EMBO reports

literature report

Of yeast and men

The evolution of PtdIns(3,4,5)P_3 synthesis

Since the identification of the lipid second messenger phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3; Traynor-Kaplan *et al*, 1988; Auger *et al*, 1989), this molecule has become a paradigm for how phosphoinositides (PIs) mediate complex signalling events in cells. PtdIns(3,4,5)P_3 can regulate chemotaxis, cell survival, translation and cytoskeletal rearrangement (Cantley, 2002), the deregulation of which contributes to the pathology of diseases such as diabetes, and breast and prostate cancer.

PtdIns(3,4,5)P_3 is a phosphorylated form of the PI phosphatidylinositol (PtdIns). All PIs have two fatty-acid chains that anchor them to membranes and a hydrophilic inositol head group that is exposed to the aqueous cytosol (Fig 1A). This head group can be phosphorylated at the 3-, 4- and 5-positions and so far, a total of seven phosphorylated derivatives of PtdIns have been reported (Fig 1A).

After the treatment of cells with certain agonists, the transient synthesis of PtdIns(3,4,5)P_3 is initiated by class I PI 3-kinases. These lipid kinases are normally inactive; however, agonist stimulation increases their ability to phosphorylate the 3-position of PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3 (Hawkins *et al,* 1992). The receptor-driven activation of class I PI 3-kinases can give rise to greater than a tenfold increase in the cellular levels of PtdIns(3,4,5)P_3 (which rarely approach 0.1% of total PI levels, making its detection difficult—even for the more hardened lipid biochemists among us). In turn, subsets of proteins that specifically recognize and bind to PtdIns(3,4,5)P_3 (mediated by specialist lipid-binding modules such as pleckstrin homology (PH) domains) are then recruited to the membrane, which often leads to their activation and further transduction of the agonist signal (Fig 1B; Lemmon, 2003).

Recently, PtdIns(3,4,5)P_3 has stepped into the scientific limelight once again, this time not because of its biological function but intriguingly because of the way it is synthesized in fission yeast. A recent article in the *Journal of Cell Biology* (Mitra *et al,* 2004) has revealed for the first time that *Schizosaccharomyces pombe* can generate the 3-phosphorylated lipid PtdIns(3,4,5)P_3. This is exciting because researchers have assumed that yeast are incapable of producing PtdIns(3,4,5)P_3. This dogma has arisen as PtdIns(3,4,5)P_3 has never been found in yeast, and yeast do not have a class I PI 3-kinase, which suggests that they lack one of the essential reaction steps necessary to generate PtdIns(3,4,5)P_3.

The paper by Mitra and co-workers begins with the identification of *ptn1*, a homologue of the mammalian phosphatase and tensin homologue (PTEN) gene. PTEN is a lipid phosphatase (Maehama *et al*, 1998), which dephosphorylates the 3-position of PtdIns(3,4,5)P_3 to generate PtdIns(4,5)P_2. When the authors tested the lipid phosphatase activity of Ptn1 *in vitro,* they found that it also dephosphorylated PtdIns(3,4,5)P_3. This led Mitra and colleagues to question why *S. pombe* would have maintained a functional PtdIns(3,4,5)P_3 phosphatase in its genome if not to regulate PtdIns(3,4,5)P_3 levels.

In mammalian cells, disruption of PTEN activity leads to elevated PtdIns(3,4,5)P_3 levels and surprisingly, examination of the phosphoinositide levels in an *S. pombe* strain lacking *ptn1* (*ptn1*Δ) revealed a dramatic increase in PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. This finding is noteworthy in itself; however, the authors went on to postulate that *S. pombe* synthesizes PtdIns(3,4,5)P_3 through a pathway that is different to the one used by mammalian cells after growth factor stimulation.

Yeast have only one PI 3-kinase homologue, Vps34, which regulates vesicle transport to the vacuole (Takegawa *et al,* 1995). When a strain lacking *vps34* was crossed with *ptn1*Δ, the authors found that levels of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 decreased. Unlike class I PI 3-kinases, Vps34 can only phosphorylate PtdIns to generate PtdIns(3)P *in vitro*, indicating that it is not the 3-phosphorylation of PtdIns(4,5)P_2, but the synthesis of PtdIns(3)P that is the important step in *S. pombe* PtdIns(3,4,5)P_3 synthesis. Mitra and colleagues then turned their attention to the kinases that might phosphorylate the 4- and 5-positions of the inositol head group. The lipid kinase Fab1 can phosphorylate PtdIns(3)P at the 5-position; however, its deletion did not affect the PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3 production in *ptn1*Δ cells, indicating that there was no role for Fab1 in PtdIns(3,4,5)P_3 synthesis. Next, they examined Its3, the *S. pombe* homologue of the mammalian phosphatidylinositol-4-phosphate 5-kinase (PIP 5-K) family, which are key enzymes in the regulation of PtdIns(4,5)P_2 levels. When a strain retaining only 10% of wild-type PIP 5-K activity was crossed with *ptn1*Δ, levels of PtdIns(4,5)P_2 were lowered as expected. Surprisingly, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 levels were also dramatically attenuated. As PtdIns(4,5)P_2 is not a substrate for Vps34, this suggests a novel role for Its3 in PtdIns(3,4,5)P_3 synthesis. (It is possible that yeast have a PtdIns(4,5)P_2 3-kinase that is yet to be identified or that PtdIns(4,5)P_2 can allosterically regulate PtdIns(3,4,5)P_3 synthesis.) *In vitro*, mammalian PIP 5-Ks can sequentially phosphorylate PtdIns(3)P at the 4- and 5-positions to generate PtdIns(3,4,5)P_3 (Zhang *et al,* 1997), and this led Mitra and colleagues to propose that PtdIns(3)P generated by Vps34 is converted to PtdIns(3,4,5)P_3 by Its3 (Fig 1C).

What is the function of PtdIns(3,4,5)P_3 in yeast? Although the *ptn1*Δ strain displayed vacuolar defects and was more susceptible to osmotic stress than wild-type yeast, neither of these observations were directly linked to elevated PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 levels. Importantly, the authors demonstrated that at least two *S. pombe* proteins can bind PtdIns(3,4,5)P_3 *in vitro*, with the intracellular localization of one of them showing some dependency on the presence of Ptn1. This raises the possibility that PtdIns(3,4,5)P_3-dependent signalling networks exist in yeast, analogous to those described in metazoan systems.

So, what are the consequences of these findings? The fact that the PIP 5-K-driven PtdIns(3,4,5)P_3 synthesis pathway is present in yeast indicates that, historically, this was the original pathway that organisms evolved to generate PtdIns(3,4,5)P_3. Class I PI-3-kinases

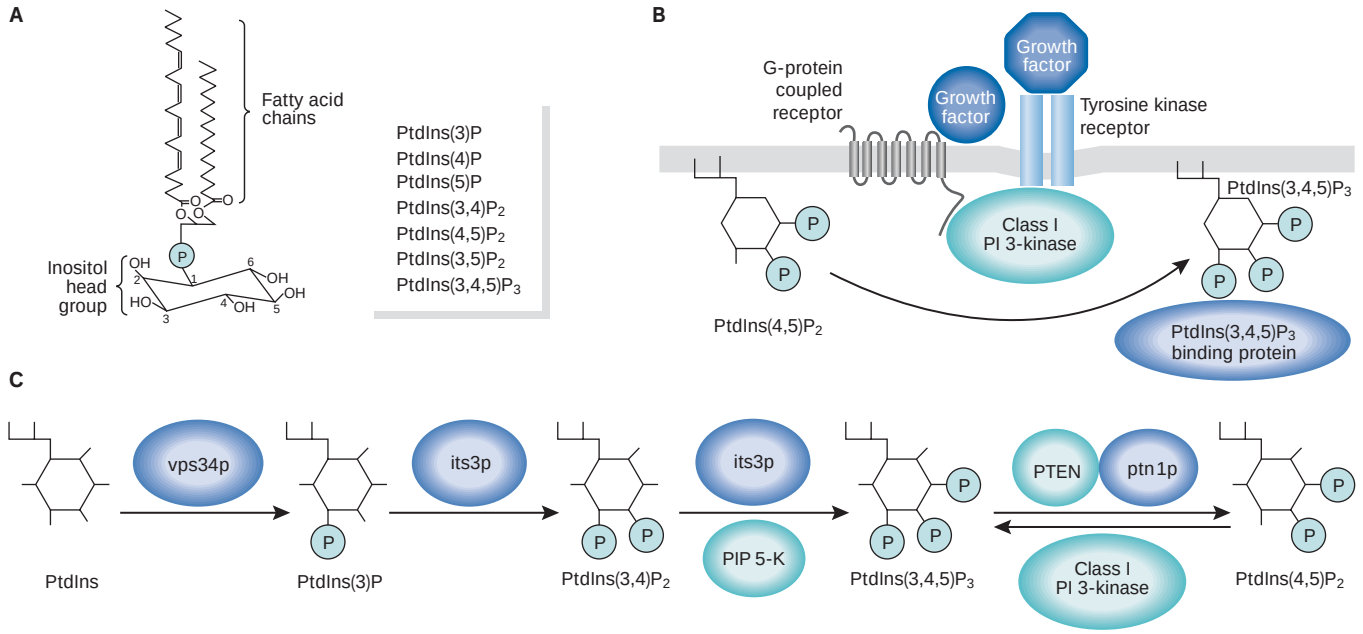

Fig 1 | Pathways of PtdIns(3,4,5)P_3 synthesis. (**A**) Phosphatidylinositol (PtdIns) is the basic building block of phosphorylated phosphoinositides (PIs). The inositol head group can be phosphorylated in the 3-, 4- and 5-positions to generate derivatives of PtdIns, which are listed on the right. **(B)** Schematic for receptor-driven PtdIns (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3) production. Agonist stimulation (tyrosine kinase or G-protein-coupled receptors) activates class I PI 3-kinase to induce phosphorylation of PtdIns(4,5)P_2 on the 3-position of the inositol group. **(C)** The proposed new pathway for PtdIns(3,4,5)P_3 production in *Schizosaccharomyces pombe*. Fission yeast enzymes are shown in blue. Their mammalian counterparts are depicted in turquoise. PIP 5-K, phosphatidylinositol-4-phosphate 5-kinase; PTEN, phosphatase and tensin homologue.

evolved much later, which suggests that the growth-factor-driven pathway found in *Caenorhabditis elegans* and mammalian cells is a 'new kid on the block'. If so, then what became of the PIP 5-K-driven PtdIns(3,4,5)P_3 pathway in mammalian cells? Intriguingly, whereas growth-factor-stimulated PtdIns(3,4,5)P_3 synthesis is dependent on class I PI 3-kinases in mammalian cells, we recently showed that certain stress agonists, such as H_2O_2, increase PtdIns(3,4,5)P_3 levels *in vivo* by the PIP 5-K-mediated 5-phosphorylation of PtdIns(3,4)P_2 (Halstead *et al*, 2001).

Finally, on a clinical note, PTEN (a tumour suppressor) deletion in human cancers leads to the activation of cell survival pathways through the upregulation of PtdIns(3,4,5)P_3 levels. In light of these new findings that connect PTEN with PIP 5-K-driven PtdIns(3,4,5)P_3 synthesis, it would be interesting to dissect how PtdIns(3,4,5)P_3 is generated in these cancer cells.

At the moment, this new, but ancient, biochemical pathway for PtdIns(3,4,5)P_3 synthesis seems to be an 'outsider' in the field of cell biology. Hopefully, with further study, it too can find a place of its own.

REFERENCES

Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC (1989) PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. *Cell* **57:** 167–175

Cantley LC (2002) The phosphoinositide 3-kinase pathway. *Science* **296:** 1655–1657

Halstead JR, Roefs M, Ellson CD, D'Andrea S, Chen C, D'Santos CS, Divecha N (2001) A novel pathway of cellular phosphatidylinositol(3,4,5)-trisphosphate synthesis is regulated by oxidative stress. *Curr Biol* **11:** 386–395

Hawkins PT, Jackson TR, Stephens LR (1992) Platelet-derived growth factor stimulates synthesis of PtdIns(3,4,5)P_3 by activating a PtdIns(4,5)P_2 3-OH kinase. *Nature* **358:** 157–159

Lemmon MA (2003) Phosphoinositide recognition domains. *Traffic* **4:** 201–213

Maehama T, Dixon JE (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* **273:** 13375–13378

Mitra P *et al* (2004) A novel phosphatidylinositol(3,4,5)P_3 pathway in fission yeast. *J Cell Biol* **166:** 205–211

Takegawa K, DeWald DB, Emr SD (1995) *Schizosaccharomyces pombe* Vps34p, a phosphatidylinositol-specific PI 3-kinase essential for normal cell growth and vacuole morphology. *J Cell Sci* **108:** 3745–3756

Traynor-Kaplan AE, Harris AL, Thompson BL, Taylor P, Sklar LA (1988) An inositol tetrakisphosphate-containing phospholipid in activated neutrophils. *Nature* **334:** 353–356

Zhang X, Loijens JC, Boronenkov IV, Parker GJ, Norris FA, Chan J, Thum O, Prestwich GD, Majerus PW, Anderson RA (1997) Phosphatidylinositol-4-phosphate 5-kinase isozymes catalyze the synthesis of 3-phosphate-containing phosphatidylinositol signaling molecules. *J Biol Chem* **272:** 17756–17761

Nullin Divecha & Jonathan R. Halstead[+] *are at the Netherlands Cancer Institute, Antoni van Leeuwenhoek ziekenhuis, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands*
[+]*Corresponding author.*
Tel: +31 20 512 2009;
Fax: +31 20 512 1989;
E-mail: j.halstead@nki.nl

Keywords: PtdInsP_3 synthesis; phosphoinositides; membrane transport; fission yeast

Submitted 22 July 2004; accepted 28 July 2004

EMBO reports (2004) **5,** 865-866. doi:10.1038/sj.embor.7400242