A protein complex that regulates PtdIns(3,5)P$_2$ levels

Robert H Michell* and Stephen K Dove

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK
*Corresponding author. School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
E-mail: r.h.michell@bham.ac.uk

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Phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P$_2$) is needed for retrograde membrane trafficking from lysosomal and late endosomal compartments and its synthesis is tightly regulated. But how cells regulate PtdIns(3,5)P$_2$ synthesis—for example, in response to hyperosmotic shock—remains unexplained. A paper from the Weisman group gives the most complete picture so far of a multi-protein complex that controls PtdIns(3,5)P$_2$ synthesis and explains how a VAC14 mutation functionally impairs the scaffold protein at the heart of the complex and causes a neurodegenerative condition in mice.

Since phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P$_2$) was discovered a decade ago (Dove et al., 1997), it has become clear that it is a ubiquitous eukaryote phosphoinositide of very low abundance. PtdIns(3,5)P$_2$ probably has multiple functions—the best characterised of which is support, and probably regulation, of retrograde membrane trafficking from lysosomal and late endosomal compartments to the Golgi complex (Dove et al., 2004; Efe et al., 2005; Michell et al., 2006; Shishheva, 2008). Genetic loss or malfunction of several proteins essential to PtdIns(3,5)P$_2$ function is deleterious both in yeast and animals (see Jin et al. (2008) for references). Only recently have the first real hints about how cellular PtdIns(3,5)P$_2$ levels are regulated—for example, how is synthesis stimulated by hyperosmotic shock—begun to emerge, and this is the problem addressed by this study (Jin et al., 2008).

The PtdIns3P 5-kinases that make PtdIns(3,5)P$_2$ are usually named Fab1p (as in yeast) or PIKfyve (in mammals). Fab1p mutants belong to a class of yeast mutants that have enlarged vacuoles and increased stress sensitivity, and well-regulated PtdIns(3,5)P$_2$ synthesis in vivo also needs the products of two other members of this gene group—Vac14p (alias ArPIKfyve in mice) and Vac7p. Proper control of PtdIns(3,5)P$_2$ synthesis also requires two other proteins involved in PtdIns(3,5)P$_2$ function: the PtdIns(3,5)P$_2$ 5-phosphatase Fig4p (Sac3 in mouse), to permit effective PtdIns(3,5)P$_2$ synthesis; and the PtdIns(3,5)P$_2$ effector protein Atdg18p, to prevent PtdIns(3,5)P$_2$ synthesis from going into overdrive (for references, see Efe et al., 2005; Michell et al., 2006). This paper starts by showing that a mutation near the N terminus of Vac14p causes the defects seen in homoygous ingls mice, which are small and have malformed nervous systems and a short lifespan (Bronson et al., 2004).

The central feature of the paper is a new model of the molecular configuration of Vac14p, which the Weisman group earlier showed to be essential for mice to develop a nervous system that can carry them beyond birth (Zhang et al., 2007). There were earlier suggestions that Vac14p contained HEAT domains (Dove et al., 2002), and this paper’s structural modelling uses many Vac14p sequences to predict that Vac14p/ArPIKfyve proteins are constructed entirely of HEAT domains, with at least 15 widely conserved. This suggests that Vac14p probably functions as the scaffold of a multi-protein complex. This study uses a combination of co-immunoprecipitation, yeast two-hybrid, imaging and co-sedimentation studies to show that Fab1p, Vac7p, Fig4p and some Atdg18p join with Vac14p to form a complex at the vacuole membrane. Others have suggested some of these associations before (Botelho et al., 2008; Sbrissa et al., 2008), but they did not envisage such a diverse assemblage.

Mutations were used to characterise the complex further. When the ingls mutation (which is in HEAT domain 4) was put into yeast VAC14, the resulting Vac14p mutant only bound Fig4p—which associated with its C-terminal half—and the cells failed to make large amounts of PtdIns(3,5)P$_2$ when hyper-osmotically stressed. Another VAC14 mutant showing poor Fab1p activation had defects in HEAT domains 2 and 3: its Vac7p and Atdg18p interactions were defective, but it interacted with Fig4p normally. Mutating a chaperone-like domain close to the centre of the FAB1 sequence (see Michell et al., 2006) prevented both the Fab1p–VAC14 interaction and localisation of Fab1p, Vac7p and Fig4p to the vacuole membrane.

These results suggest the existence of a complex of the type shown in Figure 1. Vac14p, maybe as a dimer, is the scaffold. Vac7p and Atdg18p bind close to its N terminus, Fab1p somewhat more centrally, and Fig4p somewhere on the C-terminal half of Vac14p. But much still remains to be worked out—what are the details of the interactions between the proteins in the complex, how do these interactions control the kinase activity of Fab1p, what interactions hold the complex at the vacuole membrane and how do external influences such as osmotic perturbations control PtdIns(3,5)P$_2$ synthesis by this complex?

Figure 1 A schematic representation of the PtdIns(3,5)P$_2$-synthesising complex of yeast at the vacuole membrane, as elucidated by Jin et al. (2008). PtdIns(3,5)P$_2$ is made by Fab1p, which is anchored to Vac14p, as are the interacting Fab1p regulators Vac7p, Fig4p and Atdg18p.
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