RESEARCH HIGHLIGHTS

Protein methylation: controlling endosomal trafficking

Histone methylation is typically associated with changes in gene expression. Now, Ma and colleagues (*J. Cell Biol.* **186 (3),** 343–353; 2009) report that mammalian Dpy-30 (mDpy-30), a component of several histone H3 lysine K4 methyltransferases, regulates traffic between the endosome and trans-Golgi network (TGN).

The authors found that mDpy-30 localizes to the TGN and that depleting or overexpressing mDpy-30 specifically perturbs localization of the CIMPR (cation-independent mannose 6-phosphate receptor), a cargo protein that cycles from the endosome back to the TGN. Whereas internalization of CIMPR is not altered on mDpy-30 depletion, CIMPR accumulates in endosomes near protrusions in depleted cells, suggesting a function for mDpy-30 in endosome-to-TGN recycling. Depletion of Ash2L or RbBP5, two other components of histone H3 lysine K4 methyltransferases, results in a similar accumulation of CIMPR in endosomes. BIG1, a protein that localizes to the TGN and is involved in endosome-to-TGN trafficking, was found to interact with mDpy-30. Both proteins colocalize to the TGN and BIG1 is required for the recruitment of mDpy-30 to the TGN.

Exactly how mDpy-30 functions in endosome-to-TGN trafficking remains unclear. Its localization to the TGN points to a direct role, perhaps through the methylation of BIG1 or other factors involved in endosome-to-TGN trafficking. SS

Rho GTPases unravelled

Two studies in *Nature* provide key insights into how activities of the three Rho GTPases Rac1, RhoA and Cdc42 are coupled to protrusion and retraction events at the leading edge of migrating cells (*Nature* **461,** 104–108; 2009 and *Nature* **461,** 99–103; 2009).

In the first study, Klaus Hahn and colleagues generated photoactivatable Rac1 by fusing Rac1 to a photoreactive domain of *Avena sativa* phototrophin-1; this allows precise and reversible localized activation of Rac1 in cells. Localized Rac1 activation was sufficient to drive local protrusions and to specify cell polarity, while repeated irradiation of Rac1 resulted in prolonged cell movement. The authors also showed that localized Rac1 activation triggered immediate inhibition of a RhoA biosensor.

In the second study, Danuser and colleagues used a computational 'multiplexing' approach in which cell protrusion and retraction is used as a common reference to quantitatively align imaging data of GTPase biosensors from separate experiments. They found that RhoA activity at the leading edge is synchronous with protrusion and retraction events, whereas Cdc42 and Rac1 are activated only 40s later, ~2 μm behind the leading edge. They then simultaneously imaged Cdc42 and RhoA in one cell to verify these predicted relationships. Danuser and colleagues propose that RhoA may regulate actin polymerization during protrusion events, whereas Rac1 and Cdc42 may be more important for regulating adhesion dynamics. Similarly to Hahn and colleagues, they conclude that Rac1 antagonizes RhoA function.

The technical advances of these two studies have allowed the activities of Rho GTPases to be dissected with unprecedented resolution in living cells. Both groups propose that the approaches used here to probe GTPases at the leading edge should be adaptable to other applications. AS

PAR-recruited chromatin remodeller repairs DNA

Parp1 catalyses poly(ADP)-ribose (PAR) synthesis from NAD^+. PAR-associated chromatin remodelling facilitates DNA repair. Ahel *et al.* (*Science* **325,** 1240–1243; 2009) and Gottschalk *et al.* (*Proc. Natl Acad. Sci. USA* **106,** 13770–13774) report that Alc1, which is encoded by a hepatocellular carcinoma-associated oncogene and is a member of the SNF2 family of ATPases, contains a PAR-binding macrodomain. Alc1 also binds nucleosomes *in vitro*, activating its ATPase activity, and this is probably dependent on the autoPARylation of Parp1. Indeed, both Alc1 ATPase activity and nucleosome repositioning *in vitro* is dependent on Parp1, NAD^+ and, according to Ahel *et al.*, the H4 amino-terminal tail.

Parp1 and Alc1 rapidly and transiently accumulated at sites of laser microirradiation-induced DNA damage in living cells (Alc1 recruitment was dependent on its macrodomain and Parp1). Ahel *et al.* also show that an ATPase-dead Alc1 mutant defective in nucleosome sliding and its macrodomain was retained longer at damage sites. Thus, Alc1 recruitment to damage sites requires macrodomain-mediated PAR binding and its ATPase activity is implicated in disengagement from damaged chromatin. Notably, Ahel *et al.* show that Alc1 downregulation by shRNA led to increased sensitivity to H_2O_2 and the radiomimetic drug phleomycin, whereas its overexpression caused increased damage by phleomycin. Thus, chromatin relaxation may be required for repair, but excessive opening may de-protect DNA. Ahel *et al.* went on to identify Parp1-dependent Alc1 binding to the repair proteins DNA-PK, Ku, XRCC1 and APLF, using mass spectrometry and immunoblot assays.

It remains to be seen whether Parp1-activated chromatin remodelling by Alc1 contributes to DNA replication and transcription, in addition to DNA repair. BP

Making frog eyes

A functional frog eye can be made by engineering stem cells to express seven transcription factors and grafting the cells to a frog embryo (*Plos Biol.* **7,** e1000174; 2009). Zuber and colleagues hypothesized that because all retinal cell types derive from a common retinal progenitor, pluripotent cells should in principle generate retinas if converted to retinal progenitors. Earlier work showed that a group of six eye field transcription factors (EFTFs) induced eye-like structures when overexpressed in frog embryos together with the neural patterning factor Otx2. Thus, the authors expressed the transcription factor cocktail in blastula-derived pluripotent stem cells, and compared the induced gene expression profile to that of the eye field — the neural plate area where the retina is formed. The gene profiles were similar, suggesting that the transcription factors direct the pluripotent cells to a retinal lineage. When stem cells expressing EFTFs and a fluorescent marker were transplanted into the flank of developing frog embryos, morphologically normal ectopic retinas formed. When EFTF-expressing stem cells were grafted to the eye-field area in embryos where the endogenous eye-fields had been removed, eyes containing all seven eye-specific cell types formed and the eye had neural connections with the brain. The induced eyes also were shown to be functional in the phototropic behaviour of tadpoles. Interestingly, the induced eyes continued to grow with the animal and possibly contained a retinal stem cell pool, like the eyes of normal frogs. These findings may point to a strategy for retinal repair and lay to rest a long-standing anti-Darwinian argument. CKR

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