP)-tagged *AMN1* strain showed that high-level expression was daughter-cell specific. The transcription factors responsible for *AMN1* transcription, Swi5 and Ace2, turned out to be dependent on activation by MEN. So, *AMN1* transcription is induced specifically in daughter cells after mitotic exit.

To look for the target of Amn1-mediated MEN inhibition, the Elledge group carried out another genetic screen and isolated *TEM1*. Amn1 binds directly to Tem1, both *in vitro* and *in vivo*, indicating that Amn1 might inhibit MEN through its ability to bind and inhibit Tem1 function. Binding of Tem1 to its target kinase Cdc15 is essential for activation of MEN, and the authors observed that the absence of Amn1 promotes Tem1–Cdc15 association. Conversely, overproduction of Amn1 reduced Tem1–Cdc15 association, suggesting that Cdc15 and Amn1 compete for association with Tem1. When AMN1 was overexpressed in cells arrested during mitotic exit, levels of Tem1–Cdc15 association were also reduced, indicating that Amn1 can disassemble preformed Tem1–Cdc15 complexes, thereby turning off MEN.

So finally, how is Amn1 itself turned off? The authors have evidence that SCF E3 ubiquitin ligase might be responsible for Amn1 degradation. Moreover, it is possible that Cdk1 phosphorylation targets Amn1 for degradation, which would explain its rapid degradation when cells enter S phase.

Arianne Heinrichs

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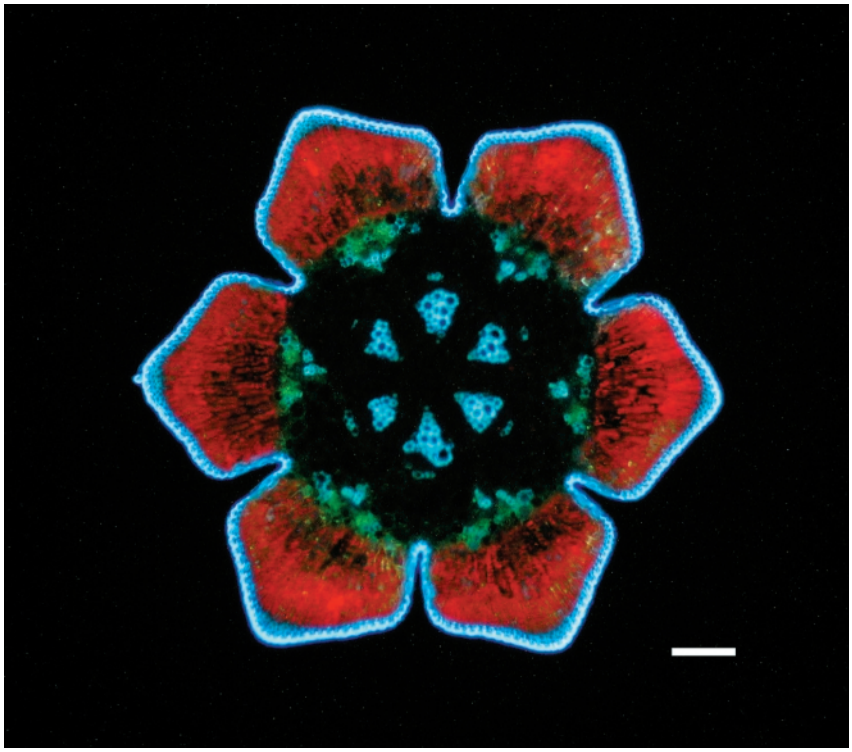
ORIGINAL RESEARCH PAPER Wang, Y. *et al.* Exit from exit: resetting the cell cycle through Amn1 inhibition of G protein signaling. *Cell* **112**, 697–709 (2003)

CELL OF THE MONTH



This month's winning image was submitted by Sergio Svistoonoff (Institut de recherche pour le développement, Montpellier, France (Sergio.Svistoonoff@mpl.ird.fr)). It shows shoots from *Allocasuarina verticillata* that are expressing green fluorescent protein (GFP) under the control of the 35S promoter.

GFP is expressed in the phloem cells. The chloroplasts of the mesophyll cells fluoresce in red, whereas the lignified cells of the epidermis and the xylem vessels appear in light blue. The image was acquired from transversal shoot sections under blue-UV light using a Leica-DMR microscope, with an epifluorescence filter A (Leica). Bar, 100 µm.

We are pleased to acknowledge the help of our two external advisors, Ariel Ruiz i Altaba (Developmental Genetics Program, New York Medical Center) and Lelio Orci (Department of Morphology, University Medical Center, Geneva).