New insights into the functions of PtdIns(3,5)P$_2$ in the pathogenesis of neurodegenerative disorders

The membrane trafficking systems in brain play an important role in the regulation of neuronal processes, such as morphology, neuronal survival and synaptic plasticity. It has been suggested that the phosphatidylinositol (Pis) located on endolysosomal membranes play a key role in controlling this trafficking systems. Recently, we have reported that PtdIns(3,5)P$_2$, which is a low abundant PIs in cells, is involved in the regulation of lysosomal degradation via vesicle transport (Tsuruta et al., 2009; Tsuruta and Dolmetsch, 2015). In this perspective, we provide an overview of the general functions of PtdIns(3,5)P$_2$, and discuss their potential role in the pathogenesis of neurodegenerative disorders.

The cellular functions of PtdIns(3,5)P$_2$: Pis are crucial for the regulation of a wide variety of cellular processes. The hydroxyl groups of the inositol ring of Pis are phosphorylated and dephosphorylated by specific phosphatidylinositol kinases and phosphatases. These enzymes control the amount of intracellular Pis at appropriate levels and regulate specific cellular functions. Recently, several groups have actively investigated PtdIns(3,5)P$_2$, which is less abundant than most other Pis, such as PtdIns(4,5)P$_2$, PtdIns(3,5)P$_2$, is predominantly localized in endosome and lysosomes, where it is involved in regulating multisvesicular bodies (MVBs) invagination, autophagy, and membrane trafficking (Shisheva, 2012; McCartney et al., 2014). Intriguingly, PtdIns(3,5)P$_2$ levels are dramatically elevated after osmotic stresses and return to the basal level within a half hour. Because this reaction is dynamic, the understanding of PtdIns(3,5)P$_2$ turnover may provide important cues into the role of lipids in regulating neuronal functions. PtdIns(3,5)P$_2$ is known to be generated from PtdIns(3)P by phosphatidylinositol 3-phosphate 5-kinase (Fab1; yeast or PIKfyve; mammals), the only lipid kinase in most eukaryotes. Fab1/PIKfyve is thought to be involved in the regulation of membrane homeostasis, and defect in its function lead to abnormal vacuole formation in both yeast and mammalian cells. Moreover, loss of the PIKfyve gene is embryonically lethal, suggesting that PtdIns(3,5)P$_2$ produced by Fab1/PIKfyve plays a vital role in maintaining membrane homeostasis. The PtdIns(3,5)P$_2$ was initially reported to be implicated in the regulation of ESCRTIII complex. After phosphorylation of PtdIns(3)P by Fab1 in yeast, PtdIns(3,5)P$_2$ associates with Vps24, which is an ESCRTIII subunit, followed by the recruitment of additional subunits of ESCRTIII to sort cargo proteins into MVBs. Another effector called Ent3p functions in MVBs sorting via an ENTH domain, which is a phosphatidylinositol phosphate-binding motif. However, the molecular mechanisms by which PtdIns(3,5)P$_2$ regulates MVBs is still controversial (Michell et al., 2006). Additionally, PtdIns(3,5)P$_2$ has been implicated in the autophagy pathway. Atg18/WIPLs (Atg18; yeast or WIPI1, WIPI2, WIPI3, and WIPI4; mammals), which are essential proteins in autophagy, recognize both PtdIns(3,5)P$_2$, and PtdIns(3)P. Interestingly, it was reported that Atg18 negatively regulates PtdIns(3,5)P$_2$ level in yeast. Moreover, PtdIns(3,5)P$_2$ has been suggested to be a potential sensor of intracellular amino acids, followed by the regulation of target of rapamycin complex 1 (TORC1) activity (Jin et al., 2014). These observations imply that both Atg18/WIPLs and PtdIns(3,5)P$_2$ are mediators that link amino acids levels to TORC1 signaling in cells.

PtdIns(3,5)P$_2$ regulates vesicle motility: Recently, we have found a novel functions of PtdIns(3,5)P$_2$ in neurons. Previously, we reported that PtdIns(3,5)P$_2$ produced by PIKfyve plays a significant role in the protection of excitotoxic neuronal cell death via regulation of the levels of the voltage-gated calcium channel Ca$_{1.2}$ (Tsuruta et al., 2009). In neurons, intracellular Ca$^{2+}$ concentration is responsible for the coordination of neuronal activity being tightly regulated through control of both N-methyl-D-aspartate receptor (NMDAR) and voltage-gated calcium channel, Ca$_{1.2}$. Our studies indicated that bath glutamate application promotes the internalization of Ca$_{1.2}$ channels from the plasma membrane leading to lysosomal degradation. Under this condition, PIKfyve associates with Ca$_{1.2}$ and synthesizes PtdIns(3,5)P$_2$ on Ca$_{1.2}$-containing vesicles, resulting in the effective transition from endosomes to lysosomes. Notably, inhibition of PIKfyve, which causes a decrease in PtdIns(3,5)P$_2$ levels, suppresses glutamate-induced internalization of surface Ca$_{1.2}$, leaving neurons vulnerable to excitotoxicity. As defects in both membrane trafficking and Ca$^{2+}$ homeostasis are implicated in many neurodegenerative disorders, PIKfyve may link between impaired trafficking systems and neurological disorders. The mechanism by which PIKfyve mediates lysosomal degradation of cargo proteins in neurons has been a long-standing unsolved question. In our recent study, we were able to shed light on the function of PIKfyve (Tsuruta and Dolmetsch, 2015). We observed that endolysosomal compartments are highly motile in neuronal dendrites. Thus, we speculated that PtdIns(3,5)P$_2$, are involved in the regulation of vesicle motility. In fact, knockdown of PIKfyve in neurons significantly decreased a motility of both endosomes and lysosomes, demonstrating that PIKfyve is required to control their motility in neurons.

The remaining question is the molecular mechanisms by which PtdIns(3,5)P$_2$, mediates vesicle movements in neurons. So far, several studies have reported that other Pis on endolysosomal compartments are associated with their own motility (Hirokawa et al., 2009). For instance, kinesin-3 family proteins, KIF1A and KIF1B, have a PH domain located at the C-terminal tail, which directly interacts with PtdIns(4,5)P$_2$, and regulates the motility of PtdIns(4,5)P$_2$-containing vesicles, including synaptic vesicles in neurons. In addition, guanylate kinase-associated kinesin (GAK1N), classified as a kinesin-3 protein KIF13B, interacts with PtdIns(3,4,5)P$_3$-containing vesicles and transports them to the edge of axons, followed by selective activation of PI3K pathways. Furthermore, the kinesin-3 family member KIF16B directly binds to PtdIns(3)P on early endosomes and transports early endosomes to the microtubule plus ends. Hence, it is plausible that Pis function as a recognition target of either motor proteins or their adaptor proteins, and that PtdIns(3,5)P$_2$ could be associated with either motor proteins or adaptor proteins, which mediates vesicle transport in neurons (Figure 1).

PIKfyve might also control membrane trafficking through the binding proteins. Recent studies have shown that members of JNK-interacting proteins (JIPs) are involved in axonal transport regulated by KIF5 proteins. Mutation in JIP homologs in both Drosophila and Caenorhabditis elegans impair axonal transport. JIP4, a novel member of the JIP family, binds to PIKfyve, and is involved in the regulation of membrane trafficking (Ikonomov et al., 2009). Therefore, PIKfyve binding proteins such as JIP4 could control vesicle movements in neurons. Apart from JIP4, other target proteins that alter the functions of either motor or adaptor proteins are thought to be potent candidates. It has been known that Fab1/PIKfyve activity is regulated by protein complex formation on vesicle, including Fig4 (Fig4; yeast or Fig4/Sac3; mammals) and Vac14 (Vac14; yeast or Vac14/Ar-PIKfyve; mammals). Fig4, which is a lipid phosphatase, dephosphorylates PtdIns(3,5)P$_2$ at 5-position and synthesizes...
PtIns(3,5)P

Interestingly, Fig4 also functions as a positive regulator of PIKfyve, demonstrating that not only PIKfyve but also Fig4 is essential to adjust the balance between PtIns(3,5)P
2 and PtIns(3)P on vesicles. Vac14 is composed of many HEAT domains and acts as a scaffold to form the protein complex. As PtIns(3,5)P
2 turnover is dynamic and rapid, these protein complexes may monitor PI3s levels on vesicles spatiotemporally and activate downstream signaling pathway, resulting in the alternation of motor protein functions. Taken together, it is possible that PtIns(3,5)P
2 on vesicles is associated with the function of motor protein either directly or indirectly.

Defect of PtIns(3,5)P
2 effectors and neurodegeneration: Recent study have demonstrated that mutation in Fig4 causes neurological disorders (Chow et al., 2007), such as Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis (ALS). Presently, it is known that two candidates, WIPI4 and SNX14, are the PtIns(3,5)P
2 effectors involved in neurodegeneration. Mutations in WIPI4, the mammalian homolog of Atg18, were reported to be associated with a severe neurodegenerative disorder—Static encephalopathy of childhood with neurodegeneration in adulthood (SENDA). SENDA is a genetic disorder characterized by abnormal accumulation of iron in the basal ganglia (Saitsu et al., 2013). Recently, several groups have reported that accumulation of ferritin clusters, iron storage structures, is observed in autophagy-deficient cells. Furthermore, NCOA4, a ferritinopathy specific autophagy cargo receptor associated with ferritin heavy chain 1, was identified. These findings implied that defects in autophagy causes impairment of ferritophagy and abnormal accumulation of iron in cells (Kaur and Debnath, 2015). In addition, recent exome sequencing studies revealed that mutation in SNX14, which is part of a family of sorting nexins, is associated with cerbellar atrophy (Akizu et al., 2015). SNX14 contains a PX domain, which recognizes PtIns(3,5)P
2 on endolysosomal membrane. Defect in SNX14 exhibits large lysosomes and impairs effective autophagosome clearance. As the SNX family consists of a diverse group of membrane trafficking proteins, impaired PtIns(3,5)P
2 may either affect the sorting ability of SNX14 or the properties of endolysosomal compartments. Interestingly, the phenotype showing larger lysosome was similar to that of PIKfyve knockdown in our experiments, implying that SNX14 could be a target of PtIns(3,5)P
2, associated with the regulation of vesicle motility.

Conclusions: The PtIns(3,5)P
2 turnover is crucial for maintaining the neuronal functions including vesicle transport. Aberrant PtIns(3,5)P
2 metabolism leads to neurodegenerative disorders. As defects in either PIKfyve or Fig4 expression lead to abnormal neuronal properties including neuronal morphology and survival, misregulation of PtIns(3,5)P
2 may alter the normal tuning of neural circuit and brain homeostasis. Although the molecular mechanism that connect impaired PtIns(3,5)P
2 to pathogenesis of neurodegenerative disorders has not been elucidated, this question is important and will need to be addressed in the future. Furthermore, it might be interesting to investigate whether control of PtIns(3,5)P
2 levels by pharmacological compounds in neurons are effective against several diseases such as Charcot-Marie-Tooth disease and ALS.

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