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Review

Regulation of autophagy by phosphatidylinositol 3-phosphate

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ABSTRACT

The simple phosphoinositide phosphatidylinositol 3-phosphate (PI(3)P) has been known to have important functions in endocytic and phagocytic traffic, and to be required for the autophagic pathway. In all of these settings, PI(3)P appears to create platforms that serve to recruit specific effectors for membrane trafficking events. In autophagy, PI(3)P may form the platform for autophagosome biogenesis.

1. Formation and consumption of PI(3)P

Phosphoinositides (PI's) are formed by the phosphorylation of phosphatidylinositol (PI) on its inositol ring. With the exception of the 2′ and 6′ positions all free OH groups of the inositol ring can be phosphorylated. The unique functional role of each type of PI within the cell can be attributed to the arrangement of phosphate groups around the inositol ring. Phosphoinositide 3-kinases (PI3 kinases) are the enzymes responsible for phosphorylating the 3′OH-position of the inositol ring of PI, and three classes of these enzymes exist in cells [1,2] Class I PI3 kinases most commonly phosphorylate PI(4,5)P_2 at its 3′OH group to produce PI(3,4,5)P_2 (often referred to as PIP3). Class III PI3 kinases are the orthologues of the yeast vesicular protein-sorting protein Vps34, and these enzymes can only utilise PI as a substrate (Fig. 1). Class II PI3 Kinase can use PI, PI(4)P and PI(4,5)P_2 as substrates, with a strong preference for PI. It is likely that the type III enzyme is responsible for the majority of PI(3)P synthesis within cells, whereas the type II enzymes appear to be involved in specialised signal-dependent settings [3]. PI(3)P can be further phosphorylated by a 4′-kinase at its 4′-position to generate PI(3,4)P_2 (Fig. 1). Additionally, PI(3)P can be phosphorylated by a PI(3)P 5-kinase known as PIKfyve in mammals (Fab1p in yeast) to form PI(3,5)P_2 [4]. 3′ Phosphatases that can act on PI(3)P include PTEN (phosphatase and tensin homologue) [5], myotubularin proteins such as Jumpy [6,7], and the yeast phosphatase, Sac1p [8].

2. Recognition of PI(3)P by specific protein domains

The generation of PI's by PI3 kinases has been found to be responsible for a diverse array of cellular signalling events, often mediated by distinct proteins that can bind to PI's. Lipid binding can affect the localization, conformation and/or activity of PI-binding proteins, and two types of PI(3)P-binding domains have been reported. One is the FYVE domain (whose name reflects the first four proteins found to contain it: Fab1p,YOTB, Vac1p and Early Endosome Antigen 1) [9,10]. At present 37 FYVE domain containing proteins have been identified in humans. These domains are known to bind to PI(3)P with high affinity (K_d = 50 nM) and they usually consist of approximately 65 amino acids with eight conserved cysteine residues. A hydrophobic loop in the FYVE domain initially interacts with the membrane in a non-specific manner. This leads to the subsequent exposure of a characteristic basic motif [(R/K) (R/K)HHCR] in the FYVE domain, which surrounds the third conserved cysteine residue, and this can bind to the inositol head group of PI(3)P [2,11]. PI(3)P can also bind to the 120 amino acid Phox homology (PX) domains. PX domains have been found in NADPH oxidase subunits, a PI3 kinase, sorting nexins, a SNARE, as well as some phospholipases and protein kinases. PI(3)P binding to PX domains is thought to occur via a pair of highly conserved basic motifs consisting of [RR(Y/F)] [11].

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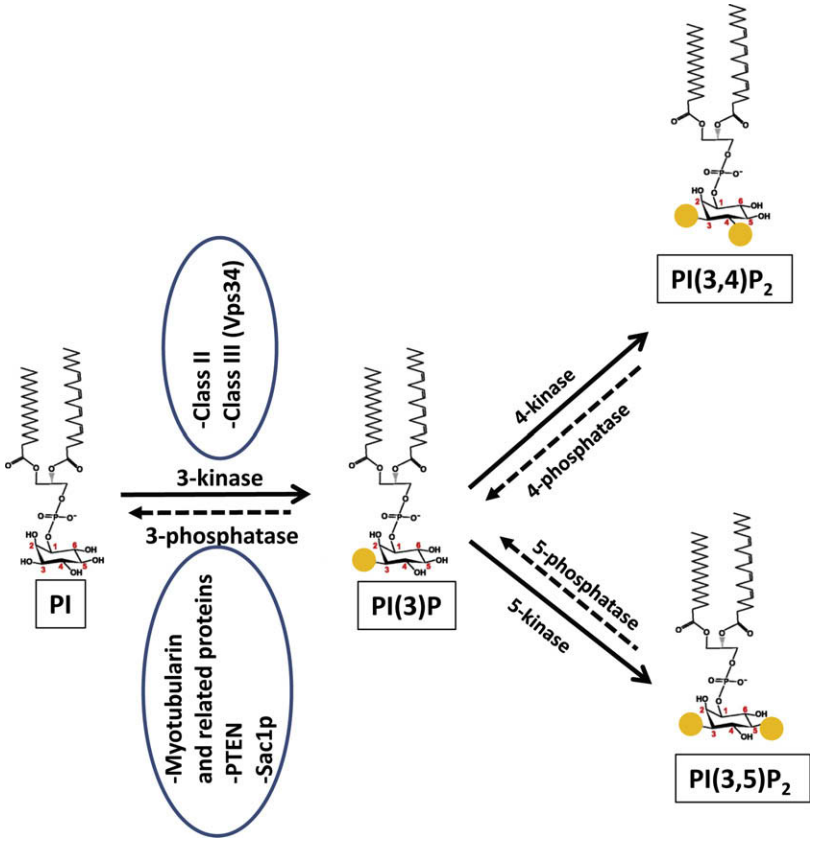

Fig. 1. Pathways for PI(3)P synthesis and conversion into PI, PI(3,4)P_2 and PI(3,5)P_2. PI(3)P can be synthesised from PI by class II and class III PI3-kinases (circled in blue). 3-Phosphatases (circled in blue) can convert PI(3)P back into PI. PI(3)P can also be converted into PI(3,4)P_2 and PI(3,5)P_2 by 4-kinases and 5-kinases, respectively.

3. Localization and role of PI(3)P within endocytic and phagocytic compartments

PI(3)P is maintained at a cellular concentration of approximately 200 μM, and is thought to be produced mainly by Vps34. Genetic and biochemical data have shown that PI(3)P regulates endocytic trafficking, and, in support of this, studies using a double FYVE domain as a probe have found that PI(3)P is enriched on early endosomes, in the internal vesicles of multivesicular endosomes and in yeast vacuoles [12]. Moreover, proteins that bind to PI(3)P are known to play a role in membrane trafficking events, directing traffic from endosomes and Golgi bodies to lysosomes (Fig. 2). Many of these proteins have FYVE domains such as EEA1, which regulates the fusion of endocytic membranes, and is recruited to early endosomes by Rab5-GTP and PI(3)P [13]. EEA1 recruitment to endosomal membranes acts to regulate proper membrane fusion by SNARE's (Soluble *N*-ethylmaleimide-sensitive factor Attachment protein Receptor's) [11]. Specifically, EEA1 has been shown to interact with at least two SNARE proteins, syntaxin6 and syntaxin13 [14]. Another FYVE domain containing protein, Rabenosyn-5, an orthologue of yeast Vac1p, is also required for early endosome fusion, and can bind to both PI(3)P and Rab5-GTP. When bound to PI(3)P Rabenosyn-5 can modulate SNARE complex formation with Rab5 and Sec1 (Vps45) proteins [15]. An adaptor protein SARA (Smad Anchor for Receptor Activation), also contains a FYVE domain which localizes it to endosomes. In endosomes SARA then acts to recruit the transcription factors Smad2 and Smad3 to the transforming growth factor β receptor [11]. Additionally, PX domain containing proteins have also been found to bind to PI(3)P and play a role in early endosomal trafficking events. For example, sorting nexins play a role in trafficking activated growth factor receptors, and sorting nexin 3 is targeted to endosomal PI(3)P pools via its PX domain [16]. The motility of endosomes along microtubules and actin filaments has also been shown to be dependent on both Rab5 and Vps34 (class III PI3 Kinase), and a kinesin motor, KIF16B, which can bind to PI(3)P via a PX domain has been identified [14].

FYVE domain containing proteins have also been found to play a role in directing target proteins to lysosomes via the formation of multivesicular endosomes (Fig. 2). For example, Fab1p, the yeast orthologue of PIKfyve, is a PI(3)P 5-kinase that also contains FYVE domains, and has been suggested to target cell surface receptors for degradation in lysosomes. Hrs (mammalian orthologue of yeast Vps27), contains multiple FYVE domains, and is also known to play a role in endosomal maturation and multivesicular endosome formation [17]. It is thought that Hrs regulates SNARE complex formation, as well as recognising ubiquitylated cargo and selecting it for degradation. Hrs is required for the formation of the ESCRT (endosomal sorting complex required for transport) complexes on endosomal membranes [14]. ESCRT complexes in turn are required for the sorting and recognition of ubiquitylated cargo protein into the internal vesicles of multivesicular endosomes. PX domain containing proteins have also been found to play a role in trafficking target proteins for degradation. For example, the yeast SNARE Vam7 is targeted to the vacuolar membrane via PI(3)P binding to its PX domain [18], here it is known to play a role in trafficking proteins to the vacuole [19].

Retrograde trafficking from endosomes to Golgi is important for the recycling of sorting receptors, such as yeast Vps10, and mammalian mannose-6 phosphate receptor. Two accessory proteins required for Vps34 activity in yeast, Vps30 and Vps38 (see below), have been shown to be essential for endosome to Golgi trafficking