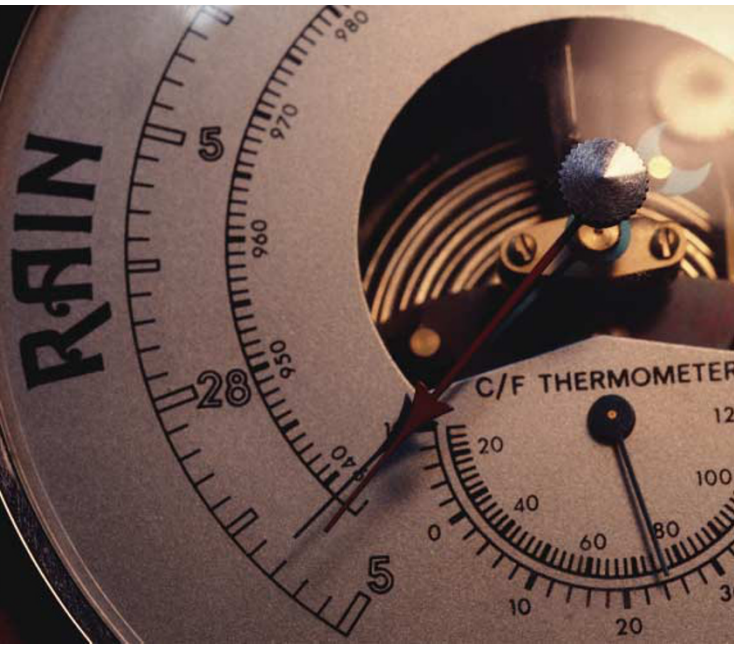
LIPID METABOLISM

Sensing change



Reporting in *Science*, Levine and colleagues provide new insights into how cells coordinate changes in the composition of their cellular membranes with lipid metabolism. In yeast, in response to inositol (a precursor of the phospholipid phosphatidylinositol), it is known that the endoplasmic reticulum (ER)-bound transcription factor Opi1 becomes active and represses the transcription of a gene, *INO1*. *INO1* encodes an enzyme that is important for inositol synthesis. But, to induce this negative-feedback loop, what signal does inositol generate and how does Opi1 sense this signal?

The authors first showed that inositol caused green-fluorescent-protein–Opi1 to translocate from its ER location to the nucleus. As the timing of this change correlated with a decrease in *INO1* mRNA levels, they proposed that this change is physiologically relevant. But what is Opi1 sensing?

By labelling the phospholipid pool in the ER with [^{32}P]-orthophosphate, Levine and co-workers showed that the nuclear translocation of Opi1 was temporally related to large changes in the levels of phosphatidylinositol and its precursors — the latter were consumed to produce the former. So, they wondered whether Opi1 might be directly sensing these changes.

It has long been thought that phosphatidic acid (PA), a precursor of phosphatidylinositol, is involved in the regulation of lipid metabolism, so the authors tested whether Opi1 could bind PA. They showed that Opi1 could indeed bind PA, both directly and specifically, and that Opi1 could detect PA-rich membranes *in vivo*.

Except for the presence of one or more basic residues, there is no known PA-binding motif. However, Levine and colleagues showed that Opi1 seems to contain two PA-binding sites — one in its carboxy-terminal half and another in a basic domain in the second quarter of the protein. When they mutated this basic domain to disrupt the PA-binding

MEMBRANE TRAFFICKING

Actin' at a new site

Actin is already known to be important in endocytosis. But, in *The Journal of Cell Biology*, Drubin and colleagues now report new roles for actin and the HIP1R linker protein in the budding from the *trans*-Golgi network (TGN) of clathrin-coated vesicles (CCVs) that are bound for lysosomes.

Actin is involved in CCV formation at the plasma membrane, and short actin filaments have also been observed close to the Golgi apparatus in mammalian cells. HIP1R functions as a linker between the actin cytoskeleton and components of the endocytic machinery, such as clathrin. So, Drubin and co-workers speculated that actin and HIP1R might be involved in vesicle-trafficking processes that occur at the TGN.

Initially, fluorescently tagged HIP1R was identified on CCVs that were closely associated with the TGN. Time-lapse microscopy then showed TGN-derived vesicles that contained both HIP1R and clathrin moving away from the TGN. These vesicles also contained the cation-dependent mannose-6-phosphate receptor (CD-MPR), which is involved in protein delivery to lysosomes.

A small interfering (si)RNA approach was then used to deplete HIP1R expression in cultured cells, which caused disruption of the TGN. Although other organelles appeared normal, the Golgi cisternae were swollen and had an accumulation of clathrin-coated buds. Furthermore, there was an increase in the size and number of lysosome-like stuctures in these cells. These observations pointed to an impairment in CCV trafficking between the TGN and lysosomes, and possibly also in lysosome function in HIP1R-depleted cells.

Actin filaments were detected associated with the TGN and CCVs in normal cells, which indicates a role for actin in CCV formation. However, HIP1R-depleted cells showed a significant increase in the number and size of these actin structures, so HIP1R might negatively regulate actin polymerization during CCV formation. Furthermore, this regulation might be required for efficient CCV release from the TGN or for subsequent movement of the CCVs. The actin-nucleating Arp2/3 complex was also identified on TGN-derived CCVs, indicating that it might stimulate, while HIP1R limits, actin assembly at these structures.

To test whether HIP1R has a role in trafficking from the TGN to lysosomes, cathepsin D — which is present in the TGN as a pro-enzyme and matures during trafficking to lysosomes — was studied using pulse-chase experiments in HIP1R-depleted cells. More of the pro-enzyme form of cathepsin D was retained in the TGN in HIP1R-depleted cells than in normal cells, an effect that was specific to the HIP1R knockdown. Treating cells with actin poisons also had a severe affect on cathepsin-D maturation, causing it to be retained in the Golgi.

The authors have therefore established a new role for actin dynamics in trafficking from the TGN to lysosomes and have shown that HIP1R is necessary for the productive coupling of actin dynamics to this pathway.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPER Carreno, S. *et al.* Actin dynamics coupled to clathrin-coated vesicle formation at the *trans*-Golgi network. *J. Cell Biol.* **165**, 781–788 (2004)