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MINIREVIEW ARTICLE

Membrane phosphoinositides and protein–membrane interactions

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Abstract Proteins with polybasic clusters bind to negatively charged phosphoinositides at the cell membrane. In this review, I have briefly discussed the types of phosphoinositides naturally found on membrane surfaces and how they recruit protein complexes for carrying out the process of signal transduction. A large number of researchers from around the world are now focusing their attention on protein–membrane binding, as these interactions have started to offer us a much better insight into the process of cell signaling. The main areas discussed in this brief review article include the phosphoinositide binding specificities of proteins and the role of their lipid binding in signaling processes downstream of membrane recruitment.

Keywords Proteins · Phosphoinositides · Lipids · Protein–membrane interactions · Biochemistry · Signal transduction

Introduction

Phospholipids play an important role in regulating the process of signal transduction at the membrane surface (Cho 2006). Protein–membrane interactions are involved in a large number of physiologically significant processes such as endocytosis, exocytosis, intracellular transport and diseases ranging from inflammation to cancer (Cho and Stahelin 2005). Lipid-binding proteins get localized to various components of the membrane machinery (depending on their lipid selectivity) and exhibit a variety of functions that are direct consequences of their membrane-selective recruitment (Varnai and Balla 2008). Some proteins are constitutively localized to intracellular membrane sites while others translocate to membrane surfaces in response to certain agonists (Saltiel and Kahn 2001). Depending on their membrane binding specificities, the peripherally binding proteins described above can be broadly divided into various classes. For example, proteins harboring PH domains generally prefer to localize to the plasma membrane which is constitutively abundant in PtdIns(4,5)P_2, or the transiently produced plasma membrane PtdIns(3,4,5)P_3, owing to the presence of positively charged amino acids such as Lys and Arg in their binding sites (Moravcevic et al. 2012). Previously, most of the lipid research was restricted to the study of membrane PtdIns(4,5)P_2 and its subsequent hydrolysis by phospholipase C (PLC) thereby yielding diacyl glycerol (DAG) and InsP_3 (the latter responsible for release of calcium from intracellular stores) (Mitchell et al. 1981), but after the discovery of PI 3-kinases by Whitman et al. (1985), the phosphoinositide community started focusing its attention on the lipid products downstream of PI3 K activation (Dove et al. 1997).

Protein interactions with membrane phosphoinositides

Several classes of phosphoinositide-binding proteins have been discovered and functionally characterized. Examples include the PtdIns3P binding FYVE (Hayakawa et al. 2006) and PX domains, the PtdIns(4,5)P_2 binding ENTH domain, the PtdIns4P binding FAPP1 PH domain, the PtdIns5P binding ING2 zinc finger and the more promiscuous

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Annexin A2t (Moravcevic et al. 2012; Gokhale et al. 2005). More recently, a PtdIns(4,5)P_2 depletion technique was pioneered by Liou et al. (2007) to study the effects of PtdIns(4,5)P_2 dephosphorylation (i.e., PtdIns(4,5)P_2 depletion) on the intracellular localization of plasma membrane binding proteins in living cells. Shears (2007) took a step further and started investigating into the role of soluble inositol phosphates and pyrophosphates ($InsP_4$ to $InsP_8$), downstream of the PLC-mediated $InsP_3$ synthesis, in regulating intracellular energy homeostasis and ion channel function (Vajanaphanich et al. 1994). Briefly, phosphoinositides that interact with various classes of signaling proteins can be divided into eight different sub-types as discussed below.

PtdIns(4,5)P_2

Membrane PtdIns(4,5)P_2 has been implicated in a host of physiological roles ranging from endocytosis to hearing (Rodriguez et al. 2012; Abe et al. 2008). Rodriguez et al. (2012) demonstrated with knockout experiments that phosphatidylinositol phosphate kinase type 1γ (PIPKIγ) is primarily responsible for the synthesis of the receptor-regulated PLC-sensitive PtdIns(4,5)P_2 pool in the cell syncytia that supports auditory hair cells in adult mice, whereas Abe et al. (2008) used PtdIns(4,5)P_2 depletion to further investigate into the involvement of this phosphoinositide in the endocytotic machinery. PtdIns(4,5)P_2 is constitutively abundant at the plasma membrane surface; however, some recent studies (Yildirim et al. 2013; Blind et al. 2012) have also started investigating into the role of nuclear PtdIns(4,5)P_2. Although the presence of nuclear pools of PtdIns(4,5)P_2 was reported earlier (Divecha et al. 1991), significant progress in that direction was made only recently. For example, Yildirim et al. (2013) recently reported the involvement of nuclear PtdIns(4,5)P_2 in RNA polymerase I transcription. Blind et al. (2012) showed that inositol polyphosphate multikinase, which functions as an inositol kinase and as a phosphoinositide 3-kinase, interacts with the nuclear receptor steroidogenic factor 1 (SF-1) and phosphorylates the bound PtdIns(4,5)P_2.

PtdIns(3,4)P_2 and PtdIns(3,5)P_2

Phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2) is produced in vivo by the PI3 K mediated phosphorylation of phosphatidylinositol-4-phosphate PI4P, by the hydrolysis of PI(3,4,5)P_3 or by the phosphorylation of PI3P (Auger et al. 1989). In a recent report, Sasaki et al. (2010) highlighted the importance of PtdIns(3,4)P_2 metabolism and showed that inositol polyphosphate phosphatase 4A (INPP4A), a PtdIns(3,4)P_2 phosphatase, acts as a suppressor of glutamate excitotoxicity in the central nervous system. The PtdIns(3,4)P_2-binding protein TAPP1 has been shown to interact with the scaffold proteins syntrophins, thereby regulating actin cytoskeletal organization (Hogan et al. 2004). Interestingly, the phosphoinositide PtdIns(3,5)P_2 has by far been the least understood of all the three "PIP_2" phospholipids. However, recent studies clearly indicate its participation in a host of processes including endolysosomal regulation, trafficking, acidification and autophagy (Ho et al. 2012). Abnormalities in PtdIns(3,5)P_2 regulation have been linked with Charcot–Marie–Tooth disease and amyotrophic lateral sclerosis (Ho et al. 2012; Chow et al. 2007).

PtdIns(3,4,5)P_3, monophosphorylated phosphoinositides and PtdIns

PtdIns(3,4,5)P_3, a lipid product of PI3 K activity, gets dephosphorylated by the phosphatase PTEN on the 3′ position of its inositol head group, thereby generating PI(4,5)P_2 and by SHIPs (SH2-containing inositol phosphatase) on the 5′ position, resulting into the formation of PI(3,4)P_2 (Auger et al. 1989; Damen et al. 1996; Stocker et al. 2002). This important phosphoinositide has been implicated in various different physiological roles such as AMPA receptor clustering (Arendt et al. 2010) and actin polymerization (Chen et al. 2012). It also functions as a membrane anchor by recruiting a large number of signaling proteins with polybasic clusters to the plasma membrane (Heo et al. 2006). Although studied to a lesser extent than PIP_3 and the PIP_2s, monophosphorylated phosphoinositides also play an important role in membrane recruitment. The FYVE domain has been previously shown to have a high affinity for membrane PI3P (Stenmark et al. 2002). The protein SARA (which harbors a FYVE domain), through its direct interaction with PI3P, rhodopsin and the SNARE protein syntaxin 3, mediates the fusion of rhodospsin-laden vesicles with nascent discs in the vertebrate rod photoreceptor (Chuang et al. 2007). Similar to what Heo et al. (2006) demonstrated a few years ago, Hammond et al. (2012) recently showed that plasma membrane PI4P contributed not only to the synthesis of PtdIns(4,5)P_2, but also to the recruitment of signaling complexes at the plasma membrane. Unlike PI3P and PI4P, there is no evidence that PI5P can be synthesized inside the cells by the phosphorylation of PI and a recent report actually suggests that PI5P is produced by the MTMR3 and inositol 4-phosphatase-mediated dephosphorylation of PI(3,5)P_2 and PI(4,5)P_2, respectively (Devereaux and Di Paolo 2013). PI5P has been shown to regulate cell migration in a recent report by Oppelt et al. (2013).

Conclusions

Membrane surfaces are predominantly comprised of phospholipids and between 1–3 % phosphoinositides. For example, early endosomes primarily involved in cargo transport are abundant in PtdIns3P, whereas the acidic late endosomes are enriched in PtdIns(3,5)P_2 (Moravcevic et al. 2012). The differential binding affinities of peripheral proteins towards these membrane phosphoinositides causes selective recruitment of various classes of proteins to the membrane surfaces and these proteins then interact with each other to form membrane scaffolds or protein complexes. These protein complexes then regulate events downstream of membrane binding (Cho 2006). For example, AKT (also referred to as PKB) gets recruited to the plasma membrane owing to its high binding affinity towards plasma membrane PtdIns(3,4,5)P_3 and subsequently gets activated by mTORC2-mediated phosphorylation. Once activated, the kinase domain of AKT regulates a large number of downstream processes that are currently being studied by several research groups around the world and the PI3 K/AKT pathway serves as an effective target for designing drugs against inflammation and cancer (Sarbassov et al. 2005). Surprisingly, there are a large number of studies that investigate into the role of lipid binding and membrane recruitment without giving due consideration to the differential binding selectivities of proteins for all of the eight naturally occurring phosphoinositides. In view of the recent reports (Heo et al. 2006; Hammond et al. 2012) highlighting the importance of PtdIns(4,5)P_2 and PI4P binding that has been observed in the case of proteins that were previously thought to bind specifically to PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 respectively, a careful re-examination of the existing literature to rule out any promiscuous lipid binding behavior seems essential.

As the area continues to proliferate rapidly, a systematic study of protein–membrane interactions and the related lipid binding selectivities/specificities seems to be yielding valuable information that is currently being used to delineate the mechanisms of various physiological processes and a host of diseases (Sudhahar et al. 2008).

Conflict of interest The author declares that he has no conflict of interest.

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