



**The many roles of type II phosphatidylinositol 4-kinases in membrane trafficking: New tricks for old dogs**

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## Summary

The type II phosphatidylinositol 4-kinases (PI4KIIIs) produce the lipid phosphatidylinositol 4-phosphate (PtdIns4P) and participate in a confusing variety of membrane trafficking and signalling roles. This review argues that both historical and contemporary evidence supports the function of the PI4KIIIs in numerous trafficking pathways, and that the key to understanding the enzymatic regulation is through membrane interaction and the intrinsic membrane environment.

By summarising new research and examining the trafficking roles of the PI4KIIIs in the context of recently solved molecular structures, I highlight how mechanisms of PI4KII function and regulation are providing insights into the development of cancer and in neurological disease. I present an integrated view connecting the cell biology, molecular regulation and roles in whole animal systems of these increasingly important proteins.

## 1. Introduction

The phosphatidylinositol 4-kinases (PI4Ks) occupy a central position in phosphatidylinositol (PtdIns) metabolism because they catalyse the first step in the biosynthesis of four of the seven phosphoinositide (PI) lipids<sup>[1]</sup> all of which are intimately involved in membrane trafficking<sup>[2, 3]</sup> Their primary enzymatic product, phosphatidylinositol 4-phosphate (PtdIns4P), is a signalling precursor and an important regulator of membrane traffic<sup>[3]</sup> that can be produced by either of the four PI4K activities. The extensively studied type III PI4Ks (PI4KIII $\alpha$  and PI4KIII $\beta$ ) were the first PI4K enzymes to be identified at the molecular level and these isoforms produce PtdIns4P at the plasma membrane (PM) and Golgi, respectively (reviewed in depth elsewhere<sup>[4, 5]</sup>). The type II PI4Ks (PI4KII $\alpha$  and PI4KII $\beta$ ) were identified more recently<sup>[6, 7]</sup> and we are only now beginning to understand in detail how they influence membrane identity and trafficking.

PI4KII was first described decades ago as a membrane-bound enzyme activated by non-ionic detergents and inhibited by adenosine<sup>[6, 7]</sup> For many years, interest in PI4K activities was driven by their requirement in PtdIns(4,5)P<sub>2</sub>-dependent signalling at the plasma membrane. However, recent years have seen profound advances in our understanding of PI4KII in membrane trafficking that have largely eclipsed the role of PI4KII in signalling. The functions of PtdIns4P have also expanded [into](#) a much broader subcellular distribution than previously envisaged. Discrete pools of PtdIns4P outside of the Golgi are now recognised on early (EE) and sorting endosomes (SEs), late endosomes (LEs), at the plasma membrane (PM)<sup>[8-10]</sup> and even on different subdomains of the *trans*-Golgi network (TGN)-endosomal system<sup>[11]</sup> (Figure 2). The PI4KII $\alpha$  and PI4KII $\beta$  enzymes have also been detected at the PM and in endosomal compartments containing PtdIns4P. Subcellular distribution of both PI4KII isoforms and the [PIs they produce](#) is therefore broad and overlapping, leading to questions of whether the enzymes have redundant functions.

We now have much information relating to the trafficking events controlled by these enzymes and the PtdIns4P they synthesise. The recently solved structures of the PI4KII isoforms place their hitherto mysterious properties into a molecular framework, thereby allowing us to rationalise their functions and explain the unique biochemistry of these two key PI kinases. This review discusses the

1 structure and function of the PI4KIIIs, the differences and similarities in their  
2 regulation and their involvement in physiological processes and disease.  
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## 5 **2. The PI4KIIIs are an ancient kinase family**

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9 The PI4KII family eluded molecular identification for many years because their  
10 gene sequences differed from those of known PI kinases.<sup>[12, 13]</sup> Discovery of the  
11 alpha isoform (PI4KII $\alpha$ ) revealed an unexpected second isoform that we termed  
12 PI4KII $\beta$ .<sup>[13]</sup> The PI4KIIIs are distinct from the PI4KIII family whose members are  
13 clearly homologous to the archetypal protein kinase PKA and the PI3Ks, thereby  
14 placing the PI4KII structures unequivocally within the PI3K/protein kinase  
15 superfamily.<sup>[5]</sup>  
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21 Three-dimensional structures of recombinant human PI4KII $\alpha$  have been  
22 reported recently<sup>[14, 15]</sup> and the data subjected to extensive molecular modelling to  
23 predict biochemical behaviour of the proteins. These studies illustrate how the  
24 enzymes differ from the phosphoinositide 3-kinase (PI3K) superfamily and how the  
25 PI4KIIIs interact with membranes. The structures also offer explanations for several  
26 unique biochemical properties of the PI4KIIIs such as sensitivity to detergent,  
27 cholesterol and adenosine that were first described during the characterisation of  
28 purified PI4KII activity. The models also provide valuable insights into the  
29 regulation of PI4KII activity.  
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37 PI4KII $\alpha$  has a flattened interface that contacts membranes, similar to  
38 features reported for the PI3Ks and phosphatidylinositol 4-phosphate 5-kinases  
39 (PIP5Ks).<sup>[16]</sup> suggesting that these evolutionarily distant proteins have retained a  
40 structural characteristic determining membrane interaction. Overall the catalytic  
41 domain of PI4KII $\alpha$  is similar to PI3Ks in that it is composed of N- and C-terminal  
42 lobes (Figure 1A) but with additional insertions not found in PI3Ks. A search for  
43 related structures showed that the closest relatives of PI4KII $\alpha$  are protein kinases  
44 such as the *Helicobacter pylori* pro-inflammatory kinase CTKA, thus indicating an  
45 ancient evolutionary relationship with the protein Ser/Thr kinases.  
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52 PI4KII $\alpha$  has a nucleotide-binding pocket distinct from those found in PI3Ks,  
53 which accounts for its insensitivity to PI3K inhibitors such as wortmannin. Another  
54 feature significantly different from the PI3Ks is a glycine-rich loop (G-loop)<sup>[14]</sup> that is  
55 likely to determine nucleotide-binding specificity and is potentially responsible for  
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1 the sensitivity of PI4KII $\alpha$  to adenosine. Co-crystallisation of PI4KII $\alpha$  in the presence  
2 of ADP indicates that the ATP binding pocket is positioned so that the terminal  $\gamma$ -  
3 phosphate is oriented in direct apposition to the membrane, where its PI substrate  
4 resides.<sup>[15]</sup>

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8 Numerous parts of the PI4KII $\alpha$  molecule interact with membranes but  
9 multiple palmitoylation performs a special role by bringing a nearby amphipathic  
10 helix and the catalytic core close to the membrane (Figure 1B). This causes the  
11 nucleotide-binding G-loop, a putative kinase activation loop, to interact with  
12 membranes along with elements that form the PI binding pocket.<sup>[14]</sup> Together,  
13 these elements stabilise critical regions of the active site leading to a more robust  
14 kinase activity. This tight interaction with the membrane and the effect of  
15 membrane association on activity, may explain why PI4KII $\alpha$  is regulated mainly by  
16 membrane composition and the availability of PtdIns substrate.<sup>[17]</sup> A second  
17 hydrophobic pocket has been identified in the C-terminal lobe of PI4KII $\alpha$ <sup>[15]</sup> and,  
18 although the physiological ligand that binds this is unknown, candidates include  
19 cholesterol, an important membrane organiser and PI4KII $\alpha$  regulator.

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21 The N-termini of the PI4KIIs lack primary sequence homology, but contain  
22 protein binding sites that determine isoform-specific functions (Figure 1A).  
23 Historically, the two PI4KII activities could not be distinguished in cell and tissue  
24 extracts and, unsurprisingly, the structure of the conserved core of PI4KII $\beta$  is very  
25 similar to that of PI4KII $\alpha$ .<sup>[18]</sup> Recently an isoform-selective small molecule inhibitor,  
26 PI-273, has been developed [that](#) is approximately 30 times more potent against  
27 PI4KII $\alpha$  than PI4KII $\beta$ .<sup>[19]</sup> This compound is capable of differentiating the two  
28 isoforms and is likely to become an important experimental tool.

## 29 **2.1 PI4KII activity is tuned by membrane environment**

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31 Evidence that PI4KII $\alpha$  activity is regulated comes from the finding that membranes  
32 containing low and high activity pools of the enzyme can be separated by  
33 subcellular fractionation.<sup>[20]</sup> It is clear from the three-dimensional structures that  
34 membrane interaction determines PI4KII activity, and that this is achieved through  
35 stable multiple S-palmitoylation.<sup>[21]</sup> This modification tightly anchors PI4KII $\alpha$  in  
36 membranes, such that only detergent treatment can release the protein.<sup>[12]</sup> Close  
37 membrane interaction renders PI4KII $\alpha$  activity exquisitely sensitive to its  
38 membrane environment,<sup>[22]</sup> being activated by cholesterol<sup>[17]</sup> and

1 phosphatidylcholine, but inhibited by acidic lipids such as phosphatidic acid.<sup>[23]</sup> The  
2 nature of this interaction is essential to understand how PI4KII $\alpha$  activity responds  
3 to a changing environment, in particular the supply of PI substrate. Thus the  
4 molecular structures support numerous observations that PI4KII $\alpha$  is regulated  
5 largely by intrinsic membrane factors.  
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9 PI4KII $\alpha$  activity is responsible for a large proportion of total PI4K activity in  
10 cell extracts but, in contrast, PI4KII $\beta$  activity is difficult to measure.<sup>[24]</sup> Unlike  
11 PI4KII $\alpha$ , a substantial proportion of PI4KII $\beta$  is extractable without the need for  
12 detergent<sup>[25]</sup> indicating that it is only partially membrane associated, and it is likely  
13 that this difference is key to the regulation of PI4KII $\beta$  activity. Unlike PI4KII $\alpha$ , the  
14 PI4KII $\beta$  isoform is dynamically palmitoylated on cysteine residues within the  
15 conserved <sub>170</sub>CCPCC<sub>174</sub> motif (Figure 1A). Membrane recruitment of PI4KII $\beta$  is  
16 regulated and occurs in response to growth factors and on expression of  
17 constitutively active Rac1.<sup>[26]</sup>  
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25 PI4KII $\beta$  binds the molecular chaperone HSP90 through a site in the C-  
26 terminal lobe, a region [that](#) is occluded in the tightly membrane bound PI4KII $\alpha$ .  
27 This interaction stabilises cytosolic PI4KII $\beta$  and prevents palmitoylation, and  
28 therefore membrane recruitment. Inhibition of the interaction releases PI4KII $\beta$   
29 leading to degradation in the proteasome (Figure 3B). However, acute inhibition of  
30 the interaction leads to rapid palmitoylation and translocation to TGN and plasma  
31 membranes. This interaction is likely to be regulated by external stimuli because  
32 treatment with EGF or PDGF is sufficient to disrupt the HSP90-PI4KII $\beta$  complex.<sup>[27]</sup>  
33 Thus, it appears that both protein turnover and membrane translocation, and  
34 therefore substrate access, are under tight control. This indicates the existence of  
35 elaborate mechanisms controlling PI4KII $\beta$  activity, and suggests that this isoform is  
36 subject to stringent regulation in a manner quite distinct from PI4KII $\alpha$ .  
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### 47 **2.3 The lipid products of PI4KII $\alpha$ can be studied at the subcellular level**

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50 Spatially distinct pools of PIs can be imaged at subcellular resolution by using  
51 recombinant probes that are now widely used in microscopy.<sup>[28]</sup> The use of these  
52 has confirmed the existence of PtdIns4P in the PM and, surprisingly, in multiple  
53 endosomal compartments (<sup>[8, 10, 29]</sup>, Figure 2).  
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PtdIns4P levels on intracellular membranes are determined by the localisation and activity of the resident PI4Ks, but a central problem in the study of PI4KII function is that the PtdIns4P produced is indistinguishable from the enzymatic products of other PI4K isoforms. Two other issues further complicate PtdIns4P experiments: remarkably, some PtdIns4P pools can supply lipid to adjacent membranes through non-vesicular traffic<sup>[30]</sup> making it technically difficult to assign a membrane compartment to an individual isoform; also, dynamic changes in PtdIns4P levels may not be detectable, for example, when PtdIns4P is rapidly converted to another PI. These cautions notwithstanding, cellular imaging and the manipulation of PIs has proved decisive in understanding PI4KII function.

The biosynthesis of PtdIns4P by different PI4K isoforms can occur in metabolically separate pools<sup>[10, 11]</sup> Connecting each of these pools to a particular PI4KII activity in the absence of effective inhibitors has been challenging; however, the Balla laboratory has adopted and developed techniques to acutely manipulate PIs in different organelles by the inducible targeting of PI phosphatases<sup>[31]</sup> Thanks to this approach, which has since been used by several investigators, we know that PtdIns4P is produced in the PM by PI4KIII $\alpha$ <sup>[8]</sup> (as was predicted by many earlier studies<sup>[10]</sup>), that PtdIns4P can regulate a PM calcium channel<sup>[32]</sup>, that PtdIns4P is present in LE and lysosomal membranes<sup>[8]</sup> and that PtdIns4P metabolism occurs during phagosomal maturation<sup>[33]</sup>

### 3. PI4KIIs have multiple roles in membrane trafficking

Early studies indicated that the PI4KIIs localise to, and traffic between, the Golgi complex and endosomes<sup>[20, 24, 34]</sup> It is now clear that most trafficking pathways involving PI4KIIs can be traced back to the TGN; however, to understand the multitude of PI4KII pathways, it is important to recognise the complexity of communication between the TGN and endosomal compartments, which are highly heterogeneous and ultimately traffic cargo to the lysosome or PM<sup>[35]</sup> The TGN is itself a highly dynamic, PtdIns4P-rich compartment at the *trans* face of the Golgi apparatus that functions as an exit point where cargo is sorted into biosynthetic and endosomal pathways. The TGN also receives backward (retrograde) traffic from endosomal compartments<sup>[36]</sup> The TGN therefore, plays critical functions as a sorting hub that coordinates many aspects of intracellular traffic, including secretion and the biogenesis of lysosomes and related organelles. PI4KII $\alpha$ , PI4KII $\beta$

1 and the PtdIns4P the enzymes produce, all function at the TGN and associated  
2 membranes, and the following sections describe how the PI4KIIIs function in these  
3 processes.  
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### 7 **3.1 How the PI4KIIIs control traffic.**

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10 PtdIns4P is a factor in the recruitment of several clathrin adaptors at the TGN and  
11 therefore in determining the sorting of proteins into distinct pathways. For example,  
12 EpsinR is a PtdIns4P-binding protein that mediates TGN-to-endosome cargo  
13 sorting<sup>[37]</sup> as are the Golgi-localised  $\gamma$ -ear-containing Arf binding proteins  
14 (GGAs)<sup>[38]</sup> which require PtdIns4P synthesised by PI4KII $\alpha$  for correct  
15 localisation.<sup>[39]</sup> PI4KII $\alpha$  is a significant PtdIns4P biosynthetic activity at the TGN  
16 and it was originally reported that depletion of the enzyme delocalises the clathrin  
17 adaptor and PtdIns4P effector AP-1<sup>[40]</sup> which principally directs TGN-to-endosomal  
18 traffic. However, subsequent studies have failed to demonstrate colocalisation or  
19 direct interaction between PI4KII $\alpha$  and AP-1.<sup>[9, 41, 42]</sup> Instead PI4KII $\alpha$  forms a direct  
20 complex with another adaptor, AP-3, via its dileucine interaction motif  
21 <sup>56</sup>ERQPLL<sup>61</sup><sup>[42]</sup> (Figure 1A). AP-3 controls traffic from EE/SE into the  
22 endolysosomal pathway.<sup>[43]</sup> The finding that PI4KII $\alpha$  is a component of AP-3  
23 containing vesicles, along with many AP-3 cargoes,<sup>[44]</sup> further supports the  
24 involvement of PI4KII $\alpha$  in EE/SE-to-LE traffic (Figure 3A).  
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36 PI4KII $\beta$  is also found in the TGN where it colocalises with AP-1, and to a  
37 lesser degree with EE and LE markers, indicating that PI4KII $\beta$  is distributed  
38 between the TGN and endosomal systems. PI4KII $\beta$  contains the N-terminal  
39 dileucine sequence <sup>25</sup>EREPLL<sup>30</sup> that specifically binds AP-1, rather than AP-3.<sup>[45]</sup>  
40 This AP-1 complex is required to localise PI4KII $\beta$  to the TGN and PI4KII $\beta$   
41 knockdown causes mislocalisation of AP-1, indicating that the proteins are  
42 functionally interdependent. Importantly, by virtue of their adaptor recognition  
43 motifs, both PI4KII isoforms are effectively cargoes that promote the recruitment of  
44 their cognate adaptors. In the case of PI4KII $\beta$ /AP-1, this depends on the  
45 biosynthetic activity of PI4KII $\beta$ ; whereas, with PI4KII $\alpha$ /AP-3, despite having a  
46 requirement for both kinase activity and an intact dileucine motif, evidence  
47 supporting a direct role for PtdIns4P in AP-3 recruitment has been elusive.  
48 However, the PI4KII isoforms synthesise spatially separate pools of PtdIns4P in  
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1 the TGN-endosomal system<sup>[11]</sup> (Figure 2) and a pool of PtdIns4P is required for  
2 AP-3 cargo trafficking.<sup>[46, 47]</sup> arguing that both PI4K-adaptor systems are dependent  
3 on PtdIns4P. In summary, it is clear that PI4KII isoforms operate in different post-  
4 TGN trafficking pathways by utilising distinct PI4KII-dependent clathrin adaptor  
5 systems, and that the PI4KII $\alpha$ /AP-3 and PI4KII $\beta$ /AP-1 complexes exhibit a similar  
6 reciprocal relationship between location and function.  
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### 10 3.2 Late endosomal traffic: degradation or recycling? 11 12 13

14 The epidermal growth factor receptor (EGFR) is a potent tyrosine kinase  
15 and significant human oncogene that, when activated, is rapidly downregulated by  
16 traffic to late endosomes (LEs). PI4KII $\alpha$  is necessary for efficient EGFR trafficking  
17 and degradation.<sup>[34]</sup> and whilst PI4KII $\alpha$  may play a role in trafficking the receptor  
18 into the LE pathway, the traffic of hydrolytic enzymes to the lysosome may also  
19 contribute indirectly to impaired degradation in siRNA-treated cells.<sup>[47]</sup> Furthermore,  
20 EGFR degradation is likely to be influenced by interaction of PI4KII $\alpha$  with the  
21 ubiquitin proteasome system. PI4KII $\alpha$  directly binds to the E3 ubiquitin ligase Itch  
22 at the TGN-endosome boundary, an interaction that increases Itch ubiquitin ligase  
23 activity whilst decreasing PI4KII $\alpha$  activity. This interaction has important functional  
24 consequences for canonical Wnt signalling because the formation of the complex  
25 has the effect of facilitating the sorting of frizzled receptors for degradation, thereby  
26 determining the output of this major developmental signalling pathway.<sup>[41]</sup>  
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38 After they exit the TGN, newly synthesised soluble hydrolases rely on  
39 mannose-6-phosphate receptors (M6PR) for delivery to lysosomes.<sup>[48]</sup> Depletion of  
40 either PI4KII $\alpha$  or PI4KII $\beta$  affects traffic of the cation-independent M6PR, which is  
41 an AP-1 cargo sorted to the LE through an AP-3 pathway, and it is likely that  
42 PtdIns4P generated by the PI4KII isoforms is responsible for this.<sup>[40, 45]</sup> PI4KII $\alpha$  is  
43 required for co-trafficking of the lysosomal hydrolase  $\beta$ -glucocerebrosidase (GBA)  
44 and its receptor, the AP-3 cargo LIMP-2. GBA is essential for the metabolism of  
45 lysosomal glucosylceramide and mutations in GBA that affect binding to LIMP-2  
46 lead to missorting and lysosomal accumulation of glucosylceramide. Interestingly,  
47 although PI4KII $\alpha$  binds AP-3, and is clearly active in SE membranes, kinase dead  
48 PI4KII $\alpha$  is able to partially rescue AP-3 trafficking defects.<sup>[42, 47]</sup> It is currently  
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1 unclear why this phenomenon occurs but it is possible that an alternative activity is  
2 responsible for generating PtdIns4P at this locus.  
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4 Phagocytic cells use endosomal trafficking to deliver internalised pathogens  
5 and cell debris to the lysosome. As with endolysosomal traffic, phagosomes  
6 undergo maturation through early and late compartments, finally fusing with  
7 lysosomes. Two phases of PtdIns4P synthesis occur on maturing phagosomes:  
8 initially a transient phase, probably the result of PI4KIII $\alpha$  activity, followed by a  
9 sustained phase that precedes lysosomal fusion that corresponds to the late stage  
10 of phagocytosis. Mature phagocytic membranes contain PI4KII $\alpha$ , which  
11 synthesises PtdIns4P during this phase of maturation.<sup>[33, 49]</sup> This PtdIns4P pool  
12 presumably mediates traffic and fusion with lysosomes, probably by recruitment of  
13 the Rab7 effector RILP. An analogous heterotypic fusion process has been  
14 described involving the Atg8 family member  $\gamma$ -aminobutyric acid receptor-  
15 associated protein (GABARAP) that binds to and colocalises with PI4KII $\alpha$  on  
16 autophagic structures, along with PtdIns4P (Figure 2).<sup>[46, 50]</sup> siRNA of PI4KII $\alpha$  or  
17 overexpression of the inactive kinase leads to the accumulation of enlarged  
18 autophagosomes, whose appearance can be phenocopied by GABARAP  
19 depletion. This phenotype can be rescued with exogenous PtdIns4P;<sup>[50]</sup> therefore,  
20 the formation of large autophagosomes may be due to preventing the local  
21 generation of PtdIns4P and the impairment of autophagosome-lysosome fusion.  
22 The parallels between the processes of phagocytosis and autophagy are clear and  
23 it is likely that the role of PI4KII $\alpha$  in heterotypic fusion is conserved.  
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### 40 **3.3 PI4KII $\alpha$ determine the fate of cargoes through TGN sorting and** 41 **endosomal exit.** 42

43 PI4KII $\alpha$  exists in varying amounts in the TGN, EE, LE, recycling  
44 compartments and at the PM (Figure 3A). Mechanistic information on the trafficking  
45 steps that determine this broad subcellular distribution is, however, scarce. We  
46 know that siRNA of PI4KII $\alpha$  results in the mislocalisation of recycling<sup>[24]</sup> and late  
47 endosomal cargoes,<sup>[34, 44]</sup> and impaired sorting of the epidermal growth factor  
48 receptor (EGFR) and transferrin to degradative and recycling fates, respectively.<sup>[9]</sup>  
49 It appears that, rather than dynamic recruitment of the enzyme, the constitutively  
50 membrane-bound PI4KII $\alpha$  is sorted, cargo-like, into different pathways through  
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1 interaction with SNAREs. PI4KII $\alpha$  binds to the R-snare VAMP3 on SEs where it  
2 facilitates retrograde delivery back to the TGN. Furthermore, the close homolog  
3 VAMP7 was identified as a component of AP-3 and PI4KII $\alpha$ -containing vesicles,<sup>[42]</sup>  
4 where it mediates fusion with the LE. Thus converging evidence supports a role for  
5 SNAREs and PI4KII $\alpha$  in sorting into retrograde recycling and LE pathways,  
6 probably determining the balance of traffic between these diverging routes (Figure  
7 3A).  
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12 The association of PI4KII with membranes derived from secretory tissues is  
13 well documented and extracts of these have proved to be rich sources of activity.<sup>[7,</sup>  
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15 <sup>12]</sup> Aside from the work describing PI4KII in the control of post-TGN and endosomal  
16 trafficking, there is also a substantial body of work supporting a role for PI4KII $\alpha$  in  
17 traffic towards the PM (Table 2; Figure 3A). An explanation for this is provided by  
18 the fact that endosomal compartments can serve as intermediates in trafficking to  
19 the plasma membrane, particularly in specialised secretory cells.<sup>[51]</sup> Such a  
20 pathway has been described originating at EE/SEs (Figure 3A) that involves the  
21 PtdIns3P-phosphatase myotubularin 1 (MTM1).<sup>[52]</sup> Hydrolysis of endosomal  
22 PtdIns3P is required during the exocytotic step returning the unloaded TfR to the  
23 plasma membrane. Failure to hydrolyse PtdIns3P results in impaired exit from  
24 endosomes, and consequently, the accumulation of exocytic cargoes. Normally,  
25 MTM1 is recruited to PtdIns3P-containing EE/SEs that also harbour PI4KII $\alpha$ .<sup>[24, 34,</sup>  
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27 <sup>53]</sup> and when PtdIns3P is hydrolysed, the PtdIns produced becomes a substrate for  
28 resident PI4KII $\alpha$ . In the context of a membrane in which the supply of PtdIns  
29 may be limited, and where the local environment is optimal for kinase activity, then  
30 the result would be increased local PtdIns4P synthesis, effectively converting  
31 PtdIns3P on EE/SEs into PtdIns4P. This is a significant switch in membrane  
32 identity because PtdIns3P-positive endosomes are unable to fuse with the PM;  
33 however, vesicles containing PtdIns4P, that arise from the closely connected RE,  
34 can (Figure 3A).<sup>[52]</sup> Generation of PtdIns4P also leads to recruitment of the exocyst  
35 complex, which interacts with PI4KII $\alpha$ . Exocyst mediates the docking and  
36 membrane insertion of exocytic and recycling cargoes such as TfR and integrin  $\beta$ 1;  
37 therefore, MTM1 and PI4KII $\alpha$  forms a conversion module controlling endosomal  
38 exocytosis by switching PI metabolism from PtdIns3P to PtdIns4P. It also shows  
39 that PI4KII $\alpha$  is a more versatile enzyme that functions, not only in trafficking to the  
40 LE and terminal processes such as lysosomal fusion but also in anterograde traffic.  
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### 3.4 PI4KII-mediated traffic is linked to the actin cytoskeleton

Numerous actin-binding proteins interact both with PIs and elements of endocytic machinery, thereby linking PI metabolism with actin dynamics in membrane trafficking processes. For example, actin polymerisation is required to propel newly endocytosed cargoes through the cytoplasm on comet-like structures,<sup>[54]</sup> and requirements for actin polymerisation have been identified in LE to lysosomal traffic and in recycling. The Wiskott-Aldrich syndrome (WAS) family of actin branching regulators are targets of PtdIns(4,5) $P_2$  that are frequently implicated in endosomal trafficking pathways.<sup>[55]</sup> A clue that PI4KII enzymes may have a conserved role in actin-dependent endocytic traffic comes from the sole PI4KII homolog of the yeast *S. cerevisiae*, Lsb6, which is a binding protein of the yeast Wiskott-Aldrich syndrome protein (WASp) homolog Las17.<sup>[56]</sup>

During phagocytosis and *Listeria monocytogenes* infection, movement through the cytoplasm is accompanied by the dynamic formation of actin-rich comets. These require the coordinated activities of actin nucleating machinery ARP2/3, Ena-VASP and WASp. Both PI4KII isoforms are recruited to internalised beads coated with the bacterial cell surface protein InIB and these pseudophagosomes become decorated with PtdIns4P.<sup>[57]</sup>

PI4KII $\alpha$  exists in complex with a large number of actin regulatory proteins including RhoGEF1 and several subunits of the actin nucleation promoting WASH complex,<sup>[41, 53]</sup> which, together with Arp2/3, regulates endosome morphology and trafficking.<sup>[58]</sup> PI4KII $\alpha$  also colocalises with fluorescent actin on EEs that contain branched actin. The close association of PI4KII $\alpha$  with actin regulatory machinery and the necessity for PtdIns4P synthesis on endosomes together suggests that PI4KII $\alpha$  participates in the synthesis of an EE pool of PtdIns(4,5) $P_2$ . In support of this, we recently demonstrated that PtdIns4P and PtdIns(4,5) $P_2$  exist in separate endosomal microdomains and that PI4KII $\alpha$  is required for correct early endosomal sorting of the EGFR and Tf through the synthesis of PtdIns4P.<sup>[9]</sup>

PtdIns(4,5) $P_2$  levels in the EE are controlled by the PtdIns(4,5) $P_2$  5-phosphatase OCRL, depletion of which leads to an N-WASp-dependent increase in endosomal F-actin and consequently the mistrafficking of TfR, EGFR and M6PR.<sup>[59]</sup> Ectopic accumulation of PtdIns4P and PtdIns(4,5) $P_2$  is also observed when PtdIns4P hydrolysis by the PtdIns4P phosphatase Sac1 is inhibited at

1 endosome-ER contacts, resulting in aberrant trafficking of retrograde cargoes <sup>[60]</sup>.  
2 Interestingly, the activity of OCRL is also required on SEs to maintain correct traffic  
3 of multiple endosomal cargoes. <sup>[59]</sup> Together this evidence demonstrates the  
4 importance of regulating the levels of endosomal PtdIns4*P* and PtdIns(4,5)*P*<sub>2</sub> and,  
5 as the principal sources of early endosomal PtdIns4*P*, the PI4KII<sub>s</sub> are likely to  
6 assume greater importance at this site in the future.  
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#### 10 11 12 **4. PI4KII<sub>s</sub> modulate Wnt signalling**

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15 Wnt ligands control major developmental signalling cascades that are frequently  
16 dysregulated in human cancers. <sup>[61]</sup> Both PI4KII<sub>α</sub> and type I<sub>β</sub> phosphatidylinositol 4-  
17 phosphate 5-kinase (PIP5KI<sub>β</sub>) bind to, and are activated by, the Wnt scaffolding  
18 protein Dvl in a multiprotein complex synthesising PtdIns(4,5)*P*<sub>2</sub>. <sup>[62]</sup> Knockdown of  
19 PI4KII<sub>α</sub> causes axis duplication in *Xenopus*. <sup>[63]</sup> a developmental defect linked to  
20 aberrant Wnt signalling. Another study showed that knockdown of PI4KII<sub>α</sub> impaired  
21 the internalisation, endosomal traffic and degradation of the Wnt receptor frizzled  
22 (Fz) 4. <sup>[41]</sup> It is therefore likely that PI4KII<sub>α</sub> is involved in at least two regulatory  
23 steps in canonical Wnt signalling: the formation of an activated signalling complex  
24 with Dvl promoting PM PtdIns(4,5)*P*<sub>2</sub> synthesis at the PM and in a sorting complex  
25 that facilitates traffic to degradative compartments.  
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Knockdown of Zebrafish PI4KII<sub>β</sub> affects Wnt target gene expression and  
causes developmental defects. In cell lines, the kinase exists in a complex with Dvl  
and axin1, both components of the canonical Wnt signalling cascade. Depletion of  
PI4KII<sub>β</sub> leads to accumulation of endocytosed Fz in LEs, probably in transit for  
degradation. This accumulation can be rescued by ectopic expression of PI4KII<sub>β</sub>  
but not with a mutant incapable of AP-1 binding, indicating that AP-1 dependent  
trafficking underlies the phenomenon. Consistent with this, the AP-1 complex is  
known to be important for traffic to basolateral domains of epithelial cells <sup>[64]</sup> in  
developing embryos and interference with AP-1 perturbs the Wnt planar cell  
polarity (PCP) pathway <sup>[65]</sup> important for organisation of embryonic tissues.

#### 5. PI4KII<sub>s</sub> are associated with cancer

1 Both elevated and reduced levels of PI4KII $\alpha$  expression are associated with  
2 cancers. For example, elevation has been detected in a diverse panel of human  
3 tumours and increasing PI4KII $\alpha$  expression was observed during the development  
4 of tumours in a mouse xenograft model.<sup>[66]</sup> Meanwhile, ectopic overexpression of  
5 PI4KII $\alpha$  but not a kinase dead mutant was sufficient to induce expression of HIF-  
6 1 $\alpha$ , an important factor allowing cells to survive the hypoxic tumour environment.  
7 The same group subsequently showed that PI4KII $\alpha$  siRNA leads to degradation of  
8 Her2 and EGFR, and overexpression to Her2 activation.<sup>[67]</sup> Whilst the data  
9 supporting the hypothesis that PI4KII $\alpha$  positively regulates EGFR levels and  
10 signalling is comprehensive and compelling, it is at odds with the established LE  
11 roles of PI4KII $\alpha$  and in regulating the degradation of the EGFR<sup>[34]</sup> and the fact that  
12 loss rather than gain of the PI4K2A gene is associated with an aggressive brain  
13 cancer.<sup>[68]</sup> However, PI4KII $\alpha$  depletion studies have revealed complex effects on  
14 both the supply of PtdIns4P for signalling and membrane trafficking.<sup>[69]</sup> Thus it is  
15 likely that these discrepancies may reflect the inherent heterogeneity of tumours;  
16 nevertheless, it is clear that the dual targeting of EGFR family kinases and PI4KII $\alpha$   
17 holds some promise as a personalised chemotherapeutic approach in certain  
18 cancers.<sup>[67]</sup>

19 PI4KII $\beta$  has been implicated in regulation of cell migration and metastasis of  
20 hepatocellular carcinoma through interaction with the tetraspanin CD81.<sup>[70]</sup> a  
21 molecule that has been shown previously to co-purify with PI4KII activity.<sup>[71]</sup> siRNA  
22 of PI4KII $\beta$  leads to the formation of invasive structures, increased extracellular  
23 matrix degradation by the matrix type I metalloproteinase (MT1-MMP) and  
24 increased motility through collagen gels, thus indicating that PI4K2B has properties  
25 consistent with a suppressor of metastasis. In the same work, oncogenomic  
26 studies identified PI4KII $\beta$  loss as a risk factor in cancer.<sup>[11]</sup>

### 27 **5.1 PI4KII $\alpha$ is linked to neurological disease**

28 Loss of PI4KII $\alpha$  in mice leads to impaired neurological function resembling human  
29 spastic paraplegia, eventually resulting in premature death.<sup>[72]</sup> It is difficult to  
30 pinpoint a single trafficking step that may account for these defects given that  
31 PI4KII $\alpha$  functions in LE, retrograde traffic and exocytosis but, in addition to axonal  
32 pathology, the mice show accumulation of lipofuscin-like deposits and membrane  
33

1 inclusions. This is consistent with deficient LE traffic or EE/SE exit to the Golgi<sup>[46, 47]</sup>  
2 (Figure 4) and such trafficking defects would explain the axonal degeneration and  
3 neuron loss in these mice.  
4

5 Aberrant endolysosomal traffic is frequently associated with late onset  
6 neurodegenerative diseases.<sup>[73]</sup> As mentioned above, PI4KII $\alpha$  is responsible for the  
7 traffic of GBA from the TGN to endosomes where the complex is routed to  
8 lysosomes. Failure of this hydrolase to reach lysosomes results in failure to  
9 catabolise glucosylceramide and the accumulation causing Gaucher disease, the  
10 most common human lysosomal storage disease.  
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12 The ability PI4KII $\alpha$  to bind AP-3 and recruit the clathrin adaptor to  
13 membranes has important implications for traffic to lysosomes and lysosomal-  
14 related organelles. Hermansky-Pudlak syndrome is a rare autosomal recessive  
15 disease characterised by albinism and bleeding disorders. Like hereditary spastic  
16 paraplegia, Hermansky-Pudlak syndrome is genetically heterogeneous and can  
17 result from mutation in a number of loci encoding proteins involved in  
18 endolysosomal traffic. Clinical presentation and histopathology shows little in  
19 common with hereditary spastic paraplegia, but like the PI4KII $\alpha$  mouse knockout,  
20 lipofuscin deposition can be observed in some tissues. The adaptor complex AP-3  
21 is just one gene affected in Hermansky-Pudlak syndrome, and PI4KII $\alpha$  associates  
22 with multiprotein complexes known to be involved in Hermansky-Pudlak  
23 syndrome.<sup>[74]</sup>  
24

25 Loss of function mutations of the MTM1 gene encoding a PI3-phosphatase  
26 leads to X-linked centronuclear myopathy (XLCNM), a severe congenital  
27 neuromuscular disease. As discussed previously, hydrolysis of endosomal  
28 PtdIns3P and salvage of the PtdIns formed by PI4KII $\alpha$  appears to be a prerequisite  
29 for exocytic trafficking to the plasma membrane (Figure 4). Specific inhibitors for  
30 the PI 3-kinase hVPS34 have been developed and were shown to restore exocytic  
31 traffic to the PM.<sup>[52]</sup> suggesting that they could form the basis for treatment of  
32 XLCNM.  
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34 Cholesterol, a membrane constituent and activator of PI4KII $\alpha$ , has long  
35 been implicated in the pathogenesis of Alzheimer's disease.<sup>[75]</sup> Steroid-like natural  
36 ginsenosides can reduce the accumulation of the amyloidogenic A $\beta$   $\square\square\square\square\square\square$  in  
37 cultured neurons and the brains of an Alzheimer's mouse model, whilst enhancing  
38 the activity of PI4KII $\alpha$  and increasing the levels of PtdIns4P and PtdIns(4,5)P<sub>2</sub> in  
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1 | cultured cells.<sup>[76]</sup> Cholesterol and sterol analogs are able to activate PI4KII $\alpha$ .<sup>[17]</sup> and  
2 | although PI4KII $\alpha$  has not been directly implicated in the pathology of Alzheimer's  
3 | disease in recent gene studies, a plausible therapeutic strategy might be the up-  
4 | regulation PI4KII $\alpha$  activity, or the inhibition of PI 4-phosphatases, to protect against  
5 | the development of AD. However, testing this hypothesis would require  
6 | exceptionally demanding longitudinal studies.  
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## 11 | **6. Conclusions: Integrating PI4KII functions**

12 | The discovery of PtdIns4*P* on early and late endosomes, as well as the TGN,  
13 | challenges the prevailing view that PtdIns3*P* is an exclusive determinant of  
14 | endosomal membrane identity. It is now clear that PtdIns4*P* synthesis by PI4KII  
15 | isoforms at these membranes performs decisive roles in cargo selection, sorting  
16 | and in directing traffic. In early endosomes, PtdIns3*P* can be converted to PtdIns4*P*  
17 | via dephosphorylation to the common PtdIns precursor to accomplish endosomal  
18 | exit and traffic toward the plasma membrane. A similar relationship between  
19 | PtdIns3*P* and PtdIns4*P* has been noted during phagosomal maturation, suggesting  
20 | that comparable conversion mechanisms exist in other trafficking pathways. Also,  
21 | local PtdIns4*P* synthesis by PI4KIIs can recruit different clathrin adaptors thereby  
22 | controlling the traffic of different cargoes into distinct pathways and adding to the  
23 | complexity of PI4KII trafficking.  
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25 | Close membrane interaction as a result of palmitoylation is the principal  
26 | determinant of PI4KII activity at membrane interfaces. Unlike all known PI kinases,  
27 | which are invariably recruited to membranes containing their substrates, PI4KII $\alpha$  is  
28 | constitutively membrane associated. The emerging model then is one in which  
29 | membrane-bound PI4KII $\alpha$  is highly sensitive to its membrane environment,  
30 | responding to activation with the local synthesis of PtdIns4*P*. PI4KII $\alpha$  activity can  
31 | be further modulated in situ through the binding of proteins such as Itch that can  
32 | regulate activity. Therefore, PI4KII $\alpha$  appears to be a sensor capable of reacting to  
33 | changes in membrane environment, such as cholesterol content and the local  
34 | availability of PtdIns, with increased synthesis of PtdIns4*P*. In this way, PI4KII $\alpha$   
35 | activity recruits PtdIns4*P* effector proteins, alters membrane identity and redirects  
36 | traffic from the EE/SE in specific directions such as toward the LE, Golgi or PM.  
37 | The implication here is that membranes containing active PI4KII $\alpha$  could respond in  
38 | a similar way to many other metabolic activities generating PtdIns.  
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1 Insight into the biology of PI4KII $\beta$  has lagged behind but it is clear that this  
2 isoform, in contrast to PI4KII $\alpha$ , can be regulated by extrinsic factors. Extracellular  
3 signals trigger membrane recruitment of the enzyme and therefore control its  
4 activity. The plasma membrane location of PI4KII $\beta$  is intriguing because it implies a  
5 function for PtdIns4P synthesis at the PM, a role currently reserved for PI4KIII $\alpha$ .  
6 Also, it is not clear how PI4KII $\beta$  is specifically targeted to the plasma membrane by  
7 palmitoylation alone, and it is possible that additional factors are required. It is  
8 likely that PI4KII $\beta$  functions in developmental Wnt signalling at the plasma  
9 membrane, possibly by maintaining epithelial cell polarity and suppressing a  
10 migratory program. In support of this, loss of the enzyme is a risk factor in human  
11 cancers, which is also consistent with the notion that PI4KII $\beta$  is highly regulated  
12 under physiological conditions.  
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27 comments on the manuscript.  
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### 33 Figure legends

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36 **Table 1. Properties of the PI4KII isozymes.** TGN, *trans*-Golgi network; EE, early  
37 endosome; SE, sorting endosome; LE, late endosome; Pg, phagosome; Lys,  
38 lysosome; Apg, autophagosome; EV, exocytic vesicle. \*A large number of potential  
39 binding proteins have been identified by affinity purification and mass  
40 spectrometry, not all of which have been characterised in detail [41, 44, 45, 53, 74].  
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### 46 Table 2. PI4KII $\alpha$ in anterograde traffic.

47 Summary of evidence showing PI4KII $\alpha$  in traffic towards the PM.  
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51 **Figure 1. Structure of the PI4KII enzymes.** **A.** The domain organisation of  
52 PI4KII $\alpha$  and PI4KII $\beta$  is highly similar. The divergent N-termini lack homology but  
53 contain interaction sites for clathrin adaptors. In PI4KII $\alpha$ , a dileucine sorting motif  
54 <sup>57</sup>ERQPLL<sup>62</sup> is recognised by AP-3 [41, 42] whilst PI4KII $\beta$  contains a similar  
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1 sequence (<sup>25</sup>EREPLL<sup>30</sup>) that specifically binds AP-1 <sup>[45]</sup>. The N-ter of PI4KII $\alpha$  also  
 2 contains the sequence <sup>15</sup>PPDY<sup>18</sup> that binds the WW3 domain of the ubiquitin ligase  
 3 Itch, which mediates multi-ubiquitination of PI4KII $\alpha$  <sup>[41]</sup>. The conserved catalytic  
 4 core is now known to be composed of amino- (N) and carboxy- (C) terminal lobes.  
 5 Both isoforms contain a conserved palmitoylation motif, <sup>174</sup>CCPCC<sup>178</sup> in PI4KII $\alpha$   
 6 and PI4KII $\beta$  <sup>170</sup>CCPCC<sup>174</sup>. HSP90, which plays a role in the stability and  
 7 membrane recruitment of PI4KII $\beta$ , binds to a region between residues 315-481 in  
 8 the C-lobe <sup>[27]</sup>. This schematic is based on the human polypeptide sequences  
 9 (NP\_060895 and NP\_060793.2). **B.** Model illustrating how the PI binding pocket of  
 10 palmitoylated PI4KII $\alpha$  interacts with membranes (adapted with permission <sup>[14]</sup>).  
 11 Boxed area indicates palmitoylation loop.  
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## 20 **Figure 2. The subcellular distribution of PtdIns4P**

21 PtdIns4P is enriched in the Golgi complex where PI4KIII $\beta$ , PI4KII $\alpha$  and PI4KII $\beta$  are  
 22 responsible for its synthesis. Imaging methods using recombinant proteins derived  
 23 from PtdIns4P binding domains have revealed pools at the plasma membrane (PM  
 24 <sup>[8]</sup>), recycling endosome (RE, <sup>[52]</sup>), early/sorting endosome (EE/SE, <sup>[9, 46]</sup>), late  
 25 endosome (LE, <sup>[8]</sup>), lysosome (Lys, <sup>[8]</sup>), autophagosome (Apg, <sup>[50]</sup>) and phagosome  
 26 (Pg, <sup>[33, 49]</sup>). Discrete PI4K activities are responsible for this distribution and it is  
 27 known that PI4KIII $\alpha$  is a major PtdIns4P biosynthetic activity at the PM (yellow).  
 28 PI4KIII $\beta$  produces a pool in the Golgi apparatus (magenta), whereas PI4KII $\alpha$  and  
 29 PI4KII $\beta$  produce PtdIns4P on separate domains of the TGN (red and green,  
 30 respectively) <sup>[11]</sup>. PI4KII $\alpha$  is also responsible for pools of PtdIns4P on RE <sup>[52]</sup>, SE,  
 31 LE and Apg membranes (blue) that are likely to be dynamically regulated.  
 32 Furthermore, PI4KII $\alpha$  generates PtdIns4P in late phagosomes.  
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50 TGN, *trans*-Golgi network; EE, early endosome; SE, sorting endosome; LE, late  
 51 endosome; Pg phagosome; Lys, lysosome; Apg, autophagosome; EV, exocytic  
 52 vesicle; RE, recycling endosome.  
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## 61 **Figure 3. The membrane trafficking functions of the type II PI 4-kinases.**

62 Both PI4KII $\alpha$  and PI4KII $\beta$  localise to the TGN and endosomes, although it is likely  
 63 that they exist in separate domains within the TGN-endosomal system. **A.** PI4KII $\alpha$   
 64 localises predominantly to the TGN and endolysosomal system but relatively minor  
 65 pools have also been detected at the PM. At the early/sorting endosome (EE/SE),  
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1 PI4KII $\alpha$  recruits AP-3 to membranes participating in traffic to the LE; however, the  
2 enzyme is also involved in retrograde traffic via a VAMP3-dependent pathway.  
3 PI4KII $\alpha$  further functions in degradative pathways by producing PtdIns4P required  
4 for heterotypic fusion of lysosomal (Lys) and autophagosomal (Apg) membranes  
5 and an analogous process occurs in phagocytic cells [33, 49]. PI4KII $\alpha$  mediates  
6 exocytic traffic from endosomal compartments including the EE/SE via the  
7 recycling endosome (RE) [52] and in a Rab27a-dependent step from the LE during  
8 HIV-1 viral assembly in T-cells [77].

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14 **B.** PI4KII $\beta$  is partially cytosolic and is dynamically palmitoylated in response to  
15 growth factors and expression of constitutively active Rac, which both lead to PM  
16 recruitment. PI4KII $\beta$  synthesises a pool of PtdIns4P that colocalises with TGN46  
17 (green; [11]). At the TGN, PI4KII $\beta$  is responsible for recruitment of the clathrin  
18 adaptor AP-1 [45]. The molecular chaperone HSP90 binds PI4KII $\beta$  preventing  
19 palmitoylation, thereby maintaining PI4KII $\beta$  in an inactive, cytosolic form whilst  
20 protecting the protein from proteosomal degradation [27].  
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#### 28 **Figure 4. PI4KII trafficking pathways of relevance to human disease.**

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30 Five main pathways are affected by loss of PI4KII function in in vitro or whole  
31 animal models: TGN-to-endosomal traffic (i) is affected in zebrafish [that](#) have lost  
32 PI4KII $\beta$ , resulting in developmental defects attributed to aberrant Wnt signalling [45];  
33 (ii), loss of PI4KII $\alpha$  impairs endosome-to-TGN traffic facilitated by VAMP3 [46];  
34 (iii) is affected by knockdown of PI4KII $\alpha$ , which impairs  
35 endolysosomal traffic (iii) is affected by knockdown of PI4KII $\alpha$ , which impairs  
36 degradation of lysosomal cargoes such as the EGFR [34], the traffic of lysosomal  
37 membrane proteins [47] and lysosome-related organelles [78] and likely causes a  
38 phenotype in mice [that](#) is reminiscent of human spastic paraplegia (HSP; [72]. Note  
39 that this phenotype may reflect a defect in a lysosomal pathway or a retrograde  
40 step (i.e., ii); (iv) endosome-to-plasma membrane traffic controlled by PI4KII $\alpha$  is  
41 affected by in human X-linked centronuclear myopathy (XLCNM) [52]; (v), LE-to-PM.  
42 PI4KII $\alpha$  is trafficked from the LE to the PM in HIV infected T-cells [77]. Note that  
43 both PI4KII $\alpha$  and PI4KII $\beta$  function in pathways that can culminate in traffic to the  
44 lysosome; therefore, knockdown of either isoform can give similar late  
45 endosomal/lysosomal phenotypes, leading some to conclude they have  
46 overlapping functions.  
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## References

- 1 [1] T. Balla, *Physiol Rev* 2013, 93, 1019.
- 2 [2] K. O. Schink, K. W. Tan, H. Stenmark, *Annu Rev Cell Dev Biol* 2016, 32, 143.
- 3 [3] M. A. De Matteis, C. Wilson, G. D'Angelo, *Bioessays* 2013, 35, 612.
- 4 [4] A. Balla, T. Balla, *Trends Cell Biol* 2006, 16, 351.
- 5 [5] S. Minogue, M. G. Waugh, *Subcell Biochem* 2012, 58, 1.
- 6 [6] M. Whitman, D. Kaplan, T. Roberts, L. Cantley, *Biochem J* 1987, 247, 165.
- 7 [7] E. S. Husebye, A. J. Letcher, D. J. Lander, T. Flatmark, *Biochim Biophys Acta*
- 8 1990, 1042, 330.
- 9 [8] G. R. Hammond, M. P. Machner, T. Balla, *J Cell Biol* 2014, 205, 113.
- 10 [9] Y. Henmi, Y. Morikawa, N. Oe, N. Ikeda, A. Fujita, K. Takei, S. Minogue, K.
- 11 Tanabe, *Mol Biol Cell* 2016, 27, 990.
- 12 [10] A. Balla, G. Tuymetova, A. Tsiomenko, P. Varnai, T. Balla, *Mol Biol Cell* 2005, 16,
- 13 1282.
- 14 [11] G. O. Alli-Balogun, C. A. Gewinner, R. Jacobs, J. Kriston-Vizi, M. G. Waugh, S.
- 15 Minogue, *Mol Biol Cell* 2016, 27, 4033.
- 16 [12] B. Barylko, S. H. Gerber, D. D. Binns, N. Grichine, M. Khvotchev, T. C. Sudhof, J. P.
- 17 Albanesi, *J Biol Chem* 2001, 276, 7705.
- 18 [13] S. Minogue, J. S. Anderson, M. G. Waugh, M. dos Santos, S. Corless, R. Cramer, J. J.
- 19 Hsuan, *J Biol Chem* 2001, 276, 16635.
- 20 [14] Q. Zhou, J. Li, H. Yu, Y. Zhai, Z. Gao, Y. Liu, X. Pang, L. Zhang, K. Schulten, F. Sun,
- 21 C. Chen, *Nat Commun* 2014, 5, 3552.
- 22 [15] A. Baumlova, D. Chalupska, B. Rozycki, M. Jovic, E. Wisniewski, M. Klima, A.
- 23 Dubankova, D. P. Kloer, R. Nencka, T. Balla, E. Boura, *EMBO reports* 2014, 15, 1085.
- 24 [16] E. H. Walker, O. Perisic, C. Ried, L. Stephens, R. L. Williams, *Nature* 1999, 402,
- 25 313; V. D. Rao, S. Misra, I. V. Boronenkov, R. A. Anderson, J. H. Hurley, *Cell* 1998, 94,
- 26 829.
- 27 [17] M. G. Waugh, S. Minogue, D. Chotai, F. Berditchevski, J. J. Hsuan, *J Biol Chem*
- 28 2006, 281, 3757.
- 29 [18] M. Klima, A. Baumlova, D. Chalupska, H. Hrebabecky, M. Dejmek, R. Nencka, E.
- 30 Boura, *Acta Crystallogr D Biol Crystallogr* 2015, 71, 1555.
- 31 [19] J. Li, Z. Gao, D. Zhao, L. Zhang, X. Qiao, Y. Zhao, H. Ding, P. Zhang, J. Lu, J. Liu, H.
- 32 Jiang, C. Luo, C. Chen, *Cancer Res* 2017.
- 33 [20] M. G. Waugh, S. Minogue, J. S. Anderson, A. Balinger, D. Blumenkrantz, D. P.
- 34 Calnan, R. Cramer, J. J. Hsuan, *Biochem J* 2003, 373, 57.
- 35 [21] D. Lu, H. Q. Sun, H. Wang, B. Barylko, Y. Fukata, M. Fukata, J. P. Albanesi, H. L.
- 36 Yin, *J Biol Chem* 2012, 287, 21856.
- 37 [22] S. Minogue, K. M. Chu, E. J. Westover, D. F. Covey, J. J. Hsuan, M. G. Waugh, *J*
- 38 *Lipid Res* 2010, 51, 2314; B. Mesmin, J. Bigay, J. Polidori, D. Jamecna, S. Lacas-Gervais,
- 39 B. Antonny, *EMBO J* 2017.
- 40 [23] H. Olsson, W. Martinez-Arias, B. Jergil, *FEBS Lett* 1993, 327, 332.
- 41 [24] A. Balla, G. Tuymetova, M. Barshishat, M. Geiszt, T. Balla, *J Biol Chem* 2002, 277,
- 42 20041.
- 43 [25] G. Jung, J. Wang, P. Wlodarski, B. Barylko, D. D. Binns, H. Shu, H. L. Yin, J. P.
- 44 Albanesi, *Biochem J* 2008, 409, 501.
- 45 [26] Y. J. Wei, H. Q. Sun, M. Yamamoto, P. Wlodarski, K. Kunii, M. Martinez, B.
- 46 Barylko, J. P. Albanesi, H. L. Yin, *J Biol Chem* 2002, 277, 46586.
- 47 [27] G. Jung, B. Barylko, D. Lu, H. Shu, H. Yin, J. P. Albanesi, *J Biol Chem* 2011, 286,
- 48 12775.
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- 1 [28] O. Idevall-Hagren, P. De Camilli, *Biochim Biophys Acta* 2015, 1851, 736.  
2 [29] G. R. Hammond, G. Schiavo, R. F. Irvine, *Biochem J* 2009, 422, 23.  
3 [30] Z. Szentpetery, P. Varnai, T. Balla, *Proceedings of the National Academy of*  
4 *Sciences of the United States of America* 2010, 107, 8225.  
5 [31] P. Varnai, B. Thyagarajan, T. Rohacs, T. Balla, *J Cell Biol* 2006, 175, 377.  
6 [32] M. K. Korzeniowski, M. A. Popovic, Z. Szentpetery, P. Varnai, S. S. Stojilkovic, T.  
7 Balla, *J Biol Chem* 2009, 284, 21027.  
8 [33] R. Levin, G. R. Hammond, T. Balla, P. De Camilli, G. D. Fairn, S. Grinstein, *Mol*  
9 *Biol Cell* 2017, 28, 128.  
10 [34] S. Minogue, M. G. Waugh, M. A. De Matteis, D. J. Stephens, F. Berditchevski, J. J.  
11 Hsuan, *Journal of cell science* 2006, 119, 571.  
12 [35] F. R. Maxfield, T. E. McGraw, *Nat Rev Mol Cell Biol* 2004, 5, 121.  
13 [36] J. S. Bonifacino, R. Rojas, *Nat Rev Mol Cell Biol* 2006, 7, 568.  
14 [37] I. G. Mills, G. J. Praefcke, Y. Vallis, B. J. Peter, L. E. Olesen, J. L. Gallop, P. J. Butler,  
15 P. R. Evans, H. T. McMahon, *J Cell Biol* 2003, 160, 213.  
16 [38] J. S. Bonifacino, *Nat Rev Mol Cell Biol* 2004, 5, 23.  
17 [39] J. Wang, H. Q. Sun, E. Macia, T. Kirchhausen, H. Watson, J. S. Bonifacino, H. L. Yin,  
18 *Mol Biol Cell* 2007, 18, 2646.  
19 [40] Y. J. Wang, J. Wang, H. Q. Sun, M. Martinez, Y. X. Sun, E. Macia, T. Kirchhausen, J.  
20 P. Albanesi, M. G. Roth, H. L. Yin, *Cell* 2003, 114, 299.  
21 [41] J. Mossinger, M. Wieffer, E. Krause, C. Freund, F. Gerth, M. Krauss, V. Haucke,  
22 *EMBO reports* 2012, 13, 1087.  
23 [42] B. Craige, G. Salazar, V. Faundez, *Mol Biol Cell* 2008, 19, 1415.  
24 [43] A. A. Peden, V. Oorschot, B. A. Hesser, C. D. Austin, R. H. Scheller, J.  
25 Klumperman, *J Cell Biol* 2004, 164, 1065.  
26 [44] G. Salazar, B. Craige, B. H. Wainer, J. Guo, P. De Camilli, V. Faundez, *Mol Biol Cell*  
27 2005, 16, 3692.  
28 [45] M. Wieffer, E. Cibrian Uhalte, Y. Posor, C. Otten, K. Branz, I. Schutz, J. Mossinger,  
29 P. Schu, S. Abdelilah-Seyfried, M. Krauss, V. Haucke, *Curr Biol* 2013, 23, 2185.  
30 [46] M. Jovic, M. J. Kean, A. Dubankova, E. Boura, A. C. Gingras, J. A. Brill, T. Balla,  
31 *Journal of cell science* 2014, 127, 3745.  
32 [47] M. Jovic, M. J. Kean, Z. Szentpetery, G. Polevoy, A. C. Gingras, J. A. Brill, T. Balla,  
33 *Mol Biol Cell* 2012, 23, 1533.  
34 [48] S. Kornfeld, I. Mellman, *Annu Rev Cell Biol* 1989, 5, 483.  
35 [49] A. Jeschke, N. Zehethofer, B. Lindner, J. Krupp, D. Schwudke, I. Haneburger, M.  
36 Jovic, J. M. Backer, T. Balla, H. Hilbi, A. Haas, *Proceedings of the National Academy of*  
37 *Sciences of the United States of America* 2015.  
38 [50] H. Wang, H. Q. Sun, X. Zhu, L. Zhang, J. Albanesi, B. Levine, H. Yin, *Proceedings of*  
39 *the National Academy of Sciences of the United States of America* 2015, 112, 7015.  
40 [51] A. L. Ang, T. Taguchi, S. Francis, H. Folsch, L. J. Murrells, M. Pypaert, G. Warren,  
41 I. Mellman, *J Cell Biol* 2004, 167, 531.  
42 [52] K. Ketel, M. Krauss, A. S. Nicot, D. Puchkov, M. Wieffer, R. Muller, D.  
43 Subramanian, C. Schultz, J. Laporte, V. Haucke, *Nature* 2016, 529, 408.  
44 [53] P. V. Ryder, R. Vistein, A. Gokhale, M. N. Seaman, M. A. Puthenvedu, V. Faundez,  
45 *Mol Biol Cell* 2013, 24, 2269.  
46 [54] S. Benesch, S. Lommel, A. Steffen, T. E. Stradal, N. Scaplehorn, M. Way, J.  
47 Wehland, K. Rottner, *J Biol Chem* 2002, 277, 37771; J. Taunton, B. A. Rowning, M. L.  
48 Coughlin, M. Wu, R. T. Moon, T. J. Mitchison, C. A. Larabell, *J Cell Biol* 2000, 148, 519; A.  
49 L. Rozelle, L. M. Machesky, M. Yamamoto, M. H. Driessens, R. H. Insall, M. G. Roth, K.  
50 Luby-Phelps, G. Marriott, A. Hall, H. L. Yin, *Curr Biol* 2000, 10, 311.  
51 [55] H. Miki, K. Miura, T. Takenawa, *EMBO J* 1996, 15, 5326.  
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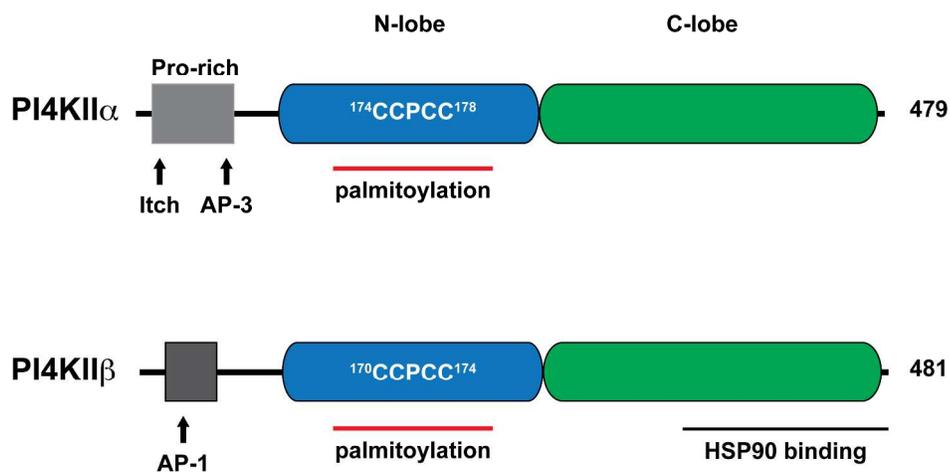
- 1 [56] F. S. Chang, G. S. Han, G. M. Carman, K. J. Blumer, *J Cell Biol* 2005, 171, 133.  
2 [57] J. Pizarro-Cerda, B. Payrastre, Y. J. Wang, E. Veiga, H. L. Yin, P. Cossart, *Cell*  
3 *Microbiol* 2007, 9, 2381.  
4 [58] S. N. Duleh, M. D. Welch, *Cytoskeleton* 2010, 67, 193.  
5 [59] M. Vicinanza, A. Di Campli, E. Polishchuk, M. Santoro, G. Di Tullio, A. Godi, E.  
6 Levtchenko, M. G. De Leo, R. Polishchuk, L. Sandoval, M. P. Marzolo, M. A. De Matteis,  
7 *EMBO J* 2011, 30, 4970.  
8 [60] R. Dong, Y. Saheki, S. Swarup, L. Lucast, J. W. Harper, P. De Camilli, *Cell* 2016,  
9 166, 408.  
10 [61] A. Klaus, W. Birchmeier, *Nat Rev Cancer* 2008, 8, 387.  
11 [62] Y. Qin, L. Li, W. Pan, D. Wu, *J Biol Chem* 2009, 284, 22544.  
12 [63] W. Pan, S. C. Choi, H. Wang, Y. Qin, L. Volpicelli-Daley, L. Swan, L. Lucast, C.  
13 Khoo, X. Zhang, L. Li, C. S. Abrams, S. Y. Sokol, D. Wu, *Science* 2008, 321, 1350.  
14 [64] J. S. Bonifacino, *J Cell Biol* 2014, 204, 7.  
15 [65] J. M. Carvajal-Gonzalez, S. Balmer, M. Mendoza, A. Dussert, G. Collu, A. C. Roman,  
16 U. Weber, B. Ciruna, M. Mlodzik, *Nat Commun* 2015, 6, 6751.  
17 [66] J. Li, Y. Lu, J. Zhang, H. Kang, Z. Qin, C. Chen, *Oncogene* 2010, 29, 2550.  
18 [67] J. Li, L. Zhang, Z. Gao, H. Kang, G. Rong, X. Zhang, C. Chen, *Protein Cell* 2014, 5,  
19 457.  
20 [68] M. G. Waugh, *Mol Neurobiol* 2016, 53, 621.  
21 [69] K. M. Chu, S. Minogue, J. J. Hsuan, M. G. Waugh, *Cell Death Dis* 2010, 1, e106.  
22 [70] A. Mazzocca, F. Liotta, V. Carloni, *Gastroenterology* 2008, 135, 244.  
23 [71] F. Berditchevski, K. F. Tolias, K. Wong, C. L. Carpenter, M. E. Hemler, *J Biol Chem*  
24 1997, 272, 2595.  
25 [72] J. P. Simons, R. Al-Shawi, S. Minogue, M. G. Waugh, C. Wiedemann, S. Evangelou,  
26 A. Loesch, T. S. Sihra, R. King, T. T. Warner, J. J. Hsuan, *Proceedings of the National*  
27 *Academy of Sciences of the United States of America* 2009, 106, 11535.  
28 [73] A. M. Schreij, E. A. Fon, P. S. McPherson, *Cell Mol Life Sci* 2016, 73, 1529.  
29 [74] G. Salazar, S. Zlatic, B. Craige, A. A. Peden, J. Pohl, V. Faundez, *J Biol Chem* 2009,  
30 284, 1790.  
31 [75] G. Di Paolo, T. W. Kim, *Nature reviews. Neuroscience* 2011, 12, 284.  
32 [76] M. S. Kang, S. H. Baek, Y. S. Chun, A. Z. Moore, N. Landman, D. Berman, H. O.  
33 Yang, M. Morishima-Kawashima, S. Osawa, S. Funamoto, Y. Ihara, G. Di Paolo, J. H. Park,  
34 S. Chung, T. W. Kim, *J Biol Chem* 2013, 288, 20868.  
35 [77] P. P. Gerber, M. Cabrini, C. Jancic, L. Paoletti, C. Banchio, C. von Bilderling, L.  
36 Sigaut, L. I. Pietrasanta, G. Duette, E. O. Freed, S. Basile Gde, C. F. Moita, L. F. Moita, S.  
37 Amigorena, P. Benaroch, J. Geffner, M. Ostrowski, *J Cell Biol* 2015, 209, 435.  
38 [78] M. Lopes da Silva, M. N. O'Connor, J. Kriston-Vizi, I. J. White, R. Al-Shawi, J. P.  
39 Simons, J. Mossinger, V. Haucke, D. F. Cutler, *Journal of cell science* 2016, 129, 2096.  
40 [79] R. L. Del Vecchio, P. F. Pilch, *J Biol Chem* 1991, 266, 13278; S. Kristiansen, T.  
41 Ramlal, A. Klip, *Biochem J* 1998, 335 ( Pt 2), 351; Z. Xu, G. Huang, K. V. Kandror, *Mol*  
42 *Endocrinol* 2006, 20, 2890.  
43 [80] Y. Ishihara, T. Furuno, M. Nakanishi, *Inflamm Res* 2006, 55, 465.  
44 [81] C. Wiedemann, T. Schafer, M. M. Burger, *EMBO J* 1996, 15, 2094; J. Burgess, L.  
45 M. Del Bel, C. I. Ma, B. Barylko, G. Polevoy, J. Rollins, J. P. Albanesi, H. Kramer, J. A. Brill,  
46 *Development* 2012, 139, 3040.  
47 [82] C. Panaretou, S. A. Tooze, *Biochem J* 2002, 363, 289.

<b>Table 1 Properties of the PI4KII isozymes</b>		
	<b>PI4KII<math>\alpha</math></b>	<b>PI4KII<math>\beta</math></b>
<b>Subcellular distribution</b>	EE, LE, Pg, PM, Apg	TGN, EE, LE, cytosolic
<b>Principal functions</b>	EE and LE traffic, Apg-Lys and Pg-Lys fusion, exocytosis	TGN-endosomal traffic
<b>Binding proteins*</b>	AP-3 <sup>(direct)</sup> , BLOC-1, exocyst complex <sup>(unknown)</sup> , Itch <sup>(direct)</sup> , GABARAPs <sup>(unknown)</sup>	HSP90 <sup>(direct)</sup> , AP-1 <sup>(direct)</sup>
<b>Membrane association</b>	Constitutively associated (palmitoylation)	Recruited in response to growth factors (PM)
<b>Upstream regulators</b>	Cholesterol, Itch (-ve)	Rac, growth factors, HSP90, Cholesterol (?)
<b>Downstream effectors</b>	AP-3	AP-1

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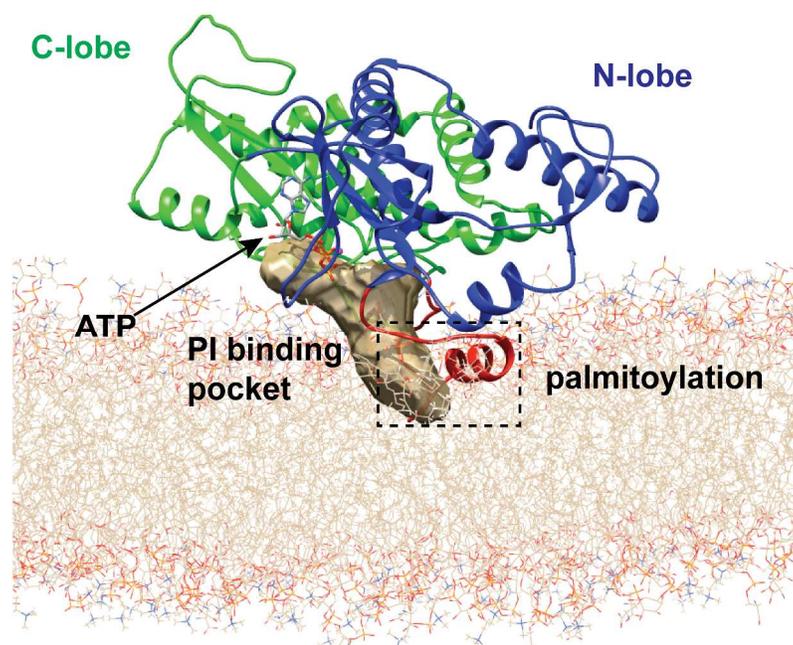
<b>Trafficking process</b>	<b>References</b>
<b>TGN&gt;PM</b>	[40]
<b>EE/SE&gt;RE&gt;PM</b>	[52]
<b>LE&gt;PM</b>	[77]
<b>Glut4 &gt;PM</b>	[79]
<b>Mast cell degranulation</b>	[80]
<b>Regulated secretion</b>	[81]
<b>Secretory granule formation</b>	[82]

Figure 1A. Minogue 2017



243x149mm (300 x 300 DPI)

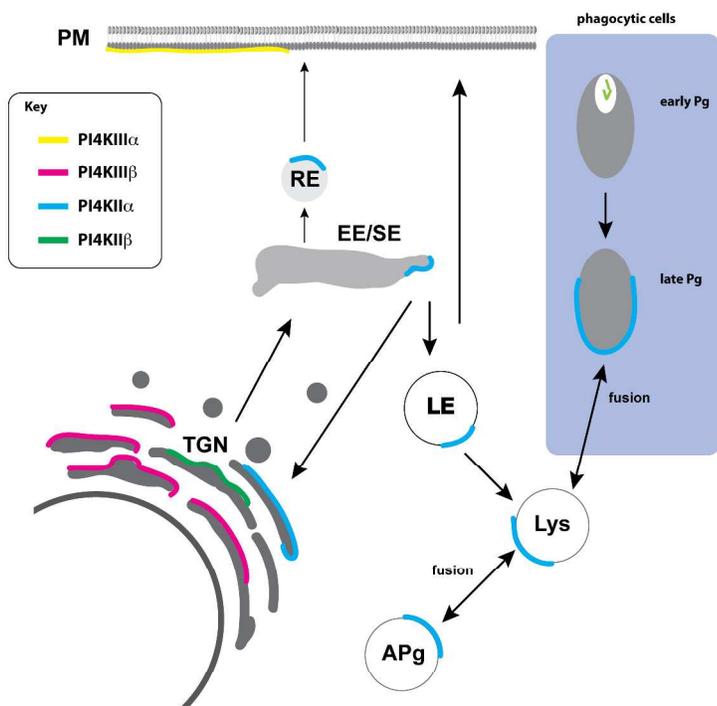
Figure 1B. Minogue 2017



293x218mm (300 x 300 DPI)

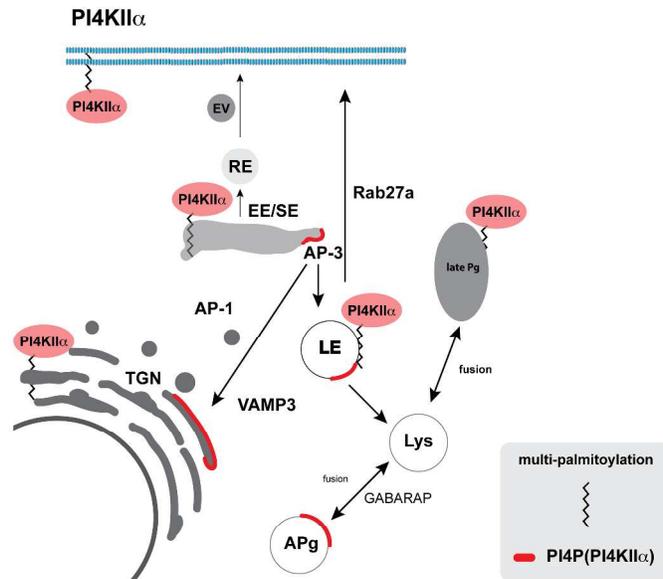
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Figure 2 (Minogue 2017)



353x330mm (300 x 300 DPI)

Figure 3A (Minogue 2017)



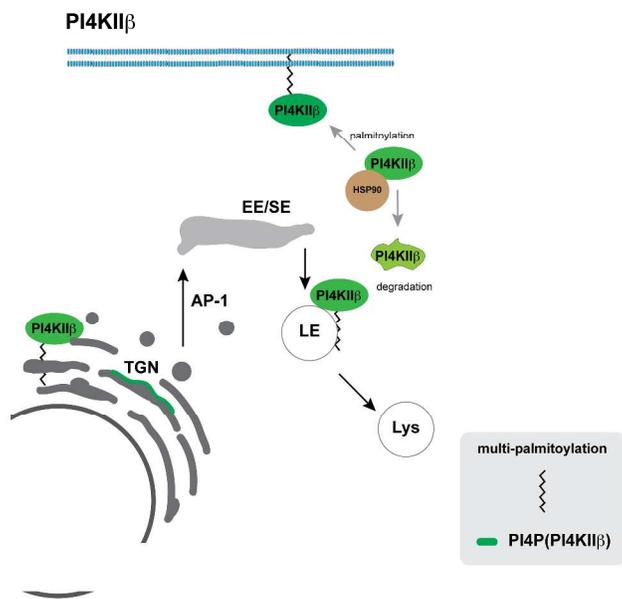
446x337mm (300 x 300 DPI)

review

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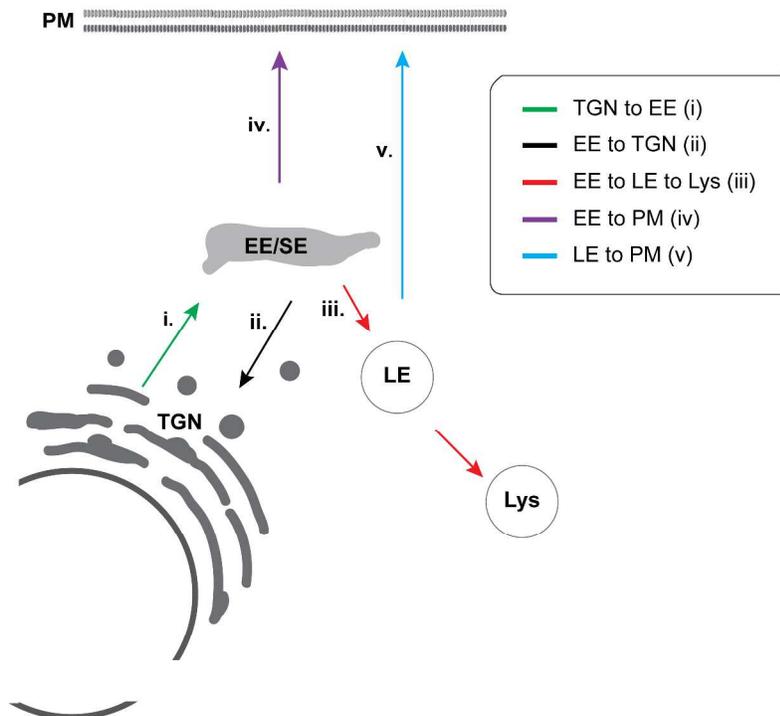
Figure 3B (Minogue 2017)



464x300mm (300 x 300 DPI)

Review

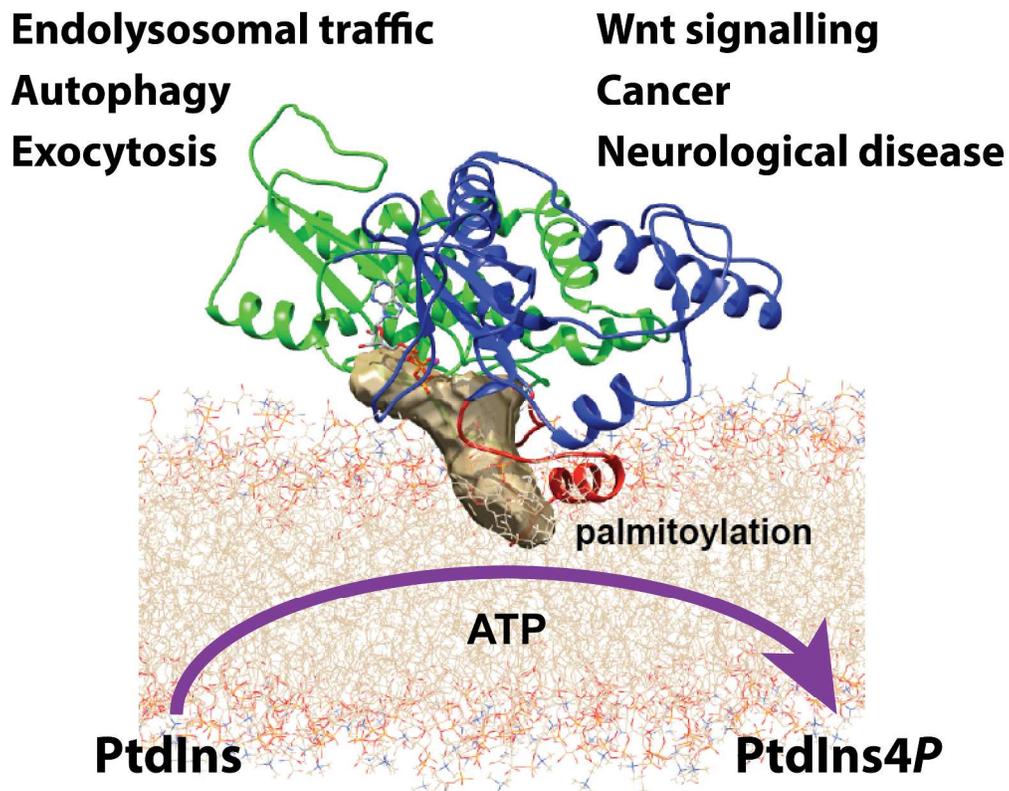
Figure 4 (Minogue 2017)



358x330mm (300 x 300 DPI)



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The type II phosphatidylinositol 4-kinases (PI4KIIs) are versatile mediators of membrane trafficking, controlling pathways that impact on disease. Recent advances suggest that the membrane lipid environment regulates enzyme activity and that the complexity of trafficking routes can be explained by the ability of these kinases to promote membrane trafficking in different directions.

269x213mm (300 x 300 DPI)