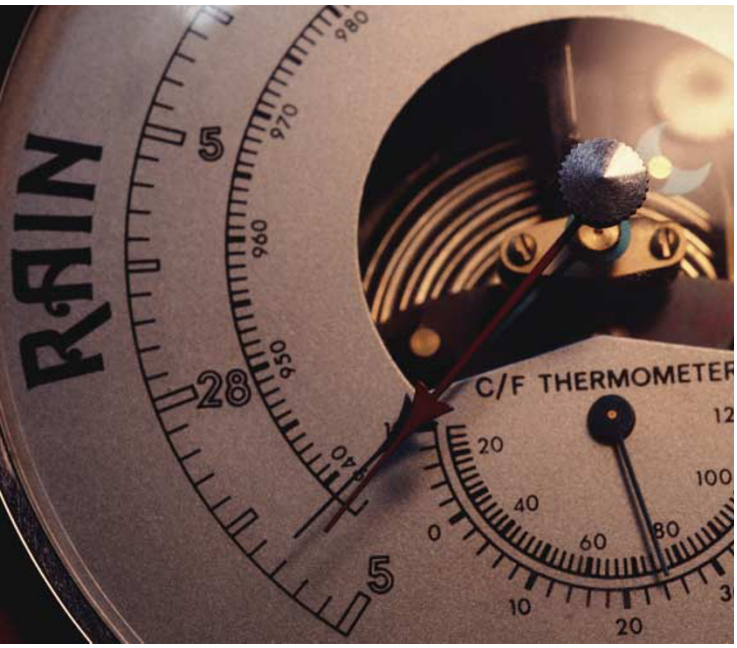
LIPID METABOLISM

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)

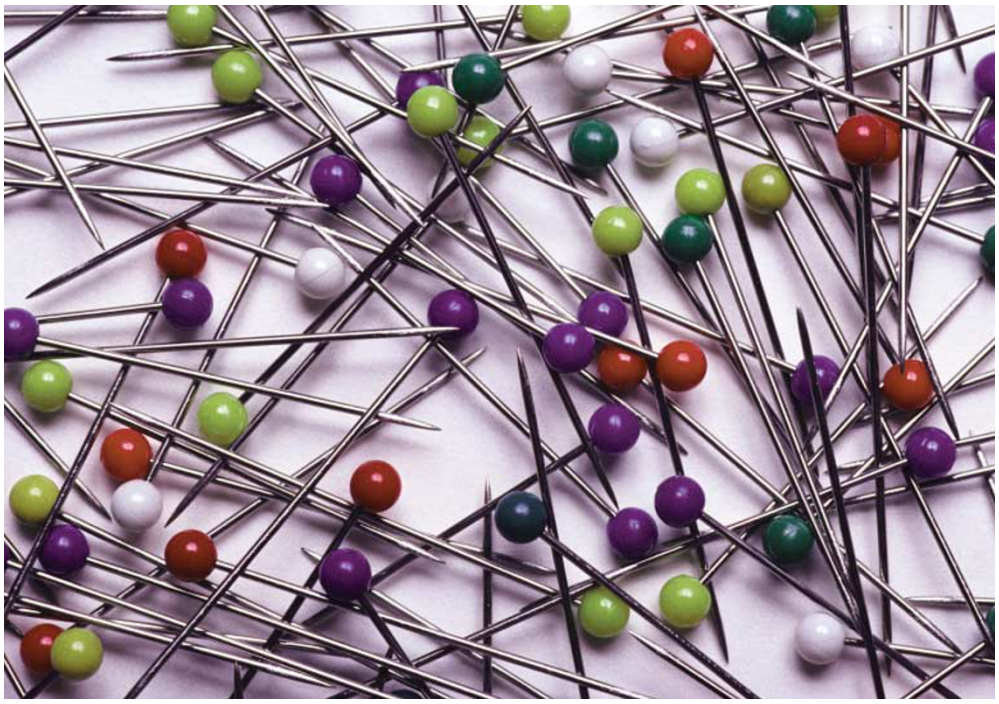
ability of Opi1, they found that Opi1 was activated and translocated to the nucleus in the absence of inositol.

So, this work has clarified the details of a negative-feedback loop that controls phospholipid metabolism in yeast. The ER pool of PA directly binds to Opi1 to maintain it in an inactive state, but in the presence of inositol, this pool is consumed. The decreasing PA levels result in the release of Opi1 from the ER and its translocation to the nucleus, where it can repress the synthesis of inositol. Although many proteins are known to bind PA, the physiological significance of this has been unclear. This study, which has shown a physiological response to changing PA levels, therefore indicates that specific pools of PA might have important signalling roles in other cells.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Loewen, C. J. R. *et al.* Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. *Science* **304**, 1644–1647 (2004)



TUMOUR SUPPRESSORS

Pinning down p73

Two recent studies, one in *Molecular Cell* and the other in *The Journal of Experimental Medicine*, have shed new light on the molecular mechanisms that regulate the function and stability of p73 — a close relative of the tumour suppressor p53.

In the first study, Giannino Del Sal and colleagues investigated whether the prolyl isomerase Pin1, which is involved in regulating the apoptotic function of p53, has a similar effect on p73 function. This turned out to be the case, as Pin1 strongly enhanced p73-dependent apoptosis in *p53*-null cells. This effect correlated with the Pin1-dependent induction of pro-apoptotic p73 target genes, including PUMA and $p53^{AIP1}$.

Pin1 recognizes phosphorylated serine or threonine residues followed by proline (pSer/pThr-Pro; where p denotes phosphorylation), and Pin1 binding induces a conformational change in its substrate. Mutational analysis of Ser/Thr-Pro sites in p73 identified three residues in the carboxyl terminus that, when mutated, decreased the Pin1-binding ability and the transcriptional activity of p73. The authors showed a Pin1-dependent conformational change in p73 *in vitro*, and found that Pin1-modified p73 has an increased affinity for the acetyl transferase p300. The Pin1-induced conformational change caused the p300-mediated acetylation of p73, which, in turn, promoted the transcriptional activation of p73 gene targets.

Pin1 binding to p73 also increased the half-life of p73, and the absence of Pin1 destabilized its substrate, both in normal and genotoxic-stress conditions. p73 is activated and stabilized in response to genotoxic stress by the Abl tyrosine kinase, through the p38 mitogen-activated protein kinase (MAPK) pathway. Del Sal and co-workers showed that the overexpression of Abl or p38 increased Pin1–p73 binding in response to genotoxic stress. And, importantly, the Abl-mediated stabilization of p73 and the activation of its apoptotic function were dependent on the presence of Pin1. So, the Del Sal group concluded that Pin1 is essential for the activation of the apoptotic response by p73, and that, given its role in the functioning of p53, Pin1 might be a common regulator of the p53 family.

Pier Paolo Pandolfi and co-workers also studied p73 stability, although from a different angle. The degradation of p53 is regulated by the ubiquitin–proteasome pathway, and the Pandolfi group showed that the same pathway is responsible for p73 degradation. They then made an interesting observation — the overexpression of the tumour suppressor promyelocytic leukaemia (PML) caused the dose-dependent accumulation of p73, which coincided with reduced levels of ubiquitylated p73.

So, PML seems to protect p73 from ubiquitylation, but how? PML is known to promote the acetylation of p53 by CREB-binding protein (CBP), and Pandolfi and colleagues showed that PML has a similar effect on p73 — it causes the p300-mediated acetylation and stabilization of p73. The authors suggest that the competition between acetylation and ubiquitylation might be responsible for regulating the steady-state levels of p73, which represents a new mechanism for the regulation of protein stability.

Given the involvement of PML in the pathogenesis of acute promyelocytic leukemia (APL), p73 might also be implicated in this disease. The authors hypothesize that the stability and activity of p73 might be compromised in APL. Finally, the findings of these studies make one wonder how p63 stability and function are regulated…

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Mantovani, F. *et al*. Pin1 links the activities of c-Abl and p300 in regulating p73 function. *Mol. Cell* **14**, 625–636 (2004) | Bernassola, F. *et al*. Ubiquitin-dependent degradation of p73 is inhibited by PML. *J. Exp. Med.* **199**, 1545–1557 (2004)

FURTHER READING Urist, M. & Prives, C. The linchpin? Pin1 meets p73. *Cancer Cell* **5**, 515–517 (2004) | Bernardi, R. *et al*. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nature Cell Biol.* **6**, 665–672 (2004)