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A protein complex that regulates PtdIns(3,5)P_2 levels

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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; Shisheva, 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 PtdIns3*P* 5-kinases that make PtdIns(3,5)P_2 are usually named Fab1p (as in yeast) or PIKfyve (in mammals). *FAB1* 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 Atg18p, 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 homozygous *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 Atg18p 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 Atg18p 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–Vac14p interaction and localisation of Fab1p, Vac14p 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 Atg18p 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?

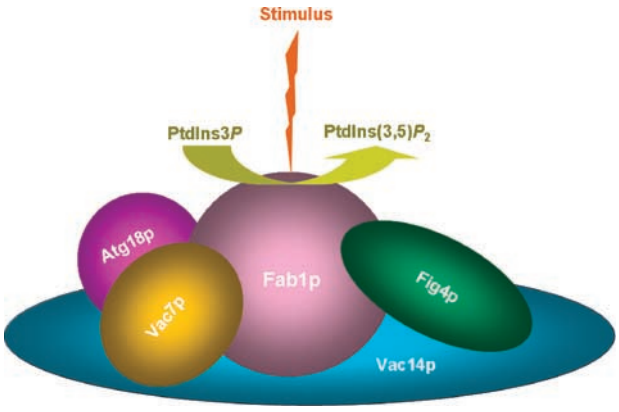

Figure 1 A schematic representation of the PtdIns(3,5)P_2-synthesising complex of yeast at the vacuole membrane, as elucidated by Jin *et al* (2008). PtdIns(3,5)P_2 is made by Fab1p, which is anchored to Vac14p, as are the interacting Fab1p regulators Vac7p, Fig4p and Atg18p.

References

Botelho RJ, Efe JA, Teis D, Emr SD (2008) Assembly of a Fab1 phosphoinositide kinase signaling complex requires the Fig4 phosphoinositide phosphatase. *Mol Biol Cell* **19:** 4273–4286

Bronson R, Perides G, Sweet H, Ward-Bailey PF, Davisson M (2004) Infantile gliosis (ingls) a new spontaneous mutation in the mouse. *MGI Direct Data Submission*, http://mousemutant.jax.org/gliosis_paper.html

Dove SK, Cooke FT, Douglas MR, Sayers LG, Parker PJ, Michell RH (1997) Osmotic stress activates phosphatidylinositol-3,5-bisphosphate synthesis. *Nature* **390:** 187–192

Dove SK, McEwen RK, Mayes A, Hughes DC, Beggs JD, Michell RH (2002) Vac14 controls PtdIns(3,5)P(2) synthesis and Fab1-dependent protein trafficking to the multivesicular body. *Curr Biol* **12:** 885–893

Dove SK, Piper RC, McEwen RK, Yu JW, King MC, Hughes DC, Thuring J, Holmes AB, Cooke FT, Michell RH, Parker PJ, Lemmon MA (2004) Svp1p defines a family of phosphatidylinositol 3,5-bisphosphate effectors. *EMBO J* **23:** 1922–1933

Efe JA, Botelho RJ, Emr SD (2005) The Fab1 phosphatidylinositol kinase pathway in the regulation of vacuole morphology. *Curr Opin Cell Biol* **17:** 402–408

Jin N, Chow CY, Liu L, Zolov SN, Bronson R, Davisson M, Petersen JL, Zhang Y, Park S, Duex JE, Goldowitz D, Meisler MH, Weisman LS (2008) VAC14 nucleates a protein complex that is essential for the acute interconversion of PI3P and PI(3,5)*P*2 in yeast and mouse. *EMBO J* **27:** 3221–3234

Michell RH, Heath VL, Lemmon MA, Dove SK (2006) Phosphatidylinositol 3,5-bisphosphate: metabolism and cellular functions. *Trends Biochem Sci* **31:** 52–63

Sbrissa D, Ikonomov OC, Fenner H, Shisheva A (2008) ArPIKfyve homomeric and heteromeric interactions scaffold PIKfyve and Sac3 in a complex to PROMOTE PIKfyve activity and functionality. *J Mol Biol* **384:** 766–779

Shisheva A (2008) PIKfyve: partners, significance, debates and paradoxes. *Cell Biol Int* **32:** 591–604

Zhang Y, Zolov SN, Chow CY, Slutsky SG, Richardson SC, Piper RC, Yang B, Nau JJ, Westrick RJ, Morrison SJ, Meisler MH, Weisman LS (2007) Loss of Vac14, a regulator of the signaling lipid phosphatidylinositol 3,5-bisphosphate, results in neurodegeneration in mice. *Proc Natl Acad Sci USA* **104:** 17518–17523