Phosphoinositides in phagolysosome and autophagosome biogenesis

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Abstract

Interconversions of phosphoinositides play a pivotal role during phagocytosis and at the subsequent stages of phagosomal maturation into the phagolysosome. Several model systems have been used to study the role of phosphoinositides in phagosomal membrane remodelling. These include phagosomes formed by inanimate objects such as latex beads, or pathogenic bacteria, e.g. *Mycobacterium tuberculosis*. The latter category provides naturally occurring tools to dissect membrane trafficking processes governing phagolysosome biogenesis. *M. tuberculosis* persists in infected macrophages by blocking Rab conversion and affecting Rab effectors. One of the major Rab effectors involved in this process is the type III phosphatidylinositol 3-kinase hVPS34. The lipid kinase hVPS34 and its enzymatic product PtdIns3P are critical for the default pathway of phagosomal maturation into phagolysosomes. Mycobacteria block PtdIns3P production and thus arrest phagosomal maturation. PtdIns3P is also critical for the process of autophagy, recently recognized as an effector of innate immunity defenses. Induction of autophagy by pharmacological, physiological, or immunological means, overcomes mycobacterial phagosome maturation block in a PtdIns3P generation dependent manner and eliminates intracellular *M. tuberculosis*. PtdIns3P and PtdIns3P-dependent processes represent an important cellular nexus where...
fundamental trafficking processes, disease causing host–pathogen interactions, and innate and adaptive immunity defense mechanisms meet.

Introduction

Phosphorylated phosphatidylinositol derivatives (phosphoinositides) play a critical role in phagolysosome biogenesis, a pathway that can be divided into two stages: (i) phagosome formation, and (ii) phagosomal maturation into a degradative organelle. The first stage, uptake of particulate objects, is a topologically and mechanically complex membrane and cytoskeleton remodelling process, consisting of phagocytic cup formation, extension, and phagosome closure, orchestrated by sequential and precisely coordinated phospholipid interconversions. A nascent phagocytic cup shows a rapid and transient PtdIns-4-phosphate 5-kinase-dependent generation of PtdIns(4,5)\(_P^2\), followed by its conversion into diacylglycerol due to mobilization of phospholipase C\(_\gamma\) [1]. These processes control actin polymerization, pseudopod extension, and, acting in concert with type I PI3K (phosphatidylinositol 3-kinase), are a prerequisite for the completion of phagocytosis [2]. The terminal stages of phagocytosis are dominated by the formation of PtdIns(3,4,5)\(_P^3\), and activation of Rho GTPases and their effectors, p21-activated and Rho-activated protein kinases. These in turn first inhibit and then activate myosin light-chain kinase and control contractile myosin activity in phagosomal completion and closure.

The second stage, phagosomal maturation into phagolysosome, is dominated by the type III PI3K and its product PtdIns3P [3–6], and effector proteins with PtdIns3P-binding domains such as PX (phox homology) and FYVE [7], including Hrs [8] and EEA1 (early endosomal autoantigen 1) [4]. In the present chapter we use the *Mycobacterium tuberculosis* phagosome as a model system to highlight the role of PtdIns3P in phagosome maturation [9], and review the recent findings indicating that another PtdIns3P-dependent process, autophagy, overcomes the mycobacterial phagosome maturation block and plays a role in innate and acquired immunity processes [10].

*M. tuberculosis* phagosome

The distinguishing properties of the *M. tuberculosis* phagosome are its incomplete luminal acidification and paucity of mature lysosomal hydrolases [11]. At least two mycobacterial glycosylated phosphatidylinositol products [4,6, 12,13] have been identified as affecting phagosome maturation. In addition, mycobacterial products endow the *M. tuberculosis* phagosome with the ability to continue fusion with early endosomes [13], and receive nutrients including transferrin-bound iron delivered by transferrin receptor [14]. Another important aspect of the mycobacterial phagosome is its inefficient antigen processing capacity [15].
The initial recognition of the mycobacterial phagosome maturation arrest [16] as a Rab conversion block [17], has lead to a search for Rab effectors in an effort to further delimit the point of mycobacterial interference with Rab-regulated intracellular trafficking processes [9]. The majority of Rab5-effectors that have been examined appear to be recruited to both the model (latex bead) and mycobacterial phagosomes [4], with the notable exceptions of a Rab5 effector EEA1 [4] and Hrs [8], both being PtdIns3P-binding endosomal regulatory proteins. PtdIns3P is generated on endosomal membranes by the action of the type III PI3K hVPS34 [18]. The reduced or altered recruitment of EEA1 and Hrs to mycobacterial phagosomes [4,8] is due to mycobacterial interference with hVPS34 recruitment to and PtdIns3P generation on phagosomes [6,12,19–21]. Figure 1 summarizes how mycobacterial factors converge upon hVPS34 and PtdIns3P. PtdIns3P, generated by hVPS34, is essential for proper membrane trafficking and sorting events within the endosomal system, leading to the formation of late endosomal/phagosomal organelles such as endosomal multivesicular bodies [22] and phagolysosomes [4,5]. An *M. tuberculosis* product, the glycosylated phosphatidylinositol lipooarabinomannan interferes with PtdIns3P production, by a process which includes blocking Ca\(^{2+}\) signalling [23,24] implicated in hVPS34 recruitment in macrophages [6]. A second product, the *M. tuberculosis* PtdIns3P-phosphatase SapM (Figure 1), reduces PtdIns3P levels on mycobacterial phagosomes by removing any residual PtdIns3P [20], thus effectively isolating the mycobacterial phagosome from late endosomal compartments.

**Figure 1** PtdIns3P is targeted by *M. tuberculosis* as a key regulatory lipid controlling phagolysosome biogenesis. LAM (lipooarabinomannan) is a heavily glycosylated phosphatidylinositol produced in copious amounts and shed by *M. tuberculosis*. LAM prevents PtdIns3P generation. SapM, secreted by *M. tuberculosis*, is a PtdIns3P phosphatase removing any PtdIns3P generated past the LAM-imposed block. *M. tuberculosis* PIM (phosphatidylinositol mannoside) stimulates phagosome-early endosome fusion.

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Autophagy: a PtdIns3P-dependent process that can overcome the mycobacterial phagosome maturation block

The recognition of PtdIns3P as a centerpiece of phagosomal maturation and mycobacterial phagolysosome biogenesis block has lead to a search of a mechanism that could boost cellular ability to generate PtdIns3P and potentially override the mycobacterial inhibition of PtdIns3P production. An outcome of these studies was a demonstration that stimulation of another PtdIns3P-dependent process, autophagy, bypassed the mycobacterial phagolysosome biogenesis block and eliminated intracellular mycobacteria. [10,25].

Autophagy is a fundamental intracellular trafficking pathway, conserved from yeast to man, playing a role in cytoplasmic maintenance [26]. There are several forms of autophagy, with macroautophagy and chaperone-mediated autophagy as the two extremes in terms of autophagic ability to degrade large portions of the cytoplasm (macroautophagy) or individual proteins (chaperone-mediated autophagy). During macroautophagy (referred to in this text as autophagy), discrete portions of the cytoplasm are corralled into a specialized double membrane vacuole, called the autophagosome, sequestered away from the rest of the cytosol, and delivered to lysosomes for degradation (Figure 2). Autophagic degradation removes defective or excess organelles, including, among others, spuriously damaged mitochondria and surplus peroxisomes. An equally important, second function of autophagy is to turn over long half-life cytosolic macromolecules, such as stable proteins. By degrading long-lived macromolecules, autophagy supports anabolic needs and cellular viability under starvation conditions [26].

The process of autophagy can be divided into an induction/signalling phase and an execution phase (Figure 2). In the induction phase, various signals are integrated by the serine/threonine kinase Tor (target of rapamycin), which acts as a central, negative regulator of autophagy. A classical physiological inducer of autophagy is amino acid starvation, which inactivates Tor and stimulates autophagy. Additional signals include growth factor receptor signalling via the Akt/PKB (protein kinase B) pathway, and energy status (AMP/ATP ratio) via AMPK (AMP-activated protein kinase). Other signals affecting Tor include hypoxia and HIF (hypoxia-inducible factor) 1-regulated events, DNA damage via p53, and ERK (extracellular-signal-regulated kinase) downstream of Ras. The execution phase of autophagy involves morphologically tractable events, and can be subdivided into initiation, elongation and maturation/flux stages. During initiation, a damaged organelle or a portion of the cytosol is surrounded by a membranous structure called the isolation membrane or phagophore. During elongation, the phagophore is enlarged by the addition of new membrane of undefined origin, possibly coming from endoplasmic reticulum or a combination of endoplasmic reticulum, Golgi and endosomes. The isolation membrane seals to form an autophagosome. During maturation (also referred to as flux), an autophagosome fuses with lysosomes forming a degradative autolysosome.

The execution stages of autophagy depend on PtdIns3P generation, and thus require the type III PI3K hVPS34 in mammals or Vps34 in yeast. The clearest role for hVPS34 in autophagy is its requirement for the autophagosomal
Figure 2 Autophagy is regulated by hVPS34 and PtdIns3P at both signalling and execution stages. The autophagy pathway is divided into the induction phase and execution phase. The execution phase is subdivided into initiation, elongation and maturation/flux. The type III PI3K hVPS34 affects the induction and execution stages: hVPS34 has been implicated in affecting Tor activity and autophagy initiation and maturation stages. Beclin (Atg6) is a subunit of the hVPS34 complex required for autophagy. Initiation: a newly forming autophagosome, termed phagophore (isolation membrane) surrounds an organelle or a section of the cytoplasm. Elongation: phagophore elongates and bends. During this stage, Atg factors form two distinct protein–protein or protein–lipid conjugates: (i) Atg5 is covalently linked to Atg12, and the resulting Atg5–Atg12 conjugate associates with Atg16. (ii) Atg8 (LC3) is converted from its cytosolic LC3-I form into a C-terminally PE (phosphatidylethanolamine)–conjugated form, LC3-II. MVB, multivesicular bodies; LE, late endosome; Lys, lysosome. Induction of autophagy by pharmacological, physiological or immunological agonists results in control of intracellular *M. tuberculosis* by sequestering and directly destroying intraphagosomal mycobacteria. Induction of autophagy may also work indirectly by enhancing hVPS34 activity, reviving the endosomal–lysosomal system, and bypassing the *M. tuberculosis*-imposed PtdIns3P block on phagosomes. Autophagy can also clear a number of other pathogens, such as cytosolic bacteria (*Shigella, Listeria, streptococci*, etc.) and certain viruses. Induction of autophagy: red symbols or highlights, inhibitors of autophagy; green symbols or highlights, activators of autophagy. Yellow coloured symbol, hVPS34 plays a dual role: during the signalling/induction phase it activates Tor in response to amino acid replete conditions, so it may inhibit autophagy; in the execution phase, Vps34 (yeast) and hVPS34 (mammals) play a positive regulatory role.
merger with the lysosomal pathway (flux/maturation). *Saccharomyces cerevisiae* Vps34 also plays a role in the formation of a specialized pre-autophagosomal structure, PAS (a PAS has not been identified in mammalian cells), and therefore most likely affects initiation of autophagy in yeast [27]. The mammalian hVPS34, according to recent reports [28,29], may play a role in Tor signalling upstream of the initiation. The hVPS34 lipid kinase interacts with Beclin 1 (yeast Atg6), an autophagy-inducing tumour suppressor required specifically for the role of hVPS34 in autophagic processes [30–32]. In yeast, two separate protein complexes containing Vps34 control autophagic and endosomal degradative pathways: Complex I, consisting of Atg6 (Beclin), Atg14 and Vps34 specializes in autophagy, whereas Complex II, with Vps38 instead of Atg14, specializes in endosomal–vacuolar sorting [33]. No obvious orthologues of Vps38 or Atg14 have been identified in mammalian cells, leaving open the question of how higher eukaryotes accomplish hVPS34 specialization in autophagosomal versus endosomal pathways.

Autophagy is a cell survival mechanism, but under certain conditions excessive or selective autophagy can cause non-apoptotic programmed cell death [34]. The two processes are balanced at least in part via Bcl-2 interactions with Beclin, a subunit of the hVPS34 complex [30]. Autophagy has been implicated in both health and disease in the context of cancer, neurodegeneration, development and aging [26]. Most recently, autophagy, in its various forms of macro-autophagy and chaperone-mediated autophagy, has been shown to contribute to innate and adaptive immunity [10,35]. In addition to the capacity to eliminate intracellular mycobacteria, autophagy represents a more general immune effector against infectious diseases [25,36–38]. Growing evidence suggests that autophagy serves as a mechanism for removal of certain viruses and intracellular bacteria, in keeping with cytoplasmic housekeeping as one of the primary functions of this pathway [10]. The immunological role of autophagy exceeds its innate defense action, as autophagy plays a role in adaptive immunity enabling antigen processing for MHC II presentation [38].

**Concluding remarks**

Our understanding of the role of phosphoinositides in phagosome and autophagosome biogenesis has advanced, but is far from being complete, as exemplified by the lack of information on PtdIns(3,5)P₂, an important phosphoinositide controlling parts of the endosomal pathway, shown by Michell and colleagues to be synthesized from PtdIns3P [39]. Historically, phosphatidylinositol lipids were first discovered in mycobacteria [40] and only subsequently recognized in eukaryotic cells. We have now come full circle in connecting the mycobacterial lipids on one side and host lipids on the other side in a struggle to control PtdIns3P-dependent trafficking processes in the host cell. This is well illustrated in the model system of *M. tuberculosis* phagosome maturation arrest, and, in a broader context, in the autophagic control of intracellular pathogens. The latter, PtdIns3P-dependent process of autophagy is
a growing field of study in the area of innate and adaptive immunity, and in cell biology in general.

**Note added in proof (received 17 October 2006)**

Since the submission of this chapter, a study has been published showing that Rab22a plays a role in Rab5-to-Rab7 conversion block on mycobacterial phagosomes [41], another study has shown that autophagy is stimulated by immunity-related p47 GTPases (LRG47/Irgm1 in mice and IRGM in humans) [42], and a third study has demonstrated a new link between autophagy and apoptosis by association with mitochondria and Bclx(L), thus triggering cytochrome c release [43].

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**References**


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