RESEARCH HIGHLIGHTS

Phosphorylation regulates HOPS-directed fusion

In yeast, the HOPS tethering complex regulates vacuolar fusion events. HOPS localizes to late endosomes, where it mediates endosome–vacuole fusion. However, when present on vacuoles, HOPS can regulate fusion of AP-3 vesicles derived from the *trans*-Golgi network. The mechanism underlying this localization-dependent specificity remained unclear. Ungermann and colleagues now show that phosphorylation of the HOPS component Vps41 affects its affinity for vesicles, and thus regulates fusion specificity (*J. Cell Biol.* **191,** 845–859; 2010).

The kinase Yck3 was previously shown to phosphorylate Vps41, and inhibition of Vps41 phosphorylation blocked AP-3 vesicle fusion and caused Vps41 to accumulate at endosomes. An intact Vps41 ALPS motif, which is known to associate with highly curved membranes and contains the Yck3 phosphorylation site, was essential for association with small vesicles. This raises the possibility that ALPS motif phosphorylation attenuates Vps41 binding to endosomes.

How then does Vps41 regulate AP-3 vesicle fusion? Vps41 possesses a binding site for the AP-3 complex, and this region was necessary for its efficient vacuolar localization. However, AP-3 could not interact with vesicle-bound Vps41. As the AP-3 pathway delivers Yck3 to vacuoles, the authors speculate that unphosphorylated Vps41 preferentially associates with endosomes and directs endosome–vacuole fusion. Once at vacuoles, Yck3-mediated phosphorylation of Vps41 lessens its affinity for vesicles and also exposes the AP-3 binding motif, permitting HOPS-directed fusion of AP-3 vesicles. EJC

Cancer cells harness metabolism through ENTPD5

Cancer cells sustain their high proliferative rates through the Warburg effect, the metabolic process of aerobic glycolysis that allows the use of glycolytic intermediates for macromolecule production. Fang *et al*. now report that the PI3K/Akt pathway increases aerobic glycolysis by upregulating ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5), an endoplasmic reticulum (ER) UDPase involved in protein *N*-glycosylation and folding (*Cell* **143,** 711-724; 2010).

The authors discovered that Akt activation through loss of the PTEN tumour suppressor leads to increased ATP hydrolysis through the transcriptional derepression of ENTPD5. Biochemical experiments demonstrated that ATP-to-AMP conversion requires the enzymatic activities of ENTPD5, the UMP/CMP kinase CMPK1 and adenylate kinase-1. This ENTPD5-dependent ATP hydrolysis cycle results in increased aerobic glycolysis and lactate production by affecting glucose influx and activating glycolysis enzymes. The proposed role of ENTPD5 in this setting is to respond to elevated Akt-driven translation rates by ensuring correct *N*-glycosylation and folding of polypeptides in the ER. Consistent with this, loss of ENTPD5 activity leads to ER stress, decreased *N*-glycosylation and growth factor receptor protein levels and growth arrest *in vivo*. Furthermore, elevated ENTPD5 correlates with Akt activity and loss of Pten in human tumour samples.

Thus, ENTPD5 illuminates one of the mechanisms employed by cancer cells to respond to the increased energetic demands of PI3K/Akt driven proliferation. AIZ

Defining pluripotency in human embryonic stem cells

A genome-wide RNAi screen to identify genes required for human embryonic stem cell (ESC) identity, reveals a role for the transcription factor PRDM14 in controlling the pluripotency programme (*Nature* **468,** 316–320; 2010). 566 candidates were identified in an initial screen looking for genes the silencing of which reduced the expression of the pluripotency gene POU5F1. Further validation using additional cell lines and stem cell markers SOX2 and NANOG generated 127 hits, among these the transcriptional regulator PRDM14 and the chromatin remodelling factor NFRKB. Expression of the transcription factors OCT4, SOX2 and KLF4 induces pluripotency in somatic cells. The authors found that adding either PRDM14 or NFRKB to the cocktail increased the fraction of induced pluripotent stem cells. Depletion of PRDM14 from human ESCs (but not from mouse ESCs) downregulates stemness gene expression, while increasing differentiation-associated genes. ChIP-sequencing analysis revealed that PDRM14 binds to the promotors of 2,755 genes where it often co-localizes with other members of the pluripotency transcription factor network OCT4, SOX2, NANOG and p300. Gene profiling showed that PRDM14 has both positive and negative effects on transcription. Furthermore, PRDM14 binds and activates the CR2 enhancer to directly control POU5F1 expression. CKR

Microtubules control cell length homeostasis

Intrinsic mechanisms control cell shape and size in unicellular organisms, but it has remained unclear to what extent such control can be exerted by single cells from multicellular organisms. A study by the groups of Baum and McKendry now show that an oriented population of microtubules drive cell extension while subsequent interactions of the microtubules with the cell cortex induces their depolymerisation to maintain cell length (*PLoS Biol.* doi: 10.1371/journal.pbio.1000542).

Growing haemocyte-derived *Drosophila* cells and HeLa cells on micro-contact-printed lines, they demonstrate that animal cells spread to a steady-state length that is independent of pattern width and cell volume. Whereas siRNA-mediated knockdown of the actin regulators SCAR/WAVE or Rac did not affect cell length, microtubule inhibitors blocked elongation, indicating that microtubules control cell length independently of the actin cytoskeleton. Microtubule inhibitors also reduced the height of neural tube epithelial cells in Zebrafish embryos. The authors observed that microtubules became progressively polarized and led cell extension, but depolymerized or bent on contact with the cell cortex. A mathematical model indicates that microtubule catastrophe indeed increases as the microtubules approach the cell cortex, such that only a few microtubules make it to the end of the cell, and this is sufficient to maintain a steady-state cell length without promoting further growth.

This study recapitulates cell spreading dynamics and the maintenance of a steady-state cell length using a simple model of microtubule-dependent cell elongation and cortical-induced catastrophe. Future research will be needed to understand how this cell intrinsic mechanism is integrated in multicellular organisms. IO

By Emily J. Chenette, Iley Ozerlat, Christina Karlsson Rosenthal & Alexia-Ileana Zaromytidou