ENDOCYTOSIS AND SIGNALLING

It's what's inside that counts

Phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) at cellular membranes can recruit and activate various proteins that activate downstream signalling pathways. However, little is known about the dynamics of PtdIns(3,4,5)P_3 production, because there has been no technique to analyse the spatial and temporal dynamics of this process quantitatively in single living cells. However, now, in *Nature Cell Biology*, Umezawa and colleagues present a new fluorescent indicator — called 'fllip' — that has allowed them to study PtdIns(3,4,5)P_3 dynamics *in vivo* based on fluorescence resonance energy transfer (FRET).

Fllip contains a pleckstrin-homology (PH) domain (which binds PtdIns(3,4,5)P_3 selectively) fused between cyan- and yellow-fluorescent-protein (CFP and YFP) variants using rigid linkers. One of these linkers contains a di-glycine motif that functions as a hinge, and an additional rigid linker attaches the indicator to a membrane-localization sequence (MLS). When the central PH domain of fllip binds PtdIns(3,4,5)P_3, a 'flip-flop-type' conformational change occurs through the flexible hinge, which can be detected as intramolecular FRET from CFP to YFP.

After showing that the PtdIns(3,4,5)P_3-binding specificity of the PH domain was unaffected by its inclusion in fllip, Umezawa and co-workers showed that fllip could be targeted to specific membranes using specific MLSs. Fllip that was observed mainly at the plasma membrane was designated fllip-pm, whereas fllip that could be seen at endomembranes (that is, at the endoplasmic reticulum (ER) and Golgi) was designated fllip-em. Furthermore, they showed that the PtdIns(3,4,5)P_3-dependent increase in FRET between CFP and YFP is dose dependent.

Next, Umezawa and colleagues studied the response of fllip-pm and fllip-em to physiological stimulation by studying the effect of platelet-derived growth factor (PDGF) on PDGF receptor (PDGFR)-expressing cells. PDGF promotes PDGFR dimerization, which results in PDGFR phosphorylation and activation. Phosphorylated PDGFR then recruits and activates phosphatidylinositol 3-kinase (PI3K), which results in PtdIns(3,4,5)P_3 production.

The authors found that, after PDGF stimulation, the levels of PtdIns(3,4,5)P_3 increased at the plasma membrane as expected, and also at endomembranes. There was, however, a delay before the increase occurred at endomembranes. In addition, the increase at endomembranes was two- to threefold greater than at the plasma membrane. So, what is the molecular mechanism behind the PtdIns(3,4,5)P_3 increase at endomembranes?

Umezawa and co-workers showed that overexpression of a dominant-negative dynamin mutant blocked the PDGF-induced increase in PtdIns(3,4,5)P_3 levels at endomembranes, but not at the plasma membrane (dynamin controls clathrin-mediated endocytosis of receptor tyrosines kinases (RTKs) such as PDGFR). They observed the same effect when they overexpressed protein tyrosine phosphatase-1B (this phosphatase is localized exclusively to the cytoplasmic surface of the ER, where it has been proposed to inactivate RTKs). These results indicate that PtdIns(3,4,5)P_3 production "…is increased at endomembranes when activated PDGFR is internalized from the plasma membrane to endomembranes by clathrin-coated endocytosis, thereby activating [PI3K] at endomembranes".

So, PtdIns(3,4,5)P_3 at endomembranes is produced *in situ*, and is not transported to this location by plasma-membrane-derived endocytic vesicles. Umezawa and colleagues have therefore suggested that endocytosed RTKs activate PI3K on endomembranes before they are downregulated at the ER. This work has addressed a long-standing question about how PtdIns(3,4,5)P_3 activates its downstream signalling pathways at intracellular compartments that are remote from the plasma membrane, and has also provided us with a new type of fluorescent indicator that could be used to study other lipid second messengers.

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URLs

pleckstrin-homology (PH) domain
http://srs.ebi.ac.uk/srs6bin/cgi-bin/wgetz?-id+4Flds1EeiJO+-e+[InterPro-AccNumber:'IPR001849']

YFP
http://ca.expasy.org/cgi-bin/nicelprot.pl?P21578

PDGF
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=5154%20or%205155%20and%20PDGF&ORG=Hs

PDGFR
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=PDGFR2%20or%205159&ORG=Hs

References and links

ORIGINAL RESEARCH PAPER Sato, M. *et al.* Production of $PtdInsP_3$ at endomembranes is triggered by receptor endocytosis. *Nature Cell Biol.* 5 Oct 2003 (doi:10.1038/ncb1054)

WEB SITE

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