Thematic Review Series: Seeing 2020: Lipids and Lipid-Soluble Molecules in the Eye

Signaling roles of phosphoinositides in the retina

Raju V. S. Rajala[1]*

[1]Departments of Ophthalmology, Physiology, and Cell Biology, and Dean McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Abstract   The field of phosphoinositide signaling has expanded significantly in recent years. Phosphoinositides (also known as phosphatidylinositol phosphates or PIPs) are universal signaling molecules that directly interact with membrane proteins or with cytosolic proteins containing domains that directly bind phosphoinositides and are recruited to cell membranes. Through the activities of phosphoinositide kinases and phosphoinositide phosphatases, seven distinct phosphoinositide lipid molecules are formed from the parent molecule, phosphatidylinositol. PIP signals regulate a wide range of cellular functions, including cytoskeletal assembly, membrane binding and fusion, ciliogenesis, vesicular transport, and signal transduction. Given the many excellent reviews on phosphoinositide kinases, phosphoinositide phosphatases, and PIPs in general, in this review, we discuss recent studies and advances in PIP lipid signaling in the retina. We specifically focus on PIP lipids from vertebrate (e.g., bovine, rat, mouse, toad, and zebrafish) and invertebrate (e.g., *Drosophila*, horseshoe crab, and squid) retinas. We also discuss the importance of PIPs revealed from animal models and human diseases, and methods to study PIP levels both in vitro and in vivo. ▣ We propose that future studies should investigate the function and mechanism of activation of PIP-modifying enzymes/phosphatases and further unravel PIP regulation and function in the different cell types of the retina.

Phosphatidylinositol (PI) is a minor constituent (~0.5–1%) of the total phospholipid pool in the cell membrane ([1]). Phosphatidylinositol consists of a head group of D-*myo*-inositol and a backbone of the trihydroxy alcohol, glycerol, in which the C1 and C2 positions of glycerol are occupied with two fatty acids (Fig. 1). The free-OH groups (3, 4, and 5) in the inositol head group undergo phosphorylation by specific phosphoinositide kinases. These phosphorylated phosphatidylinositol phosphates are collectively called phosphoinositides (PIPs).

The intracellular pools of PIPs are dynamically converted from one form to the other through the action of specific phosphoinositide kinases, and phosphoinositide phosphatases can generate seven distinct PIPs ([1–3]) (Fig. 2). These molecules include PI(3)P, PI(4)P, PI(5)P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2, and PI(3,4,5)P_3. Of these PIPs, the D3-phosphoinositides include PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3; the D4-phosphoinositides include PI(4)P and PI(4,5)P_2; and the D5-phosphoinositides include PI(5)P and PI(3,5)P_2. The D3-PIPs are formed by the action of the phosphoinositide 3-kinases (PI3Ks), which use PI, PI(4)P, and PI(4,5)P_2 to generate PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3. The D4-PIPs are formed by the action of the PI4 kinases (PI4Ks), which use PI and PI(5)P to generate PI(4)P and PI(4,5)P_2. The D5-PIPs are formed by the action of the enzyme PI5K, which uses PI and PI(3)P to generate PI(5)P and PI(3,5)P_2 ([1, 3]). These seven distinct PIPs are present in all mammalian cells ([4]), and their formation is also present in the retina/photoreceptor cells ([5–8]). The D3-, D4-, and D5-phosphoinositides regulate cytoskeletal organization, membrane fusion and budding, ciliogenesis, vesicular transport, and signal transduction ([9, 10]). PI(3)P is involved in the vesicle export from the Golgi. PI(4,5)P_2 is involved in exocytosis, cytoskeletal regulation, and regulation of phospholipase D/A_2. PI(3,5)P_2 regulates Golgi, lysosomal/endosome trafficking, and osmoprotection. PI(3,4,5)P_3 is involved in cell survival, solute transport, and regulation of ARF/Rac protein ([1, 9]).

There are many excellent reviews on phosphoinositide kinases, phosphoinositide phosphatases, and PIPs in general. This review describes the recent studies and advances in PIP lipids and their signaling in the retina. The PIP lipids from vertebrate (e.g., bovine, rat, mouse, toad, zebrafish) and invertebrate [e.g., *Drosophila*, limulus (horseshoe crab), squid] retina are discussed. The importance of PIPs revealed from animal models and human diseases and methods to study PIP levels in vitro and in vivo are also described.

PHOSPHOINOSITIDES IN THE RETINA

In 1981, Anderson and Hollyfield ([11]) reported that light stimulates the incorporation of inositol in the

*For correspondence: Raju V. S. Rajala, raju-rajala@ouhsc.edu.

Fig. 1. Structure of phosphatidylinositol (PI). D-*myo*-inositol presented as a Haworth projection (A). Phosphatidylinositol contains D-*myo*-inositol attached to a glycerol backbone and two fatty acids attached to the Cl and C2 positions of the glycerol (B).

vertebrate retina. Light has also been shown to stimulate the generation of PIs in horizontal cells of the retina (12, 13). Subsequent studies showed that light adaptation of bovine retinas in situ stimulates PI synthesis in retinal rod outer segment (ROS) membranes in vitro (5). The work described in 1983 and 1984 by Anderson and colleagues revealed a functional significance in photoreceptor horizontal cell synapses (12, 13). Light stimulates the PI metabolism in these cells, and these PIPs are important for

Fig. 2. Generation of seven distinct phosphoinositides. The inositol head group contains several free hydroxyls that undergo phosphorylation by phosphoinositide kinases and dephosphorylation by phosphoinositide phosphatases. Phosphorylation of free hydroxyls at the 3, 4, 5 positions facilitates the generation of seven PPIs. PI(4,5)P$_2$ undergoes hydrolysis by phospholipase C (PLC) generates two-second messenger signaling molecules, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$). IP$_3$ binds to IP$_3$-sensitive Ca^{2+} channels on the endoplasmic reticulum and mobilizes the release of Ca^{2+}. Both, DAG and Ca^{2+} activate protein kinase C (PKC). Activated PKC phosphorylates its downstream target proteins. MTMl, myotubularin 1 (3'phosphatase); MTMRs, myotubularin-related phospholipid phosphatase; PI(3)P, phosphatidylinositol 3-phosphate; PI(4)P, phosphatidylinositol 4-phosphate; PI(5)P, phosphatidylinositol 5-phosphate; PI(3,4)P$_2$, phosphatidylinositol 3,4-bisphosphate; PI(3,5)P$_2$, phosphatidylinositol 3,5-bisphosphate; PI(4,5)P$_2$, phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P$_3$, phosphatidylinositol 3,4,5-trisphosphate. PIP4K, phosphatidylinositol 5-phosphate 4-kinase; PI3K, phosphoinositide 3-kinase; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; PTEN, phosphatase and tensin homolog; SHIP, Src homology 2 (SH2) domain-containing inositol polyphosphate 5-phosphatase; PIKfyve, FYVE-type zinc finger containing phosphoinositide kinase; OCRL, Oculocerebrorenal inositol polyphosphate-5-phosphatase; PI4K, phosphatidylinositol 4-kinase.

synaptic ribbon formation, glutamate release, and signaling (12, 13).

Phosphatidylinositol is a comparatively large component of the membranes of most cells in metazoans ranging from 4 to 20 mol% of total phospholipid (1, 14), and analysis of phosphatidylinositol in six different mammalian species shows 4.4–6.4% mol% of total retina phospholipid (14–17). Studies have shown that retinal ROS membranes contain lower levels of PI than do retinal pigment epithelium (RPE) cells (18) and ER membranes isolated from bovine retinas (19). Recently, Wensel's laboratory reported that ROSs and fragments of inner segments contain PI(3)P at 0.0035 mol% and PI(4)P and PI(4,5)P_2 on the order of 0.04 mol%, 10-fold higher than the levels of PI(3)P of total phospholipid (14).

Phototransduction is modulated by phosphoinositides within photoreceptors (5, 20), which increase phospholipase C (PLC) activity in the outer segment membranes, increase the uptake of radiolabeled inositol and phosphoinositide turnover in photoreceptor cells (20, 21), and release inositol 1,4,5-trisphosphate (IP$_3$) from the retina (22). Furthermore, IP$_3$ receptors (23) and PLC (24, 25) are expressed in the outer segments. In invertebrates, phototransduction is mediated through PLC activation, while in the vertebrate retina, the phototransduction cascade is mediated through rhodopsin activation and subsequent hydrolysis of cGMP (26, 27). Interestingly, in the intrinsically photosensitive retinal ganglion cells, PLC-mediated hydrolysis is activated by the photopigment, melanopsin, which couples Gq to open the transient receptor potential (TRP) channels (28), suggesting evolutionarily conserved pathways that use PI(4,5)P_2 as a substrate for the modulation of phototransduction.

One of the potential functions of PLC is involvement in the translocation of arrestin from inner to outer segments of photoreceptor cells (29). Furthermore, activators of the downstream effector of PLC, protein kinase C (PKC), and PLC facilitate arrestin translocation to outer segment membranes, independent of light (29). Physiological studies also show that phototransduction is modulated by PIPs (14, 30). The cone cyclic nucleotide-gated (CNG) (31) and olfactory (32) channels are known to be inhibited by PI3K-generated PI(3,4,5)P_3.

It has been suggested that phosphoinositides play an important role in photoreceptor cell processes, such as disk morphogenesis, endocytosis, exocytosis, membrane budding, endosomal sorting, and post-Golgi vesicle trafficking. In rhodopsin trafficking, the involvement of PI(3)P and PI(4,5)P_2 has been demonstrated (33, 34). The actin-nucleating proteins Arp2 and Arp3 are demonstrated to be involved in basal disc extensions (34), and local pools of PI(4,5)P_2 may be important for their function (35, 36).

METABOLISM OF PHOSPHOINOSITIDES IN THE RETINA

There is an active PI metabolism in the vertebrate retina and ROSs (6–8, 12, 13, 20–22, 25, 37–51). Light has been shown to stimulate several enzymes that are involved in PI metabolism, such as class I PI3K (7, 52), class III PI3K (53, 54), PI synthetase (5), DAG kinase (6), phosphatidylethanolamine N-methyltransferase (55), phospholipase A2 (56), phospholipase D (57), PLC (58–61), PKC (62), lipid phosphate phosphatase (63), and DAG lipase (37). Light also modulates the second messengers generated in the retina from DAG, PC, and PA (37). Further, light activates proteins regulated by insulin signaling (64–67).

PHOSPHOINOSITIDE KINASES AND PHOSPHATASES

Forty-seven genes encode 19 phosphoinositide kinases and phosphatidylinositol phosphate (PIP) kinases (PIPKs) and 28 PIP phosphatases in mammals (2). The action of the lipid kinases, PI3K, PI4K, and PI5K, and PI3-, PI4- and PI5-specific lipid phosphatases can generate seven distinct phosphoinositide lipids in mammalian cells (1–3). The phosphoinositide kinase isoforms are divided into three major families: the PI 3-kinases (PI3Ks), PI 4-kinases (PI4Ks), and PIPKs (68).

PHOSPHOINOSITIDE 3-KINASES

The phosphoinositide 3-kinases have been broadly divided into four classes: class I, class II, class III, and class IV. Members of class I PI3K enzymes are heterodimers and consist of a p110 kDa catalytic subunit and a p85 regulatory subunit that contains two Src-homology regions capable of binding to phosphotyrosine sequences on the growth factor receptors (10). The growth factors, such as platelet-derived growth factor (PDGF), insulin, insulin-like growth factor (IGF)-1, and nerve growth factors, bind to its cognate receptor tyrosine kinase(s) and activate class I PI3Ks (69) (**Fig. 3**).

In mammals, class I PI3K-p110 catalytic subunits are encoded by four genes: *Pik3ca*, *Pik3cb*, *Pik3cg*, and *Pik3cd*, which are referred to as PI3Kα, -β, -γ, and -δ (70). The expression profile of these genes in tissues is not uniform. Ubiquitous expression of *Pik3ca* and *Pik3cb* genes has been demonstrated, whereas leukocytes specifically express *Pik3cg* and *Pik3cd* genes (70). *Pik3cg* is also expressed in cardiac tissues (71). All of the PI3Kα, -β, -γ, and -δ-p110 subunits have the phosphatidylinositol kinase domain, a catalytic domain, a C2 lipid-binding domain, and a GTPase Ras domain; these proteins all have a significant homology at the N-terminal end of the molecule (72). In mammalian cells, the catalytic subunits of p110α, p110β, and p110δ are associated with any of five different regulatory subunits, p85α, p85β, p55γ, p55α, and p50α, referred to as "p85 subunits," for
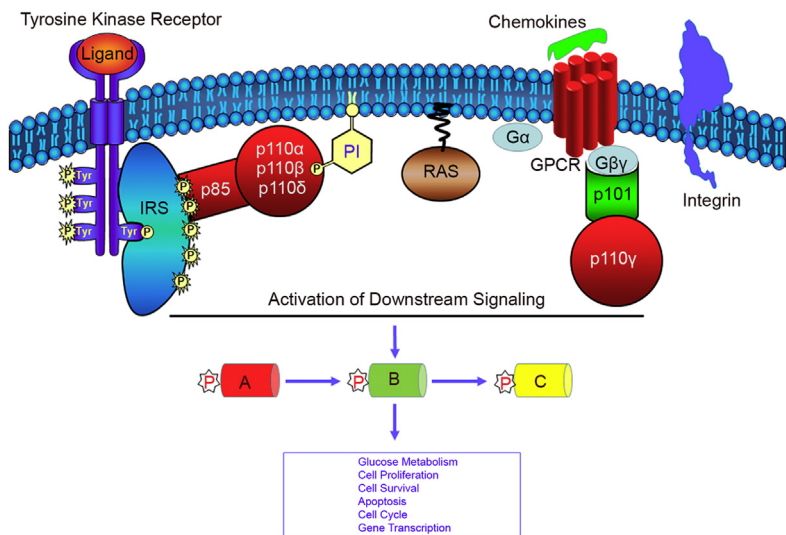
Fig. 3. Activation of phosphoinositide 3-kinases. PI3Ks are activated through tyrosine kinase receptor activation, interaction with RAS, through GPCR activation, or are integrin-mediated. The PIPs generated at the membrane attract phospholipid-binding proteins in the cytosol, activate the downstream signaling (A–C), and regulate various aspects of cellular functions. In the case of the IR, IRS adaptor proteins bind to the IR by facilitating a platform for the binding of multiple PI3K molecules and amplify the signal.

phosphorylation of the lipid substrates (73). The p85 regulatory subunits are encoded by three genes, *Pik3r1*, *Pik3r2*, and *Pik3r3* (73). *Pik3r1* encodes the p85α, p55α, and p50α subunits. Both regulatory subunits, p85α and p85β, are universally expressed in all tissues (72), including retina (74), whereas p55γ is specifically expressed in brain tissues (75). The expression of p50α and p55α has been demonstrated in fat, muscle, liver, and brain tissues (76, 77). The regulatory p85 subunits interact with a unique domain present at the N-terminal end of the p110 catalytic subunits (78). In addition to the Src homology 2 (SH2) domain, the p85 regulatory subunit also contains an SH3 domain, which is capable of binding to proline-rich sequences and also contains a region with high conservation to the breakpoint cluster region (78). Extensive studies on class I PI3K have been carried out in the retina, especially in photoreceptor cells. Functionally, class I PI3K is essential for cone photoreceptor structure and function but not for rod photoreceptor cell survival (74, 79, 80).

Research also indicates that class IA PI3Kβ activation is regulated both by the G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (81). The class I PI3Kγ lacks the p85-binding motif; hence, it interacts with p101 (82) and p84/87 adaptor proteins for regulation (83, 84). The class IB PI3Kγ is activated through GPCR signaling (Fig. 3). Upon GPCR activation by the ligand(s), heterotrimeric G protein composed of α-, β-, and γ-subunits binds to the GPCR (85). The α-subunit of G protein in its bound GDP readily exchanges with the cytosolic GTP, and upon GPCR activation dissociates from the rest of the βγ-subunits and activates the downstream effector molecules (86). The free $G_{\beta\gamma}$-subunits of heterotrimeric G proteins, mostly the G_i subtype, activate PI3K signaling (71, 87) (Fig. 3). The PI3K family of lipid kinases has also been shown to be stimulated by chemokines in regulating the chemotaxis of inflammatory T lymphocytes, eosinophils, neutrophils, and

macrophages, and PI3K signaling regulates the chemokine-activated cell migration (88).

The structure and function of class II PI3Ks are distinct from those of class I PI3Ks. The class II PI3Ks have a C-terminal C2 domain, which lacks a critical aspartate residue that coordinates the binding of Ca^{2+}. The class II PI3K binds to lipid in a Ca^{2+}-independent manner (89, 90). The class II PI3K consists of three catalytic isoforms, which include C2α, C2β, and C2γ, but is distinct from class I and III, as they do not have regulator subunits (89, 90). The class II enzyme catalyzes the conversion of PI to PI(3)P and PI(4)P to PI(3,4)P$_2$ (89, 90). PI(3,4)P$_2$ has been shown to play a role in the invagination step of clathrin-mediated endocytosis (91). The class II PI3K C2α and C2β isoforms are ubiquitously expressed in all tissues, but the expression of the C2γ isoform is restricted to hepatocytes (91). To date, there are no available studies of class II PI3K in the retina.

The structure and function of class III PI3K are distinct from those of class I and class II PI3K. Class III PI3K, also known as vacuolar protein sorting (Vps)34, was first identified in budding yeast, *Saccharomyces cerevisiae*, and screens for proteins involved in the regulation of vesicle-mediated Vps (92). Several PI(3)P binding proteins have been identified, and all are involved in protein trafficking (93). The crystal structure of Vps34 has been solved (94). Class III enzymes, which phosphorylate only PI, are heterodimers of a catalytic subunit associated with the serine/threonine-protein kinase adaptor subunit that is required for membrane recruitment (9, 10). The class III PI3K, Vps34p, is responsible for producing the majority of the cellular PI(3)P and is involved in protein trafficking through the lysosome (93). Class III PI3K Vps34 is closer to class I PI3K in terms of heterodimers of catalytic (Vps34) and regulatory (Vps15, a 150 kDa protein) subunits (95). Class III enzyme-generated PI(3)P is mainly involved in the trafficking of vesicles and proteins (96). Some studies

show that PI(3)P regulates immune cell function and phagocytosis (97, 98). Class III PI3K plays an important role in rod, bipolar, and RPE cell functions in the retina (14, 53, 99, 100).

Class IV is a collection of enzymes, which include ataxia-telangiectasia mutated, ataxia telangiectasia and Rad3-related, DNA-dependent protein kinase, and mammalian target of rapamycin (mTOR). These enzymes are occasionally referred to as the class IV PI3Ks. Unlike class I, II, and III PI3Ks, which are lipid kinases, class IV PI3Ks are protein serine/threonine kinases (101). However, in vitro, class I PI3K has been shown to have protein kinase activity (102).

INSULIN RECEPTOR-REGULATED CLASS I PI3K IN PHOTORECEPTOR CELLS

In 1997, class I phosphoinositide 3-kinase (PI3K) was reported to be responsible for the generation of D3-PIPs in retinal ROSs (7). Furthermore, tyrosine phosphorylation in vitro was shown to stimulate the PI3K activity in isolated bovine retinal outer segment membranes (8). The class I PI3K is composed of two subunits, a p110 catalytic subunit and a p85 regulatory subunit (10). With the application of p85 regulatory subunits that contain both N-terminal and C-terminal SH2 domains, Rajala and Anderson (103) identified that the insulin receptor (IR) in the retina/ROS is the receptor that regulates PI3K activity. The authors also observed that in vitro tyrosine phosphorylation enhanced the phosphorylation of the IR, which results in the recruitment of the p85-N-SH2 domain, binds to the tyrosine-phosphorylated IR, and activates PI3K (103).

In rod photoreceptors, PI3K activation is mediated through light-dependent tyrosine phosphorylation of the IR (52, 104) (**Fig. 4**). The IR activation has been shown to be independent of G protein transducin activation and dependent on the photobleaching of rhodopsin (52). This IR/PI3K activation is a noncanonical rhodopsin activation (105) (Fig. 4). In the retina, the IR is constitutively phosphorylated (106, 107). However, the phosphorylation state of the IR is regulated through dark and light adaptation (108). In dark-adapted conditions, the IR is in the inactive state because of increased protein tyrosine phosphatase 1B (PTP1B) activity, which dephosphorylates the IR and results in the reduced association of PI3K with the IR (108). Upon rhodopsin activation, adaptor protein growth factor receptor-bound protein 14 (Grb14) localized to rod inner segments in the dark translocates to the outer segments in light and undergoes tyrosine phosphorylation by a nonreceptor tyrosine kinase, Src, in a rhodopsin-dependent manner (67, 109) (Fig. 4). The Src phosphorylation was abolished in animals deficient in the photobleaching of rhodopsin (109). The tyrosine-phosphorylated-Grb14 binds to PTP1B and inactivates its activity (Fig. 4). Thus, the IR becomes active, which then activates PI3K and promotes photoreceptor
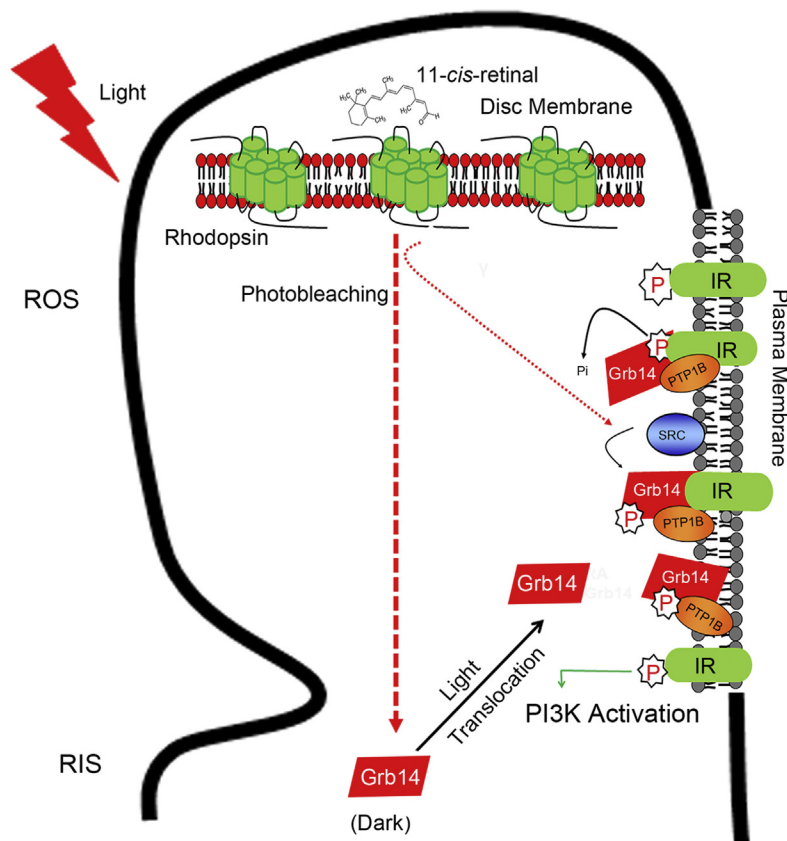

Fig. 4. Noncanonical IR signaling-mediated activation of PI3K in the retina/photoreceptor cells. The IR in the retina is constitutively activated. However, the activated (phosphorylation) state of the IR is light-dependent. Under a dark-adapted state, the IR undergoes dephosphorylation by PTP1B and keeps the IR in an inactive state. Upon illumination, rhodopsin activation facilitates the translocation of Grb14 from inner segments to outer segments, where it undergoes a light- and rhodopsin-dependent phosphorylation by a nonreceptor tyrosine kinase, Src. Phosphorylated Grb14 binds to PTP1B and inhibits its activity, thus preserving the phosphorylation of the IR, which can bind to PI3K and generate PIPs. RIS, rod inner segments; Pi, inorganic phosphate; P, phosphorylation.

survival (108–110) (Fig. 4). Conditional deletion of the IR in rod photoreceptors resulted in stress-induced photoreceptor degeneration (111). The serine/threonine kinase Akt2, the downstream effector of IR, and PI3K deletion result in stress-induced photoreceptor degeneration (112). The proteins that regulate IR singing pathway in rods are also expressed in cones (113, 114). Interestingly, deletion of the IR in cones resulted in cone degeneration without added stress (113).

INTERACTION BETWEEN PHOSPHOINOSITIDES AND PHOSPHOLIPID-BINDING PROTEINS

Several pleckstrin homology (PH) domain-containing proteins are expressed in the retina. PIPs regulate these proteins directly or indirectly. These proteins include PH domain retinal protein 1 (PHR1) (115, 116), Evectin-1 (117), Grb14 (118), IR substrate (IRS)-1 and IRS-2 (106, 119, 120), and three isoforms of Akt (Akt1, Akt2, and Akt3) (66, 106, 112). Evectin-1 is involved in the trafficking of post-Golgi membranes in photoreceptor cells (117). The PH domain retinal protein 1 PH domain is identical to serine/threonine kinase Akt and does not bind to inositol phosphates but interacts with transducin Gβγ subunits (115, 116). In the retina, the Akt-PH domain binds to PIPs (66). The PH domain of Akt binds to the cytoskeletal protein myosin II (121). This protein is mutated in patients with Usher syndrome (122). However, the interaction between myosin and Akt has not been tested in the retina. PH domain and leucine-rich repeat protein phosphatase (PHLPP) and PH domain and leucine-rich repeat protein phosphatase-like (PHLPPL) are PH domain-containing proteins known to dephosphorylate Akt isoforms (123, 124) and are also expressed in the retina (125–127).

DOWNSTREAM EFFECTS OF PI3K SIGNALING IN THE RETINA

In vivo, light stimulates the tyrosine phosphorylation of the IR, which results in the recruitment of PI3K and subsequent activation of Akt, the downstream effector of PI3K regulated through PI3K-generated PIPs (104). The addition of insulin as the ligand for the IR also stimulates the tyrosine phosphorylation of the IR, which results in the activation of PI3K ex vivo (66, 103). In the retina, light stress induces the phosphorylation of the IR, which results in the activation of the PI3K/Akt pathway through increased tyrosine phosphorylation of the IR (111). Addition of insulin growth factor-1, the ligand for IGF-1R, ex vivo to retinas stimulates the tyrosine phosphorylation of the IGF-1R, which results in the activation of the PI3K/Akt pathway (128). Interestingly, light stress, but not physiological light, stimulates the tyrosine phosphorylation of the IGF-1R that results in the activation of the PI3k/Akt downstream signaling pathway (128). In the retina, PI3K activation

stimulates serine/threonine kinase Akt in vitro and in vivo (66, 106, 112).

Akt kinase exists as three isoforms: Ak1, Akt2, and Akt3. All three isoforms are expressed in retina and rod photoreceptor cells (66, 106). Membrane binding of Akt1 is mediated through its PH domain binding to PI3K-generated PIPs (66). In transgenic *Xenopus laevis*, the PH domain fused to GFP under the control of the *Xenopus* opsin promoter binds to PIPs in a light-dependent manner (66). In dark-adapted conditions, the actin exists as a stress fiber phenotype. Upon light-illumination, reorganization of the actin cytoskeleton was found to colocalize with PIPs in a *X. laevis* model (66). The expression of a mutant version of the PH domain in which the arginine 25 residue is substituted with cysteine failed to bind to PIPs (66).

The IR/PI3K/Akt signaling proteins are also expressed in the rod inner segments. These proteins may play an important role in cellular signaling events (105). One of the functions of PI3K-activated Akt in the inner segment is to regulate hexokinase 2 as it interacts with mitochondria in the photoreceptors (125). Growth factor-mediated activation of Akt was demonstrated to increase the association of hexokinase 2 with mitochondria in normal tissues and cells (129). Akt activation inhibits the dissociation of hexokinase 2 from mitochondria and is the primary event in the induction of apoptosis (125, 129–131). The mechanism of interaction between hexokinase 2 and mitochondria is that Akt phosphorylates glycogen synthase kinase 3β (GSK3β), which renders GSK3β inactive (132, 133). In the absence of Akt activation, GSK3β is active and phosphorylates the voltage-dependent anion channel (VDAC) on serine 51 that disrupts the binding of hexokinase 2 to the VDAC (134). The PI3K-generated PIP-activated Akt prevents the release of cytochrome c from mitochondria and inhibits apoptosis (129, 135). Retinal photoreceptors are postmitotic neurons and apoptosis is detrimental; therefore, the PIP-activated Akt promotes photoreceptor survival (112, 125).

INTERACTION BETWEEN THE ROD CNG CHANNEL AND PI3K IN ROD PHOTORECEPTOR CELLS

Class I PI3Kγ lacks a p85-binding motif. Hence, it interacts with p101 (82) and p84/87 adaptor proteins for regulation (84, 136). Class I PI3Kγ is known to be regulated through Ras proteins (137) (Fig. 3). Ras belongs to a family of related proteins called small GTPases (138). These proteins have a Ras-associating (RA) domain, and PI3K interacts with this RA domain (139). In photoreceptor cells, the C-terminal region of the rod CNG channel subunit α1 (CNGA1) has 50–70% tertiary structural homology with Ras proteins (140). This domain has been named the Ras-like domain (140). In rod photoreceptor cells, PI3Kγ activation occurs through the interaction of its RA -domain with the Ras-

like domain of CNGA1 (141). The interaction of PI3Kγ with CNGA1 does not affect the channel physiology. However, PI3Kγ uses CNGA1 as an anchor to achieve a close vicinity to its substrates to generate PIPs (141).

PI3K KO PHENOTYPES IN THE RETINA AND RPE

In the retina, loss of p85α does not affect the overall morphology, but decreased PI3K activity associated with the IR is observed (142). Conditional deletion of the p85α (*pik3r1*) regulatory subunit in rod photoreceptor cells does not affect the structure of the retina (74). Mice with conditional deletion of p85α exhibited a slight delay in recovery kinetics and a delay in the translocation of rod arrestin from the inner segments to the outer segments (74). The absence of the disease phenotype in mouse rods lacking p85α could be explained by the expression of p85β in p85α KO mice (74). Interestingly, the deletion of the p85α-subunit of PI3K in cones results in age-related cone degeneration (79). The surviving cone-terminals of the cone-p85α KO mice exhibit progressive disorganization of synaptic structures (79). The loss of p85α in cones does not affect rod structure and function (79). Conditional deletion of the catalytic subunit of PI3K, p110α, in cones also resulted in cone degeneration (143). These studies highlight that PI3K signaling is indispensable for cone photoreceptor survival. These findings also suggest that other PIP-signaling pathways may regulate rod survival.

Conditional deletion of the class III PI3K *Vps34* gene in rods results in a failure in the fusion of endosomal and autophagy-related membranes with lysosomes that prompts the buildup of anomalous membrane structures (53). These mice have normal structure and function and trafficking of rhodopsin to the outer segments; however, the mice experience progressive rod degeneration by 12 weeks of age (53). The rod degeneration accompanying *Vps34* gene deletion is much faster than that of rods lacking autophagy genes, highlighting that PI(3)P is required for endosome recycling and other pathways that are necessary for rod photoreceptor survival (53). Vps34 has recently been shown to be essential for on-bipolar cell survival. Loss of this enzyme in these cells results in a significant loss of structure and function (99). This study further highlights that PI(3)P is necessary for the fusion of autophagosomes with lysosomes and maturation of late endosomes, and PI(3)P is needed for the maintenance of on-bipolar cells health (99).

In RPE, PI(3)P is essential for the fusion with autophagosomes, lysosomes, and phagosomes (100). These findings highlight that PI(3)P is essential for RPE cell health. In cone photoreceptors, the ablation of Vps34 resulted in an age-related cone degeneration (R. V. S. Rajala, unpublished observations).

PHOSPHATIDYLINOSITOL PHOSPHATE KINASES

Phosphatidylinositol 4-kinases (PI4Ks) make PI(4)P from phosphatidylinositol (3). PI(4)P is an important molecule for the generation of other phosphoinositides involved in signaling, such as PI(4,5)P_2, and is the substrate for the generation of second messengers, such as IP_3 and DAG, through the action of PLC (144). PI(4,5)P_2 also serves as a substrate for PI3K for the generation of PI(3,4,5)P_3 (10, 145). PIPKs phosphorylate the D5 position of inositol on PI(4)P and the D4 position of PI(5)P to generate PI(4,5)P_2 (146). Numerous PIPKs have been identified, purified, and cloned (147–149), and are separated into type 1 and type II based on their biochemical and immunological characteristics (10, 150). Based on substrate specificity, PIPK1 (type 1) phosphorylates PI(4)P and PIPKII (type II) phosphorylates PI(5)P (151). The mechanism of activation of PIPKs is not completely understood. Type I PIPK is activated by GTPγs (152), small G proteins (153), PA (154), heparin, and spermine (155). Spermine and PA do not affect PIPKII activity, but heparin inhibits PIPKII activity (155). Tyrosine phosphorylation has been shown to influence the activity of PIPKs (156).

In most cells, the PI(4,5)P_2 is generated through type I PIPKs and global KOs of the α, β, and γ isoforms of this kinase have been produced (157). This isoform is highly expressed in the retina (158) and other neurons (159, 160). Early postnatal mortality was reported when this kinase was deleted (161). The mouse genes that encode PIPKs are *Pip4k2a*, *Pip4k2b*, *Pip4k2c*, *Pip5k1a*, and *Pip5k1c*; all of these were detected in the retinal mouse proteome (162).

In bovine ROS membranes, type II PIPK activity is regulated by tyrosine phosphorylation (163), and PI(5)P serves as a substrate for the synthesis of PI(4,5)P_2 (163). In rodents and transgenic frog retina, PIPKIIα activity is stimulated by light, and the membrane binding of PIPKIIα to ROS proteins is tyrosine phosphorylation-dependent (164). PI(4,5)P_2 is a critical PIP and regulates several key biological processes, such as actin cytoskeletal organization, endocytosis, exocytosis, modulation of ion channels, gene expression, angiogenesis, vesicular transport, cell migration, and nuclear functions (165). PI(4,5)P_2 has been shown to activate cGMP-phosphodiesterase in ROS membranes, which results in the inhibition of ion influx through CNG channels (30, 166). In mammalian retina, the rod CNG channels, KCN1, TPR channels, and Na^+-Ca^{2+} exchange are known to be regulated by PI(4,5)P_2 (167). In photoreceptor cells, PI(4,5)P_2 regulates the biogenesis of light-sensing organelles and plays an important role in the delivery of rhodopsin-containing membranes to the ROS (33).

One of the receptor tyrosine kinases, epidermal growth factor receptor, has a cluster of basic residues in the juxtamembrane domain. PI(4,5)P_2 directly binds to

this region and has been shown to activate EGFR (168). These basic residues are also present in other growth factor receptors, such as IGF-1R, IR, FGFR1, PDGFR, VEGFR1, EPHB2, TRKA, and TRKB receptor (168). However, the activation of these receptors by PI(4,5)P$_2$ has not been studied.

PHOSPHATIDYLINOSITOL-3-PHOSPHATE 5-KINASE OR PIKfyve

In humans, FYVE finger-containing phosphoinositide kinase is encoded by the *PIKFYVE* gene (169). PIKfyve phosphorylates PI(3)P to PI(3,5)P$_2$ and PI to PI(5)P (170–172). The FYVE finger domain of PIKfyve tethers to membrane PI(3)P and is essential for membrane localization of PIKfyve to the cytosolic leaflet of endosomes (173). This binding is needed to phosphorylate PI to PI(5)P and PI(3)P to PI(3,5)P$_2$ (170). Dysregulated enzyme activity of PIKfyve results in enlarged lysosomes due to defective synthesis of PI(3,5)P$_2$, which results in defective lysosome fission events; thus, PIKfyve navigates all aspects of the vesicular and endocytic pathways (172, 174). Mutations in one of the alleles of PIKfyve are linked to Francois-Neetens corneal fleck dystrophy (175). The ablation of both alleles in the mouse is lethal (176). Changes in PIKfyve have been shown to inhibit insulin-mediated glucose uptake (177). Mice with selective gene ablation of PIKfyve in skeletal muscle show prediabetic symptoms, which include insulin resistance, hyperinsulinemia, glucose intolerance, and increased adiposity (178). In one study, inhibition of PIKfyve resulted in the prevention of myocardial apoptosis and cardiac hypertrophy mediated through the activation of sirtuin-3 (*SIRT3*), a major mitochondria NAD$^+$-dependent deacetylase in obese mice (179). Because photoreceptor cells are highly metabolic (180, 181), the PIKfyve enzyme might regulate several important functions. However, there are no available studies of this enzyme in the retina.

It was previously reported that Vps34-generated PI(3)P regulates the canonical autophagy (182). However, Vps34-independent noncanonical autophagy has been observed in sensory neurons from the PI(3)P-generated Vps34 enzyme, T-lymphocytes, and glucose-starved cells incubated with PI3K inhibitor, Wortmannin, which inhibits the Vps34 enzyme (183). PI(5)P-dependent noncanonical autophagy has been reported in cells depleted of PI(3)P (170). PIKfyve plays an important role in generating PI(5)P upon binding to PI(3)P through its FYVE domain (170). This PI(5)P can be converted to PI(4,5)P$_2$ through the action of the type II phosphatidylinositol 5-phosphate 4-kinase (PIP4K) enzyme, which was previously shown to express in rod photoreceptor cells (163). The generated PI(4,5)P$_2$ can perform various functions, including serving as a substrate for class I PI3K for the generation of PI(3,4,5)P$_3$ (10, 80, 145). In cone photoreceptor cells, the deletion of class I PI3K that makes PI(3,4,5)P$_3$ resulted in age-related cone degeneration (79, 143).

NEUROPROTECTIVE ROLES OF PI3K IN THE RETINA

Several growth factors, such as PDGF, brain-derived neurotrophic factor, insulin, IGF-1 and -2, basic fibroblast growth factor, erythropoietin (EPO), and ciliary neurotrophic factor (CNTF), promote retinal cell survival through PI3K/Akt activation. Insulin and IGF-1 have been shown to promote photoreceptor survival (106, 184), whereas PDGF, CNTF, and EPO promote the survival of ganglion cells and RPE (185). basic fibroblast growth factor-mediated activation of PI3K has been observed in Müller cells (186). PI3K exerts its neuroprotective effect through its downstream effector, Akt (66, 106, 112). IR activity is important for PI3K/Akt activation (66, 106). For the signal to be maintained for a longer period, the protein tyrosine phosphatase, PTP1B, must be inactivated. It has been shown that activated Akt phosphorylates PTP1B on serine 50 (187), which inhibits the activity of PTP1B to facilitate a positive feedback mechanism for IR/PI3K/Akt signaling. In diabetic retinopathy, PI3K activity is downregulated (188) due to increased PTP1B activity (107). Furthermore, decreased activation of mTOR and p760S6K, and increased activation of GSK3β, the downstream effector of PI3K, are downregulated in diabetic retinopathy (189).

PI3K activation through the IR inhibits caspase-mediated cell death of retinal neurons in culture (190). 17β-Estradiol protects stressed retina through PI3K activation (191, 192). The deletion of the phosphatase and tensin homolog (PTEN) results in an increased level of PI(3,4,5)P$_3$, which protects ganglion cells (193). Activation of PI3K through PDGF stimulation protects retinal pericytes under diabetic conditions (194). Activation of PI3K/Akt protects the RPE from oxidative stress (195–197). brain-derived neurotrophic factor-induced PI3K activation protects axotomized retinal ganglion cells (198). The neuroprotective function of CNTF is mediated through the PI3K pathway in vitro and in vivo (199). The PI3K/Akt pathway activated through cytokine EPO promotes adult CNS neuron regeneration (200). The PI3K pathway is necessary for nerve regrowth in the goldfish retina (201). PI3K also plays an important role in retinal development and survival of differentiated neurons in vivo (202). In addition, PI3K regulates the circadian output in the retina (203).

Constitutive activation of Akt has been observed in cone photoreceptor cells (204). In rods, Akt activation is transient under physiological light (66), hyperosmotic stress (205), oxidative damage (206), or light stress (112). In cone photoreceptor cells, PI metabolism is Ca^{2+}-dependent and essential for glutamate release and synaptic ribbon formation (207). In dark-adapted cones,

a rise in intracellular Ca^{2+} increases PI metabolism at the synaptic terminals compared with the ROS (207). These changes in cones result in the release of glutamate from the synapse, which halts PI metabolism in the adjacent horizontal cells. Under light-adapted conditions, increased PI metabolism has been observed in outer segments that support phototransduction (207). Decreased intracellular Ca^{2+} results in decreased PI metabolism in the synapse, which blocks the release of glutamate that releases the block on PI metabolism in the adjacent horizontal cells (207).

Inositol polyphosphates also play an important role in synaptic ribbon disassembly (207). Previous studies showed that the activation of PI metabolism and photoreceptor synapses under dark-adapted conditions is in parallel with the disappearance of synaptic ribbons (207). Further, inositol polyphosphates (IP$_3$) have been shown to influence synaptic ribbons in cone photoreceptors but not in rods, suggesting that the cones have an active PI metabolism in cone synapses (207). Consistent with these studies, cones lacking class I PI3K exhibit disorganization of synaptic ribbons (207). In the retina/photoreceptors, a cross-talk exists between IR/PI3K and phototransduction. The PI3K pathway is activated through photobleaching of rhodopsin but is not dependent on G protein transducin activation (52). These observations suggest that other G proteins might be involved in the activation of the PI3K pathway. Such a cross-talk between tyrosine kinases and GPCR signaling has been observed outside of the retina. Mitogen-activated protein kinases, extracellular-regulated kinases, stress-activated protein kinase (208, 209), and nonreceptor protein tyrosine kinases (210) are the best examples of this cross-talk.

PHOSPHOINOSITIDE PHOSPHATASES

To date, over 35 mammalian phosphoinositide phosphatases that downregulate many phosphoinositide signals have been identified (211). A retina proteome shows the presence of phosphoinositide phosphatases that are encoded by Mtmr2 and Synjl, Inpp1, Inpp4a, Inpp4b, and Inpp5e genes (162). Synaptojanins regulate synaptic function (212–214), which includes endocytosis and vesicle uncoating, and profound cone defects have been observed in zebrafish when this phosphatase is lacking (215–217). In cone photoreceptor cells, the phosphoinositide phosphatase, synaptojanin 1 (SynJl), has been shown to regulate autophagy and endosomal trafficking, and mutations in zebrafish cone photoreceptors show abnormal accumulation of late endosomes and autophagosomes (215, 217–219). Synaptojanin 1 has also been shown to regulate cognition and plays an important role in Down syndrome (220).

Defects in oculocerebrorenal syndrome of Lowe (OCRL) phosphatase are associated with glaucoma, a second leading cause of blindness worldwide (221, 222).

Mutations in inositol polyphosphate 5-phosphatase (INPP5)E are associated with Joubert syndrome and Bardet-Bedi syndrome (223–226), which result in retinal degeneration. Both PI(4,5)P$_2$ and PI(3,4,5)P$_3$ serve as substrates for INPP5E and the levels of PI(4,5)P$_2$ are suggested to be important for the regulation of cilium (14). The $3'$ phosphatase PTEN regulates retinal neurogenesis and Notch signaling (227). Further inactivation of PTEN in the RPE causes retinal degeneration, highlighting the importance of this phosphatase in RPE functions (228). In an animal model of retinitis pigmentosa, the deletion of PTEN in cone photoreceptors resulted in cone photoreceptor survival (229). The retinal amacrine cell number is shown to be regulated by PTEN through the modulation of Erk, TGFβ, and serine-threonine kinase Akt (230). Retinal interneuron morphogenesis and synaptic layer formation are shown to be regulated by PTEN (231). Interestingly, the deletion of PTEN together with the retinoblastoma gene resulted in penetrant bilateral retinoblastomas (232). PI3K activation triggered by insulin or other growth factors phosphorylates PI(4,5)P$_2$ to PI(3,4,5)P$_3$, which can be rapidly dephosphorylated by PTEN to generate PI(4,5)P$_2$ or by INPP5s to generate PI(3,4)P$_2$. INPP5 also hydrolyzes PI(4,5)P$_2$ to PI(4)P (211).

In mammalian cells, ten 5-phosphatases have been identified. These 5-phosphatases regulate several aspects of cellular function, including synaptic vesicle recycling, cell proliferation, embryonic development, control of hematopoietic development, and insulin-mediated signaling (233). The 5-phosphatases, OCRL and INPP5E, are mutated in Lowe and Joubert syndrome, respectively (234). Src homology 2 domain-containing inositol polyphosphate 5-phosphatase (SHIP)2 and skeletal muscle- and kidney-enriched inositol phosphatase (SKIP) are negative regulators of insulin signaling and glucose metabolism (235, 236). Insulin resistance is associated with polymorphisms in SHIP2 (237). INPP4A and INPP4B, the inositol 4-phosphatases, dephosphorylate PI(3,4)P$_2$ to PI(3)P, and these phosphatases have been shown to control neuro-excitatory cell death (238, 239). The Sac phosphatases dephosphorylate PI(3)P, PI(4)P, PI(5)P, and PI(3,5)P$_2$ to form PI (211). Degenerative neuropathy has been reported with loss of one of the Sac phosphatase genes, FIG4 gene (240). Like phosphoinositide kinases, mutations or changes in the expression of phosphatases cause many human diseases (211).

THE ROLE OF PHOSPHOINOSITIDES IN THE INVERTEBRATE RETINA

Although this review focuses on phosphoinositide metabolism in the vertebrate retina, it is important to note that PI metabolism is also important in the invertebrate retina. Visual excitation in *Limulus* (horseshoe crab) photoreceptors have been shown to be mediated by *myo*-inositol polyphosphate (241). In *Drosophila*

photoreceptor cells, light stimulated the hydrolysis of PI(4,5)P$_2$ by PLC into DAG and IP$_3$ (242–244). IP$_3$ facilitates the opening of two calcium-sensitive channels, TRP and TRP-like (218), which results in plasma membrane depolarization (245). In the vertebrate retina, cGMP modulates the channel but not the IP$_3$ (26). In squid photoreceptor membranes, PI-PLC is activated through metarhodopsin-activated G protein, Gq (246). In invertebrates, retinal degenerations result from a mutation in various enzymes of the PI cycle (243–245). In *Drosophila*, phosphatidylinositol transfer protein (PITP) is essential for photoreceptor cell survival and recovery of light stimulation (250). Further, rhodopsin inactivation is mediated by the visual arrestin, Arr2. Its translocation from the rhabdomeric cell body to the microvilli is governed by myosin III-driven PI(3,4,5)P$_3$-dependent movement (251–254). *Drosophila* Arr2 has a PH domain that binds to PI(3,4,5)P$_3$ (251), but mammalian visual arrestins do not. The light-induced trafficking of Arr2 to the microvilli, the termination of phototransduction, and recovery are impaired in *Drosophila* retinas lacking phosphoinositide biosynthesis and trafficking genes *cds* and *rdgB* or overexpression of 3′ phosphoinositide phosphatase PTEN, which dephosphorylates PI(3,4,5)P$_3$ to PI(4,5)P$_2$ (255). In *Drosophila*, light-activated TRP and TRP-like channels regulate the PI signaling cascade (256). In *Drosophila* photoreceptor membranes, PI(4)P synthesis is catalyzed by PI4KIIIα (257). Further, PIP5K is needed for the generation of PI(4,5)P$_2$ for GPCR signaling in *Drosophila* (258).

ROLES OF PHOSPHOSPHOINOSITIDE LIPIDS IN THE RETINA

Alterations in the metabolic flow of events in the retina can lead to retinal degeneration (see RETNET; https://sph.uth.edu/retnet/, May 2020). It has been reported that blue light-excited all-*trans*-retinal and 11-cis-retinal irrevocably disrupt plasma membrane-bound PI(4,5)P$_2$ and alter its function in nonvisual cells (259). This altered PI(4,5)P$_2$ increases cytosolic calcium and causes a change in cell morphology, leading to cell death (259). These studies suggest photoexcited chromophore-prompted PI(4,5)P$_2$ alteration, which leads to oxidative damage to the plasma membrane (259). This altered PI(4,5)P$_2$ function is independent of nonvisual GPCR activation (259). mTOR1 activation is mediated by lysosomal positing, while amino acids promote the recruitment of FYVE and coiled-coil domain autophagy adaptor 1 (FYCO1), a PI(3)P binding protein, to the lysosomes and facilitate the interaction between FYCO1 lysosomes and the ER that encompasses protrudin, a PI(3)P effector protein (260). These studies suggest that the Vps34-generated PI(3)P regulates mTORCl activation through lysosomal positioning (260). In addition, the cause of congenital cataracts is due to mutations in the FYCO1 gene (261). In the

retina, type 1 metabotropic glutamate receptors are coupled to the hydrolysis of polyphosphoinositide (262). Class I PI3K in the retina has a dual function in regulating cell survival and cell trafficking (263). A mathematical model of the PI signaling pathway has been constructed, and the authors argue that this model reflects the study state of the PI pathway (264). In the zebrafish retina, PITPβ is needed for the integrity of cone cell outer segments (265). PITP protein transfers the PI and PC across membranes (266). Further, mutations in the PYK2-binding domain of phosphatidylinositol transfer membrane-associated protein (PIPNM3) caused autosomal dominant cone dystrophy (CORD5) in two Swedish families (267). The cone CNG (31) and olfactory (32) channels are known to be inhibited by the PI3K-generated PI(3,4,5)P$_3$. There are no available studies on the role of PI(3,4,5)P$_3$ in rod CNG channels. Further, PLC and PI(4,5)P$_2$ levels in phototransduction are regulated by ceramide kinase (268). The components of PI signaling in photoreceptor outer segments were identified in the mid-1990s (25). PIPs and proteins that are involved in phosphorylation of these lipids have been observed in the axoneme of the bovine ROSs (269). In ROSs, transducin βγ-subunits have been shown to regulate the metabolism of PI(4,5)P$_2$ (270). Alterations in retinal structure and PI degradation by light damage and chronic lithium treatment have been reported (271).

The PI synthesis in photoreceptor cell inner segments is enhanced by light and cytidine (20, 272). In rat retina, the PI synthesis and phosphorylation are stimulated by light (41). The PI turnover is also enhanced in light (21). The lipid PI(4,5)P$_2$ has been shown to regulate blood vessel stability; this lipid could be a viable therapeutic target for angiogenesis therapies in diseases such as AMD, cancer, and proliferative diabetic retinopathy (273). RPE-mediated photoreceptor phagocytosis is regulated by phosphoinositide lipid signaling (274). In cone photoreceptor cells, CNGA3-associated mutation potentiates the PI sensitivity of cone-CNG channels by disrupting the interaction between subunits (275). In photosensitive chicken retinal ganglion cells, light has been shown to stimulate the PI cycle (276).

PHOSPHOINOSITIDES IN RETINAL CILIA

PI(4)P and PI(4,5)P$_2$ are important for the regulation of primary cilia, especially in assembly and disassembly (277–281). Phosphoinositide signaling has been coupled with proper protein trafficking and Hedgehog signaling in the primary cilia (280, 282). The 5′ PI phosphatase, INPP5E, is paired with proper ciliogenesis (280, 282).

INPP5E dephosphorylates PI(3,4,5)P$_3$ to PI(4,5)P$_2$ (282). This inositol phosphatase is important in ciliary development in zebrafish (223). Mutations in INPP5E result in Joubert syndrome, a rare disorder characterized by deformation of the midbrain, retinitis

pigmentosa, renal cysts, and polydactyly (283). INPP5E has been shown to regulate the phosphoinositide-dependent cilia transition zone function. IPP5E mutant embryos have dysfunctional Hedgehog signaling (284). In the retina, ciliopathy protein RPGR interacts with phosphodiesterase 6δ and regulates the ciliary localization of INPP5E (285).

In addition to INPP5E, OCRL is also important for the regulation of cilia (277–281, 286). Bardet-Biedl syndrome is caused by the mutations in BBSome, and this protein complex is involved in ciliary trafficking and binds to phosphoinositides (287, 288). In vitro, the PH domains of BBS5 bind to PI(3)P (287). Similar specificities in PIP binding have been observed with BBS1, -4, -5, -8, -9, and -18. A recent review of phosphoinositides mentioned the caveat that commercial PIP strips have local surface densities of PIPs, which are significantly different under physiological conditions (14). Further studies are warranted to establish that these proteins indeed bind to PIPs in vivo.

THE SIGNALING ROLE OF PHOSPHOINOSITIDES IN A CELLULAR PROCESS

The phosphorylated phosphoinositides are formed in response to growth factor stimulation or GPCR activation or are activated by other cellular cues that attract proteins from the intracellular side of the cells through protein-lipid interactions. The proteins that interact with PIPs are called phospholipid-binding or phosphoinositide binding proteins. It is essential to establish the specific lipid involved in given cellular processes. It is also important to identify the effector protein that is regulated by the PIP signal that governs the cellular functions.

Investigators have identified several phosphoinositide-binding proteins that relay the PIP signal. Generally, two groups of interacting sites exist for the stereoselective association of PIP with effector proteins (247). The first group comprises a 10–20 co-linear sequence enriched with basic and hydrophobic residues that mediate interaction with PIP (247). The second group has proteins consisting of around 120 amino acids that share homology with a well-established tertiary structure of the PH domain (247). These PH domains are extensively characterized and are well-known PIP binding modules. The PH domain sequence was first observed in the platelet PKC substrate pleckstrin (248), which contains a collinear sequence of 120 amino acids and is present in more than 100 proteins.

PI(3)P binds to FYVE and PX domain-containing proteins (247). $PI(4,5)P_2$, $PI(3.4)P_2$, and $PI(3,4,5)P_3$ bind to proteins containing PH domains (249, 289–291). PI(5)P binds to proteins containing the plant homeodomain domain (292). Several studies showed that phosphoinositides were detected qualitatively or quantitatively using the PH, PX, and FYVE domains (291, 293). The FYVE domains of EEA1 and Hrs have been used to detect PI(3)P (173, 291, 294). The PH domains of FAPP1, OSBP, and OSH2 and the P4M domain of SidM/GOLPH3 have been used to detect PI(4)P (291). The plant homeodomain domain of ING2 (292), Tam1 DH-PHc domain (295), and the VHS domain of TOM1
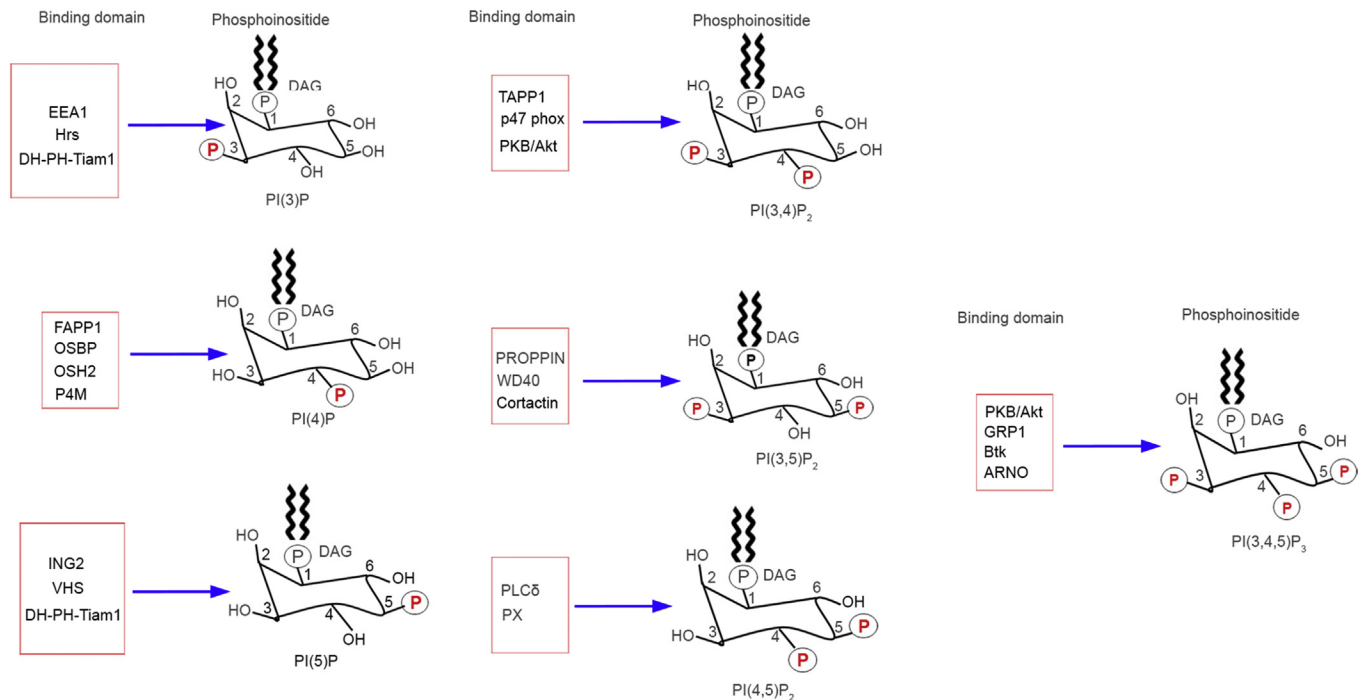

Fig. 5. Commonly used phosphoinositide-binding protein probes (domains) to determine the levels and localization of specific PIPs in vitro and in vivo.

have been used to detect PI(5)P (296). The PROPPIN domain of Atg18P and the WD40 domain of raptor have been used to detect PI(3,5)P$_2$ (297). The PH domains of TAPP1, p47phox, and PKB/Akt have been used to detect PI(3,4)P$_2$ (291). The PH domain of PLCδ1 and the PX domain of tubby have been used to detect PI(4,5)P$_2$ (291). The PH domains of PKB/Akt, GRP1, Btk, and ARNO have been used to detect PI(3,4,5)P$_3$ (249, 291). Some of these domains may bind to more than one PIP lipid (291).

Cortactin has been shown to bind to PI(3,5)P$_2$ (298). It does not contain canonical PIP binding motifs, such as PH or FYVE, but contains a stretch of basic lysine residues that may bind to PIP. This region is located within the N-terminal fourth repeat domain of cortactin (298). One study showed that the lactadherin C2 domain binds to PS (299); this domain could be used as a probe to determine the levels of PS in cells/tissues. PH domains do not always bind to PIPs, but sometimes act as platforms for protein-protein interaction domains (300, 301). Recently, the PIP binding domain has been used to quantitate the levels of PI3K-generated PIs in rod photoreceptor cells (53). A list of phospholipid-binding proteins that bind to specific PIPs commonly used as probes to study protein-lipid interaction is presented in **Fig. 5**.

METHODS TO DETECT CELLULAR LEVELS OF PHOSPHOINOSITIDES

Mass spectrometry is commonly used to detect PIPs. The PIPs can be identified through the *m/z* values of their collision-induced fragments. This approach provides a "fingerprint" for each PIP (3). By including a standard such as lysophosphatidic acid, the specific PIP can be identified and quantitated (3). Mass spectrometric methods are very sensitive and overcome the need for radioactive labeling.

Effector phosphoinositide binding proteins bind to specific PIPs. The proteins fused to reporters have been used as valuable tools to analyze specific PIPs in live or fixed cells and tissues (3, 53). Fluorescently tagged phosphoinositide-binding protein (either GFP or DsRed) or antibodies against fusion proteins have been used as probes for the immunoelectron or fluorescent microscopic detection of specific localization of PIPs (302). In rod photoreceptor cells, RPE cells, zebrafish, and transgenic *Xenopus* retina, PIP probes have been successfully employed to determine the localization of specific PIPs (53, 66, 100, 217, 218).

The fastest way of testing the potential phosphoinositide binding proteins is through protein-lipid overlay assays using purified phosphoinositide-binding proteins in vitro (290, 293, 299). Because the PIPs are localized to membranes, sometimes they may not interact with a specific phosphoinositide-binding protein in vitro. In those cases, liposomes containing specific PIPs can be used to mimic cellular membranes (3). Incubation of these liposomes with phosphoinositide-binding protein and the associated phosphoinositide-binding proteins with liposomes can be recovered by centrifugation of heavy multilamellar liposomes or affinity isolation of small unilamellar vesicles, followed by immunoblotting or radioactive counting (3). Liposome-based assays are better in terms of

measuring differential affinities of phosphoinositide-binding proteins to PIP than are protein-lipid overlay assays (3). These liposomes can be placed on the surface of the sensor chip for surface plasmon resonance spectroscopy (3). Association and dissociation constants can be calculated when the phosphoinositide-binding proteins are passed through a flow cell and bind to the immobilized PIP, which causes a change in surface refractory index (3).

Identification and measurement of phosphoinositide-metabolizing enzyme activity is another way of measuring PIPs in a given tissue or cell. These assays can be performed using radioactive ATP followed by TLC, after which the incorporated radioactivity in the PIPs can be counted (52, 104, 303). Nonradioactive enzyme assays are also available to determine the phosphoinositide-metabolizing enzymes (304). Many assays have been developed for the measurement of PI kinases and PI phosphatases in cell extracts or with purified proteins. These extracts or purified proteins can be incubated with radiolabeled PIPs, and their phosphorylated or dephosphorylated PIPs can be monitored using TLC or HPLC (305). Researchers have also developed novel mass assays to quantify phosphoinositides from cells and tissues (306–308).

Competitive ELISAs have been developed to determine the PI kinase assays (3). Several ELISAs have been developed to quantify the levels of specific PIPs using phosphoinositide-binding proteins (249, 309). The phosphoinositide-binding proteins are coupled to enzymes, peroxidase-conjugated antibodies, or antibody epitopes for the detection and quantification of various PIPs in cells and tissues (53, 309, 310). In photoreceptor cells, PI3K-generated products have been shown using phosphoinositide-binding proteins coupled with rhodopsin 1D4 antibody epitope on ELISA (53, 311). Fluorescence resonance energy transfer is another technique to determine the binding between PIP and phosphoinositide-binding proteins (3).

There are many other high-resolution techniques coupled with molecular probes to visualize the location, organization, and dynamics of PIPs (312). These techniques include single-particle tracking, fluorescence recovery after photobleaching, and fluorescence correlation spectroscopy (313). The combination of lipid probes and available microscopic techniques is very powerful, but these approaches have several potential limitations and caveats (313). There are numerous commercially available PIP-specific antibodies. A number of investigators have used these antibodies to localize PIP in cells and tissues (54, 314–316). Also, transgenic frogs (66) and zebrafish (217) models have been used to study the generation and localization of PIPs in vivo.

CONCLUSIONS AND FUTURE DIRECTIONS

Numerous studies have explored the roles of various phosphoinositides, phosphoinositide kinases, and phosphoinositide phosphatases in the vertebrate retina. The minor lipids play an important role in various aspects of photoreceptor biochemistry and physiology. These lipids are involved in protein transport, ciliogenesis, cell survival, vesicular transport, autophagy, phagocytosis, and synaptic functions. Many researchers have demonstrated that dysfunctional PI metabolism causes retinal degenerative diseases. In other cell types, various PIPs are generated through the activation receptors. However, in the retina, light stimulates the activation of phosphoinositide-metabolizing enzymes.

The mechanism of these enzyme activations is currently under investigation. Future studies should be aimed at the function and mechanism of activation of phosphoinositide-metabolizing enzymes/phosphatases and PIP function and regulation. The phosphoinositide metabolism in the cone synapse is different from that of rod synapses. Identification of phosphoinositide kinases and phosphoinositide phosphatases and identification and localization of specific PIPs in various cell types of the retina in health and disease would advance the field. ▪

Author ORCIDs
Raju V. S. Rajala ⓘ https://orcid.org/0000-0003-3783-8504

Conflict of interest

The author declares that he has no conflicts of interest with the contents of this article.

Abbreviations

CNG, cyclic nucleotide-gated; CNGA1, cyclic nucleotide-gated channel subunit α1; CNTF, ciliary neurotrophic factor; EPO, erythropoietin; FYCO1, FYVE and coiled-coil domain autophagy adaptor 1; GPCR, G protein-coupled receptor; Grbl4, growth factor receptor-bound protein 14; GSK3β, glycogen synthase kinase 3β; IGF, insulin-like growth factor; INPP5, inositol polyphosphate 5-phosphatase; IP$_3$, inositol triphosphate; IR, insulin receptor; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin; OCRL, oculocerebrorenal syndrome of Lowe; PDGF, platelet-derived growth factor; PH, pleckstrin homology; PI, phosphatidylinositol; PIKfyve, FYVE-type zinc finger containing phosphoinositide kinase; PI3K, phosphoinositide 3-kinase; PI4K, phosphatidylinositol 4-kinase; PIP, phosphatidylinositol phosphate; PI(3)P, phosphatidylinositol 3-phosphate; PI(4)P, phosphatidylinositol 4-phosphate; PI(5)P, phosphatidylinositol 5-phosphate; PI(3,4)P$_2$, phosphatidylinositol 3,4-bisphosphate; PI(3,5)P$_2$, phosphatidylinositol 3,5-bisphosphate; PI(4,5)P$_2$, phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P$_2$, phosphatidylinositol 3,4,5-trisphosphate; PIPK, phosphatidylinositol phosphate kinase; PITP, phosphatidylinositol transfer protein; PKC, protein kinase C; PLC, phospholipase C; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B; RA, Ras-associating; ROS, rod outer segment; RPE, retinal pigment epithelium; SH2, Src homology 2; SHIP, Src homology 2 domain-containing inositol polyphosphate 5-phosphatase; TRP, transient receptor potential; Vps, vacuolar protein sorting; VDAC, voltage-dependent anion channel.

REFERENCES

1. Balla, T. (2013) Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol. Rev.* **93**, 1019–1137
2. Sasaki, T., Takasuga, S., Sasaki, J., Kofuji, S., Eguchi, S., Yamazaki, M., and Suzuki, A. (2009) Mammalian phosphoinositide kinases and phosphatases. *Prog. Lipid Res.* **48**, 307–343
3. Rusten, T. E., and Stenmark, H. (2006) Analyzing phosphoinositides and their interacting proteins. *Nat. Methods.* **3**, 251–258
4. Rameh, L. E., and Cantley, L. C. (1999) The role of phosphoinositide 3-kinase lipid products in cell function. *J. Biol. Chem.* **274**, 8347–8350
5. Ghalayini, A. J., and Anderson, R. E. (1995) Light adaptation of bovine retinas in situ stimulates phosphatidylinositol synthesis in rod outer segments in vitro. *Curr. Eye Res.* **14**, 1025–1029
6. Huang, Z., Ghalayini, A., Guo, X. X., Alvarez, K. M., and Anderson, R. E. (2000) Light-mediated activation of diacylglycerol kinase in rat and bovine rod outer segments. *J. Neurochem.* **75**, 355–362
7. Guo, X., Ghalayini, A. J., Chen, H., and Anderson, R. E. (1997) Phosphatidylinositol 3-kinase in bovine photoreceptor rod outer segments. *Invest. Ophthalmol. Vis. Sci.* **38**, 1873–1882
8. Guo, X. X., Huang, Z., Bell, M. W., Chen, H., and Anderson, R. E. (2000) Tyrosine phosphorylation is involved in phosphatidylinositol 3-kinase activation in bovine rod outer segments. *Mol. Vis.* **6**, 216–221
9. Martin, T. F. (1998) Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation, and membrane trafficking. *Annu. Rev. Cell Dev. Biol.* **14**, 231–264
10. Fruman, D. A., Meyers, R. E., and Cantley, L. C. (1998) Phosphoinositide kinases. *Annu. Rev. Biochem.* **67**, 481–507
11. Anderson, R. E., and Hollyfield, J. G. (1981) Light stimulates the incorporation of inositol into phosphatidylinositol in the retina. *Biochim. Biophys. Acta.* **665**, 619–622
12. Anderson, R. E., Maude, M. B., Kelleher, P. A., Rayborn, M. E., and Hollyfield, J. G. (1983) Phosphoinositide metabolism in the retina: localization to horizontal cells and regulation by light and divalent cations. *J. Neurochem.* **41**, 764–771
13. Anderson, R. E., and Hollyfield, J. G. (1984) Inositol incorporation into phosphoinositides in retinal horizontal cells of Xenopus laevis: enhancement by acetylcholine, inhibition by glycine. *J. Cell Biol.* **99**, 686–691
14. Wensel, T. G. (2020) Phosphoinositides in retinal function and disease. *Cells.* **9**, 866
15. Broekhuyse, R. M. (1968) Phospholipids in tissues of the eye. I. Isolation, characterization and quantitative analysis by two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. *Biochim. Biophys. Acta.* **152**, 307–315
16. Anderson, R. E., Feldman, L. S., and Feldman, G. L. (1970) Lipids of ocular tissues. II. The phospholipids of mature bovine and rabbit whole retina. *Biochim. Biophys. Acta.* **202**, 367–373
17. Anderson, R. E. (1970) Lipids of ocular tissues. IV. A comparison of the phospholipids from the retina of six mammalian species. *Exp. Eye Res.* **10**, 339–344
18. Anderson, R. E., Lissandrello, P. M., Maude, M. B., and Matthes, M. T. (1976) Lipids of bovine retinal pigment epithelium. *Exp. Eye Res.* **23**, 149–157
19. Anderson, R. E., Maude, M. B., and Zimmerman, W. (1975) Lipids of ocular tissues–X. Lipid composition of subcellular fractions of bovine retina. *Vision Res.* **15**, 1087–1090
20. Schmidt, S. Y. (1983) Light- and cytidine-dependent phosphatidylinositol synthesis in photoreceptor cells of the rat. *J. Cell Biol.* **97**, 832–837

21. Schmidt, S. Y. (1983) Light enhances the turnover of phosphatidylinositol in rat retinas. *J. Neurochem.* **40**, 1630–1638
22. Jung, H. H., Reme, C. E., and Pfeilschifter, J. (1993) Light evoked inositol trisphosphate release in the rat retina in vitro. *Curr. Eye Res.* **12**, 727–732
23. Day, N. S., Koutz, C. A., and Anderson, R. E. (1993) Inositol-1,4,5-trisphosphate receptors in the vertebrate retina. *Curr. Eye Res.* **12**, 981–992
24. Ghalayini, A. J., Tarver, A. P., Mackin, W. M., Koutz, C. A., and Anderson, R. E. (1991) Identification and immunolocalization of phospholipase C in bovine rod outer segments. *J. Neurochem.* **57**, 1405–1412
25. Peng, Y. W., Rhee, S. G., Yu, W. P., Ho, Y. K., Schoen, T., Chader, G. J., and Yau, K. W. (1997) Identification of components of a phosphoinositide signaling pathway in retinal rod outer segments. *Proc. Natl. Acad. Sci. USA.* **94**, 1995–2000
26. Kaupp, U. B., and Seifert, R. (2002) Cyclic nucleotide-gated ion channels. *Physiol. Rev.* **82**, 769–824
27. Yau, K. W., and Hardie, R. C. (2009) Phototransduction motifs and variations. *Cell.* **139**, 246–264
28. Schmidt, T. M., Chen, S. K., and Hattar, S. (2011) Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends Neurosci.* **34**, 572–580
29. Orisme, W., Li, J., Goldmann, T., Bolch, S., Wolfrum, U., and Smith, W. C. (2010) Light-dependent translocation of arrestin in rod photoreceptors is signaled through a phospholipase C cascade and requires ATP. *Cell. Signal.* **22**, 447–456
30. He, F., Mao, M., and Wensel, T. G. (2004) Enhancement of phototransduction g protein-effector interactions by phosphoinositides. *J. Biol. Chem.* **279**, 8986–8990
31. Bright, S. R., Rich, E. D., and Varnum, M. D. (2007) Regulation of human cone cyclic nucleotide-gated channels by endogenous phospholipids and exogenously applied phosphatidylinositol 3,4,5-trisphosphate. *Mol. Pharmacol.* **71**, 176–183
32. Brady, J. D., Rich, E. D., Martens, J. R., Karpen, J. W., Varnum, M. D., and Brown, R. L. (2006) Interplay between PIP3 and calmodulin regulation of olfactory cyclic nucleotide-gated channels. *Proc. Natl. Acad. Sci. USA.* **103**, 15635–15640
33. Deretic, D., Traverso, V., Parkins, N., Jackson, F., Rodriguez de Turco, E. B., and Ransom, N. (2004) Phosphoinositides, ezrin/moesin, and racl regulate fusion of rhodopsin transport carriers in retinal photoreceptors. *Mol. Biol. Cell.* **15**, 359–370
34. Spencer, W. J., Lewis, T. R., Phan, S., Cady, M. A., Serebrovskaya, E. O., Schneider, N. F., Kim, K. Y., Cameron, L. A., Skiba, N. P., Ellisman, M. H., *et al.* (2019) Photoreceptor disc membranes are formed through an Arp2/3-dependent lamellipodium-like mechanism. *Proc. Natl. Acad. Sci. USA.* **116**, 27043–27052
35. Bucki, R., Wang, Y. H., Yang, C., Kandy, S. K., Fatunmbi, O., Bradley, R., Pogoda, K., Svitkina, T., Radhakrishnan, R., and Janmey, P. A. (2019) Lateral distribution of phosphatidylinositol 4,5-bisphosphate in membranes regulates formin- and ARP2/3-mediated actin nucleation. *J. Biol. Chem.* **294**, 4704–4722
36. Daste, F., Walrant, A., Holst, M. R., Gadsby, J. R., Mason, J., Lee, J. E., Brook, D., Mettlen, M., Larsson, E., Lee, S. F., *et al.* (2017) Control of actin polymerization via the coincidence of phosphoinositides and high membrane curvature. *J. Cell Biol.* **216**, 3745–3765
37. Giusto, N. M., Pasquare, S. J., Salvador, G. A., and Ilincheta de Boschero, M. G. (2010) Lipid second messengers and related enzymes in vertebrate rod outer segments. *J. Lipid Res.* **51**, 685–700
38. Anderson, R. E., Maude, M. B., Kelleher, P. A., Maida, T. M., and Basinger, S. F. (1980) Metabolism of phosphatidylcholine in the frog retina. *Biochim. Biophys. Acta.* **620**, 212–226
39. Anderson, R. E., Kelleher, P. A., and Maude, M. B. (1980) Metabolism of phosphatidylethanolamine in the frog retina. *Biochim. Biophys. Acta.* **620**, 227–235
40. Anderson, R. E., Maude, M. B., Pu, G. A., and Hollyfield, J. G. (1985) Effect of light on the metabolism of lipids in the rat retina. *J. Neurochem.* **44**, 773–778
41. Schmidt, S. Y. (1983) Phosphatidylinositol synthesis and phosphorylation are enhanced by light in rat retinas. *J. Biol. Chem.* **258**, 6863–6868
42. Choe, H. G., Ghalayini, A. J., and Anderson, R. E. (1990) Phosphoinositide metabolism in frog rod outer segments. *Exp. Eye Res.* **51**, 167–176
43. Ghalayini, A. J., Guo, X. X., Koutz, C. A., and Anderson, R. E. (1998) Light stimulates tyrosine phosphorylation of rat rod outer segments In vivo. *Exp. Eye Res.* **66**, 817–821
44. Brown, J. E., Blazynski, C., and Cohen, A. I. (1987) Light induces a rapid and transient increase in inositol-trisphosphate in toad rod outer segments. *Biochem. Biophys. Res. Commun.* **146**, 1392–1396
45. Das, N. D., Yoshioka, T., Samuelson, D., and Shichi, H. (1986) Immunocytochemical localization of phosphatidylinositol-4,5-bisphosphate in dark- and light-adapted rat retinas. *Cell Struct. Funct.* **11**, 53–63
46. Ferreira, P. A., Shortridge, R. D., and Pak, W. L. (1993) Distinctive subtypes of bovine phospholipase C that have preferential expression in the retina and high homology to the norpA gene product of Drosophila. *Proc. Natl. Acad. Sci. USA.* **90**, 6042–6046
47. Gehm, B. D., and Mc Connell, D. G. (1990) Phosphoinositide synthesis in bovine rod outer segments. *Biochemistry.* **29**, 5442–5446
48. Gehm, B. D., Pinke, R. M., Laquerre, S., Chafouleas, J. G., Schultz, D. A., Pepperl, D. J., and McConnell, D. G. (1991) Activation of bovine rod outer segment phosphatidylinositol-4,5-bisphosphate phospholipase C by calmodulin antagonists does not depend on calmodulin. *Biochemistry.* **30**, 11302–11306
49. Hayashi, F., and Amakawa, T. (1985) Light-mediated breakdown of phosphatidylinositol-4,5-bisphosphate in isolated rod outer segments of frog photoreceptor. *Biochem. Biophys. Res. Commun.* **128**, 954–959
50. Jelsema, C. L. (1989) Regulation of phospholipase A2 and phospholipase C in rod outer segments of bovine retina involves a common GTP-binding protein but different mechanisms of action. *Ann. N. Y. Acad. Sci.* **559**, 158–177
51. Millar, F. A., Fisher, S. C., Muir, C. A., Edwards, E., and Hawthorne, J. N. (1988) Polyphosphoinositide hydrolysis in response to light stimulation of rat and chick retina and retinal rod outer segments. *Biochim. Biophys. Acta.* **970**, 205–211
52. Rajala, A., Anderson, R. E., Ma, J. X., Lem, J., Al Ubaidi, M. R., and Rajala, R. V. (2007) G-protein-coupled receptor rhodopsin regulates the phosphorylation of retinal insulin receptor. *J. Biol. Chem.* **282**, 9865–9873
53. He, F., Agosto, M. A., Anastassov, I. A., Tse, D. Y., Wu, S. M., and Wensel, T. G. (2016) Phosphatidylinositol-3-phosphate is light-regulated and essential for survival in retinal rods. *Sci. Rep.* **6**, 26978
54. Rajala, R. V., Rajala, A., Morris, A. J., and Anderson, R. E. (2014) Phosphoinositides: minor lipids make a major impact on photoreceptor cell functions. *Sci. Rep.* **4**, 5463
55. Roque, M. E., Salvador, G. A., and Giusto, N. M. (1999) Light activation of phosphatidylethanolamine N-methyltransferase in rod outer segments and its modulation by association states of transducin. *Exp. Eye Res.* **69**, 555–562
56. Castagnet, P. I., and Giusto, N. M. (1993) Properties of phospholipase A2 activity from bovine retinal rod outer segments. *Exp. Eye Res.* **56**, 709–719
57. Salvador, G. A., and Giusto, N. M. (2006) Phospholipase D from photoreceptor rod outer segments is a downstream effector of RhoA: evidence of a light-dependent mechanism. *Exp. Eye Res.* **83**, 202–211
58. Giusto, N. M., Pasquare, S. J., Salvador, G. A., Castagnet, P. I., Roque, M. E., and Ilincheta de Boschero, M. G. (2000) Lipid metabolism in vertebrate retinal rod outer segments. *Prog. Lipid Res.* **39**, 315–391
59. Ghalayini, A., and Anderson, R. E. (1984) Phosphatidylinositol 4,5-bisphosphate: light-mediated breakdown in the vertebrate retina. *Biochem. Biophys. Res. Commun.* **124**, 503–506
60. Ghalayini, A. J., and Anderson, R. E. (1992) Activation of bovine rod outer segment phospholipase C by arrestin. *J. Biol. Chem.* **267**, 17977–17982
61. Ghalayini, A. J., Weber, N. R., Rundle, D. R., Koutz, C. A., Lambert, D., Guo, X. X., and Anderson, R. E. (1998) Phospholipase Cgammal in bovine rod outer segments: immunolocalization and light-dependent binding to membranes. *J. Neurochem.* **70**, 171–178
62. Natalini, P. M., Mateos, M. V., Ilincheta de Boschero, M. G., and Giusto, N. M. (2014) A novel light-dependent activation of DAGK and PKC in bovine photoreceptor nuclei. *Exp. Eye Res.* **125**, 142–155

ASBMB

63. Pasquaré, S. J., Salvador, G. A., Roque, M. E., and Giusto, N. M. (2000) Effect of light on phosphatidate phosphohydrolase activity of retina rod outer segments: the role of transducin. *Arch. Biochem. Biophys.* **379**, 299–306

64. Balasubramanian, N., and Slepak, V. Z. (2003) Light-mediated activation of Rac-1 in photoreceptor outer segments. *Curr. Biol.* **13**, 1306–1310

65. Natalini, P. M., Mateos, M. V., Ilincheta de Boschero, M. G., and Giusto, N. M. (2016) Insulin-related signaling pathways elicited by light in photoreceptor nuclei from bovine retina. *Exp. Eye Res.* **145**, 36–47

66. Li, G., Rajala, A., Wiechmann, A. F., Anderson, R. E., and Rajala, R. V. (2008) Activation and membrane binding of retinal protein kinase Balpha/Akt1 is regulated through light-dependent generation of phosphoinositides. *J. Neurochem.* **107**, 1382–1397

67. Rajala, A., Daly, R. J., Tanito, M., Allen, D. T., Holt, L. J., Lobanova, E. S., Arshavsky, V. Y., and Rajala, R. V. (2009) Growth factor receptor-bound protein 14 undergoes light-dependent intracellular translocation in rod photoreceptors: functional role in retinal insulin receptor activation. *Biochemistry.* **48**, 5563–5572

68. Brown, J. R., and Auger, K. R. (2011) Phylogenomics of phosphoinositide lipid kinases: perspectives on the evolution of second messenger signaling and drug discovery. *BMC Evol. Biol.* **11**, 4

69. Cantley, L. C. (2002) The phosphoinositide 3-kinase pathway. *Science.* **296**, 1655–1657

70. Zhao, L., and Vogt, P. K. (2008) Class I PI3K in oncogenic cellular transformation. *Oncogene.* **27**, 5486–5496

71. Hirsch, E., Lembo, G., Montrucchio, G., Rommel, C., Costa, C., and Barberis, L. (2006) Signaling through PI3Kgamma: a common platform for leukocyte, platelet and cardiovascular stress sensing. *Thromb. Haemost.* **95**, 29–35

72. Hirsch, E., Costa, C., and Ciraolo, E. (2007) Phosphoinositide 3-kinases as a common platform for multi-hormone signaling. *J. Endocrinol.* **194**, 243–256

73. Geering, B., Cutillas, P. R., Nock, G., Gharbi, S. I., and Vanhaesebroeck, B. (2007) Class IA phosphoinositide 3-kinases are obligate p85-p110 heterodimers. *Proc. Natl. Acad. Sci. USA.* **104**, 7809–7814

74. Ivanovic, I., Allen, D. T., Dighe, R., Le, Y. Z., Anderson, R. E., and Rajala, R. V. (2011) Phosphoinositide 3-kinase signaling in retinal rod photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **52**, 6355–6362

75. Pons, S., Asano, T., Glasheen, E., Miralpeix, M., Zhang, Y., Fisher, T. L., Myers, M. G., Jr., Sun, X. J., and White, M. F. (1995) The structure and function of p55PIK reveal a new regulatory subunit for phosphatidylinositol 3-kinase. *Mol. Cell. Biol.* **15**, 4453–4465

76. Antonetti, D. A., Algenstaedt, P., and Kahn, C. R. (1996) Insulin receptor substrate 1 binds two novel splice variants of the regulatory subunit of phosphatidylinositol 3-kinase in muscle and brain. *Mol. Cell. Biol.* **16**, 2195–2203

77. Inukai, K., Anai, M., Van Breda, E., Hosaka, T., Katagiri, H., Funaki, M., Fukushima, Y., Ogihara, T., Yazaki, Y., Kikuchi, M., *et al.* (1996) A novel 55-kDa regulatory subunit for phosphatidylinositol 3-kinase structurally similar to p55PIK Is generated by alternative splicing of the p85alpha gene. *J. Biol. Chem.* **271**, 5317–5320

78. Hiles, I. D., Otsu, M., Volinia, S., Fry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., and Totty, N. F. (1992) Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell.* **70**, 419–429

79. Ivanovic, I., Anderson, R. E., Le, Y. Z., Fliesler, S. J., Sherry, D. M., and Rajala, R. V. (2011) Deletion of the p85alpha regulatory subunit of phosphoinositide 3-kinase in cone photoreceptor cells results in cone photoreceptor degeneration. *Invest. Ophthalmol. Vis. Sci.* **52**, 3775–3783

80. Rajala, R. V. (2010) Phosphoinositide 3-kinase signaling in the vertebrate retina. *J. Lipid Res.* **51**, 4–22

81. Murga, C., Fukuhara, S., and Gutkind, J. S. (2000) A novel role for phosphatidylinositol 3-kinase beta in signaling from G protein-coupled receptors to Akt. *J. Biol. Chem.* **275**, 12069–12073

82. Stephens, L. R., Eguinoa, A., Erdjument-Bromage, H., Lui, M., Cooke, F., Coadwell, J., Smrcka, A. S., Thelen, M., Cadwallader, K., Tempst, P., *et al.* (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell.* **89**, 105–114

83. Suire, S., Hawkins, P., and Stephens, L. (2002) Activation of phosphoinositide 3-kinase gamma by Ras. *Curr. Biol.* **12**, 1068–1075

84. Voigt, P., Brock, C., Nurnberg, B., and Schaefer., M. (2005) Assigning functional domains within the p101 regulatory subunit of phosphoinositide 3-kinase gamma. *J. Biol. Chem.* **280**, 5121–5127

85. Weis, W. I., and Kobilka, B. K. (2018) The molecular basis of G protein-coupled receptor activation. *Annu. Rev. Biochem.* **87**, 897–919

86. Smrcka, A. V. (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cell. Mol. Life Sci.* **65**, 2191–2214

87. Murga, C., Laguinge, L., Wetzker, R., Cuadrado, A., and Gutkind, J. S. (1998) Activation of Akt/protein kinase B by G protein-coupled receptors. A role for alpha and beta gamma subunits of heterotrimeric G proteins acting through phosphatidylinositol-3-OH kinasegamma. *J. Biol. Chem.* **273**, 19080–19085

88. Curnock, A. P., Logan, M. K., and Ward, S. G. (2002) Chemokine signalling: pivoting around multiple phosphoinositide 3-kinases. *Immunology.* **105**, 125–136

89. Margaria, J. P., Ratto, E., Gozzelino, L., Li, H., and Hirsch, E. (2019) Class II PI3Ks at the intersection between signal transduction and membrane trafficking. *Biomolecules.* **9**, 104

90. Gulluni, F., De Santis, M. C., Margaria, J. P., Martini, M., and Hirsch, E. (2019) Class II PI3K functions in cell biology and disease. *Trends Cell Biol.* **29**, 339–359

91. Posor, Y., Eichhorn-Grunig, M., and Haucke, V. (2015) Phosphoinositides in endocytosis. *Biochim. Biophys. Acta.* **1851**, 794–804

92. Backer, J. M. (2008) The regulation and function of class III PI3Ks: novel roles for Vps34. *Biochem. J.* **410**, 1–17

93. Marat, A. L., and Haucke, V. (2016) Phosphatidylinositol 3-phosphates–at the interface between cell signalling and membrane traffic. *EMBO J.* **35**, 561–579

94. Miller, S., Tavshanjian, B., Oleksy, A., Perisic, O., Houseman, B. T., Shokat, K. M., and Williams, R. L. (2010) Shaping development of autophagy inhibitors with the structure of the lipid kinase Vps34. *Science.* **327**, 1638–1642

95. Stjepanovic, G., Baskaran, S., Lin, M. G., and Hurley, J. H. (2017) Vps34 kinase domain dynamics regulate the autophagic PI 3-kinase complex. *Mol. Cell.* **67**, 528–534.e3

96. Mayinger, P. (2012) Phosphoinositides and vesicular membrane traffic. *Biochim. Biophys. Acta.* **1821**, 1104–1113

97. Gillooly, D. J., Simonsen, A., and Stenmark, H. (2001) Phosphoinositides and phagocytosis. *J. Cell Biol.* **155**, 15–17

98. Kale, S. D., Gu, B., Capelluto, D. G., Dou, D., Feldman, E., Rumore, A., Arredondo, F. D., Hanlon, R., Fudal, I., Rouxel, T., *et al.* (2010) External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell.* **142**, 284–295

99. He, F., Nichols, R. M., Kailasam, L., Wensel, T. G., and Agosto, M. A. (2019) Critical role for phosphatidylinositol-3 kinase Vps34/PIK3C3 in ON-bipolar cells. *Invest. Ophthalmol. Vis. Sci.* **60**, 2861–2874

100. He, F., Agosto, M. A., Nichols, R. M., and Wensel, T. G. (2020) Multiple phosphatidylinositol(3)phosphate roles in retinal pigment epithelium membrane recycling. *bioRxiv.* https://doi.org/10.1101/2020.01.09.899815

101. Takai, H., Wang, R. C., Takai, K. K., Yang, H., and de Lange, T. (2007) Tel2 regulates the stability of PI3K-related protein kinases. *Cell.* **131**, 1248–1259

102. Thomas, D., Powell, J. A., Green, B. D., Barry, E. F., Ma, Y., Woodcock, J., Fitter, S., Zannettino, A. C., Pitson, S. M., Hughes, T. P., *et al.* (2013) Protein kinase activity of phosphoinositide 3-kinase regulates cytokine-dependent cell survival. *PLoS Biol.* **11**, e1001515

103. Rajala, R. V., and Anderson, R. E. (2001) Interaction of the insulin receptor beta-subunit with phosphatidylinositol 3-kinase in bovine ROS. *Invest. Ophthalmol. Vis. Sci.* **42**, 3110–3117

104. Rajala, R. V., McClellan, M. E., Ash, J. D., and Anderson, R. E. (2002) In vivo regulation of phosphoinositide 3-kinase in retina through light-induced tyrosine phosphorylation of the insulin receptor beta-subunit. *J. Biol. Chem.* **277**, 43319–43326

105. Rajala, A., and Rajala, R. V. S. (2020) A non-canonical rhodopsin-mediated insulin receptor signaling pathway in retinal photoreceptor neurons. *Cell Biol. Int.* **44**, 1020–1027

106. Reiter, C. E., Sandirasegarane, L., Wolpert, E. B., Klinger, M., Simpson, I. A., Barber, A. J., Antonetti, D. A., Kester, M., and Gardner, T. W. (2003) Characterization of insulin signaling in rat retina in vivo and ex vivo. *Am. J. Physiol. Endocrinol. Metab.* **285**, E763–E774

107. Rajala, R. V., Wiskur, B., Tanito, M., Callegan, M., and Rajala, A. (2009) Diabetes reduces autophosphorylation of retinal insulin receptor and increases protein-tyrosine phosphatase-1B activity. *Invest. Ophthalmol. Vis. Sci.* **50**, 1033–1040

108. Rajala, R. V., Tanito, M., Neel, B. G., and Rajala, A. (2010) Enhanced retinal insulin receptor-activated neuroprotective survival signal in mice lacking the protein-tyrosine phosphatase-1B gene. *J. Biol. Chem.* **285**, 8894–8904

109. Basavarajappa, D. K., Gupta, V. K., Dighe, R., Rajala, A., and Rajala, R. V. (2011) Phosphorylated Grbl4 is an endogenous inhibitor of retinal protein tyrosine phosphatase 1B, and light-dependent activation of Src phosphorylates Grbl4. *Mol. Cell. Biol.* **31**, 3975–3987

110. Rajala, R. V., and Anderson, R. E. (2010) Rhodopsin-regulated insulin receptor signaling pathway in rod photoreceptor neurons. *Mol. Neurobiol.* **42**, 39–47

111. Rajala, A., Tanito, M., Le, Y. Z., Kahn, C. R., and Rajala, R. V. (2008) Loss of neuroprotective survival signal in mice lacking insulin receptor gene in rod photoreceptor cells. *J. Biol. Chem.* **283**, 19781–19792

112. Li, G., Anderson, R. E., Tomita, H., Adler, R., Liu, X., Zack, D. J., and Rajala, R. V. (2007) Nonredundant role of Akt2 for neuroprotection of rod photoreceptor cells from light-induced cell death. *J. Neurosci.* **27**, 203–211

113. Rajala, A., Wang, Y., and Rajala, R. V. (2016) Activation of oncogenic tyrosine kinase signaling promotes insulin receptor-mediated cone photoreceptor survival. *Oncotarget.* **7**, 46924–46942

114. Rajala, A., Dighe, R., Agbaga, M. P., Anderson, R. E., and Rajala, R. V. (2013) Insulin receptor signaling in cones. *J. Biol. Chem.* **288**, 19503–19515

115. Xu, S., Ladak, R., Swanson, D. A., Soltyk, A., Sun, H., Ploder, L., Vidgen, D., Duncan, A. M., Garami, E., Valle, D., *et al.* (1999) PHR1 encodes an abundant, pleckstrin homology domain-containing integral membrane protein in the photoreceptor outer segments. *J. Biol. Chem.* **274**, 35676–35685

116. Xu, S., Wang, Y., Zhao, H., Zhang, L., Xiong, W., Yau, K. W., Hiel, H., Glowatzki, E., Ryugo, D. K., and Valle, D. (2004) PHR1, a PH domain-containing protein expressed in primary sensory neurons. *Mol. Cell. Biol.* **24**, 9137–9151

117. Krappa, R., Nguyen, A., Burrola, P., Deretic, D., and Lemke, G. (1999) Evectins: vesicular proteins that carry a pleckstrin homology domain and localize to post-Golgi membranes. *Proc. Natl. Acad. Sci. USA.* **96**, 4633–4638

118. Rajala, R. V., Chan, M. D., and Rajala, A. (2005) Lipid-protein interactions of growth factor receptor-bound protein 14 in insulin receptor signaling. *Biochemistry.* **44**, 15461–15471

119. Rajala, R. V., McClellan, M. E., Chan, M. D., Tsiokas, L., and Anderson, R. E. (2004) Interaction of the retinal insulin receptor beta-subunit with the P85 subunit of phosphoinositide 3-kinase. *Biochemistry.* **43**, 5637–5650

120. Yi, X., Schubert, M., Peachey, N. S., Suzuma, K., Burks, D. J., Kushner, J. A., Suzuma, I., Cahill, C., Flint, C. L., Dow, M. A., *et al.* (2005) Insulin receptor substrate 2 is essential for maturation and survival of photoreceptor cells. *J. Neurosci.* **25**, 1240–1248

121. Tanaka, M., Konishi, H., Touhara, K., Sakane, F., Hirata, M., Ono, Y., and Kikkawa, U. (1999) Identification of myosin II as a binding protein to the PH domain of protein kinase B. *Biochem. Biophys. Res. Commun.* **255**, 169–174

122. Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., and Weston, M. D. (1995) Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature.* **374**, 60–61

123. Brognard, J., Sierecki, E., Gao, T., and Newton, A. C. (2007) PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. *Mol. Cell.* **25**, 917–931

124. Gao, T., Furnari, F., and Newton, A. C. (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol. Cell.* **18**, 13–24

125. Rajala, A., Gupta, V. K., Anderson, R. E., and Rajala, R. V. (2013) Light activation of the insulin receptor regulates mitochondrial hexokinase. A possible mechanism of retinal neuroprotection. *Mitochondrion.* **13**, 566–576

126. Rajala, R. V., Kanan, Y., and Anderson, R. E. (2016) Photoreceptor neuroprotection: regulation of Akt activation through serine/threonine phosphatases, PHLPP and PHLPPL. *Adv. Exp. Med. Biol.* **854**, 419–424

127. Kanan, Y., Matsumoto, H., Song, H., Sokolov, M., Anderson, R. E., and Rajala, R. V. (2010) Serine/threonine kinase Akt activation regulates the activity of retinal serine/threonine phosphatases, PHLPP and PHLPPL. *J. Neurochem.* **113**, 477–488

128. Dilly, A. K., and Rajala, R. V. (2008) Insulin growth factor 1 receptor/PI3K/AKT survival pathway in outer segment membranes of rod photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **49**, 4765–4773

129. Majewski, N., Nogueira, V., Bhaskar, P., Coy, P. E., Skeen, J. E., Gottlob, K., Chandel, N. S., Thompson, C. B., Robey, R. B., and Hay, N. (2004) Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol. Cell.* **16**, 819–830

130. Majewski, N., Nogueira, V., Robey, R. B., and Hay, N. (2004) Akt inhibits apoptosis downstream of BID cleavage via a glucose-dependent mechanism involving mitochondrial hexokinases. *Mol. Cell. Biol.* **24**, 730–740

131. Gottlob, K., Majewski, N., Kennedy, S., Kandel, E., Robey, R. B., and Hay, N. (2001) Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev.* **15**, 1406–1418

132. Feng, J., Lucchinetti, E., Ahuja, P., Pasch, T., Perriard, J. C., and Zaugg, M. (2005) Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3beta. *Anesthesiology.* **103**, 987–995

133. Pastorino, J. G., Hoek, J. B., and Shulga, N. (2005) Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity. *Cancer Res.* **65**, 10545–10554

134. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature.* **378**, 785–789

135. Robey, R. B., and Hay, N. (2006) Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. *Oncogene.* **25**, 4683–4696

136. Suire, S., Coadwell, J., Ferguson, G. J., Davidson, K., Hawkins, P., and Stephens, L. (2005) p84, a new Gbetagamma-activated regulatory subunit of the type IB phosphoinositide 3-kinase p110gamma. *Curr. Biol.* **15**, 566–570

137. Rubio, I., Rodriguez-Viciana, P., Downward, J., and Wetzker, R. (1997) Interaction of Ras with phosphoinositide 3-kinase gamma. *Biochem. J.* **326**, 891–895

138. Wennerberg, K., Rossman, K. L., and Der, C. J. (2005) The Ras superfamily at a glance. *J. Cell Sci.* **118**, 843–846

139. Patel, M., and Cote, J. F. (2013) Ras GTPases' interaction with effector domains: Breaking the families' barrier. *Commun. Integr. Biol.* **6**, e24298

140. Rajala, R. V., Rajala, A., and Gupta, V. K. (2012) Conservation and divergence of Grb7 family of Ras-binding domains. *Protein Cell.* **3**, 60–70

141. Gupta, V. K., Rajala, A., and Rajala, R. V. (2015) Non-canonical regulation of phosphatidylinositol 3-kinase gamma isoform activity in retinal rod photoreceptor cells. *Cell Commun. Signal.* **13**, 7

142. Rajala, R. V., McClellan, M. E., Ash, J. D., and Anderson, R. E. (2003) Regulation of retinal phosphoinositide 3-kinase activity in p85alpha-subunit knockout mice. *Adv. Exp. Med. Biol.* **533**, 369–376

143. Rajala, R. V., Ranjo-Bishop, M., Wang, Y., Rajala, A., and Anderson, R. E. (2015) The p110alpha isoform of phosphoinositide 3-kinase is essential for cone photoreceptor survival. *Biochimie.* **112**, 35–40

144. Streb, H., Irvine, R. F., Berridge, M. J., and Schulz, I. (1983) Release of Ca2+ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature.* **306**, 67–69

145. Rajala, R. V., and Anderson, R. E. (2010) Focus on molecules: phosphatidylinositol-4,5-bisphosphate (PIP2). *Exp. Eye Res.* **91**, 324–325

146. Anderson, R. A., Boronenkov, I. V., Doughman, S. D., Kunz, J., and Loijens, J. C. (1999) Phosphatidylinositol phosphate kinases,

a multifaceted family of signaling enzymes. *J. Biol. Chem.* **274**, 9907–9910

147. Divecha, N., Brooksbank, C. E., and Irvine, R. F. (1992) Purification and characterization of phosphatidylinositol 4-phosphate 5-kinases. *Biochem. J.* **288**, 637–642

148. Moritz, A., De Graan, P. N., Ekhart, P. F., Gispen, W. H., and Wirtz, K. W. (1990) Purification of a phosphatidylinositol 4-phosphate kinase from bovine brain membranes. *J. Neurochem.* **54**, 351–354

149. Boronenkov, I. V., and Anderson, R. A. (1995) The sequence of phosphatidylinositol-4-phosphate 5-kinase defines a novel family of lipid kinases. *J. Biol. Chem.* **270**, 2881–2884

150. Loijens, J. C., Boronenkov, I. V., Parker, G. J., and Anderson, R. A. (1996) The phosphatidylinositol 4-phosphate 5-kinase family. *Adv. Enzyme Regul.* **36**, 115–140

151. Rameh, L. E., Tolias, K. F., Duckworth, B. C., and Cantley, L. C. (1997) A new pathway for synthesis of phosphatidylinositol-4,5-bisphosphate. *Nature.* **390**, 192–196

152. Smith, C. D., and Chang, K. J. (1989) Regulation of brain phosphatidylinositol-4-phosphate kinase by GTP analogues. A potential role for guanine nucleotide regulatory proteins. *J. Biol. Chem.* **264**, 3206–3210

153. Martin, A., Brown, F. D., Hodgkin, M. N., Bradwell, A. J., Cook, S. J., Hart, M., and Wakelam, M. J. (1996) Activation of phospholipase D and phosphatidylinositol 4-phosphate 5-kinase in HL60 membranes is mediated by endogenous Arf but not Rho. *J. Biol. Chem.* **271**, 17397–17403

154. Jenkins, G. H., Fisette, P. L., and Anderson, R. A. (1994) Type I phosphatidylinositol 4-phosphate 5-kinase isoforms are specifically stimulated by phosphatidic acid. *J. Biol. Chem.* **269**, 11547–11554

155. Bazenet, C. E., Ruano, A. R., Brockman, J. L., and Anderson, R. A. (1990) The human erythrocyte contains two forms of phosphatidylinositol-4-phosphate 5-kinase which are differentially active toward membranes. *J. Biol. Chem.* **265**, 18012–18022

156. Cochet, C., Filhol, O., Payrastre, B., Hunter, T., and Gill, G. N. (1991) Interaction between the epidermal growth factor receptor and phosphoinositide kinases. *J. Biol. Chem.* **266**, 637–644

157. Choi, S., Thapa, N., Tan, X., Hedman, A. C., and Anderson, R. A. (2015) PIP kinases define PI4,5P2 signaling specificity by association with effectors. *Biochim. Biophys. Acta.* **1851**, 711–723

158. Sakagami, H., Katsumata, O., Hara, Y., Tamaki, H., and Fukaya, M. (2014) Preferential localization of type I phosphatidylinositol 4-phosphate 5-kinase γ at the periactive zone of mouse photoreceptor ribbon synapses. *Brain Res.* **1586**, 23–33

159. Wenk, M. R., Pellegrini, L., Klenchin, V. A., Di Paolo, G., Chang, S., Daniell, L., Arioka, M., Martin, T. F., and De Camilli, P. (2001) PIP kinase Igamma is the major PI(4,5)P(2) synthesizing enzyme at the synapse. *Neuron.* **32**, 79–88

160. Wright, B. D., Loo, L., Street, S. E., Ma, A., Taylor-Blake, B., Stashko, M. A., Jin, J., Janzen, W. P., Frye, S. V., and Zylka, M. J. (2014) The lipid kinase PIP5K1C regulates pain signaling and sensitization. *Neuron.* **82**, 836–847

161. Di Paolo, G., Moskowitz, H. S., Gipson, K., Wenk, M. R., Voronov, S., Obayashi, M., Flavell, R., Fitzsimonds, R. M., Ryan, T. A., and De Camilli, P. (2004) Impaired PtdIns(4,5)P2 synthesis in nerve terminals produces defects in synaptic vesicle trafficking. *Nature.* **431**, 415–422

162. Zhao, L., Chen, Y., Bajaj, A. O., Eblimit, A., Xu, M., Soens, Z. T., Wang, F., Ge, Z., Jung, S. Y., He, F., *et al.* (2016) Integrative subcellular proteomic analysis allows accurate prediction of human disease-causing genes. *Genome Res.* **26**, 660–669

163. Huang, Z., Guo, X. X., Chen, S. X., Alvarez, K. M., Bell, M. W., and Anderson, R. E. (2001) Regulation of type II phosphatidylinositol phosphate kinase by tyrosine phosphorylation in bovine rod outer segments. *Biochemistry.* **40**, 4550–4559

164. Huang, Z., Anderson, R. E., Cao, W., Wiechmann, A. F., and Rajala, R. V. (2011) Light-induced tyrosine phosphorylation of rod outer segment membrane proteins regulate the translocation, membrane binding and activation of type ii alpha phosphatidylinositol-5-phosphate 4-kinase. *Neurochem. Res.* **36**, 627–635

165. Doughman, R. L., Firestone, A. J., and Anderson, R. A. (2003) Phosphatidylinositol phosphate kinases put PI4,5P(2) in its place. *J. Membr. Biol.* **194**, 77–89

166. Womack, K. B., Gordon, S. E., He, F., Wensel, T. G., Lu, C. C., and Hilgemann, D. W. (2000) Do phosphatidylinositides modulate vertebrate phototransduction? *J. Neurosci.* **20**, 2792–2799

167. Hille, B., Dickson, E. J., Kruse, M., Vivas, O., and Suh, B. C. (2015) Phosphoinositides regulate ion channels. *Biochim. Biophys. Acta.* **1851**, 844–856

168. Michailidis, I. E., Rusinova, R., Georgakopoulos, A., Chen, Y., Iyengar, R., Robakis, N. K., Logothetis, D. E., and Baki, L. (2011) Phosphatidylinositol-4,5-bisphosphate regulates epidermal growth factor receptor activation. *Pflugers Arch.* **461**, 387–397

169. Shisheva, A., Sbrissa, D., and Ikonomov, O. (1999) Cloning, characterization, and expression of a novel Zn2+-binding FYVE finger-containing phosphoinositide kinase in insulin-sensitive cells. *Mol. Cell. Biol.* **19**, 623–634

170. Vicinanza, M., Korolchuk, V. I., Ashkenazi, A., Puri, C., Menzies, F. M., Clarke, J. H., and Rubinsztein, D. C. (2015) PI(5)P regulates autophagosome biogenesis. *Mol. Cell.* **57**, 219–234

171. Cabezas, A., Pattni, K., and Stenmark, H. (2006) Cloning and subcellular localization of a human phosphatidylinositol 3-phosphate 5-kinase. *PIKfyve/Fab1. Gene.* **371**, 34–41

172. Hasegawa, J., Strunk, B. S., and Weisman, L. S. (2017) PI5P and PI(3,5)P2: minor, but essential phosphoinositides. *Cell Struct. Funct.* **42**, 49–60

173. Stenmark, H., Aasland, R., and Driscoll, P. C. (2002) The phosphatidylinositol 3-phosphate-binding FYVE finger. *FEBS Lett.* **513**, 77–84

174. Nicot, A. S., and Laporte, J. (2008) Endosomal phosphoinositides and human diseases. *Traffic.* **9**, 1240–1249

175. Boisset, G., Polok, B. K., and Schorderet, D. F. (2008) Characterization of pip5k3 fleck corneal dystrophy-linked gene in zebrafish. *Gene Expr. Patterns.* **8**, 404–410

176. Ikonomov, O. C., Sbrissa, D., Delvecchio, K., Xie, Y., Jin, J. P., Rappolee, D., and Shisheva, A. (2011) The phosphoinositide kinase PIKfyve is vital in early embryonic development: preimplantation lethality of PIKfyve-/= embryos but normality of PIKfyve+/- mice. *J. Biol. Chem.* **286**, 13404–13413

177. Liu, Y., Lai, Y. C., Hill, E. V., Tyteca, D., Carpentier, S., Ingvaldsen, A., Vertommen, D., Lantier, L., Foretz, M., Dequiedt, F., *et al.* (2013) Phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) is an AMPK target participating in contraction-stimulated glucose uptake in skeletal muscle. *Biochem. J.* **455**, 195–206

178. Ikonomov, O. C., Sbrissa, D., Delvecchio, K., Feng, H. Z., Cartee, G. D., Jin, J. P., and Shisheva, A. (2013) Muscle-specific Pikfyve gene disruption causes glucose intolerance, insulin resistance, adiposity, and hyperinsulinemia but not muscle fiber-type switching. *Am. J. Physiol. Endocrinol. Metab.* **305**, E119–E131

179. Tronchere, H., Cinato, M., Timotin, A., Guitou, L., Villedieu, C., Thibault, H., Baetz, D., Payrastre, B., Valet, P., Parini, A., *et al.* (2017) Inhibition of PIKfyve prevents myocardial apoptosis and hypertrophy through activation of SIRT3 in obese mice. *EMBO Mol. Med.* **9**, 770–785

180. Rajala, R. V. S. (2020) Aerobic glycolysis in the retina: functional roles of pyruvate kinase isoforms. *Front. Cell Dev. Biol.* **8**, 266

181. Rajala, A., Soni, K., and Rajala, R. V. S. (2020) Metabolic and non-metabolic roles of pyruvate kinase M2 isoform in diabetic retinopathy. *Sci. Rep.* **10**, 7456

182. Simonsen, A., and Tooze, S. A. (2009) Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J. Cell Biol.* **186**, 773–782

183. McAlpine, F., Williamson, L. E., Tooze, S. A., and Chan, E. Y. (2013) Regulation of nutrient-sensitive autophagy by uncoordinated 51-like kinases 1 and 2. *Autophagy.* **9**, 361–373

184. Biswas, S. K., Zhao, Y., Nagalingam, A., Gardner, T. W., and Sandiraegarane, L. (2008) PDGF- and insulin/IGF-1-specific distinct modes of class IA PI 3-kinase activation in normal rat retinas and RGC-5 retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci.* **49**, 3687–3698

185. Hollborn, M., Bringmann, A., Faude, F., Wiedemann, P., and Kohen, L. (2006) Signaling pathways involved in PDGF-evoked cellular responses in human RPE cells. *Biochem. Biophys. Res. Commun.* **344**, 912–919

186. Hollborn, M., Jahn, K., Limb, G. A., Kohen, L., Wiedemann, P., and Bringmann, A. (2004) Characterization of the basic fibroblast growth factor-evoked proliferation of the human Muller cell line, MIO-M1. *Graefes Arch. Clin. Exp. Ophthalmol.* **242**, 414–422

187. Ravichandran, L. V., Chen, H., Li, Y., and Quon, M. J. (2001) Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability

to dephosphorylate the insulin receptor. *Mol. Endocrinol.* **15**, 1768–1780

188. Reiter, C. E., Wu, X., Sandirasegarane, L., Nakamura, M., Gilbert, K. A., Singh, R. S., Fort, P. E., Antonetti, D. A., and Gardner, T. W. (2006) Diabetes reduces basal retinal insulin receptor signaling: reversal with systemic and local insulin. *Diabetes.* **55**, 1148–1156

189. Reiter, C. E., and Gardner, T. W. (2003) Functions of insulin and insulin receptor signaling in retina: possible implications for diabetic retinopathy. *Prog. Retin. Eye Res.* **22**, 545–562

190. Barber, A. J., Nakamura, M., Wolpert, E. B., Reiter, C. E., Seigel, G. M., Antonetti, D. A., and Gardner, T. W. (2001) Insulin rescues retinal neurons from apoptosis by a phosphatidylinositol 3-kinase/Akt-mediated mechanism that reduces the activation of caspase-3. *J. Biol. Chem.* **276**, 32814–32821

191. Yu, X., Rajala, R. V., McGinnis, J. F., Li, F., Anderson, R. E., Yan, X., Li, S., Elias, R. V., Knapp, R. R., Zhou, X., *et al.* (2004) Involvement of insulin/phosphoinositide 3-kinase/Akt signal pathway in 17 beta-estradiol-mediated neuroprotection. *J. Biol. Chem.* **279**, 13086–13094

192. Cao, W., Rajala, R. V., Li, F., Anderson, R. E., Wei, N., Soliman, C. E., and McGinnis, J. F. (2003) Neuroprotective effect of estrogen upon retinal neurons in vitro. *Adv. Exp. Med. Biol.* **533**, 395–402

193. Park, K. K., Liu, K., Hu, Y., Smith, P. D., Wang, C., Cai, B., Xu, B., Connolly, L., Kramvis, I., Sahin, M., *et al.* (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science.* **322**, 963–966

194. Stitt, A. W., Hughes, S. J., Canning, P., Lynch, O., Cox, O., Frizzell, N., Thorpe, S. R., Cotter, T. G., Curtis, T. M., and Gardiner, T. A. (2004) Substrates modified by advanced glycation endproducts cause dysfunction and death in retinal pericytes by reducing survival signals mediated by platelet-derived growth factor. *Diabetologia.* **47**, 1735–1746

195. Wang, K., and Spector, A. (2000) Alpha-crystallin prevents irreversible protein denaturation and acts cooperatively with other heat-shock proteins to renature the stabilized partially denatured protein in an ATP-dependent manner. *Eur. J. Biochem.* **267**, 4705–4712

196. Defoe, D. M., and Grindstaff, R. D. (2004) Epidermal growth factor stimulation of RPE cell survival: contribution of phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways. *Exp. Eye Res.* **79**, 51–59

197. Yang, P., Peairs, J. J., Tano, R., and Jaffe, G. J. (2006) Oxidant-mediated Akt activation in human RPE cells. *Invest. Ophthalmol. Vis. Sci.* **47**, 4598–4606

198. Nakazawa, T., Tamai, M., and Mori, N. (2002) Brain-derived neurotrophic factor prevents axotomized retinal ganglion cell death through MAPK and PI3K signaling pathways. *Invest. Ophthalmol. Vis. Sci.* **43**, 3319–3326

199. Ikeda, K., Tatsuno, T., Noguchi, H., and Nakayama, C. (2004) Ciliary neurotrophic factor protects rat retina cells in vitro and in vivo via PI3 kinase. *Curr. Eye Res.* **29**, 349–355

200. Kretz, A., Happold, C. J., Marticke, J. K., and Isenmann, S. (2005) Erythropoietin promotes regeneration of adult CNS neurons via Jak2/Stat3 and PI3K/AKT pathway activation. *Mol. Cell. Neurosci.* **29**, 569–579

201. Lavie, Y., Dybowski, J., and Agranoff, B. W. (1997) Wortmannin blocks goldfish retinal phosphatidylinositol 3-kinase and neurite outgrowth. *Neurochem. Res.* **22**, 373–378

202. Pimentel, B., Rodriguez-Borlado, L., Hernandez, C., and Carrera, A. C. (2002) A Role for phosphoinositide 3-kinase in the control of cell division and survival during retinal development. *Dev. Biol.* **247**, 295–306

203. Ko, M. L., Jian, K., Shi, L., and Ko, G. Y. (2009) Phosphatidylinositol 3 kinase-Akt signaling serves as a circadian output in the retina. *J. Neurochem.* **108**, 1607–1620

204. Johnson, L. E., van Veen, T., and Ekstrom, P. A. (2005) Differential Akt activation in the photoreceptors of normal and rd1 mice. *Cell Tissue Res.* **320**, 213–222

205. Rajala, R. V., Ivanovic, I., and Dilly, A. K. (2009) Retinal insulin receptor signaling in hyperosmotic stress. *Vitam. Horm.* **80**, 583–612

206. Chen, J., Wang, L., Chen, Y., Sternberg, P., and Cai, J. (2009) Phosphatidylinositol 3 kinase pathway and 4-hydroxy-2-nonenal-induced oxidative injury in the RPE. *Invest. Ophthalmol. Vis. Sci.* **50**, 936–942

207. Schmitz, F., and Drenckhahn, D. (1993) Li(+)-induced structural changes of synaptic ribbons are related to the phosphoinositide metabolism in photoreceptor synapses. *Brain Res.* **604**, 142–148

208. Luttrell, L. M., van Biesen, T., Hawes, B. E., Koch, W. J., Krueger, K. M., Touhara, K., and Lefkowitz, R. J. (1997) G-protein-coupled receptors and their regulation: activation of the MAP kinase signaling pathway by G-protein-coupled receptors. *Adv. Second Messenger Phosphoprotein Res.* **31**, 263–277

209. Luttrell, L. M., Daaka, Y., and Lefkowitz, R. J. (1999) Regulation of tyrosine kinase cascades by G-protein-coupled receptors. *Curr. Opin. Cell Biol.* **11**, 177–183

210. Lev, S., Hernandez, J., Martinez, R., Chen, A., Plowman, G., and Schlessinger, J. (1999) Identification of a novel family of targets of PYK2 related to Drosophila retinal degeneration B (rdgB) protein. *Mol. Cell. Biol.* **19**, 2278–2288

211. Dyson, J. M., Fedele, C. G., Davies, E. M., Becanovic, J., and Mitchell, C. A. (2012) Phosphoinositide phosphatases: just as important as the kinases. *Subcell. Biochem.* **58**, 215–279

212. Verstreken, P., Koh, T. W., Schulze, K. L., Zhai, R. G., Hiesinger, P. R., Zhou, Y., Mehta, S. Q., Cao, Y., Roos, J., and Bellen, H. J. (2003) Synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating. *Neuron.* **40**, 733–748

213. Chi, Y., Zhou, B., Wang, W. Q., Chung, S. K., Kwon, Y. U., Ahn, Y. H., Chang, Y. T., Tsujishita, Y., Hurley, J. H., and Zhang, Z. Y. (2004) Comparative mechanistic and substrate specificity study of inositol polyphosphate 5-phosphatase Schizosaccharomyces pombe Synaptojanin and SHIP2. *J. Biol. Chem.* **279**, 44987–44995

214. Chang-Ileto, B., Frere, S. G., Chan, R. B., Voronov, S. V., Roux, A., and Di Paolo, G. (2011) Synaptojanin 1-mediated PI(4,5)P2 hydrolysis is modulated by membrane curvature and facilitates membrane fission. *Dev. Cell.* **20**, 206–218

215. Holzhausen, L. C., Lewis, A. A., Cheong, K. K., and Brockerhoff, S. E. (2009) Differential role for synaptojanin 1 in rod and cone photoreceptors. *J. Comp. Neurol.* **517**, 633–644

216. Van Epps, H. A., Hayashi, M., Lucast, L., Stearns, G. W., Hurley, J. B., De Camilli, P., and Brockerhoff, S. E. (2004) The zebrafish nrc mutant reveals a role for the polyphosphoinositide phosphatase synaptojanin 1 in cone photoreceptor ribbon anchoring. *J. Neurosci.* **24**, 8641–8650

217. George, A. A., Hayden, S., Stanton, G. R., and Brockerhoff, S. E. (2016) Arf6 and the 5′phosphatase of synaptojanin 1 regulate autophagy in cone photoreceptors. *BioEssays.* **38 (Suppl. 1)**, S119–S135

218. Brockerhoff, S. E. (2011) Phosphoinositides and photoreceptors. *Mol. Neurobiol.* **44**, 420–425

219. George, A. A., Hayden, S., Holzhausen, L. C., Ma, E. Y., Suzuki, S. C., and Brockerhoff, S. E. (2014) Synaptojanin 1 is required for endolysosomal trafficking of synaptic proteins in cone photoreceptor inner segments. *PLoS One.* **9**, e84394

220. Voronov, S. V., Frere, S. G., Giovedi, S., Pollina, E. A., Borel, C., Zhang, H., Schmidt, C., Akeson, E. C., Wenk, M. R., Cimasoni, L., *et al.* (2008) Synaptojanin 1-linked phosphoinositide dyshomeostasis and cognitive deficits in mouse models of Down's syndrome. *Proc. Natl. Acad. Sci. USA.* **105**, 9415–9420

221. Prosseda, P. P., Luo, N., Wang, B., Alvarado, J. A., Hu, Y., and Sun, Y. (2017) Loss of OCRL increases ciliary PI(4,5)P(2) in Lowe oculocerebrorenal syndrome. *J. Cell Sci.* **130**, 3447–3454

222. Luo, N., Conwell, M. D., Chen, X., Kettenhofen, C. I., Westlake, C. J., Cantor, L. B., Wells, C. D., Weinreb, R. N., Corson, T. W., Spandau, D. F., *et al.* (2014) Primary cilia signaling mediates intraocular pressure sensation. *Proc. Natl. Acad. Sci. USA.* **111**, 12871–12876

223. Luo, N., Lu, J., and Sun, Y. (2012) Evidence of a role of inositol polyphosphate 5-phosphatase INPP5E in cilia formation in zebrafish. *Vision Res.* **75**, 98–107

224. Travaglini, L., Brancati, F., Silhavy, J., Iannicelli, M., Nickerson, E., Elkhartoufi, N., Scott, E., Spencer, E., Gabriel, S., Thomas, S., *et al.* (2013) Phenotypic spectrum and prevalence of INPP5E mutations in Joubert syndrome and related disorders. *Eur. J. Hum. Genet.* **21**, 1074–1078

225. Plotnikova, O. V., Seo, S., Cottle, D. L., Conduit, S., Hakim, S., Dyson, J. M., Mitchell, C. A., and Smyth, I. M. (2015) INPP5E interacts with AURKA, linking phosphoinositide signaling to primary cilium stability. *J. Cell Sci.* **128**, 364–372

226. Xu, W., Jin, M., Hu, R., Wang, H., Zhang, F., Yuan, S., and Cao, Y. (2017) The Joubert syndrome protein Inpp5e controls

ciliogenesis by regulating phosphoinositides at the apical membrane. *J. Am. Soc. Nephrol.* **28**, 118–129

227. Jo, H. S., Kang, K. H., Joe, C. O., and Kim, J. W. (2012) Pten coordinates retinal neurogenesis by regulating Notch signalling. *EMBO J.* **31**, 817–828

228. Kim, J. W., Kang, K. H., Burrola, P., Mak, T. W., and Lemke, G. (2008) Retinal degeneration triggered by inactivation of PTEN in the retinal pigment epithelium. *Genes Dev.* **22**, 3147–3157

229. Venkatesh, A., Ma, S., Le, Y. Z., Hall, M. N., Ruegg, M. A., and Punzo, C. (2015) Activated mTORC1 promotes long-term cone survival in retinitis pigmentosa mice. *J. Clin. Invest.* **125**, 1446–1458

230. Tachibana, N., Cantrup, R., Dixit, R., Touahri, Y., Kaushik, G., Zinyk, D., Daftarian, N., Biernaskie, J., McFarlane, S., and Schuurmans, C. (2016) Pten regulates retinal amacrine cell number by modulating Akt, Tgfβ, and Erk signaling. *J. Neurosci.* **36**, 9454–9471

231. Sakagami, K., Chen, B., Nusinowitz, S., Wu, H., and Yang, X. J. (2012) PTEN regulates retinal interneuron morphogenesis and synaptic layer formation. *Mol. Cell. Neurosci.* **49**, 171–183

232. Xie, C., Lu, H., Nomura, A., Hanse, E. A., Forster, C. L., Parker, J. B., Linden, M. A., Karasch, C., and Hallstrom, T. C. (2015) Co-deleting Pten with Rb in retinal progenitor cells in mice results in fully penetrant bilateral retinoblastomas. *Mol. Cancer.* **14**, 93

233. Hopkins, B. D., Hodakoski, C., Barrows, D., Mense, S. M., and Parsons, R. E. (2014) PTEN function: the long and the short of it. *Trends Biochem. Sci.* **39**, 183–190

234. Conduit, S. E., Dyson, J. M., and Mitchell, C. A. (2012) Inositol polyphosphate 5-phosphatases; new players in the regulation of cilia and ciliopathies. *FEBS Lett.* **586**, 2846–2857

235. Ijuin, T., and Takenawa, T. (2003) SKIP negatively regulates insulin-induced GLUT4 translocation and membrane ruffle formation. *Mol. Cell. Biol.* **23**, 1209–1220

236. Ijuin, T., Yu, Y. E., Mizutani, K., Pao, A., Tateya, S., Tamori, Y., Bradley, A., and Takenawa, T. (2008) Increased insulin action in SKIP heterozygous knockout mice. *Mol. Cell. Biol.* **28**, 5184–5195

237. Hao, Y. M., Liu, Q. J., Wang, R. Y., Cao, Y. P., Zhang, Y., and Zuo, L. F. (2015) Single nucleotide polymorphisms on SHIP2 is associated with type 2 diabetes mellitus in Chinese Han population. *Eur. Rev. Med. Pharmacol. Sci.* **19**, 129–137

238. Sasaki, J., Kofuji, S., Itoh, R., Momiyama, T., Takayama, K., Murakami, H., Chida, S., Tsuya, Y., Takasuga, S., Eguchi, S., *et al.* (2010) The PtdIns(3,4)P(2) phosphatase INPP4A is a suppressor of excitotoxic neuronal death. *Nature.* **465**, 497–501

239. Ji, L., Kim, N. H., Huh, S. O., and Rhee, H. J. (2016) Depletion of inositol polyphosphate 4-phosphatase II suppresses callosal axon formation in the developing mice. *Mol. Cells.* **39**, 501–507

240. Lenk, G. M., Berry, I. R., Stutterd, C. A., Blyth, M., Green, L., Vadlamani, G., Warren, D., Craven, I., Fanjul-Fernandez, M., Rodriguez-Casero, V., *et al.* (2019) Cerebral hypomyelination associated with biallelic variants of FIG4. *Hum. Mutat.* **40**, 619–630

241. Brown, J. E., Rubin, L. J., Ghalayini, A. J., Tarver, A. P., Irvine, R. F., Berridge, M. J., and Anderson, R. E. (1984) myo-Inositol polyphosphate may be a messenger for visual excitation in Limulus photoreceptors. *Nature.* **311**, 160–163

242. Hardie, R. C., and Raghu, P. (2001) Visual transduction in Drosophila. *Nature.* **413**, 186–193

243. Wang, T., and Montell, C. (2007) Phototransduction and retinal degeneration in Drosophila. *Pflugers Arch.* **454**, 821–847

244. Borst, A. (2009) Drosophila's view on insect vision. *Curr. Biol.* **19**, R36–R47

245. Hardie, R. C. (2001) Phototransduction in Drosophila melanogaster. *J. Exp. Biol.* **204**, 3403–3409

246. Suzuki, T., Narita, K., Yoshihara, K., Nagai, K., and Kito, Y. (1995) Phosphatidyl inositol-phospholipase C in squid photoreceptor membrane is activated by stable metarhodopsin via GTP-binding protein. *Gq. Vision Res.* **35**, 1011–1017

247. Fruman, D. A., Rameh, L. E., and Cantley, L. C. (1999) Phosphoinositide binding domains: embracing 3-phosphate. *Cell.* **97**, 817–820

248. Baig, A., Bao, X., Wolf, M., and Haslam, R. J. (2009) The platelet protein kinase C substrate pleckstrin binds directly to SDPR protein. *Platelets.* **20**, 446–457

249. Furutani, M., Tsujita, K., Itoh, T., Ijuin, T., and Takenawa, T. (2006) Application of phosphoinositide-binding domains for the detection and quantification of specific phosphoinositides. *Anal. Biochem.* **355**, 8–18

250. Milligan, S. C., Alb, J. G., Jr., Elagina, R. B., Bankaitis, V. A., and Hyde, D. R. (1997) The phosphatidylinositol transfer protein domain of Drosophila retinal degeneration B protein is essential for photoreceptor cell survival and recovery from light stimulation. *J. Cell Biol.* **139**, 351–363

251. Lee, S. J., Xu, H., Kang, L. W., Amzel, L. M., and Montell, C. (2003) Light adaptation through phosphoinositide-regulated translocation of Drosophila visual arrestin. *Neuron.* **39**, 121–132

252. Lee, S. J., and Montell, C. (2004) Light-dependent translocation of visual arrestin regulated by the NINAC myosin III. *Neuron.* **43**, 95–103

253. Strissel, K. J., and Arshavsky, V. Y. (2004) Myosin III illuminates the mechanism of arrestin translocation. *Neuron.* **43**, 2–4

254. Frechter, S., and Minke, B. (2006) Light-regulated translocation of signaling proteins in Drosophila photoreceptors. *J. Physiol. Paris.* **99**, 133–139

255. Stocker, H., Andjelkovic, M., Oldham, S., Laffargue, M., Wymann, M. P., Hemmings, B. A., and Hafen, E. (2002) Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science.* **295**, 2088–2091

256. Katz, B., and Minke, B. (2018) The Drosophila light-activated TRP and TRPL channels - Targets of the phosphoinositide signaling cascade. *Prog. Retin. Eye Res.* **66**, 200–219

257. Liu, C. H., Bollepalli, M. K., Long, S. V., Asteriti, S., Tan, J., Brill, J. A., and Hardie, R. C. (2018) Genetic dissection of the phosphoinositide cycle in Drosophila photoreceptors. *J. Cell Sci.* **131**, jcs214478

258. Chakrabarti, P., Kolay, S., Yadav, S., Kumari, K., Nair, A., Trivedi, D., and Raghu, P. (2015) A dPIP5K dependent pool of phosphatidylinositol 4,5 bisphosphate (PIP2) is required for G-protein coupled signal transduction in Drosophila photoreceptors. *PLoS Genet.* **11**, e1004948

259. Ratnayake, K., Payton, J. L., Lakmal, O. H., and Karunarathne, A. (2018) Blue light excited retinal intercepts cellular signaling. *Sci. Rep.* **8**, 10207

260. Hong, Z., Pedersen, N. M., Wang, L., Torgersen, M. L., Stenmark, H., and Raiborg, C. (2017) PtdIns3P controls mTORC1 signaling through lysosomal positioning. *J. Cell Biol.* **216**, 4217–4233

261. Chen, J., Ma, Z., Jiao, X., Fariss, R., Kantorow, W. L., Kantorow, M., Pras, E., Frydman, M., Pras, E., Riazuddin, S., *et al.* (2011) Mutations in FYCO1 cause autosomal-recessive congenital cataracts. *Am. J. Hum. Genet.* **88**, 827–838

262. Romano, M. R., Di Menna, L., Scarselli, P., Mascio, G., Madonna, M., Notartomaso, S., Puliti, A., Bruno, V., Battaglia, G., and Nicoletti, F. (2016) Type-1, but not type-5, metabotropic glutamate receptors are coupled to polyphosphoinositide hydrolysis in the retina. *Neurochem. Res.* **41**, 924–932

263. Azadi, S., Brush, R. S., Anderson, R. E., and Rajala, R. V. (2016) Class I phosphoinositide 3-kinase exerts a differential role on cell survival and cell trafficking in retina. *Adv. Exp. Med. Biol.* **854**, 363–369

264. Olivença, D. V., Uliyakina, I., Fonseca, L. L., Amaral, M. D., Voit, E. O., and Pinto, F. R. (2018) A mathematical model of the phosphoinositide pathway. *Sci. Rep.* **8**, 3904

265. Ile, K. E., Kassen, S., Cao, C., Vihtehlic, T., Shah, S. D., Mousley, C. J., Alb, J. G., Jr., Huijbregts, R. P., Stearns, G. W., Brockerhoff, S. E., *et al.* (2010) Zebrafish class 1 phosphatidylinositol transfer proteins: PITPbeta and double cone cell outer segment integrity in retina. *Traffic.* **11**, 1151–1167

266. Hsuan, J., and Cockcroft, S. (2001) The PITP family of phosphatidylinositol transfer proteins. *Genome Biol.* **2**. https://doi.org/10.1186/gb-2001-2-9-reviews3011

267. Köhn, L., Kadzhaev, K., Burstedt, M. S., Haraldsson, S., Hallberg, B., Sandgren, O., and Golovleva, I. (2007) Mutation in the PYK2-binding domain of PITPNM3 causes autosomal dominant cone dystrophy (CORD5) in two Swedish families. *Eur. J. Hum. Genet.* **15**, 664–671

268. Dasgupta, U., Bamba, T., Chiantia, S., Karim, P., Tayoun, A. N., Yonamine, I., Rawat, S. S., Rao, R. P., Nagashima, K., Fukusaki, E., *et al.* (2009) Ceramide kinase regulates phospholipase C and phosphatidylinositol 4, 5, bisphosphate in phototransduction. *Proc. Natl. Acad. Sci. USA.* **106**, 20063–20068

269. Baranova, L. A., Grits, A. N., and Volotovskii, I. D. (1996) Phosphorylation of proteins and phosphoinositides in the axoneme

of the bovine rod outer segments. The effect of structural factor [Article in Russian] *Biofizika.* **41**, 1258–1263

270. Grigor'ev, I. V., Grits, A. I., Artamonov, I. D., and Volotovskii, I. D. (1993) Beta-gamma-transducin controls the metabolism of phosphatidylinositide 4,5-diphosphate in rod outer segments. [Article in Russian] Dokl. *Akad. Nauk.* **331**, 235–237

271. Pfeilschifter, J., Reme, C., and Dietrich, C. (1988) Light-induced phosphoinositide degradation and light-induced structural alterations in the rat retina are enhanced after chronic lithium treatment. *Biochem. Biophys. Res. Commun.* **156**, 1111–1119

272. Schmidt, S. Y. (1983) Cytidine metabolism in photoreceptor cells of the rat. *J. Cell Biol.* **97**, 824–831

273. Im, E., and Kazlauskas, A. (2007) PtdIns-4,5–P2 as a potential therapeutic target for pathologic angiogenesis. *Expert Opin. Ther. Targets.* **11**, 443–451

274. Mustafi, D., Kevany, B. M., Genoud, C., Bai, X., and Palczewski, K. (2013) Photoreceptor phagocytosis is mediated by phosphoinositide signaling. *FASEB J.* **27**, 4585–4595

275. Cioffi, D. L., and Rich, T. C. (2013) Feedback regulation of cone cyclic nucleotide channels by phosphoinositides. Focus on "CNGA3 achromatopsia-associated mutation potentiates the phosphoinositide sensitivity of cone photoreceptor CNG channels by altering intersubunit interactions". *Am. J. Physiol. Cell Physiol.* **305**, C131–C132

276. Contín, M. A., Verra, D. M., Salvador, G., Ilincheta, M., Giusto, N. M., and Guido, M. E. (2010) Light activation of the phosphoinositide cycle in intrinsically photosensitive chicken retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci.* **51**, 5491–5498

277. Phua, S. C., Nihongaki, Y., and Inoue, T. (2018) Autonomy declared by primary cilia through compartmentalization of membrane phosphoinositides. *Curr. Opin. Cell Biol.* **50**, 72–78

278. Xu, W., Jin, M., Huang, W., Wang, H., Hu, R., Li, J., and Cao, Y. (2019) Apical PtdIns(4,5)P(2) is required for ciliogenesis and suppression of polycystic kidney disease. *FASEB J.* **33**, 2848–2857

279. Park, J., Lee, N., Kavoussi, A., Seo, J. T., Kim, C. H., and Moon, S. J. (2015) Ciliary phosphoinositide regulates ciliary protein trafficking in drosophila. *Cell Rep.* **13**, 2808–2816

280. Garcia-Gonzalo, F. R., Phua, S. C., Roberson, E. C., Garcia 3rd, G., Abedin, M., Schurmans, S., Inoue, T., and Reiter, J. F. (2015) Phosphoinositides regulate ciliary protein trafficking to modulate hedgehog signaling. *Dev. Cell.* **34**, 400–409

281. Vieira, O. V., Gaus, K., Verkade, P., Fullekrug, J., Vaz, W. L., and Simons, K. (2006) FAPP2, cilium formation, and compartmentalization of the apical membrane in polarized Madin-Darby canine kidney (MDCK) cells. *Proc. Natl. Acad. Sci. USA.* **103**, 18556–18561

282. Nakatsu, F. (2015) A phosphoinositide code for primary cilia. *Dev. Cell.* **34**, 379–380

283. Lee, J. E., and Gleeson, J. G. (2011) Cilia in the nervous system: linking cilia function and neurodevelopmental disorders. *Curr. Opin. Neurol.* **24**, 98–105

284. Dyson, J. M., Conduit, S. E., Feeney, S. J., Hakim, S., DiTommaso, T., Fulcher, A. J., Sriratana, A., Ramm, G., Horan, K. A., Gurung, R., *et al.* (2017) INPP5E regulates phosphoinositide-dependent cilia transition zone function. *J. Cell Biol.* **216**, 247–263

285. Rao, K. N., Zhang, W., Li, L., Anand, M., and Khanna, H. (2016) Prenylated retinal ciliopathy protein RPGR interacts with PDE6delta and regulates ciliary localization of Joubert syndrome-associated protein INPP5E. *Hum. Mol. Genet.* **25**, 4533–4545

286. Bielas, S. L., Silhavy, J. L., Brancati, F., Kisseleva, M. V., Al-Gazali, L., Sztriha, L., Bayoumi, R. A., Zaki, M. S., Abdel-Aleem, A., Rosti, R. O., *et al.* (2009) Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. *Nat. Genet.* **41**, 1032–1036

287. Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peränen, J., Merdes, A., Slusarski, D. C., Scheller, R. H., Bazan, J. F., Sheffield, V. C., *et al.* (2007) A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell.* **129**, 1201–1213

288. Jin, H., White, S. R., Shida, T., Schulz, S., Aguiar, M., Gygi, S. P., Bazan, J. F., and Nachury, M. V. (2010) The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell.* **141**, 1208–1219

289. Dowler, S., Currie, R. A., Campbell, D. G., Deak, M., Kular, G., Downes, C. P., and Alessi, D. R. (2000) Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *Biochem. J.* **351**, 19–31

290. Lemmon, M. A. (2008) Membrane recognition by phospholipid-binding domains. *Nat. Rev. Mol. Cell Biol.* **9**, 99–111

291. Idevall-Hagren, O., and De Camilli, P. (2015) Detection and manipulation of phosphoinositides. *Biochim. Biophys. Acta.* **1851**, 736–745

292. Gozani, O., Karuman, P., Jones, D. R., Ivanov, D., Cha, J., Lugovskoy, A. A., Baird, C. L., Zhu, H., Field, S. J., Lessnick, S. L., *et al.* (2003) The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell.* **114**, 99–111

293. Lemmon, M. A. (2003) Phosphoinositide recognition domains. *Traffic.* **4**, 201–213

294. Stenmark, H. (2011) Harald Stenmark: hands on FYVE-fingers. Interview by Caitlin Sedwick. *J. Cell Biol.* **192**, 544–545

295. Viaud, J., Lagarrigue, F., Ramel, D., Allart, S., Chicanne, G., Ceccato, L., Courilleau, D., Xuereb, J. M., Pertz, O., Payrastre, B., *et al.* (2014) Phosphatidylinositol 5-phosphate regulates invasion through binding and activation of Tiam1. *Nat. Commun.* **5**, 4080

296. Boal, F., Mansour, R., Gayral, M., Saland, E., Chicanne, G., Xuereb, J. M., Marcellin, M., Burlet-Schiltz, O., Sansonetti, P. J., Payrastre, B., *et al.* (2015) TOM1 is a PI5P effector involved in the regulation of endosomal maturation. *J. Cell Sci.* **128**, 815–827

297. Tamura, N., Oku, M., Ito, M., Noda, N. N., Inagaki, F., and Sakai, Y. (2013) Atg18 phosphoregulation controls organellar dynamics by modulating its phosphoinositide-binding activity. *J. Cell Biol.* **202**, 685–698

298. Hong, N. H., Qi, A., and Weaver, A. M. (2015) PI(3,5)P2 controls endosomal branched actin dynamics by regulating cortactin-actin interactions. *J. Cell Biol.* **210**, 753–769

299. Shirey, C. M., Scott, J. L., and Stahelin, R. V. (2017) Notes and tips for improving quality of lipid-protein overlay assays. *Anal. Biochem.* **516**, 9–12

300. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) Binding of G protein beta gamma-subunits to pleckstrin homology domains. *J. Biol. Chem.* **269**, 10217–10220

301. Rodriguez, M. M., Ron, D., Touhara, K., Chen, C. H., and Mochly-Rosen, D. (1999) RACK1, a protein kinase C anchoring protein, coordinates the binding of activated protein kinase C and select pleckstrin homology domains in vitro. *Biochemistry.* **38**, 13787–13794

302. Balla, T., and Varnai, P. (2009) Visualization of cellular phosphoinositide pools with GFP-fused protein-domains. *Curr. Protoc. Cell Biol.* Chapter 24: Unit 24.4

303. Kaplan, D. R., Whitman, M., Schaffhausen, B., Pallas, D. C., White, M., Cantley, L., and Roberts, T. M. (1987) Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell.* **50**, 1021–1029

304. Gray, A., Olsson, H., Batty, I. H., Priganica, L., and Peter, D. C. (2003) Nonradioactive methods for the assay of phosphoinositide 3-kinases and phosphoinositide phosphatases and selective detection of signaling lipids in cell and tissue extracts. *Anal. Biochem.* **313**, 234–245

305. Munnik, T. (2013) Analysis of D3-,4-,5-phosphorylated phosphoinositides using HPLC. *Methods Mol. Biol.* **1009**, 17–24

306. Chicanne, G., Severin, S., Boscheron, C., Terrisse, A. D., Gratacap, M. P., Gaits-Iacovoni, F., Tronchère, H., and Payrastre, B. (2012) A novel mass assay to quantify the bioactive lipid PtdIns3P in various biological samples. *Biochem. J.* **447**, 17–23

307. Ghosh, A., Sharma, S., Shinde, D., Ramya, V., and Raghu, P. (2019) A novel mass assay to measure phosphatidylinositol-5-phosphate from cells and tissues. *Biosci. Rep.* **39**. https://doi.org/10.1042/BSR20192502

308. Letcher, A. J., Schell, M. J., and Irvine, R. F. (2010) A femtomole-sensitivity mass assay for inositol hexakisphosphate. *Methods Mol. Biol.* **645**, 61–71

309. Tsujita, K., Itoh, T., Ijuin, T., Yamamoto, A., Shisheva, A., Laporte, J., and Takenawa, T. (2004) Myotubularin regulates the function of the late endosome through the gram domain-phosphatidylinositol 3,5-bisphosphate interaction. *J. Biol. Chem.* **279**, 13817–13824

310. Oikawa, T., Yamaguchi, H., Itoh, T., Kato, M., Ijuin, T., Yamazaki, D., Suetsugu, S., and Takenawa, T. (2004) PtdIns(3,4,5)P3 binding is necessary for WAVE2-induced formation of lamellipodia. *Nat. Cell Biol.* **6**, 420–426

≋ASBMB

311. Rajala, A., McCauley, A., Brush, R. S., Nguyen, K., and Rajala, R. V. (2020) Phosphoinositide lipids in ocular tissues. *Biology (Basel).* **9**, 125

312. Irino, Y., Tokuda, E., Hasegawa, J., Itoh, T., and Takenawa, T. (2012) Quantification and visualization of phosphoinositides by quantum dot-labeled specific binding-domain probes. *J. Lipid Res.* **53**, 810–819

313. Maekawa, M., and Fairn, G. D. (2014) Molecular probes to visualize the location, organization and dynamics of lipids. *J. Cell Sci.* **127**, 4801–4812

314. Chen, R., Kang, V. H., Chen, J., Shope, J. C., Torabinejad, J., Dewald, D. B., and Prestwich, G. D. (2002) A monoclonal antibody to visualize PtdIns(3,4,5)P(3) in cells. *J. Histochem. Cytochem.* **50**, 697–708

315. Prestwich, G. D. (2004) Phosphoinositide signaling; from affinity probes to pharmaceutical targets. *Chem. Biol.* **11**, 619–637

316. Hammond, G. R., Schiavo, G., and Irvine, R. F. (2009) Immunocytochemical techniques reveal multiple, distinct cellular pools of PtdIns4P and PtdIns(4,5)P(2). *Biochem. J.* **422**, 23–35