Salmonella vacuole maturation: PIKfyve leads the way

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In this issue of *The EMBO Journal*, a study from Rohan Teasdale's laboratory provides evidence that PIKfyve, a PtdIns D5-kinase, is required for maturation of *Salmonella*-containing vacuole (SCV) and *Salmonella* replication. This report sheds light on a novel mechanism that mediates fusion of both macropinosomes and SCV with lysosomal/late endosomal organelles.

Bacterial intracellular pathogens have developed an impressive set of mechanisms to usurp trafficking and signalling pathways in a wide range of host cells (Alonso and Garcia-del Portillo, 2004). Therefore, a mechanistic understanding of host–pathogen interactions does also reveal fundamental cell biology processes (Cossart *et al*, 1996). The study of Kerr and collaborators in this issue of *The EMBO Journal* is another intriguing example of what can be learned from the host–pathogen interface (Kerr *et al*, 2010).

Phosphoinositides (PtdIns) are short-lived phosphorylated forms of phosphatidylinositol that associate with membranes controlling several intracellular processes, including vesicular trafficking. Many intracellular pathogens, including *Salmonella* spp., have evolved strategies to manipulate the PtdIns metabolism for their own benefit (Pizarro-Cerda and Cossart, 2004).

PIKfyve is a kinase that binds PI3P via its FYVE domain and phosphorylates PtdIns in the D-5 position (Shisheva, 2008). The intracellular functions of PIKfyve and its product PI(3,5)P₂ are pleiotropic and still controversial, probably due to its multiple activities (Dove *et al*, 2009). Using 4D live-cell imaging, Kerr and collaborators described for first time that PIKfyve is required for the fusion between macropinosomes and late endocytic organelles in epithelial cells. Notably, this step is independent from Rab7's recruitment into the macropinosome (Bucci *et al*, 2000).

Salmonella invades epithelial cells by inducing macropinocytic uptake (Garcia-del Portillo and Finlay, 1994). Given its role in macropinosome–late endosome fusion, the authors hypothesized that PIKfyve could also be required for *Salmonella* vacuole maturation. Three different approaches confirm this hypothesis: siRNA, expression of dominant-negative kinase and pharmacological inhibition. Functionally, SCV fuses with late endocytic compartments and their acidification is important for the expression/activation of the *Salmonella* pathogenicity island 2 (SPI2) type III secretion system (Beuzon *et al*, 1999; Waterman and Holden,

2003). Here the authors show elegantly that inhibition of PIKfyve impairs acidification and consequently activation of SPI2.

The most significant finding, however, is that the direct impact of PIKfyve inhibition in epithelial cells impairs intracellular replication and *Salmonella*-induced filaments formation, an event associated with adaptation of *Salmonella* to the intracellular environment (Figure 1). In contrast, impairment of PIKfyve activity in macrophages somehow prevents bactericidal activity of professional phagocytes. As mechanisms of entry (invasion versus phagocytosis) likely have an impact on SCV maturation, additional studies will be necessary to dissect this phenomenon.

SCV maturation through late endosomal and lysosomal content delivery is still controversial (Brumell and Grinstein, 2004; Drecktrah *et al*, 2006). Although in this study SCV recruited lysosomal dextran and LAMP-1 via PIKfyve,

Figure 1 Model of the role of PIKfyve in the development of SCV. After internalization *Salmonella* is located in a spacious vacuole (SCV) rich in PI3P. PIKfyve likely leads to production/enrichment of PI(3,5)P₂ and PI5P. Consequently, the SCV becomes fusogenic with lysosomal/late endosomal compartments containing LAMP-1. This fusion allows acidification, SPI-2 effector induction and *Salmonella*-induced filament (SIF) formation. The result is an appropriate niche for *Salmonella* replication.

it would be interesting to study whether lysosomal hydrolases and mannose-6-phosphate receptors are also recruited in a PIKfyve-dependent manner. Importantly, this work suggests that SCV might retain some of the molecular signatures of macropinosomes in terms of PtdIns content at later points of the infection, adding a new perspective to the already complex dynamic process that can lead to the establishment of *Salmonella*'s replicative niche.

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Conflict of interest

The author declares that he has no conflict of interest.