MEMBRANE TRAFFIC

When to let go

Rab GTPases associate with the surfaces of distinct, intracellular, membrane compartments through their prenyl groups, and they regulate vesicle formation, motility, docking and fusion. Prenylated Rabs are also present in the cytosol, where they are maintained in an inactive state by guanine nucleotide dissociation inhibitor (GDI). Rab9 binds GDI with high affinity, so how does it know when to let go? Suzanne Pfeffer's group previously proposed that a specific factor might catalyse the dissociation of Rab–GDI complexes to allow Rabs to be transferred from GDI onto membranes. And now, in *Nature*, this group shows that Yip proteins might fulfil this role.

In mammalian cells, there are at least five Yip-type proteins, and the human Yip3 homologue is known to interact with numerous, prenylated Rabs and to interact weakly with GDI. The authors therefore studied the effect of human Yip3 — an integral membrane protein — on Rab9–GDI complexes. GDI blocks the release of GDP from Rab9, but when Yip3 was added to Rab9–GDI complexes, Rab9 exchanged nucleotide at the same rate as free Rab9 protein. So, Yip3 does not function as a guanine nucleotide exchange factor. Instead, using gel filtration experiments, the authors showed that Yip3 functions as a GDI-displacement factor (GDF) for Rab9.

Pfeffer and co-workers also showed that membranes from Yip3-overexpressing cells recruited significantly more Rab9 from Rab9–GDI complexes than membranes from wild-type cells. This effect was specific (anti-Yip3 antibodies markedly reduced the recruitment of Rab9) and catalytic (Rab9 was recruited to a level that was 15-fold greater than the level of Yip3 in the membranes).

Yip3 is localized to the late Golgi and endocytic pathway, so the authors investigated whether Yip3 preferentially catalyses the dissociation of GDI from endocytic Rabs, rather than endoplasmic-reticulum

STEM CELLS

ID required

Decision-making can be a difficult process and often a little help is needed. Embryonic stem (ES) cells can choose to differentiate along numerous cell lineages or undergo self-renewal, so how do they decide what to do? *Ex vivo* stem-cell proliferation is regulated by leukaemia inhibitory factor (LIF), but when ES cells are grown in serum-free cultures, other unknown factors are required. Now, Austin Smith and colleagues report in *Cell* that inhibitor of differentiation (ID) proteins — induced by the bone morphogenic protein (BMP)–SMAD pathway — collaborate with LIF to ensure that ES cells opt for self-renewal.

Mouse ES cells grown in serum-free media undergo neural differentiation and, although addition of Lif to the media initially reduces the number of differentiating cells, the undifferentiated stem-cell population declines with successive passaging in the presence of Lif alone. BMPs are known to antagonize neural differentiation, so the authors added Bmp2 or Bmp4 to Lif-containing ES cultures. Lif plus Bmp maintained pure populations of undifferentiated, diploid ES cells even after extended passage, and withdrawal of both factors allowed neural differentiation to resume. Importantly, Bmp alone produced epithelial-like cells, which indicated that the self-renewal response to Bmp is Lif dependent. The Bmp-related growth and differentiation factor-6 (Gdf-6), but not activin or transforming growth factor-β1 (Tgf-β1), also supports self-renewal in the presence of Lif, indicating that this effect is not a general feature of the TGF-β superfamily.

SMAD transcription factors are the principal downstream regulators of BMPs, so Smith and colleagues investigated Smad activation in the ES cells by immunoblotting and found increased phosphorylation of Smad1 in the presence of Bmp4. But does Smad1 activation by BMP support self-renewal? The inhibitory SMAD family members Smad6 and Smad7 were introduced into the ES cells and — in the presence of Lif — produced fewer and smaller ES cell colonies, which expanded poorly after passage compared with wild-type cells. *ID* genes are induced by the BMP–SMAD pathway, and *Id1*, *Id2* and *Id3* expression were strongly induced by Bmp and Gdf — but not by Lif — in the ES cells. Neither activin or Tgf-β1 induced *Id* expression, confirming that the proliferative response is specific to the BMP–SMAD pathway.

Smith and co-workers then introduced Id1, Id2 or Id3 into the ES cells to determine if overexpression restricted neural differentiation. The resulting *Id*-transfectants remained Lif dependent under serum-free conditions, no longer required Bmp for self-renewal and had identical properties to parental ES cells cultured in Lif plus Bmp. Removal of Lif caused the *Id*-transfectants to differentiate into epithelial-like cells, similar to the parental ES cells exposed to Bmp alone. Using revertible *Id* expression constructs, they confirmed that when Id was expressed it prevents neural differentiation in the absence of Lif although the cells can differentiate along alternate lineages. This ability to control stem-cell decisions *ex vivo* has important implications for the field of stem-cell therapy, which has been hindered by the inability to maintain stem-cell self-renewal in serum-free cultures.

Emma Croager

References and links

ORIGINAL RESEARCH PAPER Ying, Q-L. *et al.* BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **115**, 281–292 (2003)

WEB SITE

Austin Smith's laboratory:
http://www.iscr.ed.ac.uk/AGS_group.html