MEMBRANE TRAFFIC

FYVE fingers grab endosomes

Over the years, many investigators have set out to look for a protein regulator of their favourite protein and instead found a phosphoinositide. Most labs have therefore developed at least a peripheral interest in this family of lipids. So they will be happy to read in the *EMBO Journal* that the laboratories of Harald Stenmark and Rob Parton have devised a new probe to study the cellular localization of phosphatidylinositol-3-phosphate (PtdIns(3)P).

The products of phosphatidylinositol-3-OH kinases function in processes as diverse as signal transduction, cytoskeletal organization and apoptosis. PtdIns(3)P is particularly interesting for membrane traffic aficionados, as it regulates transport along the endocytic pathway in all species where this has been studied. One of its activities is to recruit proteins that contain PtdIns(3)P-binding FYVE finger domains to membranes. But to which membranes?

Gillooly *et al.* reasoned that if PtdIns(3)P binds FYVE domains, then FYVE domains should bind PtdIns(3)P. They built a probe (2XFYVE) consisting of two FYVE domains from Hrs, a protein that acts in the endocytic pathway. A series of control experiments showed that 2XFYVE binds selectively to PtdIns(3)P, both *in vivo* and *in vitro*. The probe effectively competes with endogenous proteins for PtdIns(3)P binding when transfected into cells, and can be used for immunofluorescence as well as for immunoelectron microscopy studies.

The next step was to use 2XFYVE to localize PtdIns(3)P in the cell. The FYVE finger protein EEA1 is known to localize exclusively to early endosomes, where it is involved in membrane fusion. So it was clear from the start that there must be substantial amounts of PtdIns(3)P in the membrane of early endosomes. This was confirmed in this study — 2XFYVE, shown in red in the picture, colocalized extensively with EEA1, shown in green.

More surprisingly, immunoelectron microscopy using 2XFYVE revealed that PtdIns(3)P is also present on internal membranes of multivesicular late endosomes. This observation led the authors to speculate that the PtdIns(3)P-containing intralumenal vesicles arise from invagination of the endosomal membrane. This could sequester PtdIns(3)P away from the surface, stopping it from recruiting cytoplasmic proteins such as EEA1 to late endosomes. The origin of the convoluted morphology of multivesicular endosomes is still mysterious, and 2XFYVE might prove a useful tool to study this process.

This probe is not the first on the market. The pleckstrin homology (PH) domain of phospholipase Cδ1 and the PH domains of ARNO and Bruton's tyrosine kinase have been used to detect PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, respectively. But these probes cannot be used for electron microscopy, and neither of them shows the exquisite specificity for its target that 2XFYVE seems to have.

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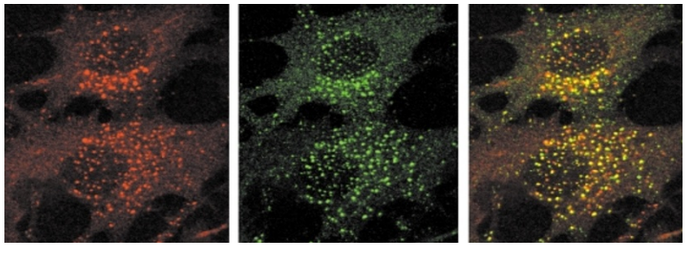
References and links

ORIGINAL RESEARCH PAPER Gillooly, D. J. *et al*. Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J.* **17**, 4577–4588 (2000)

FURTHER READING Corvera, S., D'arrigo, A. & Stenmark, H. Phosphoinositides in membrane traffic. *Curr. Opin. Cell Biol.* **11**, 460–465 (1999)



IN BRIEF

APOPTOSIS

P53AIP1, a potent mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53.

Oda, K. *et al. Cell* **102**, 849–862 (2000)

The star of this story — *p53AIP1* — is a newly cloned gene, the protein product of which localizes to the mitochondria. It causes apoptotic cell death by dissipating the mitochondrial transmembrane potential, and it could help to mediate p53-dependent apoptosis. After severe DNA damage, phosphorylation of a specific residue (serine 46) on p53 leads to apoptosis. The authors show that substitution of Ser 46 not only inhibits p53-dependent apoptosis, but also blocks expression of p53AIP1.

TRANSLOCATION

Two intermembrane space TIM complexes interact with different domains of Tim23p during its import into mitochondria.

Davis, A. J. *et al. J. Cell Biol.* **150**, 1271–1282 (2000)

How are mitochondrial proteins targeted for either insertion into the mitochondrial inner membrane or translocation into the matrix? Davis *et al*. show that the inner membrane protein Tim23p interacts with both known intermembrane space TIM complexes before reaching the Tim22p inner membrane translocon. But only its interaction with one of these complexes — Tim9p–Tim10p — is essential for correct targeting, leaving the mystery of what the Tim8p–Tim13p complex does intact.

STEM CELLS

Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells.

Schuldiner, M. *et al. Proc. Natl Acad. Sci. USA* **97**, 11307–11312 (2000)

Until now there has been no systematic attempt to determine how growth factors affect the lineage choice of embryonic stem cells. This broad study correlates different growth-factor treatments with cell morphology and expression of markers for 11 tissues, derived from all three germ layers. It shows that what you put in biases, but doesn't absolutely determine, what you get out, and has obvious implications for stem-cell therapy.

TECHNOLOGY

Harnessing the ubiquitination machinery to target the degradation of specific cellular proteins.

Zhou, P. *et al. Mol. Cell* **6**, 751–756 (2000)

This paper uses a neat trick to functionally 'knock out' stable proteins — by targeting them for proteasomal degradation. By engineering specific protein–protein interaction domains into one component of the SCF complex, a multimeric ubiquitin-conjugating machine, Zhou and colleagues can send proteins to their death in both yeast and mammalian cells, and can measure the phenotypic consequences.