The Great Escape: How phosphatidylinositol 4-kinases and PI4P promote vesicle exit from the Golgi (and drive cancer).

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Abstract.

Phosphatidylinositol 4-phosphate (PI4P) is a membrane glycerophospholipid and a major regulator of the characteristic appearance of the Golgi complex as well as its vesicular trafficking, signalling and metabolic functions. Phosphatidylinositol 4-kinases, and in particular the PI4KIIIβ isoform, act in concert with PI4P to recruit macromolecular complexes to initiate the biogenesis of trafficking vesicles for several Golgi exit routes. Dysregulation of Golgi PI4P metabolism and the PI4P protein interactome features in many cancers and is often associated with tumour progression and a poor prognosis. Increased expression of PI4P binding proteins such as GOLPH3 or PITPNC1, induces a malignant secretory phenotype and the release of proteins that can remodel the extracellular matrix, promote angiogenesis and enhance cell motility. Aberrant Golgi PI4P metabolism can also result in the impaired post-translational modification of proteins required for focal adhesion formation and cell matrix interactions, thereby potentiating the development of aggressive metastatic and invasive tumours. Altered expression of the Golgi-targeted PI 4-kinases, PI4KIIIβ, PI4KIIα and PI4KIIβ, or the PI4P phosphate Sac1, can also modulate oncogenic signalling often through effects on TGN-endosomal trafficking. A Golgi trafficking role for a PIP 5-kinase has been recently described, indicating that PI4P is not the only functionally important phosphoinositide at this subcellular location. This review charts new developments in our understanding of phosphatidylinositol 4-kinase function at the Golgi and how PI4P-dependent trafficking can be deregulated in malignant disease.

Key words: Golgi, cancer, phosphatidylinositol, 4-kinase, PI4P, GOLPH3, PITPNC1.

Abbreviations: ACBD3 - Acyl-coenzyme A binding domain containing protein 3; CERT - ceramide transfer protein; DAG - diacylglycerol; DNA-PKcs DNA-dependent protein kinase; EMT - Epithelial-mesenchymal transition, GOLPH3 – Golgi phosphoprotein 3, OSBP -oxysterol binding protein, PI – phosphatidylinositol; PI4P – phosphatidylinositol 4-phosphate, PI4KIIIβ – phosphatidylinositol 4-kinase IIIβ, PI(4,5)P_2 - phosphatidylinositol (4,5)-bisphospate, PH - pleckstrin homology, Prostate apoptosis response-4 (PAR-4), TGN – *trans*-Golgi network.

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Introduction

Phosphoinositides, also known as inositol phospholipids or PIPs, regulate a vast array of cellular functions including receptor-mediated signalling, cell movement, cell proliferation and intracellular vesicle trafficking. There are seven potential phosphorylated derivatives of phosphatidylinositol, but at the Golgi complex, a single mono-phosphorylated species called phosphatidylinositol 4-phosphate (PI4P) predominates and has important regulatory roles in vesicle trafficking. At the Golgi, PI4P is synthesised by the ATP-dependent phosphorylation of phosphatidylinositol on the D4 position in a reaction catalysed by Golgi-targeted lipid kinases, termed phosphatidylinositol 4-kinases (PI 4-kinases) (reviewed in (1)). Four different PI 4-kinases are expressed in most human cells and the phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ) isoform is especially important at the Golgi compartment. The other isoforms, including the homologous PI4KIIIα enzyme and the structurally distinct type II PI 4-kinases (2), PI4KIIα and PI4KIIβ, have also been assigned Golgi functions (3-8), although these tend to be less well understood or widely accepted. The biochemical reactions involved in generating PI4P on Golgi membranes are depicted in Figure 1. In recent years, it has become slowly apparent that in some tumours, mutations that cause alterations to Golgi PI4P or the proteins that bind to it, can result in defective vesicular trafficking, increased secretion and ultimately more aggressive cancers. It may be useful to be aware from the outset that this a research area beset with several contentious areas, an occasionally conflicting literature and alternative views on some key topics. Nevertheless, the purpose of this review is to describe current thinking on Golgi PI4P metabolism and trafficking, and to explain how these processes can become dysfunctional in malignant disease.

The Golgi complex as a phosphoinositide-dependent trafficking and signalling hub

The Golgi apparatus is a perinuclear organelle consisting of cisternae organised into cis, medial and trans stacks. In mammalian cells these membranous sub-compartments are arranged in ribbon-like structures (reviewed in (9)). (10)). Vesicle biogenesis and cargo sorting mainly occurs in an extensive post- cisternal, tubular vesicular compartment termed the *trans*-Golgi network or TGN. For the purposes of this review the *cis*-, *medial*- and *trans*–Golgi compartments together with the associated TGN will be collectively referred to as the Golgi complex. Proteins and lipids synthesised in the endoplasmic reticulum are delivered to the cis-face of the Golgi whence they are transported through the Golgi stacks and post-translationally modified by Golgi-resident enzymes such as glycosyltrasferases, lipid transferases, phosphatases and kinases, and all of these functions are influenced by either PI4P or PI 4-kinases (for examples see (4, 8, 11-16)).

PI4P and PI 4-kinases are also crucial in driving the process of vesicle biogenesis at the TGN through a variety of mechanisms, these are :

1. PI4P enrichment on the outer lipid layer of TGN membranes may induce a degree of curvature that promotes vesicle formation (17).
2. PI4P on the cytosol-facing, outer leaflet of the membrane bilayer can recruit specific-binding proteins such as GOLPH3 or arfaptins that induce further membrane deformation and bilayer asymmetry through the insertion of membrane intercalating domains that are envisaged to form wedge like structures that induce vesicle budding (18, 19).

3. PI4P together with the Golgi-resident Arf-1 small GTPase can recruit a range of lipid transport proteins such as oxysterol binding protein (OSBP) that greatly modify the lipid composition of the TGN, and increase levels of cholesterol and sphingomyelin which are also major components of nascent trafficking vesicles. FAPP2, a PI4P-recruited, glucosylceramide transfer protein, is especially noteworthy in that its association with membranes also induces their tubulation (20, 21) .

4. PI 4-kinases such as PI4KIIIβ can directly bind and recruit trafficking proteins such as the small GTPase Rab11 (22-24) and this is important for specifying the directionality of vesicle traffic from the TGN.

5. From yeast to mammals, PI 4-kinases and PI4P have been implicated in the recruitment of adaptor proteins such as the Golgi-associated, gamma adaptin ear containing, ARF binding protein (GGA) complexes (6, 25-27) that bind and select specific-classes of post-translationally modified-cargo for inclusion into, clathrin-coated transport vesicles. Hence, PI4P modulates Golgi function and vesicle trafficking in multiple ways.

The Golgi complex, PI4P and cancer – an overview.

In some tumours the changes to Golgi trafficking, signalling and morphology (28-35) can be so pronounced that the term "onco-Golgi" has been proposed to capture its altered status in malignant disease (36). Relevant to this concept, gene mutations that lead to increased expression of Golgi-targeted PI4P binding proteins or PI 4-kinases can be associated with a distended Golgi morphology (37) and the anterograde trafficking of proteins (38-41) that drive angiogenesis (42, 43), tumour invasion (44-47) and metastasis (48-51). Specifically, these disease-inducing changes correlate with gene copy number increases (52) and the overexpression of Golgi-associated PI 4-kinases such as PI4KIIIβ (53, 54) and PI4P-binding proteins such as GOLPH3 and PITPNC1 (41, 55) (see Table 1). These protein expression changes can upregulate PI4P generation and/or vesicle biogenesis, and are key to development of the malignant secretory (41) and senescence-associated secretory phenotypes (31) (Figure 3) which feature in more aggressive and difficult to treat cancers.

Enhanced secretory flux is not the only means through which mutation-induced changes to Golgi phosphoinositide metabolism can be potentially oncogenic. Alterations to Golgi-endosomal trafficking can also impair endosomal biogenesis and/or functioning and thus alter the kinetics of degradative trafficking and intracellular signalling by oncogenic receptors such as the EGFR (53, 56-58) or, enhance the endosomal delivery of tumour promoting molecules to the plasma membrane and cell exterior (59, 60). Furthermore, there are recent clear demonstrations that Golgi-associated PI4P is itself an important intracellular signalling molecule. Golgi PI4P is itself a substrate for receptor-activated phospholipase Cε resulting in the generation of a signalling pool of diacylglycerol (DAG) that can recruit PKD and/or PKC and activate downstream effector pathways such as the ERK or MAP kinase cascades (61, 62). PI 4-kinase activity at the TGN also contributes to a minor but functionally important pool of this lipid at the plasma membrane that is involved in agonist-stimulated receptor signalling and KCNQ2/3 channel function (63, 64). Finally, proteins of the PI4P trafficking machinery can act as binding partners for regulators of the ERK and MAP kinase signalling cascades as has been demonstrated for both OSBP and PI4KIIIβ (16, 65, 66).

These multiple roles for PI4P and by extension Golgi-resident PI 4-kinases, illustrate how this single lipid species integrates the trafficking, metabolic and signalling roles that characterise this particular organelle (63). The emerging mechanisms through which Golgi PI4P can evoke a repertoire of cancer-promoting trafficking defects will be explored in ensuing sections of this review.

PI4KIIIβ – functions at the TGN and in cancer.

Of the four mammalian PI 4-kinases, PI4KIIIβ (encoded by the PI4KB gene on chromosome 1q) has the most widely accepted and best understood role in generating both PI4P and vesicular carriers at the Golgi complex. PI4KIIIβ is required for the constitutive formation of large, uncoated, tubular vesicles destined for the plasma membrane (67-70), AP-1 clathrin-mediated transport to endosomes (25, 26) and in some cell types, Ca^{2+}-dependent exocytosis (71-96) . Very recently, ablated PI4KIIα and PI4KIIIβ expression were demonstrated to induce a process called GOMED or **Go**lgi **me**mbrane-associated **d**egradation pathway, which is a mode of organelle-digestion separate from autophagy that does not require either ATG5 or ATG7, and results in Golgi degradation in response to nutrient deprivation (97). In this way PI4KIIIβ controls the biogenesis of a range of secretory, endosomal and autophagic vesicles, and all of these trafficking pathways have the potential to impact on tumourigenesis.

Several independent lines of evidence including PI4KB gene copy number analyses (54, 98, 99) and immunohistochemical studies (53), have revealed that increased PI4KIIIβ expression is common in cancer. Furthermore, acute loss of PI4KIIIβ expression or inhibition of its catalytic activity can result in decreased cell proliferation, reduced cell survival and Golgi structural abnormalities (53, 68, 100, 101). These observations combined suggest that amplified PI4KIIIβ functions may be oncogenic and that targeted inhibition of this enzyme may have chemotherapeutic applications. Very importantly, PI4KIIIβ activity at the TGN has been implicated in senescence-induced secretion (31) - a phenotype associated with oncogene-induced senescence. Senescence-induced secretion can mediate the development of drug resistance in advanced cancers through the exocytosis of paracrine and autocrine factors that over an extended time period re-activate formally senescent tumours. This finding adds an additional complexity regarding PI4KIIIβ expression in cancer, since it may mean that in some early cancer stages increased PI4KIIIβ expression is associated with senescence but later in the disease the scenario changes and the enzyme becomes key to the establishment of a trafficking phenotype with a poor prognosis. Does this mean that these particular secretory cancer phenotypes would be amenable to PI4KIIIβ inhibition? It is probably too early yet to say definitively that this is a feasible therapeutic route or even to know how to identify such phenotypes in a clinical setting. However, several small molecule inhibitors of PI4KIIIβ have already been identified as efficacious anti-viral (102-107) or anti-apicomplexa drugs useful for treating malaria (108-112) and cryptosporidiosis (113). The use of these compounds to combat infectious diseases is beyond the scope of this review but there appears to be at least some potential for repurposing these existing molecules (106, 114, 115) as anti-cancer drugs.

Membrane recruitment and regulation of PI4KIIIβ at the Golgi.

Detailed structural analyses have revealed that PI4KIIIβ is recruited to the Golgi through interactions with the late-Golgi resident protein ACBD3 and this facilitates access to substrate PI on the

cytoplasmic face of *trans*-Golgi and TGN membranes (Figure 4). Golgi recruitment of this enzyme by the small GTPase Arf1 (68), the clathrin adaptor GGA2 (25) and NCS-1 (75, 76, 95) have also been reported, suggesting that different mechanisms may exist for PI4KIIIβ membrane association. However, the most comprehensive and detailed structural evidence currently available supports a key role for ACBD3 in targeting PI4KIIIβ to the TGN (24).

This raises the important issue as to how PI4KIIIβ catalytic activity is regulated? Initial investigations indicated that GTPase Arf1 and 14-3-3 could stimulate PI4KIIIβ activity directly but the latest evidence does not support such a mechanism (116). However, the current literature indicates that whilst 14-3-3 proteins can bind PKD1-phosphorylated PI4KIIIβ (117) to form a 2:2 heterocomplex. this does not stimulate the catalytic activity of the enzyme but rather delays its proteolytic degradation (116, 118). Alternatively in the later stages of vesicle biogenesis , this heterocomplex can recruit the CtBP1-S/BARS scaffolding protein to promote the scission of carriers from the TGN (70). Newer, structural and biochemical insights, instead support a different model for the recruitment and activation of PI4KIIIβ (Figure 3) where the enzyme is both recruited and activated by binding ACBD3 at the TGN (119). However, rather than inducing an activating conformational change in PI4KIIIβ, ACBD3 promotes PI4P synthesis by bringing the enzyme into direct contact with its membrane-associated PI substrate.

Significantly, the non-catalytic properties of PI4KIIIβ, and especially its capacity to form reversible hetero-complexes (24) with a subset of trafficking effector proteins, are also required for PI4KIIIβ -dependent vesicle formation at the TGN. Important in this regard is the ability of PI4KIIIβ to reversibly bind the small GTPase Rab11a (22, 23). Rab11a is localised to membranes of the exocytic pathway, which includes the TGN and recycling endosomes. In its activated GTP-bound state, Rab11a functions to recruit motor proteins such as myosin V to mediate the movement of transport vesicles along microtubules and thus away from the Golgi (120). Rab11 directly binds PI4KIIIβ and the x-ray crystal structure of the PI4KIIIβ:Rab11 complex has been solved (23, 24, 121). Rab11 effectors such as FIP3 can also associate with this heterocomplex (24) indicating that PI4KIIIβ can act as a scaffold or nucleating factor, to selectively recruit a specific subset of vesicular trafficking proteins that mediate directional traffic to the plasma membrane along the exocytic route. A simplified model for the early steps in PI4KIIIβ recruitment to the TGN is presented in Figure 4.

Variety of PI4KIIIβ roles and binding partners with relevance to cancer

Interestingly, in endothelial cells, a PI4KIIIβ and Rab11B pathway is required for the anterograde trafficking of integrin α5β1 and fibronectin to the cell surface via a post-TGN recycling compartment (122) thus revealing a role for this isoform in maintaining the extracellular matrix, which is relevant to tumour growth and angiogenesis. However, Rab11a and Rab11b are structurally distinct (123), they have functionally separable activities in vesicle trafficking and they do not co-localise (124). This indicates that PI4KIIIβ may interact with other Rab proteins and not just Rab11a. In concordance with this finding, PI4KIIIβ can be recruited by Golgi-localised Rab30 to promote Group A Streptococcus-containing autophagosome-like vacuole formation (125). This suggests that Rab GTPase binding to PI4KIIIβ is not highly isoform selective and may be context dependent. It is noteworthy that Rab11 (126-129), PI4KIIIβ (53) and ACBD3 (130) have all separately been reported to be upregulated in a range of cancers and this may imply that the entire PI4KIIIβ interactome at the Golgi is potentially oncogenic. These proteins can also mediate intracellular signalling; PI4KIIIβ and Rab11 co-operate to stimulate the activity of AKT in breast cancer cells although this occurs on endosomes rather than at the Golgi complex (53). Furthermore, both PI4KIIIβ and Rab11 are involved in trafficking to the midbody during cytokinesis and thus defects in this pathway have

the potential to induce mitotic defects that are common in many cancers (101, 131, 132). Specifically with regards to cancer biology, eukaryotic elongation factor 1 α2 (eEF1A2), itself a putative oncoprotein (133), has been reported to bind and activate PI4KIIIβ resulting in a doubling of PI4P production and the promotion of filopodia formation (134). This is an actin-based structural reorganization of the plasma required for cell motility and is relevant for both cancer cell invasion and metastasis (135). However, these potential oncogenic properties of PI4KIIIβ do not directly relate to its Golgi trafficking functions and suggest that a least in some instances, non-Golgi functions of this enzyme are also important for cancer progression.

A special case: Ca^{2+} regulation of Golgi PI4P synthesis by PI4KIIIβ

Another possible means for regulating Golgi PI4KIIIβ is through increased intracellular Ca^{2+}, which can occur subsequent to channel opening or receptor-activated phospholipase C signalling and is a well-established pathway to evoke secretion from specialised neuronal tissue and exocrine glands. There are three calcium-binding proteins known to modulate PI4KIIIβ activity; these are calneuron-1, calneuron-2 and NCS-1 (72, 83). Calneuron-1 and -2 are Golgi-localised proteins that co-localise with PI4KIIIβ at the TGN in unstimulated cells. Calneurons profoundly inhibit PI4KIIIβ activity at low physiological Ca^{2+} concentrations and this decreases post-Golgi carrier formation (83). However, at higher intracellular Ca^{2+} concentrations this inhibition is relieved as calneuron is displaced from PI4KIIIβ by the activating protein NCS-1. NCS-1 also binds Ca^{2+} but with lower affinity than calneuron. The net effect of this calneuron/NCS-1 switch is that both PI4KIIIβ activity and exocytosis are inhibited at low Ca^{2+} concentrations and activated as Ca^{2+} levels rise. Similar to the Rab11–ACBD3-PI4KIIIβ trafficking axis, increased expression of NCS-1 correlates with increased tumour aggressiveness and a poor prognosis (136, 137) although this may relate to effects on Ca^{2+} signalling and not necessarily to its anterograde trafficking function (138, 139).

Role of the type II PI 4-kinases, PI4KIIα and PI4KIIβ, in Golgi transport.

PI4KIIIβ is not the only potential source of PI4P for Golgi vesicle trafficking. A structurally distinct class of PI 4-kinases termed the type II PI 4-kinases (PI4KII) have also been assigned roles in both anterograde and Golgi-endosomal trafficking (reviewed in (2)). Consisting of the of the highly homologous PI4KIIα (140, 141) and PI4KIIβ (142) isoforms, the PI4KIIs mainly localise to the TGN and endosomes. The PI4KIIs do not overlap with PI4KIIIβ at the Golgi (8) indicating that different PI 4-kinases sustain PI4P generation in physically segregated TGN sub-compartments. However, compared to PI4KIIIβ, the Golgi-specific functions of the PI4KIIs are less well established and there are more reports of these enzymes controlling trafficking at endosomal membranes (56-59, 143-145) than at the TGN (5-7, 146). Nevertheless, as both PI4KII isoforms can exacerbate oncogenic processes their proposed roles in anterograde and TGN-endosomal trafficking will be described here.

Some of the most revealing work concerning the physiological roles of type II PI 4-kinases in anterograde trafficking has emerged from two studies on the single *Drosophila* PI4KII orthologue (147). In *Drosophila* salivary glands there are 2 main intracellular pools of the PI4KII enzyme. One pool of the enzyme is active at the TGN where it participates in secretory vesicle formation while a separate late endosomal pool is involved in retrograde trafficking between late endosomes and the TGN. Therefore, a least in this cell type and organism, PI4KII has both TGN and endosomal functions, which is important to note given the somewhat discordant literature concerning the steady state

localisations and sites of action of the mammalian orthologues. Moreover, in *Drosophila* the TGN pool of PI4KII is required for normal secretory granule size but not secretion *per se*, which still occurred with PI4KII null mutants (147). Specifically in this experimental model, PI4KII catalytic activity was required for vesicular cargo sorting. Strikingly different to a number of reports on mammalian PI4KIIs (5, 143, 148-152), the *Drosophila* orthologue was not required for optimal functioning of either the AP-1 and/or AP-3 clathrin adaptor complexes, which strongly suggests that not all trafficking functions requiring this enzyme necessarily proceed via these clathrin-dependent routes. Furthermore, a later study on PI4KII in *Drosophila* neurons (153) reported that the enzyme was not involved in synaptic vesicle formation but rather in endocytosis and recycling at the plasma membrane. Importantly, these insights reveal that at least in *Drosophila,* PI4KII functioning in exocytosis is not observed in all cell types.

PI4KIIα in intracellular vesicular transport

In mammals, the PI4KIIα protein (140, 141) is constitutively associated with TGN and endosomal membranes (5, 57, 60, 142, 154-158) via cholesterol-dependent, post-translational palmitoylation (159, 160) catalysed by Golgi-resident palmitoyl transferases (161). PI4KIIα is always membrane bound and its activity is very sensitive to alterations in membrane cholesterol levels (4, 161-163), which are mainly determined via by PI4P-cholesterol transfer proteins such as OSBP (164). PI4KIIα is also found associated with a variety of post-Golgi transport vesicles destined for the plasma membrane such as synaptic vesicles (149, 165), Rab11-positive recycling endosomes (60), Glut4 transport vesicles (166) and CARTS (167, 168), which points to possible functions in the formation or functioning of this these different types of post-Golgi carriers. However, despite an early report that this isoform could control AP-1 dependent clathrin-coated vesicle formation at the Golgi (169), and a subsequent demonstration that PI4KIIα-derived PI4P could recruit GGA adaptors (6), there is no clear agreement in the literature that PI4KIIα is required for vesicle exit from the TGN in mammalian cells. There is even evidence that PI4KIIα overexpression can have the opposite effect and reduce secretion by directly binding to and inhibiting PKD1, and consequently PI4KIIIβ-dependent trafficking (167). Instead, most of the most recent evidence is consistent with post-Golgi endosomal roles for PI4KIIα in cargo sorting on endosomes via the AP-3 (143, 148-152, 170-172) or ubiquitination pathways (58). Alternatively, following nutrient deprivation, PI4KIIα can be recruited to autophagososmal membranes where it is involved in regulating fusion with lysosomes (173, 174).

Although slightly beyond the focus of this review as it does not strictly relate to Golgi exit, endosomal PI4KIIα may in certain circumstances also mediate forward trafficking to the plasma membrane via an endosomal secretory route. PI4KIIα localized to Rab11 positive recycling endosomes can directly bind the octameric exocyst protein complex that physically docks the endosome at the plasma membrane and thereby facilitates the delivery of cargo to this location (60). There is also evidence that PI4KIIα can mediate anterograde trafficking in neurones via an endosomal pathway. PI4KIIα contains an N-terminal, dileucine LL, acidic cluster, AP-3 clathrin adaptor binding motif. AP-3 can also bind PI4P. Therefore both the enzyme and its lipid product are required for optimal recruitment of AP-3 to intracellular membranes (143). In neuronal cells PI4KIIα and AP-3 co-localise on axonally-targetted transport vesicles (148, 149, 172). In neurones, synaptic-vesicles can be formed from endosomes via recruitment of the neuronal-specific AP-3 adaptor complex (AP3B) (175) which means that at least in this context PI4KIIα is involved in a clathrin-dependent forward trafficking pathway though not via a Golgi-targetted pool of the enzyme.

This all leads to the question as to what exactly is the role of the TGN pool of PI4KIIα in mammalian cells? The answer seems to be that PI4KIIα is required for cargo processing and its

correct packaging into exiting vesicles (6, 147, 176). In concordance with this general idea, a recent paper reported that Golgi-associated PI4KIIα forms a complex with integrin α3β1 and that the formation of this complex is required for the post-translation N-glycan sialylation of a range of cell surface proteins with important functions in oncogenic signalling such as the EGF receptor (146). While the mechanism underlying PI4KIIα control of sialyation remains to be elucidated these findings support the narrative that at the Golgi, as in endosomes (58, 143), PI4KIIα co-ordinates protein modification and selection processes that channel cargoes along different trafficking routes.

PI4KIIα as a putative oncoprotein

There are multiple indications that PI4KIIα is an oncoprotein. PI4KIIα is overexpressed in many carcinomas including for example, those affecting the breast, thyroid, pancreas, liver, lung, uterus, prostate and colon (42, 177). Overexpression of this enzyme is associated with increased cell proliferation in vitro, enhanced tumorigenesis and angiogenesis in xenografts, and the increased secretion of pro-angiogenic factors such as VEGF (42). The x-ray crystal structure of PI4KIIα has now been described (178-180) and this has aided the rational design of the first wave of isoform-specific PI4KIIα inhibitors (177, 181) with potential applications as an anti-breast cancer chemotherapeutics (182). However, it is important to bear in mind that many of the pro-oncogenic signalling effects of PI4KIIα may relate to endosomal dysfunction and upregulated signalling (57, 58, 100, 146, 183, 184) as opposed to just defective Golgi exit.

PI4KIIβ in Golgi trafficking and cancer.

PI4KIIβ was initially characterised as an endosomal protein (142) but there has since been one report that its functions at the TGN on the AP-1 clathrin vesicular trafficking route to endosomes (7). Similar to the mechanism previously described for AP-3 recruitment by the PI4KIIα isoform (143), a di-leucine dileucine LL motif in the non-catalytic N-terminal region of PI4KIIβ directly binds the γ2δ1 components of the AP-1 adaptor complex thereby localising this clathrin adaptor complex to the TGN. This PI4KIIβ interaction with AP-1 is important for canonical Wnt signalling to β-catenin and may involve the formation of a heterocomplex consisting of AP-1, PI4KIIβ and the Wnt pathway proteins Dvl and axinin. Defective PI4KIIβ:AP-1 formation leads to missorting of internalized Frizzled receptors within the endosomal system and this is the underlying cause for defective Wnt signalling in PI4KIIβ -depleted cells. The canonical Wnt signalling pathway is of major importance in carcinogenesis and alterations to PI4KIIβ expression may therefore impact on this tumourigenic signalling axis.

However, there is no real consensus on the steady state intracellular localisation of PI4KIIβ or even that it is required for TGN-endosomal trafficking. Indeed, mechanisms have been proposed for receptor-stimulated recruitment of a cytosolic pool of this enzyme to the plasma membrane (185) and also for an endosomal-resident pool of PI4KIIβ that supresses anterograde trafficking of matrix metalloproteases and therefore inhibits extracellular matrix proteolysis and cell invasion (59). Furthermore, Carloni and colleagues demonstrated that PI4KIIβ co-immunopreciptates with the tetraspannin CD81 and that formation of this heterocomplex was important both for signalling via ERK MAP Kinase cascade and cytoskeletal rearrangements via actinin-4 with relevance to cell motility in hepatocellular carcinoma (186-189). Very recently, and similar to the other Golgi PI 4-kinases (100), PI4KIIβ expression has been shown to be anti-apoptotic (190). PI4KIIβ co-immunoprecipitates with the tumour suppressor Prostate apoptosis response-4 (PAR-4) and this interaction protects cells from apoptosis by suppressing the nuclear functions of PAR-4 (190). However, neither the subcellular location where the anti-apoptotic PI4KIIIβ:PAR-4

complex forms nor its relevance to Golgi-endosomal trafficking are known. Given the multiplicity of roles and localisations proposed for PI4KIIβ, it seems likely that the trafficking and cancer-relevance of the Golgi-localised pool have yet to be fully understood.

Negative regulation of Golgi complex PI4P levels and the role of the Sac1 phosphatase and OSBP

Under certain circumstances, such as nutrient deprivation, Golgi PI4P levels fall due to the activity of Sac1 – a D4 phosphatase that catalyses the dephosphorylation of PI4P and its conversion to PI, and when this occurs, secretion also decreases (16, 63, 191-194). Therefore, phosphoinositide--dependent Golgi exit is inhibited by Sac1 activity at the TGN (16). Inhibition of Sac1 activity with H_2O_2 can have the opposite effect on Golgi exit with concomitant increases in TGN-associated PI4P and anterograde vesicular transport (195, 196). In this respect, Golgi PI 4-kinase and Sac1 phosphatase activities are mutually antagonistic for Golgi trafficking (48), and the relative levels of their respective enzymatic activities reciprocally determine the overall flux of vesicle traffic out of the Golgi complex.

Sac1 access to PI4P at the TGN – three different models.

At steady state, Sac1 (191, 192) predominately localises to the ER and early Golgi cisternae and not to the TGN. This raises the question as to how does an ER localised enzyme gain access to its PI4P substrate when it is located on another membrane and on a different organelle? One such mechanism may proceed via the COPII vesicular trafficking route from the ER to TGN followed by subsequent retrieval to the ER via COPI vesicles, and there is strong evidence that starvation-induced PI4P depletion occurs using this vesicular transport mechanism (16, 197). Furthermore, human Sac1 protein contains a C-terminal dilysine COPI binding motif that facilitates retrieval from the Golgi to the ER, and a separate N-terminal 14-3-3 protein binding site which enables interactions with COPII vesicles and thus anterograde ER-Golgi transport (197). In this way, interactions with coat proteins can facilitate bi-directional ER-Golgi vesicular transport of Sac1 and dephosphorylation of Golgi PI4P when nutrients are in short supply.

However, a separate mechanism for constitutive Sac1 regulation of PI4P at the TGN has been proposed that does not depend on the enzyme being trafficked in vesicles between the ER and Golgi. In this non-vesicular model, ER-localised Sac1 rapidly degrades TGN-derived PI4P which has been transferred to the ER and exchanged for ER-synthesised cholesterol by the lipid transfer protein OSBP at TGN-ER, inter-organelle, membrane contact sites (164, 198). A recent comprehensive investigation concluded that ER-localised Sac1 acting is required to maintain a PI4P-depleted ER and therefore a secretory-competent PI4P gradient in the Golgi (164, 198, 199) that also underpins the directionality of trafficking in the secretory pathway. There is a variation on this model where Sac1 is thought to directly access PI4P at the TGN across membrane contact sites without it being first transferred to the ER. This is sometimes referred to as the *trans* model of Sac1 activity and is thought to be only possible where the inter-organelle distance is exceeding small, in the region of approximately 5nm, and requires the presence of the FAPP1 protein to stabilise these very tight connections by coincidentally binding PI4P at the TGN and VAP proteins at the ER (200) . Hence, whilst the molecular mechanisms through which Sac1 can access PI4P may vary depending on cellular metabolic status it is nevertheless clear that increased Sac1 phosphatase activity at the TGN can reduce PI4P levels to such an extent that Golgi vesicular exit also decreases.

Decreased Sac1 expression is oncogenic.

Sac1 expression is altered in some cancers and interestingly this is linked to tumour staging and disease progression (48). As an example, in breast cancer tissue, moderate overexpression of Sac1 has been noted in early stage (more treatable) tumours but expression subsequently decreases in more advanced tumours, which tend to be more invasive and metastatic, suggesting that loss of Sac1 correlates with a more aggressive phenotype (48). In terms of cancer development, it seems likely that loss of Sac1 expression or mutations that inhibit Sac1 catalytic activity could potentially promote PI4P-dependent secretion or augmented trafficking of cell surface proteins that regulate cell adhesion and motility (12, 47). Indeed, there is one study showing that Sac1 depletion can cause mislocalisation of the normally Golgi-resident enzymes N-acetylglucosamine transferase-I and mannosidase to peripheral compartments (12). Reduced Sac1 expression also results in increased trafficking of the pro-metastatic glycoproteins CD44 and variant CD44 to the cell surface together with ezrin proteins required for focal adhesion integrity (48). Similarly, another study revealed that loss of Sac1 caused mislocalisation of the cell-cell adhesion proteins E-cadherin and β-catenin and peripheral actin cytoskeletal disorganisation, which are hallmarks of the early phases of epithelial–mesenchymal transition (EMT); a process that foreshadows the onset of amplified cell motility, tissue invasion and ultimately metastasis (47). In these circumstances, Sac1 loss increases Golgi to plasma membrane trafficking through deregulating TGN PI4P levels. However, this may not be the only consequence of reduced Sac1 levels in cancer cells. Sac1 reductions lead to major derangement of Golgi architecture as evidenced in varying degrees of Golgi stack vesicularisation and dispersal, and significantly, increased cell death through a mechanism not involving apoptosis (201). These particular studies also found that Sac1 loss of function in human cells induced mitotic spindle disorganisation and cytokinetic defects that predispose cells to chromosomal instability and aneuploidy, which are key features of many cancers (202, 203). A later study concerning PI4KIIIβ and its inhibitory calneuron protein (CaBP7) demonstrated that both proteins were required for lysosomal localisation and cytokinesis during mitosis (204). Hence, there are different lines of evidence indicating that during particular points of the cell cycle, elements of the Golgi phosphoinositide trafficking transiently delocalise and have temporally- restricted functions during cytokinesis. Hence, not all of the cancer-promoting roles of the Golgi phosphoinositide homeostatic system can be understood solely in terms of alterations to the rate of vesicle formation at the TGN.

A little on lipid transfer proteins and cancer.

As mentioned in preceding sections another crucial role for Golgi PI4P is in the regulation of non-phosphoinositide lipid levels such as cholesterol, ceramide and sphingomyelin at the TGN through the activity of lipid transfer proteins. PI4P is required for the non-vesicular, protein-based transfer of lipids such as cholesterol and ceramide directly from their site of synthesis at the ER to the TGN. These lipid exchange processes are rapid and highly compartmentalized at ER-TGN membrane contact sites, thereby circumventing stepwise vesicular transfer through the Golgi stacks. In addition to OSBP, other lipid transfer proteins that support vesicle biogenesis include ceramide transfer protein (CERT) (11, 205), ORP9 (206), and ORP10 (207). The TGN targeting of these lipid transfer proteins require direct binding to both PI4P and the TGN-localised small GTPase ARF-1 by modular pleckstrin homology (PH) domains found on all of these proteins. Recently, a homeostatic feedback loop has been identified whereby increases to TGN sphingomyelin results in reduced PI4P levels at the TGN (208) and this provides more evidence for multiple layers of cross regulation between these different lipids at the Golgi complex. OSBP family proteins are also targets of the anti-cancer ORPphillin compounds such as OSW1 and schweinfurthin A (209, 210), and their putative roles in tumourigenesis have been extensively discussed elsewhere (211).

GOLPH3 - a Golgi PI4P effector and oncoprotein.

The most convincing and wide-ranging evidence for PI4P co-involvement in both Golgi exit and cancer is illustrated in the case of Golgi phosphoprotein 3 (GOLPH3) (39, 212, 213). GOLPH3 is the human orthologue of the yeast vesicular trafficking protein Vps74 (15, 214, 215). GOLPH3 has a high affinity for PI4P binding versus other phosphoinositides and this property determines its targeting to the Golgi complex (15). Very recently it has been shown that a hydrophobic β-loop structure of GOLPH3 can readily insert into membranes where it induces membrane curvature and tubulation both in vitro and in cells (18). At the TGN, GOLPH3 recruits an unconventional mysosin protein called Myosin-18A, which in turn binds the cytoskeletal protein F-actin (212, 216). Therefore, assembly of the GOLPH3 / Myosin-18A / F-actin molecular complex creates a tripartite protein bridge connecting Golgi PI4P with the cytoskeleton. This complex could be conceptualised as a spring-like structure that exerts a mechanical force on Golgi membranes. This gives rise to the characteristic cisternal membrane architecture but also facilitates the stretching or pulling forces required for separation of vesicles from the Golgi (212, 217) by Myosin-18A "walking" along actin fibers. The expression of just GOLPH3 and Myosin-18A alone appear to be sufficient to induce membrane deformation and vesicle release at the TGN which make it likely that they define a separate PI4P dependent anterograde trafficking pathway that is independent of both Rab11 and clathrin adaptors such as AP-1.

Very strikingly, altered expression of GOLPH3 and Myosin-18A are associated with a range of malignant phenotypes including tumour secretion (41), increased trafficking to the leading edge of migratory cells and consequently augmented cell motility, loss of cell-cell adhesion and invasion (39, 45, 49, 50, 218-225). However, while this model is strongly supported by a wealth of experimental data, there is at least one report indicating that Myosin-18A is neither localised to the Golgi nor required for its characteristic ribbon like structure (226). There are also some parallels evident with the PI4KIIIβ, Rab11 ACBD3 vesicular trafficking axis where overexpression of individual components may also be carcinogenic and related too altered Golgi trafficking.

DNA damage, one of the most important causes of cancer initiation, can directly lead to GOLPH3 phosphorylation via activation of DNA-dependent protein kinase (DNA-PKcs) (227). DNA-PKcs phosphorylates GOLPH3 on threonine 149 leading to enhanced association with Myosin-18A, Golgi fragmentation and dispersal, decreased anterograde trafficking but augmented cell survival. Hence there is a clear mechanistic pathway linking a known carcinogenic event with abnormal PI4P-dependent membrane trafficking (55). In addition, other studies have indicated that the range of non-trafficking GOLPH3 functions in cancer can be attributed to signalling activation, particularly of the AKT-mTOR signalling axis (34, 39, 44, 49, 220-225, 228-235), and also that extra-Golgi pools of this protein that vary in size and impact depending on cell type (236). Hence, similar to the story with the Golgi PI 4-kinases, the entire repertoire of GOLPH3 dysfunctionality in cancer is unlikely to be restricted to augmented flux through the secretory pathway. A number of non-Golgi roles have been evidenced for GOLPH3 that may add to its tumourigenicity such as the delayed trafficking and degradation of the EGFR (231); binding an activation of the JAK2-STAT3 transcription factors (237); as well as effects on autophagy (238, 239) and mitochondrial function (239, 240). How a single protein can mediate such disparate functions (241) is not yet clear and many of these relationships require further validation as they may be indirect, cell type or context specific.

GOLPH3 regulates retrograde trafficking in mammalian cells

The proposed role of GOLPH3 in oncogenic secretion is heavily predicated on its anterograde trafficking functions, however, there is also substantial evidence that this protein operates in the

retrograde intra-Golgi and Golgi-ER retrieval pathways that maintain the characteristic lipid and protein gradients of the secretory pathway (15, 242, 243). GOLPH3 facilitates retrograde, COPI dependent, intra-Golgi trafficking of glycosyl transferases such as α-2,6-sialyltransferase 1 (215, 244, 245). This is an important function, since recycling of Golgi-glycosyltransferases to their steady-state localisations at either cis, medial or trans compartments is necessary to maintain the functionality of the secretory pathway (215, 244, 245). Furthermore, the x-ray crystal structure of the heterocomplex formed by the yeast GOLPH3 orthologue VPS74 bound to the N-terminal phosphatase-containing domain of yeast Sac1 enzyme has been solved, and supports the idea that GOLPH3 and the Sac1 PI4P phosphatase act to decrease PI4P levels in early Golgi compartments (242, 243). GOLPH3 possesses an N-terminal COPI binding site necessary for direct association with retrograde Golgi trafficking vesicles and furthermore GOLPH3 can also directly bind the Golgi-resident POMGnT1 glycosyltransferase, indicating a direct mechanism for maintaining Golgi post-translational modification capacity (14). These results introduce potential twists in the developing GOLPH3 story in cancer and indicate that in addition to forward trafficking to the plasma membrane, GOLPH3 also maintains the intra-Golgi secretory PI4P gradient and the functional compartmentation of the Golgi

GOLPH3L – a cancer promoting protein that may regulate GOLPH3 trafficking functions.

In terms of direct regulation of GOLPH3 secretory functions, a structurally related protein called GOLPH3L is expressed in some specialised secretory tissues where it is thought to antagonise the functions of GOLPH3 by competing for PI4P binding (37). However, GOLPH3L does not bind Myosin-18A and is therefore, not directly involved in F-actin mediated vesicle release (37). GOLPH3L may then potentially represent an important endogenous, negative regulator of the secretory cancer pathway. However, increased expression of GOLPH3L has been reported in rhabdomyosarcoma (246); epithelial ovarian (247) and cervical (248) cancers; and correlates with an increased risk of developing squamous cell carcinoma (249). Furthermore, increased expression of GOLPH3L in cervical cancer cells is associated with chemotherapeutic resistance to cisplatin and activation of the anti-apoptotic nuclear NF-κB signalling pathway (247). If anything, these findings are counterintuitive given that high levels of GOLPH3L should counteract GOLPH3-driven oncogenesis. However, in the absence of concomitant GOLPH3 overexpression, these results from independent studies may point towards a PI4P independent function for GOLPH3L, perhaps related to structural elements not found in GOLPH3. More studies are needed to understand the molecular basis for GOLPH3L dysfunction in cancer and in particular to evaluate the extent to which GOLPH3L carcinogenesis relates to its PI4P binding properties or through outcompeting GOLPH3 at the TGN.

PITPNC1, a PI4P-binding oncoprotein and a mediator of malignant secretion.

Halberg and colleagues recently identified phosphatidylinositol transfer protein cytoplasmic 1 (PITPNC1), also known as RdgBβ, as a TGN targeted, PI4P binding protein, whose expression is upregulated in a range of metastatic cancers (41). They found that increased PITPNC1 expression led to malignant secretion of MMP9 (a matrix metalloprotease which proteolyses the ECM); ADAM10 (a plasma membrane anchored metalloprotease or sheddase); HTRA1 (a serine protease with an established role in EMT); the signalling molecules FAM3C (an interleukin-like EMT inducer) and Platelet-derived growth factor subunit A (41). PITPNC1-induced secretion of these proteins is likely to remodel the tumour microenvironment and to enhance the proliferative, angiogenic, invasive and metastatic potential of adjacent cells. The authors demonstrated that PITPNC1 recruited Rab1C and subsequently GOLPH3 to the TGN, with GOLPH3 acting as the effector to drive forward vesicular

trafficking. This is a significant finding as PI4P is also present on non-Golgi membranes and co-incident interactions with RAB1C could specify GOLPH3 targeting to the TGN.

In addition to promoting a pro-metastatic secretory phenotype, a separate study on gastric cancer revealed that PITPNC1 can upregulate the expression of genes involved in fatty acid catabolism thus enabling cells that are detached from the ECM to maintain intracellular ATP levels and avoid detachment-induced cell death, a process referred to as anoikis (250, 251). In adipocytes associated with the omentum in gastric cancer, PITPNC1 overexpression protects against anoikis through upregulated expression of the plasma membrane fatty acid transporter CD36 and the mitochondrial carnitine palmitoyltransferase enzyme 1B – the rate-limiting enzyme for long chain fatty acid β-oxidation (252). PITPNC1 overexpression was additionally associated with increased nuclear localisation of the PPARγ transcription factor although the molecular mechanism underlying this translocation event was not demonstrated. Through this metabolic rewiring function PITPNC1 expression may facilitate metastasis by fuelling the survival of detached cells which are compromised in their ability to generate ATP via glycolysis.

Whilst PITPNC1 functions in PI4P-dependent secretion and cancer are supported by a wealth of experimental evidence, it is interesting that hitherto PITPNC1 was not thought be a Golgi-recruited protein but rather a cytosolic phosphatidylinositol transfer protein that could be recruited to membranes by directly binding the Angiotensin II receptor-associated protein (253, 254) and phosphatidic acid (255). Hence, the newly described roles of PITPNC1 in cancer and Golgi trafficking do not align well with its previously described properties, suggesting that further work may be needed to better understand or reconcile these apparent differences. Furthermore, PI transfer proteins more generally may have a role in promoting GOLPH3 association with the TGN. Xie and colleagues (256) found that two PI transfer proteins, PITPNA and PITPNB, are required to ensure adequate PI4P supply for Golgi association of GOLPH3 and that this is important for apical vesicle trafficking in neural stem cells and the process of neurogenesis. However, the authors of that particular study concluded that the PI transfer proteins were acting through a mechanism that was independent of their generic lipid transfer activities. PITPs and can stimulate membrane–associated PI 4-kinase activity in vitro (257) and are known to stimulate both secretory (258-263) and retrograde Golgi trafficking (264).These new links with GOLPH3 and anterograde trafficking open up novel avenues to understand the roles of PI transfer proteins in secretory transport.

Does PI(4,5)P_2 have a role at the Golgi?

Up to this point, this review has concentrated on PI4P and the idea that it as a functionally important lipid in its own right at the Golgi. However, there is some evidence, albeit less abundant, that PI4P 5-kinases (PIPK's) (265) may also participate in trafficking out of the Golgi. PIP 5-kinases catalyse the formation of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P_2) through the ATP-dependent phosphorylation of PI4P on the D5 position of the inositol headgroup (Figure 1). PI(4,5)P_2 has well established functions as a substrate for both agonist-stimulated phospholipase C and receptor–activated Class I phosphoinositide 3-kinases at the plasma membrane. Other plasma membrane roles for PI(4,5)P_2 include protein recruitment, ion channel regulation, membrane trafficking and cytoskeletal regulation (reviewed in (266, 267)). However, up to very recently there had been little progress in characterising a Golgi-specific role for this lipid despite initial reports that an ARF1 stimulated PIP kinase resided there (68, 268) alongside 1 -5 % of the total cellular PI(4,5)P_2 compliment (268).

OCRL as a potential source of PI4P and suppressor of PI(4,5)P_2 on Golgi membranes

One possible explanation for the low level of PI(4,5)P_2 at the Golgi could be the presence of PI(4,5)P_2 5-phosphatases which can dephosphorylate this lipid to generate the more abundant PI4P (Figure 1). Two structurally related phosphoinositide 5-phosphtases have been localised to the Golgi, OCRL and INPP5B. OCRL can be recruited to the Golgi complex by binding the Golgi-resident Rab1 and Rab6 small GTPases (269). Similarly, INPP5B (270) can bind several Golgi-localised Rab GTPases including Rab33B, Rab8A, Rab6 and Rab6A. Loss of function mutations in OCRL give rise to the X-linked multisystem disease oculocerebrorenal syndrome of Lowe or a milder related condition called Dent's disease (271, 272). Both of these inherited conditions are examples of ciliopathies and are not associated with an increased risk of cancer. Both OCRL and INPP5B are also found in other compartments such as endosomes (273) and there are only a limited number of publications characterising their Golgi-specific roles. In terms of Golgi 5-phosphatase activities, INPP5B participates in retrograde trafficking from early Golgi to the ER (270) and OCRL has been implicated in vesicle transport from the TGN to endosomes (274) and in the negative regulation of apical cargo delivery in polarised cells (265). Whether these 5-phosphatases act to limit a transient PI(4,5)P_2-requiring trafficking step or indeed entirely suppress PI(4,5)P_2 function at the Golgi is not entirely clear, but their specific recruitment mechanisms and highly compartmentalised intra-Golgi functions do indicate that very tight control of PI(4,5)P_2 is a salient feature of Golgi exit pathways. The idea of a small, transient, but functionally important PI(4,5)P_2 pool seems plausible in light of a recent study that unveiled a role for PIP5K1α in generating PI(4,5)P_2 as a PLC substrate during AP-1 clathrin-coated vesicle biogenesis at the TGN (275). Interestingly, PIP5K1α was found to also interact with F-actin and so facilitate vesicle release from the TGN.

Although OCRL is not a recognised oncoprotein it has been implicated in processes relevant to cancer. OCRL is required for the successful completion of cytokinesis (276), a non-Golgi process that is often impaired during tumour growth (276, 277). Interestingly the PI4P phosphatase Sac1 (201) and PI4KIIIβ orthologues (101, 131, 132, 204, 278-281) also possess mitotic functions. These findings point towards an evolutionary conserved repurposing of the intracellular PI4P metabolic machinery during cell division when the Golgi is known to reversibly fragment and disperse (reviewed in (282)).

PIP5K1α – a PI4P 5-kinase with links to Golgi exit and cancer

The recent revelation that the PIP 5-kinase isoform PIP5K1α can mediate TGN-endosome carrier formation is very interesting in light of the emerging importance of this this enzyme in malignant disease. As an example, PIP5K1α is overexpressed in prostate cancer and is a poor prognostic indicator for this disease (283-285). Moreover, ISA-2011B - a small molecule inhibitor of PIP5K1α, is an effective anti-cancer agent in both prostate (285) and triple-negative breast cancer models (286). However, PIP5K1α has oncogenic functions outside of Golgi trafficking including the regulation of p53 oncoprotein stability in the nucleus (287) and KRAS signalling (288). This may imply that the proposed oncogenic properties of PIP5K1α may not be wholly attributable to its newly described role in AP-1/clathrin carrier formation at the TGN. Furthermore, the genes encoding PIP5K1α (PIP5K1A), PI4KIIIβ (PI4KB), AKT3 and GOLPH3L are all present on chromosome 1q, which is frequently amplified in a range of cancers (98, 289, 290). It is tempting to speculate that at least two of the phosphoinositide metabolising enzymes of the newly identified PIP5K1α/ AP-1/clathrin trafficking route could be overexpressed in some cancers due to chromosomal abnormalities, thereby linking karyotypic anomalies with trafficking defects. There is already a precedent for chromosomal copy number alterations leading to phosphoinositide dysfunction on intracellular

membranes. In Down's syndrome, there is increased expression of endosomal PI(4,5)P_2 phosphatase Synaptojanin1 and associated defective functions in intracellular trafficking due to an extra copy of the SYNJ1 gene being present due to trisomy 21 (291-293). However, it remains to be demonstrated that pro-oncogenic trafficking changes in cancer cells can be induced by copy number increases to chromosome 1.

Final overview

PI4P is required for a plethora of Golgi vesicular trafficking events that maintain the signalling and structural competencies of the plasma membrane and endosomes. As we learn more about the enzymes that sustain these vesicular trafficking routes it is becoming apparent that Golgi-targeted PI 4-kinases, and especially the PI4KIIIβ isoform, regulate the secretory pathway and Golgi vesicle transport at several levels. However, progress in this research area is still slow and there are a number of unresolved questions and disagreements in the literature on fundamental issues such as the regulation of PI4P levels at the TGN by Sac1, and the functional importance of PI4KIIs at the Golgi. While the mechanisms that regulate PI4P at the Golgi are controversial it is nevertheless clear that in cancer cells, gene or chromosomal amplifications leading to the increased expression of proteins such as GOLPH3 or PITPNC1, can potentiate the development of oncogenic trafficking phenotypes. In this way, cancer-associated changes to Golgi PI4P trafficking impacts on the steady-state proteomes and functionalities of intracellular degradative and recycling compartments, the cell surface, and the cell exterior through ECM remodelling. This spectrum of transformative biochemical changes promotes tumour progression and metastasis - a leading cause of death in many cancers. Drugs that inhibit the functions or formation of Golgi PI4P may therefore, have some potential for the treatment of advanced cancers and small molecule inhibitors of individual enzymes in this pathway are currently being evaluated. Ultimately, successful chemotherapeutic intervention may depend on precision targeting approaches that integrate karyotypic profiling with gene or protein expression data in order to identify malignant lesions likely to respond to inhibitors of PI4P-dependent vesicle exit from the Golgi.

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Figure Legends.

Figure 1.

Lipid kinase and phosphatase regulation of PI4P on Golgi membranes. PI4P is formed at the Golgi by PI 4-kinase enzymes that catalyse the phosphorylation of substrate PI phospholipid on the D4 hydroxyl group of the inositol head group in a reaction that utilises ATP. The main Golgi PI 4-kinase is PI4KIIIβ. PI4KIIα activity has also been reported at the TGN and at least one study has implicated PI4KIIβ as being functionally important on these membranes. Most of the available evidence indicates that PI4P synthesis by PI4KIIIβ is most important for generating the pool of PI4P required for vesicular trafficking. A small proportion (1 – 5 %) of the total cellular $PI(4,5)P_2$ content is present at the Golgi. $PI(4,5)P_2$ is synthesised by the D5 phosphorylation of PI4P by PIP kinases. Recently the PIPK1α isoform has been implicated in $PI(4,5)P_2$ generation at the Golgi. $PI(4,5)P_2$ can be dephosphorylated on by the D5 phosphoinositide phosphatase OCRL to PI4P. It is important to note that different to PI4KIIIβ, only minor pools of PIPK1α and OCRL are normally present at the Golgi. PI4P can be dephosphorylated back to PI by Sac1, which is a PI4P-specific D4 phosphatase. Sac1 is mainly present at the ER where it dephosphorylates TGN-sourced PI4P transferred by OSBP lipid transfer proteins. Under conditions of nutrient deprivation Sac1 may be trafficked to the TGN where it dephosphorylates PI4P and inhibits vesicle trafficking.

Figure 2.

Constitutive vesicle trafficking pathways from the Golgi involving PI4P. The Golgi apparatus continually supplies the plasma membrane, endosomes and lysosomes with modified lipids and proteins that maintain their respective structures and functionalities. These constitutive trafficking pathways include, large uncoated tubular vesicular carriers destined for the plasma membrane, clathrin-coated vesicles transporting cargo to the endosomes and COPI coated vesicles transferring material in a retrograde pathway back to earlier Golgi cisternae and the endoplasmic reticulum to maintain the functional integrity of the secretory pathway, and its characteristic protein and lipid gradients. PI4P synthesized by the PI4KIIIβ PI 4-kinase isoform is required for the formation of large tubular vesicles that traffic to the plasma membrane. However, there is no overall agreement concerning the PI 4-kinase isoform required for AP-1 clathrin vesicle trafficking to endosomes with three isoforms PI4KIIIβ, PI4KIIα and PI4KIIβ, implicated in different studies. Recently the PI4P 5-kinase, PIP5K1α has been reported to be involved in the generation AP-1 clathrin-coated carriers at the TGN. The formation of COPI vesicles has not yet been shown to require PI4P. However, this pathway is important for the retrieval of the Sac1 PI4P phosphatase back to the ER and to a lesser extent the cis-Golgi. This is important since it maintains PI4P depletion in the early secretory pathway and ensures that PI4P is only available for vesicle trafficking at the TGN. Sac1 can directly bind the PI4P-binding GOLPH3 oncoprotein and this interaction facilitates retrograde trafficking of the phosphatase via the COPI route. Although not shown here, GOLPH3 can also mediate anterograde trafficking to the plasma membrane through a distinct route that also requires PI4P synthesised by PI4KIIIβ.

Figure 3.

A hypothetical model for chromosomal instability and DNA damage modulating malignant secretion via PI4P and GOLPH3. As tumours become established, they accumulate chromosomal structural abnormalities due to defective mitosis and DNA damage, and both of these processes have the potential to affect secretory vesicle exit from the Golgi. (i) 1q - the long arm of chromosome 1, is commonly amplified in a range of carcinomas. This chromosomal region contains

genes encoding several proteins that regulate TGN vesicle trafficking including PI4KIIIβ, GOLPH3L and PIP5K1α. As this chromosomal segment is amplified to give multiple copies of chromosome 1q there is concomitant amplification of the PI4KB gene encoding for PI4KIIIβ. (ii) Increased PI4KB copy number results in increased expression of the PI4KIIIβ protein (structure from PDB code 4WAG) and (iii) increased levels of PI4P at the TGN. Higher levels of PI4P at the Golgi recruit PI4P binding oncoproteins that also drive section such as GOLPH3 and PITPNC1. Expression of these proteins could also be increased through gene copy number increases in a manner analogous way to PI4KB. (iv) GOLPH3 forward trafficking can inhibited through DNA damage leading to activation of DNA–dependent protein kinase, phosphorylation of GOLPH3 on threonine 143 and consequently Golgi dispersal and reduced vesicle transport to the plasma membrane. This indicates that PI4P-dependent vesicle trafficking can be differentially modulated by genomic changes in cancer. (v) Super activation of PI4P-mediated vesicle formation leads to hyper-functioning of anterograde trafficking and malignant secretion, which drives tumour progression through extra cellular matrix remodelling, cell detachment, invasion, proliferation and tumour angiogenesis.

Figure 4.

General scheme for PI4KIIIβ membrane recruitment and early steps in trafficking. (i) The PI4KIIIβ interacting proteins ACBD3 and the small GTPase Rab11a, together with its lipid substrate PI are all constitutively associated with the TGN membrane. (ii) PI4KIIIβ is recruited to the membrane by binding to Golgi-resident ACBD3. Once in contact with the membrane and its enzymatic substrate, PI4KIIIβ can catalyse the ATP dependent phosphorylation of PI to generate PI4P. (iii) PI4KIIIβ in turn binds the Rab11a small GTPase, to form a protein heterocomplex. (iv) The PI4P product can bind and recruit further proteins to the Golgi such as the OSBP and FAPP2 lipid transfer proteins via their PI4P-specific PH domains. Golgi targeting of these proteins requires co-incident interaction with the Golgi-resident ARF1 protein. PI4P synthesized by PI4KIIIβ is also required for other Golgi vesicle exit routes that depend on the recruitment of PI4P binding proteins such as the GOLPH3 and the AP-1/clathrin pathways. Rab11 binds and recruits effector proteins such as FIP3, facilitating interactions with dynein motor complexes and microtubules, and subsequent vesicle transport away from the Golgi.

Table 1. Summary of the expression and functional properties of the main PI4P pathway genes implicated in Golgi trafficking and cancer

Gene	Chromosome location	Protein	Expression Change in Cancer	Biochemical Activity	Main Trafficking Pathways
PI4KB	1q21	PI4KIIIβ	Increased	PI 4-Kinase	Constitutive anterograde (67) and Ca^{2+} calneuron-regulated secretion (95).
PI4K2A	10p12.2	PI4KIIα	Can be Increased or decreased	PI 4-kinase	AP-3, AP-1, GGA clathrin (5, 6, 143) routes. Endosomal and anterograde trafficking (60).
PI4K2B	4p15.2	PI4KIIβ	Can be Increased or decreased	PI 4-kinase	AP-1 clathrin (7)
SAC1ML	3p21.31	Sac1	Increased but also decreased in advanced tumours	PI4P 4-phosphatase	Negative regulation of Golgi exit (16, 47)
PIP5K1A	1q21.3	PIP5K1α	Increased	PI4P 5-kinase	TGN-endosome AP-1 (275)
GOLPH3	5p13.3	GOLPH3	Increased	PI4P binding	Secretion (212)
GOLPH3L	1q21.3	GOPLH3L	Increased	PI4P binding	Secretion (37)
PITPNC1	17q24.2	PITPNC1 or RDGBβ	Increased	PI4P binding	Secretion (41)

Figure 1

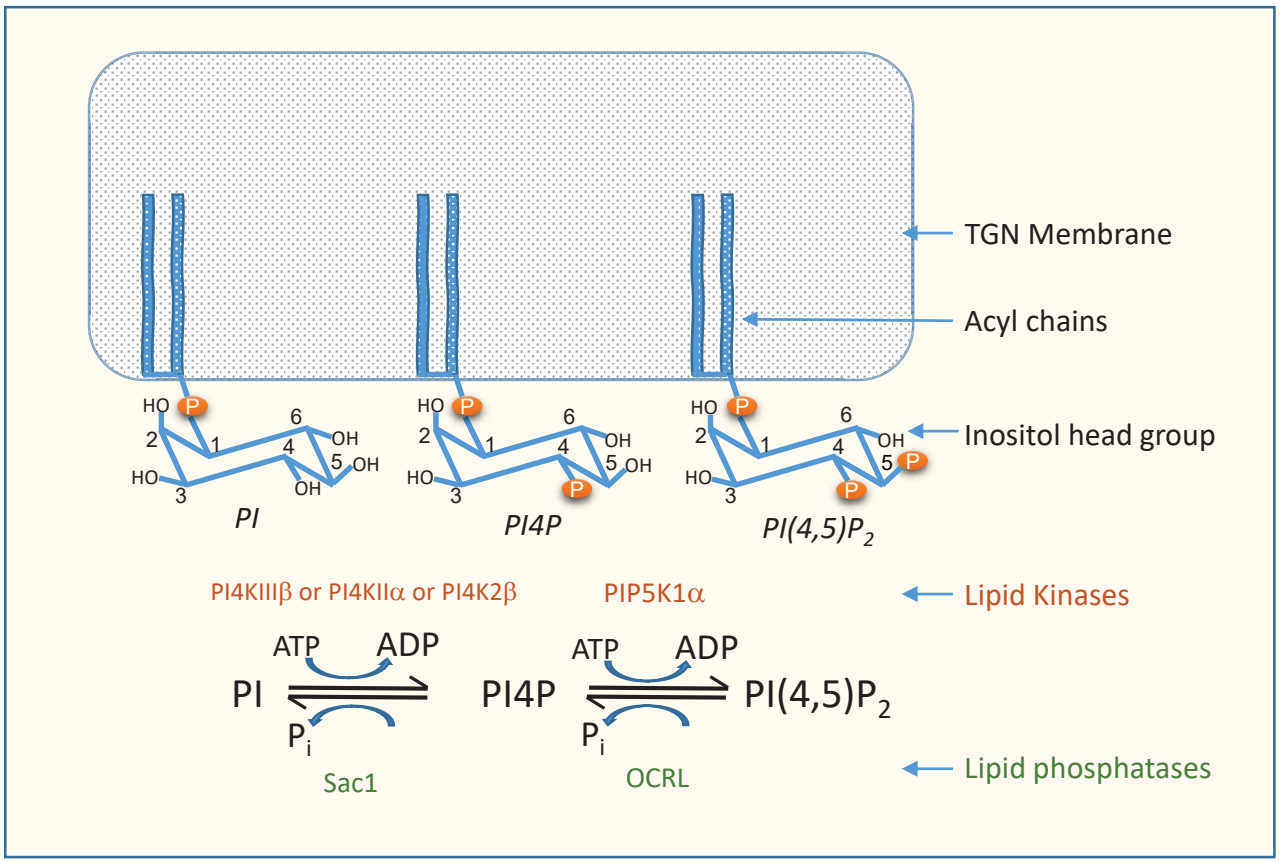

TGN Membrane
Acyl chains
Inositol head group
PI
PI4P
PI(4,5)P2
PI4KIIIβ or PI4KIIα or PI4K2β
PIP5K1α
Lipid Kinases
ATP
ADP
PI
Pi
PI4P
PI(4,5)P2
Sac1
OCRL
Lipid phosphatases

Figure 2

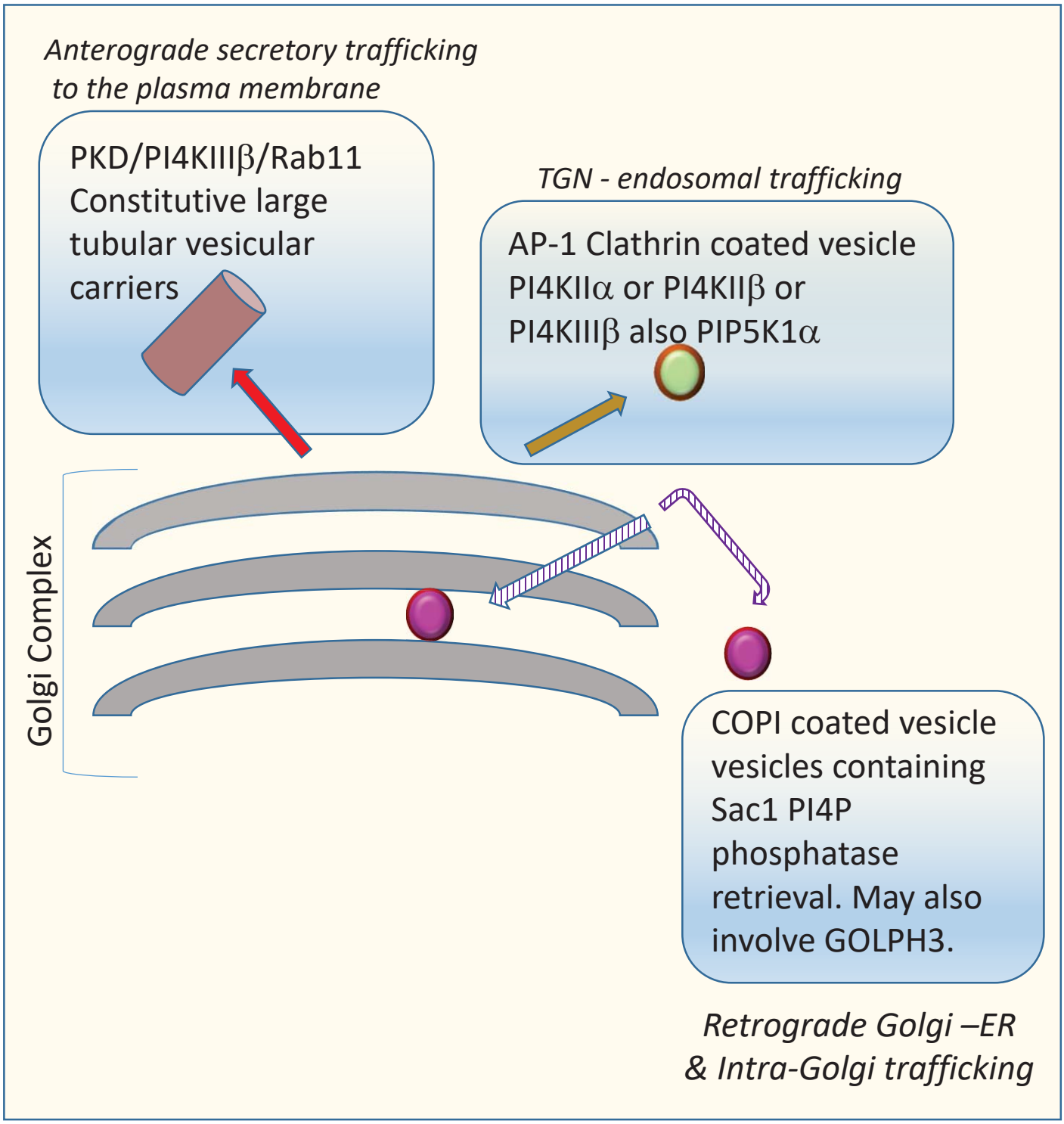

Anterograde secretory trafficking to the plasma membrane
PKD/PI4KIIIβ/Rab11 Constitutive large tubular vesicular carriers
TGN - endosomal trafficking
AP-1 Clathrin coated vesicle PI4KIIα or PI4KIIβ or PI4KIIIβ also PIP5K1α
Golgi Complex
COPI coated vesicle vesicles containing Sac1 PI4P phosphatase retrieval. May also involve GOLPH3.
Retrograde Golgi –ER & Intra-Golgi trafficking

Figure 3

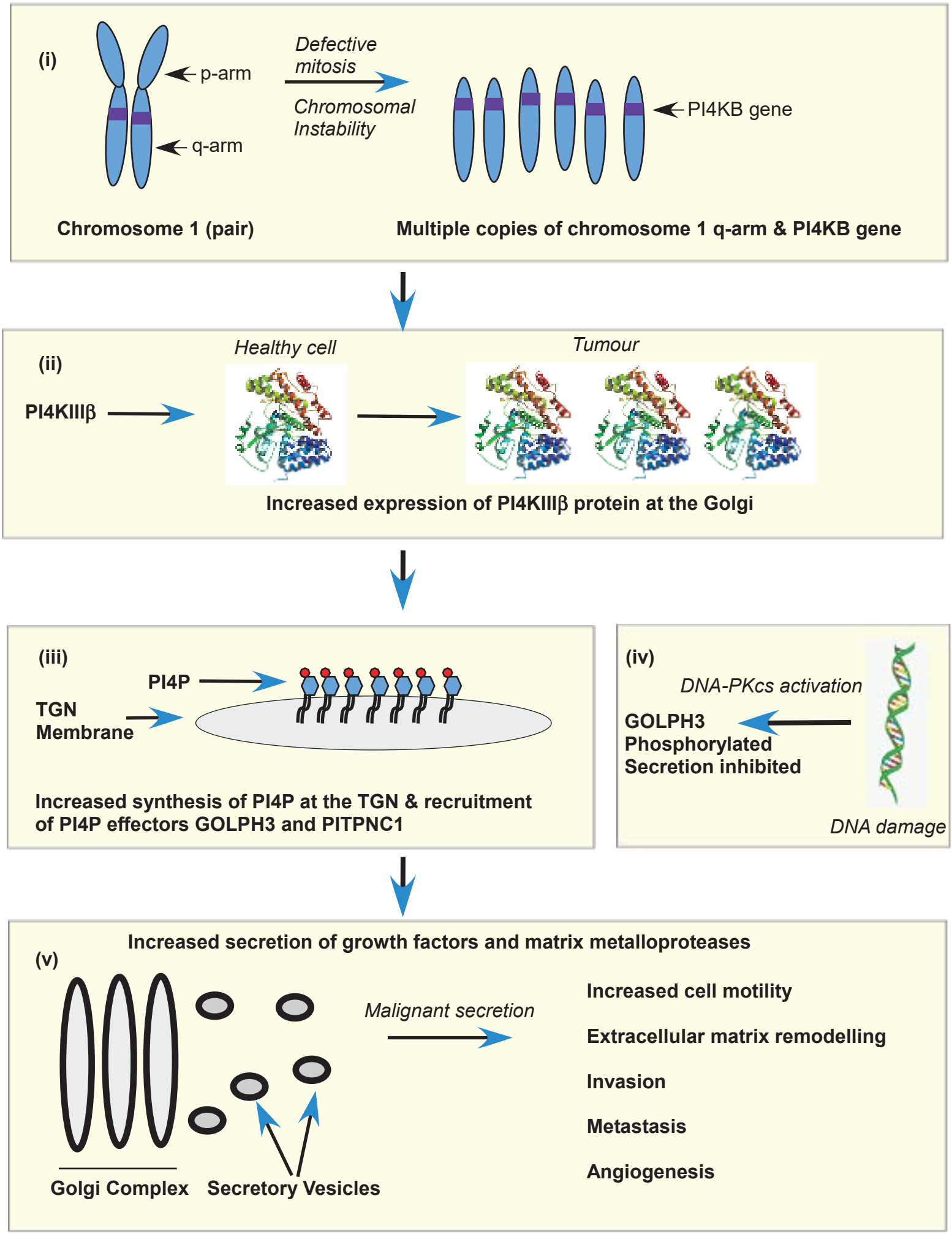
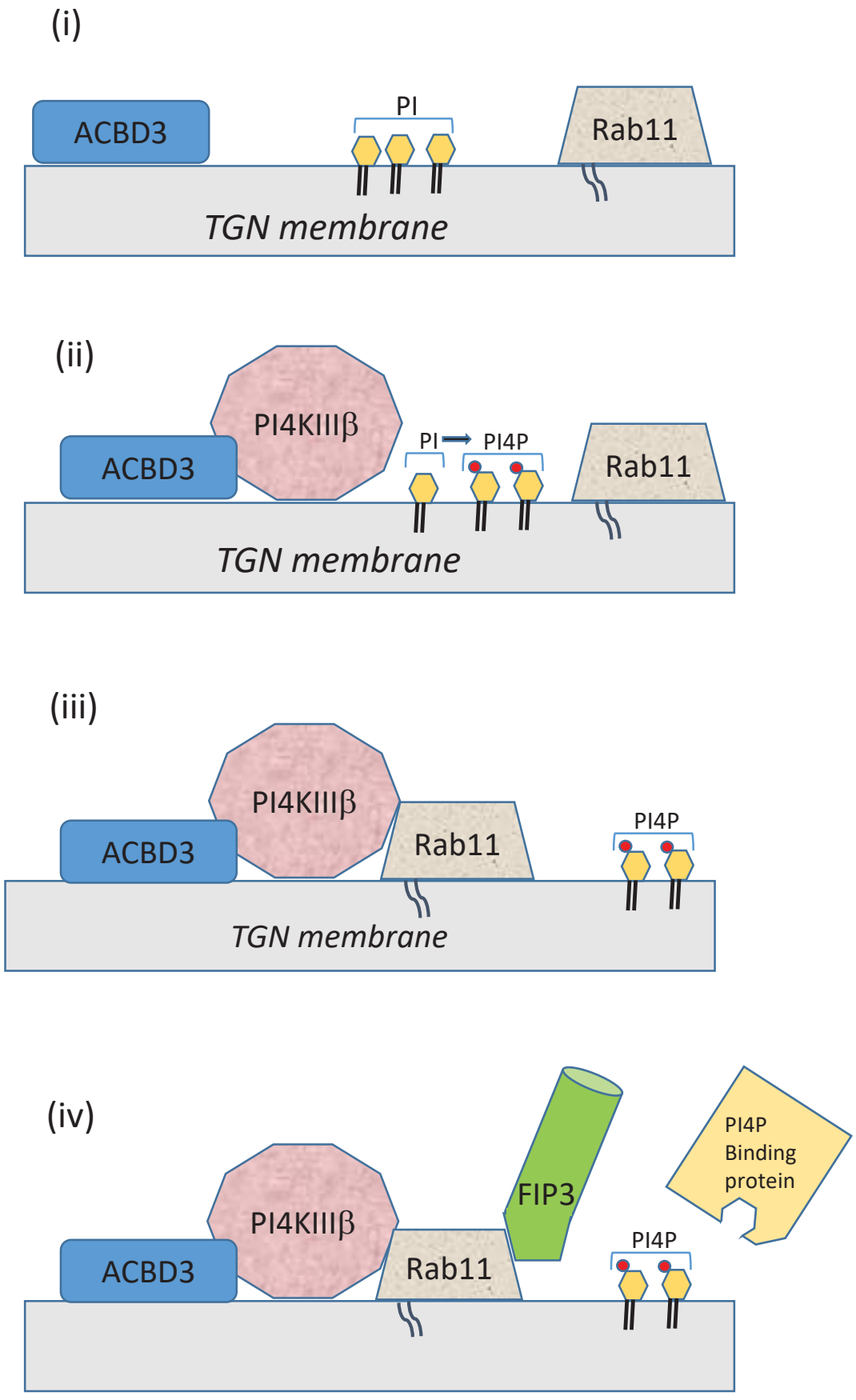

(i)
p-arm
q-arm
Defective mitosis
Chromosomal Instability
PI4KB gene
Chromosome 1 (pair)
Multiple copies of chromosome 1 q-arm & PI4KB gene
(ii)
PI4KIIIβ
Healthy cell
Tumour
Increased expression of PI4KIIIβ protein at the Golgi
(iii)
PI4P
TGN Membrane
Increased synthesis of PI4P at the TGN & recruitment of PI4P effectors GOLPH3 and PITPNC1
(iv)
DNA-PKcs activation
GOLPH3 Phosphorylated
Secretion inhibited
DNA damage
Increased secretion of growth factors and matrix metalloproteases
(v)
Malignant secretion
Golgi Complex
Secretory Vesicles
Increased cell motility
Extracellular matrix remodelling
Invasion
Metastasis
Angiogenesis

Figure 4


(i)
ACBD3
PI
Rab11
TGN membrane
(ii)
PI4KIIIβ
ACBD3
PI
PI4P
Rab11
TGN membrane
(iii)
PI4KIIIβ
ACBD3
Rab11
PI4P
TGN membrane
(iv)
PI4KIIIβ
ACBD3
Rab11
FIP3
PI4P
PI4P
Binding
protein