CHROMATIN

Keep it level

During DNA replication, histone metabolism and DNA synthesis must be carefully controlled, because an excess of histones is toxic to the cell. And, scientists have now identified Rad53 kinase as a key controller of the delicate balance between these processes.

Rad53 functions in the DNA-damage response and is essential for deoxyribonucleotide triphosphate (dNTP) production. To study the role of Rad53 in histone metabolism, Akash Gunjan and Alain Verreault overexpressed histones in wild-type and *rad53*Δ budding yeast strains and found that excess histones were far more toxic to *rad53*Δ mutant cells than to wild-type cells.

To understand why *rad53*Δ mutants were sensitive to histone overexpression, the authors followed the fate of the overexpressed histones. In cells that were arrested in G1 phase, overexpressed histone H3 was rapidly degraded in wild-type cells, but not in *rad53*Δ cells. When histone overexpression was induced in S-phase cells, a fraction of those histones was incorporated into chromatin. Both wild-type and *rad53*Δ cells incorporated similar amounts of histone H3 into chromatin, whereas unincorporated histones were quickly degraded in wild-type cells but not in the *rad53*Δ strain. Rad53 is therefore specifically needed to trigger the degradation of the excess histones that are not packaged into chromatin.

Newly synthesized histones normally associate with histone chaperones during nucleosome assembly. The amounts of histone H3 associated with the histone chaperones CAF-1 and Hir2 were much higher in *rad53*Δ mutant cells compared with wild-type cells, which confirmed that *rad53*Δ mutant cells accumulated newly synthesized histones. The treatment of cells with genotoxic agents that slow or block DNA replication also led to an increase in histones that were bound to histone chaperones.

The authors showed that a reduction in histone H3/H4 gene dosage suppressed the slow-growth phenotype of *rad53*Δ mutant cells. Other damaging effects of histone overexpression — including mitotic chromosome loss and DNA-damage sensitivity — were also reduced, which shows that excess histones interfere with several distinct processes that require access to genetic information.

CELLULAR MICROBIOLOGY

Phagosome escapology

Mycobacterium marinum causes a systemic tuberculosis-like disease in its natural hosts — fish and frogs — but can also cause disease in immunocompromised humans, and it has been established as a useful model for tuberculosis. Research recently published in the *Journal of Experimental Medicine* shows that, in common with other intracellular pathogenic microbes, *M. marinum* can escape from the phagosome and exploit the cytoskeleton of eukaryotic cells to move inside cells and spread through tissues.

Mycobacterium tuberculosis, the classic intracellular pathogen that causes the chronic disease tuberculosis, enters cells — usually macrophages — by endocytosis, and then arrests the development of the phagosome so that it doesn't fuse with lysosomes and acidify. Bacilli replicate inside this modified compartment, hidden from antibody and complement immune surveillance. Glycoconjugates released by the intracellular bacilli help to induce and maintain the granuloma — a tissue response that limits bacterial spread but ensures persistence of the infection. Compared with *M. tuberculosis*, *M. marinum* can also result in latent long-term infections with associated granuloma formation, but has a much shorter generation time. In addition, it can be safely studied using ordinary laboratory facilities and has numerous animal-infection models. Studying *M. marinum* might therefore provide answers to some of the unresolved questions about *M. tuberculosis* pathogenesis.

During painstaking scrutiny using time-lapse video microscopy of the phagosome maturation process after infection with *M. marinum*, Stamm *et al.* noticed that although most of the bacilli were in membrane-bound phagosomes, some bacilli were motile in the cytoplasm and had dense tails of polymerized actin. Importantly, other well-studied intracellular bacterial pathogens, including *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia rickettsii*, can escape from phagosomes and use actin-based motility to move through the cytoplasm, and spread from cell to cell. By 48 h post-infection, as many as 20% of the intracellular bacilli had exited the phagosome and become motile. Most infected cells eventually contained some motile bacilli, which were always located in the cytoplasm. *Listeria* and *Shigella* use independently evolved tactics to polymerize host-cell actin, and this study showed that *M. marinum* uses a mechanism most similar to that used by *Shigella*. Using polymerized actin, single motile cells were tracked and were seen to exit one cell and enter a neighbouring cell in a macrophage monolayer. Clear evidence that this is biologically relevant was obtained by visualizing the spread of fluorescently labelled bacilli in an infected monolayer — bacilli were able to infect neighbouring cells and spread even when extracellular bacteria were killed with a broad-spectrum antibiotic.

M. tuberculosis is thought to spread only when the granuloma bursts and hasn't been reported to use actin-based motility as a means of spreading in tissues. Although published evidence for the escape of *M. tuberculosis* from the phagosome is scarce, in the light of this report it might be worth investigating whether this strategy operates after initial infection has been established *in vivo* to help tuberculosis bacilli spread.

Susan Jones, Associate Editor,
Nature Reviews Microbiology

References and links

ORIGINAL RESEARCH PAPER Stamm, L. M. *et al.* *Mycobacterium marinum* escapes from phagosomes and is propelled by actin-based motility. *J. Exp. Med.* **198**, 1361–1368 (2003)

FURTHER READING Fehrenbacher, K. *et al.* Actin comet tails, endosomes and endosymbionts. *J. Exp. Biol.* **206**, 1977–1984 (2003)

WEB SITE
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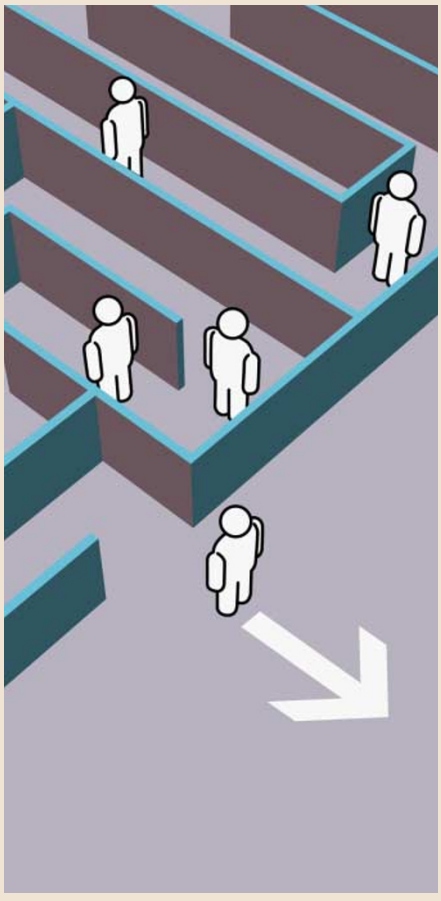
But how does Rad53 sense excess histones and target them for degradation? Gunjan and Verreault found that overexpressed histones associated transiently with Rad53. By contrast, higher levels of histones were associated with a kinase-defective mutant. This indicates that the Rad53 kinase activity is needed to avoid the accumulation of histones in a Rad53-containing complex, by triggering their degradation. Whether histone phosphorylation has a role in their degradation is not clear. Also, whether Rad53 binds histones directly or through histone chaperones remains to be determined. To elucidate the precise mechanism of this Rad53-dependent surveillance system for histone levels, researchers will undoubtedly focus on proteins that function downstream of Rad53.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Gunjan, A. & Verreault, A. A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*. *Cell* **115**, 537–549 (2003)



MEMBRANE TRAFFIC

Stimulating curves

You'll often hear women fretting about their curvaceous figures, but it now seems that larger curves are more stimulating — well, at least in membrane-trafficking events. Membranes are deformed into buds, and subsequently vesicles, by protein coats that dissociate after vesicle formation. The assembly/disassembly of the coatomer-protein-(COP)I coat is linked to the GTP/GDP cycle of the small G-protein Arf1, which specifically interacts with COPI. COPI-coat disassembly is triggered by the GTPase-activating protein ArfGAP1, which catalyses the hydrolysis of the GTP that is bound to Arf1. But what controls the timing of this event? In *Nature*, Antonny and colleagues now highlight the involvement of membrane curvature.

The authors had previously suggested that loose lipid packing — like that in the outer leaflet of a bud — favours the interaction between Arf1–GTP and ArfGAP1. So, they reasoned that, if this is true, ArfGAP1 activity should vary when Arf1–GTP is associated with liposomes of different radii but identical lipid composition. They therefore prepared such liposomes and monitored the change in tryptophan fluorescence that occurs as a result of the Arf1–GTP to Arf1–GDP transition, and they found that the smaller the liposome radius — that is, the more curved the membrane — the faster the inactivation of Arf1.

But does membrane curvature directly affect COPI-coat disassembly? To answer this question, Antonny and co-workers used a light-scattering assay, which allowed them to observe three sequential signals that corresponded to the addition of GTP, then COPI and then ArfGAP1 to a minimal system. The signals reflect Arf1–GTP recruitment to liposomes (small light-scattering increase), COPI recruitment to liposomes (large increase) and ArfGAP1-induced COPI-coat disassembly (decrease of signal to the initial level). They applied this assay to liposomes of decreasing radii, and found that, whereas the COPI coat on large liposomes was quite resistant to ArfGAP1, the coat on small liposomes dissociated within seconds of the addition of a catalytic amount of ArfGAP1.

Ionic complexes between fluoride and aluminium (AlF_x) can imitate a γ-phosphate group destined for hydrolysis, and the interaction between AlF_x and Arf1 requires ArfGAP1. In the presence of AlF_x, stable COPI-coated vesicles are produced. So, Antonny and colleagues reasoned that the stabilizing effect of AlF_x on COPI might be the result of a stable interaction between Arf1–GDP, AlF_x and ArfGAP1, which, in turn,

might depend on membrane curvature. They tested this hypothesis using the light-scattering assay, and their results indicate that, if the membrane is sufficiently curved, AlF_x stabilizes a ternary complex of Arf1–GDP, ArfGAP1 and COPI, which prevents COPI-coat disassembly. Furthermore, their data allowed them to conclude that the activity of ArfGAP1 depends on whether or not it can penetrate the COPI-coat-facing leaflet of the membrane bilayer to access its substrate, Arf1–GTP.

Antonny and co-workers, therefore, propose a model in which "…the remarkable sensitivity of ArfGAP1 to membrane curvature determines a spatial and temporal programme for GTP hydrolysis in a COPI bud". COPI polymerization to form a coat increases membrane curvature, which eventually allows ArfGAP1 to access and hydrolyse Arf1–GTP. The decrease in Arf1–GTP in the coat is probably compensated for by increased interactions between the COPI-coat subunits as the coat becomes more rounded. However, Arf1–GTP at the flatter edge of the bud is inaccessible to ArfGAP1. It might, therefore, help to keep the COPI coat in a metastable state, so that it can disassemble as soon as membrane fission occurs.

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References and links

ORIGINAL RESEARCH PAPER Bigay, J. *et al.* Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. *Nature* **426**, 563–566 (2003)

FURTHER READING Kirchhausen, T. Three ways to make a vesicle. *Nature Rev. Mol. Cell Biol.* **1**, 187–198 (2000)

WEB SITE

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http://www.ipmc.cnrs.fr/pages/antonny.htm