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Focus on Molecules: Phosphatidylinositol-4,5-bisphosphate (PIP₂)

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1. Structure

Phosphatidylinositol-4,5-bisphosphate or PtdIns (4,5) P₂, also known simply as PI-4,5-P₂, is a minor phospholipid component of cell membranes. PI-4,5-P₂ is enriched in the inner leaflet of the plasma membrane, where it is an important substrate for a number of important signaling proteins. PI-4,5-P₂ is a critical second messenger that regulates a myriad of diverse cellular activities, including modulation of the actin cytoskeleton, endocytosis, exocytosis, ion channel activity, gene expression, angiogenesis, cell migration, vesicle trafficking, focal adhesion formation, and nuclear events. Two subfamilies of phosphatidylinositol (PI) phosphate kinases (PIPK), types I and II, allow the synthesis of PI-4,5-P₂ from independent pools of substrate, PI-4-P and PI-5-P respectively. In retina, type II PIPK is the major isoform responsible for the generation of PI-4,5-P₂.

2. Function

Starting in the late 1970's, PI-4,5-P₂ received a lot of attention as the substrate for cleavage by the enzyme phospholipase C (PLC), which produces the two classical second messengers, soluble inositol-1,4,5-trisphosphate (IP₃) and membrane-delimited 1,2-diacylglycerol (DAG) (Figure 1). The role of IP₃ was established by Streb et al. (Streb et al., 1983) in their classic paper that showed elevations in IP₃ caused intracellular release of bound calcium. Subsequently, DAG was found to stimulate protein kinase C (PKC), a family of serine/threonine kinases that phosphorylate a number of cellular proteins. Activation of the PLC/PKC cascade affects a variety of cellular events, including secretion, phagocytosis, smooth muscle contraction, proliferation, neurotransmission, and metabolism. In 1989, Auger et al (Auger et al., 1989) discovered the receptor-mediated conversion of PI-4,5-P₂ to phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P₃) in platelet-derived growth factor (PDGF)-stimulated smooth muscle cells. Only in the 1990's was a signaling role recognized for PI-4,5-P₂ itself. Thus, PI-4,5-P₂ is at the center of three important metabolic processes that control a myriad of cellular functions.

Our laboratory has shown that light stimulates the activities of phosphoinositide 3-kinase (PI3K), PIPKII α , and PLC γ enzymes in retinal rod outer segment membranes (Rajala, 2010). The PIP₃ generated by phosphorylation of PI-4,5-P₂ serves as a second messenger to recruit specific phospholipid-binding proteins to the plasma membrane and controls the

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activity and subcellular localization of a diverse array of signal transduction molecules (Rajala, 2010). Phospholipid-binding proteins bind to PIP₃ through their plextrin homology (PH) domains. One of these proteins, the serine/threonine protein kinase B (PKB)/Akt, is a key mediator of signal transduction processes downstream of PI3K (Rajala, 2010). The activated Akt phosphorylates multiple proteins on serine and threonine residues of substrates that include glycogen synthase kinase 3 (GSK3), ribosomal protein S6 kinase (p70S6K), BAD (Bcl-2_{XL}-antagonist causing cell death), IKK (I κ B kinase), eNOS (endothelial nitric oxide synthase), mTOR, 4E-BP (eukaryotic translation initiation factor 4E binding protein), forkhead transcriptional factor, and caspase 9. Through phosphorylation of these targets, Akt carries out its role as a key regulator of a variety of critical cell functions including glucose metabolism, cell proliferation, and survival (Rajala, 2010).

In retinal rod outer segment membranes, PI-4,5-P₂ has been shown to activate cGMP phosphodiesterase (PDE), which ultimately leads to reduction of current flow through cyclic nucleotide-gated channels. PI-4,5-P₂ was also shown to modulate the mammalian rod cyclic nucleotide gated channels, as well as other ion channels (KCNQ and TRP channels) and transporters (Na⁺-Ca²⁺ exchanger) in the plasma membranes of other tissues. In the invertebrate retina, light-stimulated hydrolysis of PI-4,5-P₂ is the initial event that leads to IP₃-induced release of bound intracellular stores of calcium and subsequent depolarization of the plasma membrane. This does not occur in vertebrate rods and cones, where hydrolysis of cGMP is the driving force in visual transduction.

In photoreceptors PI-4,5-P₂, moesin, actin, and rac1 act in concert with rab8 to regulate tethering and fusion of rhodopsin-bearing transport carriers. Consequentially, they are necessary for rhodopsin-laden membrane delivery to the rod outer segments, thus controlling the critical steps in the biogenesis of the light-detecting organelle. Recently, it has been shown that arrestin translocation can be stimulated by the activators of PLC and PKC. *Drosophila* arrestin has a PI-binding domain that binds PI-3,4,5-P₃ and controls the movement of arrestin.

3. Disease involvement

TULP1 is a candidate gene for retinitis pigmentosa-14 (RP). Mutation in *TULP1* is a rare cause of recessive RP and TULP1 plays an essential role in the physiology of photoreceptors. The Tubby domain was first identified in the tubby protein implicated in mature-onset obesity. Spanning approximately 260 amino acids, the Tubby domain has a remarkable dual binding function as it is capable of interacting with both DNA and PI. The Tubby domain of the tubby and TULP proteins binds with high specificity to bisphosphorylated phosphoinositides that are phosphorylated at the 4-position on the inositol ring, such as PI-4,5-P₂ (Santagata et al., 2001). This allows the Tubby domain to function downstream of receptors such as the 5HT_{2C} serotonin receptor. 5HT_{2C} activation leads to stimulation of trimeric G-proteins that activate PLC. PLC hydrolysis of PI-4,5-P₂ releases the Tubby domain from the membrane, from whence it translocates into the nucleus. Once in the nucleus, the Tubby domain binds DNA, allowing the tubby protein amino-terminal transcription factor-like activation domain to promote transcription. Mutations in the tubby domain failed to bind PIP₂ and resulted in the disease phenotype.

Deletion of two PH-binding proteins, Akt2 and IRS-2, in the retina results in photoreceptor degeneration. We recently found that the cone-specific deletion of PI3K resulted in an age-related cone degeneration (Rajala, 2010). Recent work from Dr. Connie Cepko's laboratory showed that systemic administration of insulin delayed the death of cone photoreceptors. This protection could be due to the activation of PI3K-generated PIP₃ and the subsequent activation of Akt and their downstream effectors in the retina. In *Drosophila*, inactivation of

phototransduction is achieved by myosin III-driven, PIP₃-mediated translocation of visual arrestin, Arr2, from photoreceptor inner segments to outer segments. Light-induced translocation of Arr2 to the outer segment and phototransduction termination and recovery were impaired in invertebrate retinas that were deficient in *cds* and *rdgB* (phosphoinositides biosynthesis and trafficking, resp.) or that overexpressed PTEN, which dephosphorylates PIP₃.

4. Future studies

Much remains to be learned about the homeostatic mechanisms that regulate the cellular levels of PI-4,5-P₂, which fluctuate rapidly in response to a variety of extra- and intra-cellular signals. Clearly a future challenge is to unravel the broader scope of the biological functions of PI-4,5-P₂ in the retina, a task that is not made easier by the presence of a number of proteins with PH-domains and the functional redundancies of phosphoinositides.

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Figure 1.

Generation and hydrolysis of PI-4,5-P₂. PI-4-P, phosphatidylinositol-4-phosphate; PI-5-P, phosphatidylinositol-5-phosphate; PIPK, phosphatidylinositol phosphate kinase; PI-4,5-P₂, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C; PI-3,4,5-P₃, phosphatidylinositol-3,4,5-trisphosphate; DAG, 1,2-diacylglycerol; IP₃, inositol-1,4,5-trisphosphate.