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.

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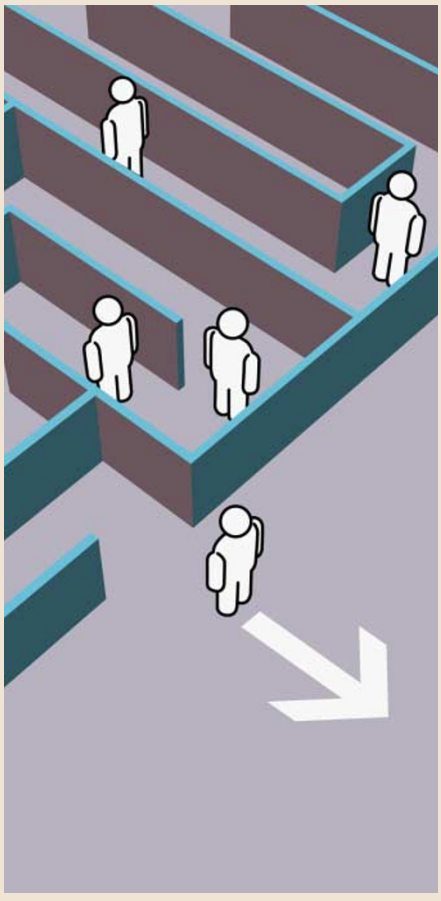

MEMBRANE TRAFFIC

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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WEB SITE

Bruno Antonny's laboratory:
http://www.ipmc.cnrs.fr/pages/antonny.htm