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Phosphatidylinositol 4-Kinases and PI4P Metabolism in the Nervous System: Roles in Psychiatric and Neurological Diseases

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Abstract The four mammalian phosphatidylinositol 4-kinases, together with the $PI(4,5)P_2$ depleting 5-phosphatases of the oculocerebrorenal syndrome of Lowe and synaptojanin families, modulate neuronal pools of PI4P lipid and regulate intracellular membrane trafficking in the endocytic and secretory pathways. Dysfunctions in these enzymes have been associated with a broad spectrum of disorders including schizophrenia, bipolar disorder, Lowe syndrome, age-related neurodegeneration, Alzheimer's disease and Down syndrome. Recent work has shown that reduced expression of individual phosphatidylinositol 4-kinase isozymes is associated with impaired survival of specific neuronal populations within the CNS. Furthermore, alterations to the concentrations of different phosphoinositide lipid species in the brain and, in particular, the ratio of PI4P to $PI(4,5)P_2$ can have deleterious effects on clathrin-dependent membrane trafficking both in the Golgi–endosomal pathway and at the plasma membrane. In this article, we focus on the cell biology, biochemistry and neuronal functions of the phosphatidylinositol 4-kinases and their emerging roles in psychiatric and neurological pathologies.

Keywords Phosphatidylinositol · 4-Kinase · Endosome · Alzheimer's · Schizophrenia · Down syndrome · Clathrin · 22q11 · Golgi

Abbreviations

Aβ	Amyloid β peptide
ARF	ADP-ribosylation factor
NCS-1	Neuronal calcium sensor-1
TGN	*Trans*-Golgi network
PAO	Phenylarsine oxide
PI	Phosphatidylinositol
PI4P	Phosphatidylinositol 4-phosphate
$PI(4,5)P_2$	Phosphatidylinositol (4,5)-bisphosphate
$PI(3,4,5)P_3$	Phosphatidylinositol (3,4,5)-trisphosphate
PI 3-kinase	Phosphoinositide 3-kinase
PI4K	Phosphatidylinositol 4-kinase
PI4KIII	Type III PI 4-kinase
PICALM	Phosphatidylinositol clathrin assembly lymphoid myeloid leukaemia
PIPK	PI4P 5-kinase

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Introduction

Phosphoinositide lipids regulate key cellular functions including endocytosis, signalling and secretion. In neuronal systems, well-studied areas include the roles of phosphatidylinositol (4,5)-bisphosphate ($PI(4,5)P_2$) in synaptic vesicle recycling [1, 2] and ion channel regulation (for examples, see [3–8]) and the function of phosphatidylinositol (3,4,5)-trisphosphate ($PI(3,4,5)P_3$) in neuronal cell survival mediated through Akt activation [9]. Both $PI(4,5)P_2$ generation by phosphatidylinositol 4-phosphate 5-kinases (PIPK) and the subsequent generation of $PI(3,4,5)P_3$ by the phosphoinositide 3-kinases are dependent on an initial phosphorylation of phosphatidylinositol (PI) on the D4 position by one of the four mammalian PI 4-kinase enzymes synthesising phosphatidylinositol 4-phosphate (PI4P) [10, 11]. Indeed, there are now numerous examples showing a requirement for PI 4-kinase activity in the maintenance of $PI(4,5)P_2$ pools necessary for ion channel regulation [12–22].

Furthermore, PI4P can function on intracellular membranes in the recruitment of clathrin adaptor proteins such as AP-3 [23], AP-1 [24] and GGAs [25] during Golgi–endosomal trafficking. In this way, PI 4-kinases have the potential to regulate many phosphoinositide-dependent trafficking and signalling functions. Yet despite being the first committed phosphorylation step in the pathway that synthesises PI(4,5)P_2 and PI(3,4,5)P_3 (Fig. 1), the neurobiological roles of PI4P are only slowly emerging.

In addition to its synthesis by PI 4-kinases, neuronal PI4P levels can potentially be augmented by the action of phosphoinositide 5-phosphatases such as the synaptojanins [2, 26–29] and oculocerebrorenal syndrome of Lowe (OCRL) [30] which dephosphorylate PI(4,5)P_2 on the D5 position to produce PI4P. Synaptojanin also contains a sac1 PI4P phosphatase domain [28, 31, 32] and thus has the potential to also decrease PI4P levels in neuronal tissue. Recent evidence suggests that PI4P levels on intracellular membranes, and particularly at the *trans*-Golgi network and endosomes, have important neuronal functions and that deregulated PI4P generation can have neuropathological consequences. In this article, we evaluate and discuss recent trends in this newly emerging area.

Four mammalian PI 4-kinases have been cloned and characterised; they are the type III PI 4-kinases (PI4KIII) which are inhibited by high micromolar concentrations of the PI 3-kinase inhibitors wortmannin and LY 294002, and the type II PI 4-kinases (PI4KII) which are wortmannin-insensitive but can be inhibited by low micromolar concentrations of adenosine [11]. The two PI4KIIs, PI4KIIα (Fig. 2) and PI4KIIβ are highly homologous ~55-kDa enzymes [33], whereas the larger PI4KIIIs consisting of the 230 kDa PI4KIIIα and 92 kDa PI4KIIIβ isoforms form a distinct protein family with greater homology to the PI 3-kinase family of enzymes in both catalytic and non-catalytic domains [10]. Despite their structural and biochemical differences, all the four mammalian PI 4-kinases synthesise the same PI4P lipid product. However, as the PI 4-kinases are targeted to different subcellular membranes, there are also isoform-dependent differences in the rates of PI4P synthesis at

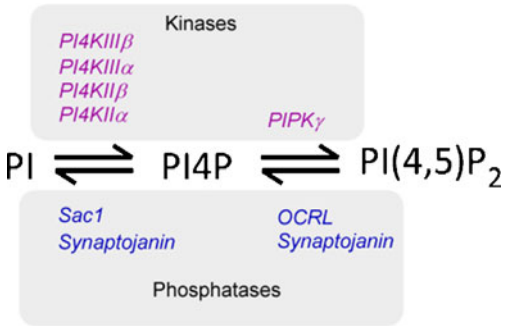

Fig. 1 PI kinases and phosphatases involved in the metabolism of PI4P in the CNS

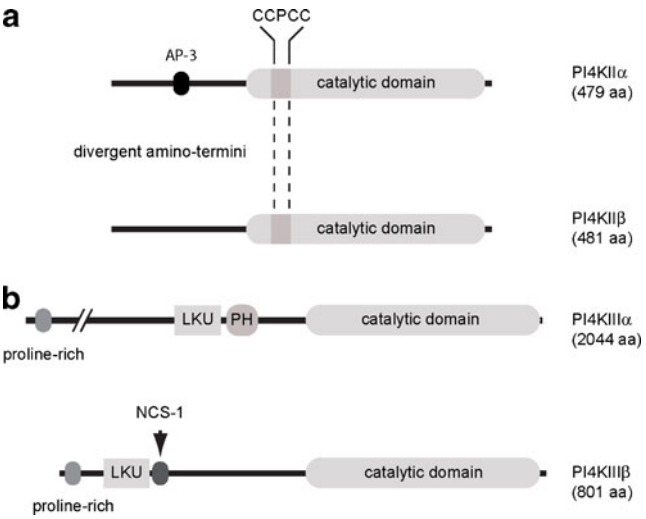

Fig. 2 Diagram illustrating the main structural features of the PI 4-kinases (adapted from [11]). **a** The PI4KII isoforms share homology in the conserved catalytic core. A conspicuous feature of the enzymes is a conserved cysteine-rich region in the kinase domain that undergoes palmitoylation and mediates membrane targeting to cholesterol-rich membrane microdomains. The N-terminal regions of the PI4KIIs are the least similar: whereas PI4KIIα is proline-rich, amphiphillic in character and contains an AP-3-binding motif $_{57}$ERQPLL$_{62}$, the N-terminus of PI4KIIβ contains a large number of acidic residues. **b** Domain organisation of mammalian PI4KIIIs. The *catalytic domains* of PI4KIIIα and PI4KIIIβ display minimal homology to the PI4KIIs but are more homologous with the PI3K family of enzymes especially in the conserved lipid kinase unique (*LKU*) domain. PI4KIIIα contains an N-terminal proline-rich domain and an internal PH domain. Non-catalytic domains in PI4KIIIβ include a proline-rich sequence and a binding site for NCS-1

different intracellular locations [34–37] and subsequently highly compartmentalised roles for individual isoforms in PI4P-dependent signalling and trafficking [34, 38].

All of the PI 4-kinases are expressed in the brain and, with the exception of the PI4KIIβ isoform, detailed immunohistochemical analyses are available on their distributions within the CNS. While there is no single systematic study comparing the distributions of all the PI 4-kinases in the CNS, there are some trends in their neuroanatomical expression patterns that are worth considering.

Mapping of PI4KIII expression by in situ mRNA hybridisation [39], and by light and electron microscopy, [40] has revealed that PI4KIIIα and PI4KIIIβ are localised in neurons throughout the central nervous system. There are some differences in PI4KIII isoform distribution in that PI4KIIIα was more highly expressed in spinal cord and cerebral cortex neurons, whereas PI4KIIIβ was most intensely immunostained in Bergman glia in the molecular layer of the cerebellar cortex. In addition, the hippocampus expresses high levels of PI4KIIIα [40] and an mRNA differential display study has demonstrated that expression of this isozyme is selectively decreased in ischemia-induced delayed neuronal death in the CA1 area of this brain region [41]. Interestingly, relatively

intense staining for PI4KIIIβ has also been reported in the CA1 subregion of the hippocampus although there is no evidence that levels of this protein are reduced following ischaemia [42]. Separately, there is evidence for a correlation between learning impairment and depressed G protein-coupled receptor (GPCR)-induced phosphoinositide signalling in the hippocampus of aged animals [43]. Since both PI4KIIIα and PI4KIIIβ are known to supply PI4P substrate during GPCR-activated phospholipase C signalling [34, 38], their hippocampal enrichments may indicate a potential role for these enzymes in muscarinic and metabotropic receptor signalling in this brain region.

Studies on the PI4KIIs have shown that with the exception of white matter, PI4KIIα is expressed at varying levels in neurons and astrocytes throughout the brain with high levels in Purkinje cells and in Bergman glia of the cerebellar molecular layer [44]. A separate detailed immunocytochemical analysis of PI4KIIα expression in the hippocampus reported high expression of the enzyme in pyramidal cells and in the molecular layer of the dentate gyrus, the dentate hilius and the stratum lucidium of the CA3 region [45]. Immunohistochemical results from the Human Protein Atlas [46–48] have shown that PI4KIIβ distribution in the cerebellum mirrors that of both PI4KIIα and PI4KIIIβ with highest expression in the molecular layer. Overall, though, staining for PI4KIIβ is strongest in hippocampal neurons. It is worth noting that mRNA in situ hybridisation studies from the Allen Brain Atlas [49] also support hippocampal and cerebellar localisations for all the PI4K isoforms. Together these findings show that multiple PI 4-kinase isoforms can be expressed at different levels in a single neuroanatomical location (Fig. 3), and also that all of these enzymes can be localised to neurons.

Subcellular Localisations of Pi 4-Kinases in Neurons of the CNS

Detailed ultrastructural studies on the localisation of the PI4KIIIs in neurons from the ventral horn of the spinal cord demonstrated that PI4KIIIα immunoreactivity was associated mainly with rough endoplasmic reticulum, mitochondrial outer membrane, occasionally on multivesicular bodies and close to synaptic specialisations [40]. PI4KIIIβ was also found associated with the rough endoplasmic reticulum, mitochondria and the Golgi complex [40]. In non-neuronal cells, confocal imaging studies have shown that PI4KIIIα is an endoplasmic reticulum enzyme while PI4KIIIβ is mainly found associated with Golgi membranes [50, 51]. Furthermore, a mitochondrial localisation for either PI4KIII isoform has not been reported in non-neuronal cells. Of the PI4KIIs, detailed neuronal subcellular localisation data is available only for the PI4KIIα isoform where it localises to dendrites, the Golgi and synaptic vesicles [45, 52]. Therefore, similar to non-neuronal cells, each PI 4-kinase isoform exhibits a distinct pattern of membrane localisation in neurons which also indicates that PI4P synthesis is likely to be highly compartmentalised within individual cells of the CNS (Fig. 4).

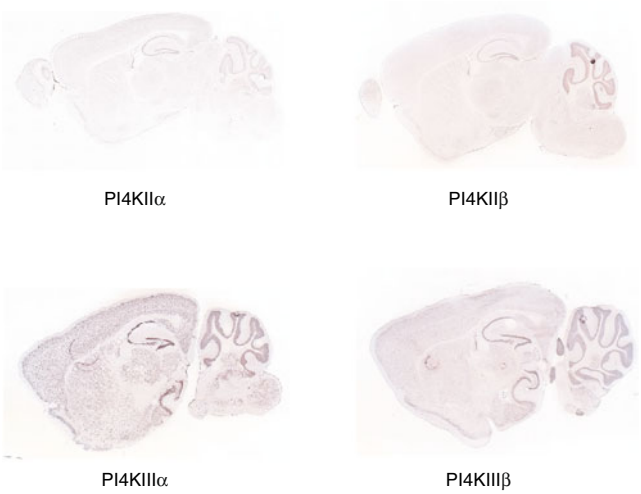

Fig. 3 In situ hybridisation images showing the distribution and expression of PI 4-kinase mRNAs in sagittal sections of mouse brain [49]. Note that mRNAs for all PI 4-kinase isoforms are highly expressed in the cerebellum and in the hippocampus. Data from Allen Mouse Brain Atlas, Seattle (WA): Allen Institute for Brain Science ©2009 (http://mouse.brain-map.org)

Neuronal Functions and Dysfunctions of the PI 4-Kinases

Early work from the Martin laboratory identified PI 4-kinase activity on secretory vesicles involved in the supply of PI4P substrates to PI4P 5-kinases [53, 54]. In the intervening years,

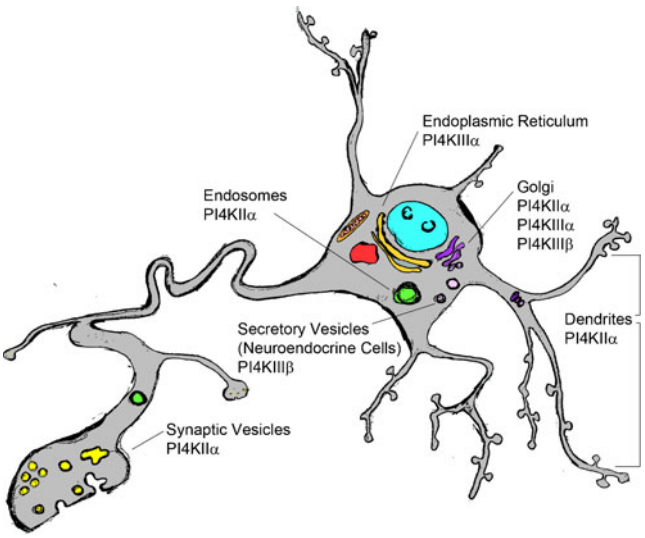

Fig. 4 Intracellular localisations of PI 4-kinase isoforms in neurons. There is no systematic or comprehensive study detailing the subcellular distributions of the four mammalian PI 4-kinases in neurons and no data at all is available for PI4KIIβ. This schematic diagram therefore depicts proposed PI 4-kinase localisations using information derived from separate reports that used different methods and different neuronal cell types

there has been some progress in understanding the functions of different PI 4-kinases in neuronal vesicle trafficking events.

In the absence of isoform-selective PI 4-kinase inhibitors, many early investigations used phenylarsine oxide (PAO) to inhibit PI4P synthesis. PAO is now known to react with vicinal thiol groups on a variety of enzymes to inhibit their catalytic activity [55–57], but nevertheless it was employed, albeit as a nonspecific intervention, to gain some initial insights into neurophysiological functions of the PI 4-kinases. As an example, sensitivity to PAO inhibition was used to infer a role for PI4P in the retrograde axonal transport of neurotrophin-4 [58] and nerve growth factor [59] in both sympathetic and sensory neurons, suggesting that PI 4-kinases may regulate multiple vesicle trafficking processes. It is important to bear in mind that there has been disagreement as to whether PAO is a more potent inhibitor of PI4KIIs or PI4KIIIs, particularly in light of earlier work which inferred a role for PI4KII activity on chromaffin granule membranes in regulated secretion [60]. This controversy has been more recently addressed by a detailed analysis [61] using purified recombinant PI 4-kinases which revealed that PAO was most selective for PI4KIIIα inhibition when added at low 1–5-μM concentrations. At higher PAO concentrations exceeding ~10 μM, there was pronounced inhibition of PI4P generation by three isoforms PI4KIIIα, PI4KIIIβ and PI4KIIα [61]. The recent discoveries of better isoform-selective small molecule inhibitors of the PI4KIIIs [62–64] have the potential to open up new avenues of research into the roles of these enzymes in the regulation of exocytosis and retrograde trafficking.

Studies on synaptic vesicle pools in synaptosomes derived from mature central nerve terminals determined that repetitive synaptic vesicle recycling did not require phosphoinositides, but that PI 4-kinases were required for the transfer of a non-releasable reserve pool of synaptic vesicles to a pool which was readily releasable in response to hypertonic stimulation [65]. Another interesting finding is there may also be cargo-specific requirements for PI 4-kinase activity in stimulated exocytosis, in that noradrenaline release requires phosphoinositide synthesis but glutamate or GABA release do not [66]. Therefore, PI 4-kinases and particularly PI4KIIIs have very specific functions restricted to particular points in the synaptic vesicle cycle, and these roles may be further restricted to particular neurotransmitters.

As regards the regulation of PI 4-kinases on secretory vesicles, PI4KIIIβ represents the best characterised example with insights being gained mainly from PC12 neuroendocrine cells [67, 68] but with some important observations also being derived from studies on non-neuronal cell lines [69–73]. On secretory vesicle membranes, the lipid kinase activity of PI4KIIIβ is stimulated by interaction with neuronal calcium sensor 1 (NCS-1) [67–83]. NCS-1 is a Ca^{2+}-binding EF hand protein which can activate membrane-associated PI4KIIIβ in response to elevated cytosolic Ca^{2+} concentrations, thereby providing some rationalisation of the relationship between Ca^{2+}-sensitive exocytosis and PI4P synthesis. Additionally, both NCS-1 and PI4KIIIβ are targeted to intracellular membranes via interactions with ADP-ribosylation factor (ARF) proteins [75, 84], and PI4KIIIβ interactions with ARF-1 are important for regulatory exocytosis [70].

While there has been significant progress in understanding the functions of PI4KIIIβ, NCS-1 and ARF-1 in the regulation of stimulated secretion, there is also some evidence that the PI4KIIα isozyme may also function on this pathway. Indeed, PI4KIIα activity is particularly enriched on synaptic [52] and secretory vesicles [85], and it is noteworthy that the enzyme was originally purified and cloned from a secretory vesicle membrane fraction as found by Barylko and colleagues [86]. PI4KIIα is required for the recruitment of AP-1 at the *trans*-Golgi network (TGN) and thus the formation of clathrin-coated vesicles [24]. A more recent work specifically investigating the role of PI4KIIα in neuronal vesicle trafficking has shown that the enzyme cooperates with the BLOC-1 and AP-3 complexes to regulate trafficking from the cell body to the nerve terminal [45]. In light of the recent precedent that both PI4KIIα and PI4KIIIβ isozymes are required for correct Golgi–lysosomal trafficking of the Gaucher disease enzyme β-glucocerebrosidase [87], it seems likely that different PI 4-kinases may control distinct biochemical steps in neuronal vesicle trafficking. Table 1 shows the emerging roles for PI 4-kinases in neuronal disease.

Emerging Neuropathological Roles for the PI 4-Kinases

Schizophrenia

Even though there is still much to be learned about the role of the different PI 4-kinases in normal neuronal physiology,

Table 1 Emerging roles for PI 4-kinases in neuronal disease

PI4K isozyme	Pathology
PI4KIIα	Knock-out mice develop neurodegeneration similar to autosomal recessive hereditary spastic paraplegia [44].
	Transports dysbindin, a schizophrenia susceptibility protein [45]
	Inhibited by nM concentrations of Aβ protein [99, 100]
PI4KIIβ	Potential role in schizophrenia [92]
PI4KIIIα	Schizophrenia [88]
	22q11.2 deletion syndrome [89]
	Downregulated in hypoxia-induced neuronal cell death [41]
	Downregulated following chronic ethanol treatment [93]

there are nevertheless a number of reports implicating these enzymes in psychiatric and neurological diseases. One emerging story is that PI4KIIIα and PI4KIIα may be important in schizophrenia. The gene for PI4KIIIα, termed *PI4K3A*, maps to chromosome 22q11, a locus which is of high interest in terms of understanding the genetic basis of mental illness. Individuals with a hemizygous deletion in this chromosomal region in what is known as 22q11.2 deletion syndrome are more susceptible to a number of psychiatric conditions including depression, autism, bipolar disorder and schizophrenia.

In a study involving a cohort of 310 Dutch patients, Jungerius and colleagues [88] identified a significant association between three intronic *PI4K3A* single nucleotide polymorphisms and schizophrenia. It is noteworthy that this association was later confirmed in a different study in patients with 22q11.2 deletion syndrome [89]. However, a similar study on a Japanese group of patients did not replicate these findings [90]. Therefore, compensatory mechanisms due to differences in genetic background and possibly related to ethnic variation may affect the linkage between *PI4K3A* and schizophrenia. In a separate study which investigated the frequencies of *PI4K3A* polymorphisms [91] in schizophrenia and bipolar disorder, there was some evidence for the occurrence of two rare but possibly functional variants in a few patients; the first could possibly result in the creation of a CREB transcription factor binding site in the promoter region and the second had the potential to disrupt splicing of the *PI4K3A* gene.

While most work has been focused on the possible role of the PI4KIIIα isoform in schizophrenia, there is some evidence that other PI 4-kinases may have a role in this disease. A potential association between the PI4KIIβ isoform and schizophrenia was suggested in a study centred on a large Scottish family cohort [92], and the PI4KIIα isoform is involved in vesicular transport of dysbindin, a schizophrenia susceptibility protein [45]. This leads to the now familiar conclusion that multiple PI 4-kinases may be involved in schizophrenia although it is not yet clear if such an association is only limited to a subset of patients.

PI4KIIIα in Chronic Alcohol Consumption

Altered PI4KIIIα expression has been reported in rat hippocampus following chronic ethanol treatment. Using cDNA microarrays to analyse gene expression, Saito et al. [93] found that the expression of PI4KIIIα was downregulated 1.6-fold in the brains of ethanol-treated rats. Several other proteins including profilin, synaptophysin, ARF-1 and dynamin-1 also exhibited a similar degree of downregulation, leading to the suggestion that cytoskeletal and vesicular trafficking processes may be particularly sensitive to perturbation by ethanol [93]. PI4KIIIα is not known to regulate post-Golgi vesicle trafficking, but emerging evidence from other experimental models suggesting a pro-survival signalling role for this isoform via the MAPK/ERK pathway may be worth investigating in a neuronal context. In particular, knockdown of PI4KIIIα in zebrafish morphilinos gave rise to anomalous brain development which seems likely to be due to defective growth factor-stimulated MAPK and PI 3-kinase signalling [94]. Also noteworthy in this regard is a siRNA kinase screen in medulloblastoma-derived cell lines which identified PI4KIIIα as a protein required to sustain cell proliferation and in underlying resistance to the chemotherapeutic reagent cisplatin [95]. These results combined with other emerging insights into the role of PI 4-kinases in cell survival [96] suggest that further work is warranted to investigate the possible targeting of these enzymes in brain cancers difficult to treat such as medulloblastomas which have poor survival rates.

Neuronal Cell Death Following Ischemia

In a rat model for transient forebrain ischemia, induced by the four-vessel occlusion method, PI4KIIIα expression is specifically downregulated 30–80 % in CA1 pyramidal neurons but not in other brain regions [41]. This event precedes the delayed neuronal apoptosis that occurs in these neurons following ischemic shock and correlated with a reduction in PI(4,5)P_2 levels. Significantly, recombinant overexpression of wild-type but not catalytically inactive PI4KIIIα in neuroblastoma cells could rescue hypoxia-induced cell death, thus demonstrating that PI4KIIIα-catalysed PI4P generation was essential for cell survival under these conditions [41].

Alzheimer's Disease

Both phosphoinositde levels [97] and PI 4-kinase activity are reduced by up to 50 % in the brains of patients with Alzheimer's disease [98]. Intriguingly, pathologically relevant nanomolar concentrations of amyloid β (Aβ) protein can inhibit PI4KII activity in a neuronal plasma membrane preparation [99] with concomitant, augmented glutamate toxicity. In a later study, it was demonstrated that the suppression of PI4P synthesis and glutamate neurotoxicity by Aβ protein could be antagonised with a simple Ile-Gly-Leu tripeptide [100]. Similar to the key Alzheimer's proteases BACE [101] and γ-secretase [102], the catalytic activity of PI4KIIα is highly sensitive to membrane cholesterol levels, and this enzyme is also targeted to cholesterol- and glycosphingolipid-rich microdomains of the TGN and endosomes [103–105]. Recently, it has been reported that γ-secretase activity is strongly inhibited by phosphatidylinositol in vitro [106]. Furthermore, single nucleotide polymorphisms in *PICALM,* a gene encoding phosphatidylinositol clathrin assembly lymphoid myeloid leukaemia (PICALM), are strongly associated with Alzheimer's disease [107, 108]. PICALM binds PI(4,5)P_2

and regulates clathrin-mediated endocytosis of amyloid precursor protein and its subsequent trafficking to endosomes where it is proteolytically processed into Aβ [109, 110]. Thus PICALM functionally links PI4P metabolism with endocytic trafficking and amyloid plaque formation [109, 110]. Together these observations suggest a common sterol-sensitive pathway that may link amyloid protein processing with PI4P metabolism and possibly in the upstream production of $PI(4,5)P_2$ [26].

Neuropathology in PI4KIIα Knock-out Mice

Acute RNAi-induced inhibition of any of the PI 4-kinase isozymes in cell lines can result in defective phosphoinositide signalling and aberrant intracellular trafficking. Thus, it could have been expected that PI 4-kinase knock-out animals would exhibit multiple abnormalities. To date, only the pi4k2a gene encoding the PI4KIIα isozyme has been knocked out in mice [44]. Surprisingly however, generation of a PI4KIIα gene trap knock-out mouse showed that homozygous−/− animals were viable and initially developed normally [44]. As the animals aged, a progressive neurological phenotype developed, with the mice exhibiting a spastic gait, nodding tremor and incontinence. These characteristics resemble the progression of autosomal recessive hereditary spastic paraplegia. Histological analysis of aged animals revealed a marked decrease in the number of Purkinje cells, along with axonal defects in both the ascending and descending tracts of the spinal cords [44]. The appearance of lipofuscin deposits in affected mice suggests that loss of PI4KIIα expression induces a cumulative failure in endo-lysosomal trafficking, since defects in this pathway characteristically give rise to endosomal storage diseases [87]. However, further investigations are required to establish whether there exists a link between early onset neuropathy and defective, PI4KIIα-dependent, intracellular trafficking.

The progressive nature of the defects in PI4KIIα knock-out mice suggests that there is initial compensation for the loss of the enzyme perhaps through functional redundancy of the other PI 4-kinase isotypes. While it is not known whether pro-survival neuronal signalling was inhibited or if membrane trafficking was defective in the knock-out mouse model, it is clear that, over time, the continued expression of PI4KIIα is essential for the viability of particular cell populations in the CNS.

Neuronal Dysfunction Controlled by other PI4P Modulators

While perturbations of the PI 4-kinase isoforms themselves are sufficient to induce neuronal dysfunction, PI4P concentrations can also be modulated by PI4P 5-kinase phosphorylation to generate $PI(4,5)P_2$ by, or conversely via phosphatase-mediated D5 dephosphorylation of $PI(4,5)P_2$ to generate PI4P. There is now strong evidence that deregulation of either pathway of PI4P metabolism can lead to neuronal dysfunction and disease.

OCRL

OCRL is a phosphoinositide D5 phosphatase, capable of producing PI4P through the dephosphorylation of $PI(4,5)P_2$ (reviewed in [111]). OCRL is deleted or mutated in individuals suffering from Lowe syndrome and in Dent's disease [112]. This is an X-linked disorder and, along with the associated ophthalmological and renal symptoms, there is a distinct neuronal phenotype, with affected boys suffering from varying degrees of intellectual impairment, seizures and maladaptive behavioural issues. OCRL is typically found on endosomes and at the Golgi of non-neuronal cell lines. Defective OCRL leads to $PI(4,5)P_2$ accumulating on early endosomes and consequently enhanced N-WASP-mediated F-actin accumulation and defective trafficking [113], thus indicating that alterations to the PI4P:$PI(4,5)P_2$ balance on intracellular membranes can have pathological consequences. Very little is known about the role of PI4P in the endosomal pathway, and this may be partly due to the technical difficulties in imaging non-Golgi PI4P pools with currently available anti-PI4P antibodies and PI4P-specific PH domain-binding proteins [114]. Nevertheless, work involving the expression of recombinant, catalytically inactive PI4KIIα, has implicated PI4P generation by this isoform in the recruitment of AP-3 to late endosomes in non-neuronal cells [23]. More recently, Larimore and colleagues have shown that PI4KIIα operating in conjunction with the AP-3 and the BLOC-1 complexes, mediates trafficking of synaptic-like microvesicles from the cell body to both neurites and nerve terminals [45]. These new insights suggest that changes to PI4P concentrations on intracellular membranes have the potential to alter the dynamics of cargo delivery to synaptic membranes, but further work is needed to evaluate the degree to which compartmentalised changes to PI 4-kinase catalytic activity can alter localised PI4P:$PI(4,5)P_2$ ratios and to establish if this impacts on intra-neuronal vesicular trafficking.

OCRL also contains a clathrin-binding motif within a non-phosphoinositide-binding PH domain [115], a non-functional Rho-GAP domain, and domains which can interact with endosomal trafficking proteins such as APPL1 and Rab GTPases (reviewed in [116]), suggesting that the protein functionally integrates PI4P generation with intracellular vesicle trafficking. A splice variant of OCRL, termed OCRLa, is only expressed in the brain, has a higher affinity for clathrin binding than the more ubiquitously expressed OCRLb variant, and is found associated with clathrin-coated intermediates [117], again suggesting an important linkage between clathrin-dependent trafficking and PI4P generation in the CNS. In concordance with this, OCRL has been co-purified with neuronal clathrin-coated vesicles from synaptosomal

preparations [118] and has been imaged in association with late stage clathrin-coated endocytic pits [119]. Further work is needed to evaluate the relative contributions of altered PI4P metabolism and the non-catalytic endosomal functions of OCRL in neurological disease particularly since missense mutations within the APPL1 binding region, situated outside the phosphatase domain, are sufficient to induce the OCRL neuropathology [120].

Synaptojanin 1

Synaptojanin 1, the main neuronal PI(4,5)P_2 D5 phosphatase, which also contains a PI4P phosphatase sac1 domain, is essential for synaptic vesicle endocytosis in neurons and is thus essential for maintenance of neuronal transmission. Knock-out mice deficient in Synaptojanin 1 die shortly after birth and exhibit numerous neurological defects including severe weakness, ataxia and convulsions. These mice have elevated levels of PI(4,5)P_2, and accumulate a large number of clathrin-coated intermediates [2] which are infrequently observed in wild-type animals.

Synaptojanin is one of the genes present on chromosome 21, the trisomy of which results in excess production of synaptojanin 1 in individuals suffering from Down syndrome [121]. In a mouse model, increased gene dosage of synaptojanin 1 led to a 15–20 % increase in PI(4,5)P_2 mass in the brains of affected mice [122] and impaired cognitive performance when assessed by the Morris water maze task. Neurons cultured from mouse models of Down syndrome, trisomic for synaptojanin 1, were found to possess significantly enlarged early endosomes [123]. This effect was recapitulated in neuroblastoma cell lines through the overexpression of tagged synaptojanin 1 [123]. Since synaptojanin possesses both D5 and D4-phosphatase activities, excesses of this enzyme have the potential to increase either PI4P or PI levels in cells. Indeed, both activities are required for synaptic vesicle recycling, indicating a requirement for both PI4P and PI(4,5)P_2 in this event [32]. However, the membrane concentrations of different phosphoinositide species present on the enlarged early endosomes have not yet been determined.

Synaptojanin is also thought to play a role in the progression of synaptic dysfunction in Alzheimer's disease [26]. Through their action on synaptojanin 1, Aβ peptides can acutely and chronically destabilise the metabolism of PI(4,5)P_2 in primary cortical cultures, implicating synaptojanin and Aβ oligomers in pathophysiological progression of this disease. This implies that normal synaptojanin levels of expression are essential for both the highly specialised, neuron-specific, synaptic vesicle cycle, as well as the ubiquitous membrane trafficking pathways found in other tissues. As mentioned previously, Aβ proteins have also been shown to inhibit PI4KIIα activity [99, 100] in the nanomolar range all of which suggests that alterations to the PI:PI4P:PI(4,5)P_2 ratio in neurons may be an important factor in the aetiology of Alzheimer's disease.

PIPKIγ

Three PIPK enzymes are capable of generating PI(4,5)P_2 through phosphorylation of PI4P on the D5 position. Of these three isoforms, PIPKIγ is the dominant form found in the nervous system, where it plays a critical role in synaptic transmission [124, 125], embryonic neural tube closure, adherens junction formation and neuronal migration [126]. Indeed murine genetic studies have revealed that loss of PIPKIγ results in either embryonic [126] or early postnatal lethality [1, 127], and also that a single allele of PIPKIγ is sufficient to ensure development to adulthood and to maintain neuronal PI(4,5)P_2 levels [127].

A lipid kinase-inactivating, single point mutation in PIPKIγ resulting in the substitution of aspartic acid with asparagine at amino acid 253 was found to be the cause of lethal congenital contractural syndrome type 3 (LCCS3), a disease which causes foetal or neo-natal mortality [128]. Interestingly, the PIPKIγ knock-out mouse did not replicate the muscle wasting and joint contracture of LCCS3, although it did cause early postnatal lethality [1]. Detailed studies of the presynapse of these knock-out mice revealed a significant defect and delay in the reformation of synaptic vesicles following exocytosis, in conjunction with pronounced rapid exocytic depression during periods of intense stimulation [1]. It is noteworthy that these PIPKIγ-deficient synapses produced significantly more and larger endosomes in response to elevated stimulation. These are reminiscent of activity-dependent bulk endocytosis profiles, which are induced in central nervous synapses in response to strong synaptic stimulus [129]. The resulting smaller recycling pool and delayed synaptic vesicle recycling of PIPKIγ-deficient synapses may indicate a defect in the generation of single synaptic vesicles which bud from these large bulk endosomes. This implicates a hitherto uninvestigated role for PI4P-derived phosphoinositides in the generation of synaptic vesicle membranes from bulk endocytic structures.

Conclusions and Future Perspectives

PI 4-kinases and PI4P are beginning to be implicated across a wide range of neuronal functions and pathologies, but this remains a very underdeveloped field of study. Future work, perhaps on transgenic models with conditional CNS expression of PI 4-kinase structural variants may be key to understanding the catalytic and non-catalytic functions of these enzymes in neuronal vesicle trafficking and synaptic transmission. The recent availability of small molecule inhibitors particularly of the PI4KIIIs [62–64] may facilitate a more

meaningful analysis of the neuronal functions of this class of enzymes and facilitate the design of novel chemotherapeutics with potential applications in the treatment of neurological and psychiatric diseases. On the other hand, the current dearth of isoform-specific inhibitors directed against the PI4KIIs, PIPKs and PI(4,5)P_2 phosphatases is reflected in a continued reliance on recombinant and genetic strategies to understand the enzymology and regulation of neuronal PI4P pools. Finally, new approaches to manipulate and detect levels of PI4P in different subcellular compartments [8, 20, 38, 114] are likely to be extremely important in dissecting specific roles for this phospholipid in the CNS.

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