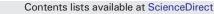
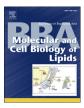
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# Review Phosphoinositides in endocytosis $\stackrel{\text{\tiny}}{\leftarrow}$

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#### ABSTRACT

The internalization and subsequent endosomal trafficking of proteins and membrane along the endocytic pathway is a fundamental cellular process. Over the last two decades, this pathway has emerged to be subject to extensive regulation by phosphoinositides (Pls), phosphorylated derivatives of the minor membrane phospholipid phosphatidylinositol. Clathrin-mediated endocytosis (CME) is the endocytic mechanism characterized in most detail. It now represents a prime example of a process spatiotemporally organized by the interplay of PI metabolizing enzymes. The most abundant PI, phosphatidylinositol-4,5-bisphosphate [PI(4,5)P<sub>2</sub>], serves as a denominator of plasma membrane identity and together with cargo proteins is instrumental for the initiation of clathrin-coated pit (CCP) formation. During later stages of the process, the generation of phosphatidylinositol-3,4-bisphosphate [PI(3,4)P<sub>2</sub>] and the dephosphorylation of PI(4,5)P<sub>2</sub> regulate CCP maturation and vesicle uncoating. Here we provide an overview of the mechanisms by which PIs are made and consumed to regulate CME and other endocytic pathways and how conversion of PIs *en route* to endosomes may be accomplished. Mutations in PI converting enzymes are linked to multiple diseases ranging from mental retardation and neurodegeneration, to inherited muscle and kidney disorders suggesting that the tight control of PI levels along the endocytic pathway plays a critical role in cell physiology. This article is part of a Special Issue entitled Phosphoinositides.

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#### 1. Introduction

While phosphoinositides (PIs) have been known to regulate Ca<sup>2+</sup> and growth factor signaling as well as cellular transformation since the 1980s [1,2] a landmark study by Emr and colleagues in yeast provided the first evidence for a function of PIs in membrane traffic [3]. A link between PIs and endocytosis emerged from the observation that inositol-1,4,5-trisphosphate (IP<sub>3</sub>), the second messenger that releases  $Ca^{2+}$  from intracellular stores, inhibits the self-association of the endocytic clathrin adaptor AP-2 in vitro [4]. The finding that AP-2 as well as the brain-specific endocytic adaptor AP180 can bind to inositol-polyphosphates [5,6] suggested that lipid signaling metabolites may directly regulate endocytic vesicle coats [7]. Subsequent work established a role for PI(4,5)P2 in clathrin-coated vesicle (CCV) formation [8] including dynamin-mediated membrane fission [9,10]. Over the last 15 years, we have gathered detailed knowledge on the mechanisms by which PIs are synthesized and turned over at endocytic sites and how their association with endocytic proteins may govern cargo selection as well as membrane deformation and internalization. PI(4,5)P<sub>2</sub> is essential for endocytic vesicle formation by clathrin-mediated

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http://dx.doi.org/10.1016/j.bbalip.2014.09.014 1388-1981/© 2014 Elsevier B.V. All rights reserved. endocytosis (CME) and via macropinosomes. Both of these pathways also are regulated by the activities of phosphatidylinositol-3-kinases (PI3Ks), while PI 5-phosphatases are crucial for the final stages of vesicle formation. The endosomal system in contrast is marked by PI(3)P, which is required for homotypic endosomal fusion, endosomal sorting, and for the formation of intraluminal vesicles along the multivesicular body (MVB) pathway *en route* to lysosomes.

Here, we provide an overview of the different roles of PIs in endocytosis with a focus on CME. We also summarize the current state of knowledge with respect to the role of PIs in clathrin-independent internalization routes such as macropinocytosis. Finally, we suggest a speculative model as to how conversion of PIs on endocytic membranes *en route* to endosomes may occur and how dysfunction of this conversion system could lead to disease.

#### 1.1. PIs and membrane identity

Given that the cytoplasmic leaflets of distinct cellular compartments are differentially enriched in distinct PI species, PIs have been postulated to serve as denominators of membrane identity [11,12], a feature intimately linked to their role in membrane traffic. The differential distribution of PIs is recognized by proteins harboring specific lipidbinding modules [13] thereby aiding the targeting of these proteins and their associated factors to their correct subcellular destination. The characteristic PI of the plasma membrane is PI(4,5)P<sub>2</sub>, which regulates a wide range of physiological processes including signal

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transduction, endocytosis, actin dynamics and cell migration, as well as the function of ion channels [14,15].  $PI(4,5)P_2$  is synthesized locally from PI(4)P by PI(4)P-5-kinases (PIP5Ks) [16], and for a long time plasma membrane PI(4)P was considered a mere precursor to  $PI(4,5)P_2$ . However, depletion of plasma membrane PI(4)P or genetic disruption of plasma membrane PI(4)P synthesis was shown to interfere with the localization and function of plasma membrane proteins, in spite of the fact that total  $PI(4,5)P_2$  levels were not significantly decreased [17, 18]. These studies indicate a crucial role of PI(4)P in conferring plasma membrane identity that is independent of its role as a precursor of  $PI(4,5)P_2$ .

Other subcellular compartments are believed to contain comparably low levels of  $PI(4,5)P_2$  and instead are enriched in other PIs [12]. For example, PI(4)P is highly enriched at the Golgi complex in addition to the plasma membrane, whereas PI(3)P predominates in the endosomal system. Among the endosomal processes shown to depend on PI(3)Pare the homotypic fusion of early endosomes, the endosomal sorting of endocytosed proteins as well as the formation of intraluminal vesicles for degradative sorting within MVBs [19]. Class III PI3K, hVps34, is believed to produce the majority of PI(3)P, yet accumulating evidence suggests that a fraction of endosomal PI(3)P may be synthesized by class II PI3Ks, either by phosphorylation of PI or indirectly via dephosphorylation of  $PI(3,4)P_2$ , another major product of class II PI3Ks [20–22].

As membranes exchange between compartments conversion of their PI identity has to occur. For example, fission of endocytic vesicles from the plasma membrane and subsequent fusion with early endosomes must be accompanied by PI conversion of the internalizing membrane from  $PI(4,5)P_2$  to PI(3)P. How this is accomplished precisely is not completely understood but recent data indicate that part of this conversion may already occur at the cell surface through acquisition of  $PI(3,4)P_2$  [22], possibly followed by hydrolysis of its 4-phosphate moiety to yield PI(3)P.

#### 1.2. Phosphoinositide regulation of clathrin-mediated endocytosis

CME is a fundamental cell biological process that is of central importance to various aspects of cellular physiology ranging from nutrient uptake and signal transduction to synaptic transmission and development [23,24]. During CME small portions of the plasma membrane are internalized into small 80 nm to 140 nm sized vesicles coated with clathrin (CCVs). CCVs are formed by the assembly of a bilayered protein coat on the cytoplasmic face of the plasma membrane. At its core, this coat consists of the heterotetrameric adaptor protein AP-2, comprising  $\alpha$ -,  $\beta$ 2-,  $\mu$ 2-, and  $\sigma$ 2-adaptin, surrounded by hexagonal and pentagonal assemblies of clathrin triskelia, composed of three tightly associated heavy and light chains [25]. AP-2, together with a host of other adaptors and accessory proteins, forms the membrane-proximal layer of the coat that directly contacts the membrane and binds to transmembrane cargo, e.g. receptors or channels. Cargo-specific adaptors enable the internalization of a large variety of distinct cargoes via CME by recognizing recurring internalization motifs. For example, AP-2 via its  $\mu$ - and  $\sigma$ subunits recognizes tyrosine-based as well as dileucine-based motifs within the cytoplasmic domains of receptors or other types of membrane proteins. In addition, more specialized dedicated adaptors sort subsets of cargo, i.e. arrestins enable CME of G protein-coupled receptors [26].

Clathrin and AP-2 serve as central protein interaction hubs within the endocytic protein network [27,28]. The clathrin terminal domain (CTD), a globular seven-bladed  $\beta$ -propeller fold at the distal end of each triskelion leg, and the appendage domains of AP-2 $\alpha$ - and  $\beta$ , via recognition of simple degenerate peptide motifs, recruit a large variety of endocytic proteins [25] that drive progression of the pathway (Fig. 1). These endocytic proteins include cargo adaptors, membrane deforming scaffolds, actin modulatory factors — and PI phosphatases and kinases [28]. The first PI metabolizing enzyme to be implicated in CME was the 5-phosphatase synaptojanin, an enzyme highly enriched at nerve terminals, where synaptic vesicles undergo local exo-endocytic cycling [29–32]. Genetic ablation of synaptojanin 1 leads to increased levels of PI(4,5)P<sub>2</sub> and an accumulation of CCVs at synapses [33]. This suggests a crucial role for PI(4,5)P<sub>2</sub> in stabilizing clathrin coats at membranes. Conversely, knockout of PIP5KI $\gamma$ , the major PI(4,5)P<sub>2</sub>-synthesizing enzyme at synapses [34], causes reduced PI(4,5)P<sub>2</sub> levels and concomitant impairments in both exocytic neurotransmitter release and in the endocytic recycling of synaptic vesicle membranes [34]. Furthermore, constitutive [35] or rapamycin-induced membrane recruitment of an inositol 5-phosphatase [36] results in the enzymatic depletion of PI(4,5)P<sub>2</sub> and a complete loss of clathrin-coated pits (CCPs) from the plasma membrane [37], suggesting that CCPs can neither form nor persist in the absence of PI(4,5)P<sub>2</sub>.

#### 1.2.1. PI(4,5)P<sub>2</sub>-binding proteins within the CME proteome

The stringent requirement for  $PI(4,5)P_2$  in endocytosis is reflected in the abundance of  $PI(4,5)P_2$ -binding proteins within the endocytic proteome. These include the early-acting clathrin adaptors AP-2 [38-41] (Figs. 1 and 2A), AP180/CALM [42,43] (Figs. 1 and 2B), and epsins 1-3, which recognize ubiquitylated cargo [42,44], the NPxY-motif adaptors disabled 2 (Dab2), autosomal recessive hypercholesterolemia (ARH), the integrin adaptor Numb [45,46], and  $\beta$ -arrestins, adaptors for the CME of G-protein coupled receptors (GPCRs) [47,48] (Fig. 1). In addition, PI(4,5)P<sub>2</sub> associates with and recruits membrane remodeling proteins of the Bin/Amphiphysin/Rvs (BAR)-domain superfamily, e.g. Fer/Cip4 homology domain-only (FCHo) 1/2 [49], which in conjunction with AP-2 and clathrin acts during early stages of CCP formation. Lateacting BAR domain proteins such as endophilin and amphiphysin can associate with a variety of negatively charged lipids including PIs [50]. An exception is the PX-BAR domain scaffold sorting nexin 9 (SNX9) [51–53], which via its PX domain specifically binds to  $PI(4,5)P_2$  and to  $PI(3,4)P_2$  (as well as to PI(3)P, a lipid absent from the plasma membrane in most cell types) (Fig. 2C). Finally,  $PI(4,5)P_2$  aids the function of the fissioning GTPase dynamin [54-56], possibly by positioning the molecule towards the membrane during assembly at the neck of invaginated CCPs. Thus, in addition to clathrin and AP-2, PI(4,5)P<sub>2</sub> serves as the third interaction hub within the endocytic network (Fig. 1). Due to its specific enrichment in the plasma membrane, PI(4,5)P<sub>2</sub> ensures compartmental fidelity of CCP assembly. Cargo internalized by CME frequently cycles between the cell surface and internal membranes, yet assembly of endocytic coats occurs only at the cell surface. Such compartmental specificity may be achieved by a coincidence detection system that capitalizes on the ability of early-acting endocytic adaptors to associate with transmembrane cargo and with  $PI(4,5)P_2$  in the same membrane, effectively limiting the site of endocytic CCV formation to the plasma membrane.

#### 1.2.2. PI(4,5)P<sub>2</sub> synthesis and CCP nucleation

CME depends on the general abundance of  $PI(4,5)P_2$  in the plasma membrane but further creates its own, dedicated pool of  $PI(4,5)P_2$ . PI(4,5)P<sub>2</sub> is synthesized from PI(4)P by type I PIP5K  $\alpha$ ,  $\beta$ , and  $\gamma$  [15]. All three isoforms of PIP5KI [57,58] can associate with the  $\mu$ -subunit of the AP-2 adaptor complex [59], while the p90 isoform of PIP5KIy in addition can bind to AP-2B. PIP5KIy is present in 6 different splice variants [15]; its p90 isoform of PIP5KI  $\gamma$  (now referred to as PIP5KI $\gamma$ -v2) carries a 28 amino acid carboxy (C)-terminal splice insert that interacts with both the  $\mu$ 2 subunit and the  $\beta$ 2-appendage of AP-2 [60–62]. Binding of AP-2-cargo complexes to PIP5KIy-v2 results in a potent stimulation of its kinase activity [59,63], suggesting a crucial role for this complex in the initial stages of CCP formation. Consistent with this scenario, enhanced PI(4,5)P<sub>2</sub> synthesis increases the rate of CCP nucleation [64] and single molecule imaging suggests that AP-2, perhaps together with FCHo1/2 [49], is among the first endocytic proteins arriving at newly forming CCPs [65]. Newly recruited AP-2 when bound to Download English Version:

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