thematic review

Thematic Review Series: Lipids and Lipid Metabolism in the Eye

Lipid second messengers and related enzymes in vertebrate rod outer segments

Norma M. Giusto,[1] Susana J. Pasquaré, Gabriela A. Salvador, and Mónica G. Ilincheta de Boschero

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), B8000FWB Bahía Blanca, Argentina

Abstract Rod outer segments (ROSs) are specialized light-sensitive organelles in vertebrate photoreceptor cells. Lipids in ROS are of considerable importance, not only in providing an adequate environment for efficient phototransduction, but also in originating the second messengers involved in signal transduction. ROSs have the ability to adapt the sensitivity and speed of their responses to ever-changing conditions of ambient illumination. A major contributor to this adaptation is the light-driven translocation of key signaling proteins into and out of ROS. The present review shows how generation of the second lipid messengers from phosphatidylcholine, phosphatidic acid, and diacylglycerol is modulated by the different illumination states in the vertebrate retina. Findings suggest that the light-induced translocation of phototransduction proteins influences the enzymatic activities of phospholipase D, lipid phosphate phosphatase, diacylglyceride lipase, and diacylglyceride kinase, all of which are responsible for the generation of the second messenger molecules.—Giusto, N. M., S. J. Pasquaré, G. A. Salvador, and M. G. Ilincheta de Boschero. **Lipid second messengers and related enzymes in vertebrate rod outer segments. *J. Lipid Res.* 2010.** 51: **685–700.**

Supplementary key words diacylglycerol • diacylglycerol kinase • diacylglycerol lipase • lipid phosphate phosphatase • monoacylglycerol • phosphatidic acid • phospholipase D

The vertebrate photoreceptor cell is composed of different cellular compartments [outer segment (OS), inner segment (IS), and synaptic terminal (ST)], all of which differ in their lipid and protein content (1, 2). The photoreceptor is a highly specialized cell that responds to light stimulus and transmits this response to adjoining neurons for ultimate relay to the visual centers of the brain. The outer segment of the cell is comprised of a stack of many hundreds of densely packed discs, each of which represents a double layer of infolded plasma membrane. It has been suggested that phospholipid moieties in these membranes are metabolically active and are closely involved in the generation of physiological mediators. Modifications in the metabolism of photoreceptor membrane glycerolipids have been linked to the transduction of visual stimuli (3). Rod outer segment (ROS) membranes are composed of 50% protein and 50% lipid, by weight (2). Phospholipids and cholesterol represent nearly 90–95% and 4–6% (w/w) of total lipids, respectively. Rhodopsin accounts for at least 80–85% (w/w) of the total proteins (4–6) in disc ROS membranes. The rest are mainly proteins involved in phototransduction. The composition of mammalian ROS membrane phospholipids indicates that the major phospholipids are phosphatidylethanolamine (PE) and phosphatidylcholine (PC), along with relatively large amounts of phosphatidylserine (PS) (15%). Small amounts (less than 1% each) of sphingomyelin, phosphatidylinositol (PI) and phosphatidic acid (PA) are found in these membranes (7–13). In vertebrates, cholesterol (exclusively as the free sterol) is the principal neutral lipid, comprising

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Agencia Nacional de Promoción Científica y Tecnológica (FONCyT) and the Secretaría General de Ciencia y Tecnología, Universidad Nacional del Sur, Argentina.

Manuscript received 9 October 2009 and in revised form 14 October 2009.

Published, JLR Papers in Press, October 14, 2009
DOI 10.1194/jlr.R001891

Abbreviations: AT, acyltransferase; Cer, ceramide; C1P, ceramide 1-phosphate; DAG, diacylglycerol; DAGL, diacylglycerol lipase; DAGK, diacylglycerol kinase; DHA, docosahexaenoic acid; DRM, detergent-resistant membrane; DROS, ROS from dark-adapted retinas; Gt, transducin; Gtα, α subunit of transducin; Gtβγ, βγ subunits of transducin; IP3, inositol 1,4,5-trisphosphate; IS, inner segment; LPA, lysophosphatidic acid; LPP, lipid phosphate phosphatase; LROS, ROS from light-adapted retinas; MAG, monoacylglycerol; OS, outer segment; PA, phosphatidic acid; PC, phosphatidylcholine; PDE, cGMP phosphodiesterase; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PI(4,5)P2, phosphatidylinositol-4,5-bisphosphate; PIK, phosphatidylinositol kinase; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PTx, pertussis toxin; RK, rhodopsin kinase; ROS, rod outer segment; Sph, sphingosine; S1P, sphingosine 1-phosphate.

[1] To whom correspondence should be addressed.
e-mail: ngiusto@criba.edu.ar

about 8–10 mol% of total lipids and approximately 50–60 mol% of neutral lipids (2).

About half of the phospholipid acyl chains in rod disc membranes are made of docosahexaenoic acid (DHA, 22:6n3) (7, 10, 12, 14–19). These DHA chains provide either maximal fluidity or maximal free volume of acyl chain packing in disc membranes, which is responsible for optimal visual function (2, 20–24). A lack of PUFAs causes abnormalities in visual function (25, 26). On the other hand, it has been reported that cholesterol mediates the function of rhodopsin, a G protein-coupled receptor, by influencing membrane lipid properties, thus reducing acyl chain packing free volume rather than interacting specifically with rhodopsin (27). Detergent-resistant membranes (DRMs) have been isolated from bovine ROS (28, 29). The DRMs represented 8% and 3% of total ROS lipid and protein, respectively. In general, DRMs were more enriched in saturated fatty acids than ROS membranes. DRMs obtained from ROSs were enriched in FFAs and a specific PC fraction that was almost devoid of PUFAs. DRMs contained less PE and PS. Ceramide (Cer) from ROS contained PUFAs but no saturated fatty acids; the converse was true for Cer from DRMs. DHA was diminished in DRM PS and was not detected in DRM FFAs although it was equally abundant in ROS and DRM PE (30). Based on the intringuing lipid composition of ROS membranes and the active phospholipid metabolism, this review explores the main findings on lipid second messengers and their related enzymes in purified ROS from vertebrate photoreceptor cells.

PROTEINS, LIPIDS, AND PHOTOTRANSDUCTION

Signal transduction in outer segments of vertebrate photoreceptors is mediated by a series of reactions among multiple polypeptides forming protein-protein complexes within or on the surface of the disc and plasma membranes. The individual components in the activation reactions include the photon receptor rhodopsin and the products derived from light absorption, the heterotrimeric G protein, transducin (Gt), the four subunits of cGMP phosphodiesterase, PDE6, and the four subunits of cGMP-gated cation channel. Recovery involves membrane complexes with additional polypeptides including Na^+/Ca^{2+}, K^+ exchanger (NCKX2), rhodopsin kinases (RK1 and RK7), arrestin, guanylate cyclases, guanylate cyclase activating proteins (GCAP1 and GCAP2), the GTPase accelerating complex of RGS9-1, (G(β5L)) and the membrane anchor (R9AP). Modes of membrane binding by these polypeptides include transmembrane helices, fatty acyl or isoprenyl modifications, polar interactions with lipid head groups, nonpolar interactions of hydrophobic side chains with lipid hydrocarbon phase, and both polar and nonpolar protein-protein interactions (31).

Photoreceptor cells have a remarkable ability to adapt the sensitivity and speed of their responses to ever-changing conditions of ambient illumination. Temporal resolution of vision also requires a rapid inactivation of the components of the phototransduction cascade so that the cell can quickly respond to the next light event (32). A major contributor to this adaptation is the light-driven translocation mechanism of key signaling proteins into and out of the photoreceptor OS. This mechanism contributes to light adaptation not only in vertebrates (33, 34) but also in *Drosophila* (35). Proteins such as transducin, arrestin, recoverin, and protein phosphatase 2A (33, 36–38) have been reported to be involved in this mechanism. They redistribute in rods in response to bright light. Arrestin predominates at rod inner segments under darkness and shifts to ROS during light exposure. Gtα and βγ subunits shift in the opposite direction in response to light (39–41) and a significant reduction of recoverin is produced in ROS by light (32). In addition to these longitudinal transport processes, lateral translocation of proteins within disc membranes and into DRMs or lipid rafts has come into focus (24, 28, 29, 42). It has been observed that light exposure induces a GTP-dependent translocation of PDE away from the edges of the discs toward the disc center (43). DRMs were shown to contain several signaling proteins, such as Gt and its effector, PDE6, the shorter splice variant of arrestin p44, and the RGS9-Gβ5L complex (28, 29), rhodopsin, recoverin (44), and c-Src (30, 45). Protein redistribution in DRMS is thus a consequence of a light-dependent translocation mechanism.

Phototransduction is the most significant physiological function of photoreceptor cells. Results from several laboratories have reported the presence of lipid components involved in signaling functions in ROS and the modulation of these components by light (18, 46–48). Phototransduction in flies represents the fastest G-protein-signaling cascade known. Unlike vertebrates, *Drosophila melanogaster* phototransduction proceeds via a phospholipase C (PLC)-triggered cascade of PI lipid modifications. The role of lipid messengers such as diacylglycerol (DAG), PA, and phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] levels are key components in the fly phototransduction process (49–51). Experimental evidence from previous research using the visual signaling pathway as a model system links the changes in phospholipid acyl chain composition associated with n-3 fatty acid deficiency to the downregulation of individual steps in a G-protein-coupled receptor (GPCR) signaling pathway (22). In this connection, a large body of research demonstrates that the efficiency of the rhodopsin-dependent steps of the visual process is exquisitely sensitive to membrane lipid composition, particularly to the content of n-3 polyunsaturates (22, 23, 52–56). The earliest events in G-protein-coupled signaling, receptor conformation change, receptor-G-protein binding, and PDE activity are reduced in membranes lacking n-3 polyunsaturated acyl chains. It was observed that the reconstitution of PDE6 and activated Gtα on the surface of large unilamellar vesicles containing $PI(4,5)P_2$, stimulated PDE activity nearly 4-fold above the level observed in the absence of phosphoinositides. PDE activity was found to be similarly stimulated by phosphatidylinositol-3,4-bisphosphate [$PI(3,4)P_2$], and phosphatidylinositol- 4-phosphate [PI(4)P]. Incubation of ROS membranes with phosphoinositide-specific

PLC decreased G protein stimulated activation of endogenous PDE6 (57). The cGMP-gated cation channel of ROS was found to be inhibited by PI(4,5)P_2 (58). Specific interactions between phospholipids and proteins involved in phototransduction such as PC, PE, and PDE6 and between PS and Gtα were observed (59). Among phospholipid classes, DHA-PA was found to display the highest binding to rhodopsin, thus suggesting a possible modulatory role of free DHA and DHA-PA in visual transduction (60).

The regulation of G-protein cellular localization and signaling is a lipid-mediated mechanism (61–63). The majority of G-protein subunits (Gγs) are modified with a thioether-linked isoprenoid geranylgeranyl (C20) attached to Cys residues within the C-terminal "CAAX" box (C is Cys, A is any aliphatic amino acid, and X is the carboxyl terminal residue in which the single cysteine residue is modified with either farnesyl or geranylgeranyl) (64). In contrast, rod-specific Gtγ1 carries the farnesyl moiety (C15), which facilitates light-dependent translocation of transducin from the OS to the inner compartments, thereby contributing to light adaptation (65). Gα-subunits are typically modified with an amide-linked fatty acid myristate (C14:0) at the extreme N-terminal Gly residue and/or with a thioester-linked palmitate (C16:0) at Cys residues near the N termini (61, 66). Uniquely, rod Gtα is heterogeneously fatty N-acylated with C12:0, C14:0, C14:1, and C14:2 moieties (67). The low hydrophobicity of Gtα acyl residues also assists light-dependent translocation of Gt between the outer segment and inner regions of rods (39).

LIGHT REGULATION OF ENZYMES RELATED TO THE GENERATION OF SECOND LIPID MESSENGERS

The activities of enzymes involved in ROS phospholipid turnover such as PLC (3, 46, 68, 69), phospholipase A2 (PLA2) (70), phosphatidylethanolamine N-methyltransferase (PEMT) (71), diacylglycerol kinase (DAGK) (72–75), lipid phosphate phosphatase (LPP) (76), diacylglycerol lipase (DAGL) (77, 78), PI synthase (79), phosphoinositide-3-kinase (PI3K) (80, 81), and PLD (82) have been reported to be modulated by light. Detailed studies on PI3K signaling are described by Rajala in the thematic review in this series on PI3K in the vertebrate retina.

Figure 1 shows the main enzymatic pathways involved in second lipid messenger generation in vertebrate ROS.

PLC

PI(4,5)P_2 breakdown by PLC and its modulation by light have been described in ROS membranes (46, 68, 83–88). PLC produces DAG and inositol 1,4,5-trisphosphate (IP_3). Receptors for IP_3 have not been found in ROS disc membranes. The IP_3-dependent release of Ca^{2+} ions from discs is activated by guanosine trisphosphate and Gtβγ. The increase in calcium concentration in the medium also activates the IP_3-dependent release of Ca^{2+} ions from discs (89). DAG-activated PKC is present in ROS (90–92) and phosphorylates ROS proteins in a light-dependent manner (93). Using immunocytochemistry with antibodies against various phosphoinositide-specific PLC isozymes, Peng et al. (48) found PLC β4-like immunoreactivity in ROS and also observed that this isoform colocalizes with Gα11. ROS from light-adapted retinas (LROS) presented a higher PLCγ1 activity than that observed in ROS from dark-adapted retinas (DROS). In addition, more PLCγ1 was associated with LROS (69). Similar differences were found in rat ROS following in vivo light exposure. It was reported that PLC-γ1 activity in vitro is increased by an allosteric mechanism that involves tyrosine phosphorylation in intact cells (94, 95). It was also reported that increasing PA levels in the cell effectively lowers the PLC-γ1 K_m for PI(4,5)P_2. Tyrosine phosphorylation and PA may work together to maximally activate PLC-γ1 (96). A direct interaction between arrestin and soluble PLC in ROS has also been reported. The addition of the soluble ROS protein arrestin to ROS membranes activated PLC 2- to 3.4-fold (68).

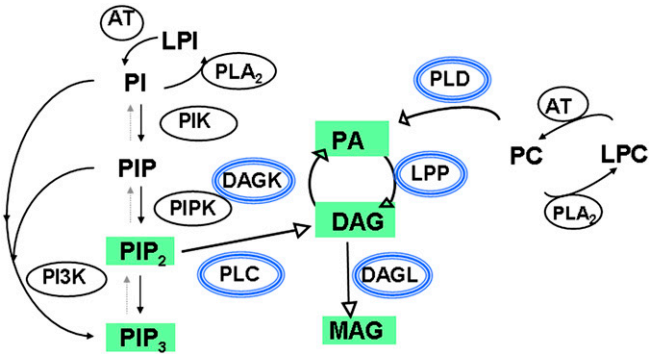

Fig. 1. Lipid second messengers and related enzymes in vertebrate rod outer segments. This figure shows lipid second messenger pathways described in ROS obtained from vertebrate retinas. Enzymatic pathways discussed in the present review are highlighted in blue ovals and their bioactive products are highlighted in green squares. AT, acyltransferase; DAG, diacylglycerol; DAGK, diacylglycerol kinase; DAGL, diacylglycerol lipase; LPC, lysophosphatidylcholine; LPI, lysophosphatidylinositol; LPP, lipid phosphate phosphatase; MAG, monoacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PI, phosphatidylinositol; PIK, phosphatidylinositol kinase; PIP, phosphatidylinositol monophosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol trisphosphate; PIPK, phosphatidylinositol monophosphate kinase; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D.

Phototransduction in invertebrates is mediated by a PLCβ-dependent opening of transient receptor potential (TRP) and TRP-like channels (97). PLCβ cleaves PI(4,5)P_2 and the DAG formed is further cleaved by DAGL to produce PUFAs and MAG. Products derived from PLC and DAGL activities have been found to be involved in the regulation of TRP and TRP-like channel activation (49, 98). In addition, it has been reported that DAG may either directly or indirectly activate channels (99) and that it also stimulates an eye-specific PKC (100).

PLA2 and AT

The acylation-deacylation cycle is also regulated by light. The presence of a light-modulated PLA2 activity in ROS

membranes has been reported (70, 101). A direct link between stimulation of PLA2 and activation of a specific G-protein has been demonstrated in ROS of bovine retina where Gt has been shown to couple the light activation of rhodopsin to stimulation of both cGMP PDE and PLA2 (101, 102). In DROS, PLA2 has been found to be stimulated upon exposure to light (70, 103). Addition of GTPγS to dark-adapted ROS led to a marked increase in PLA2 activity mimicking the effect of light. However, GTPγS added to light-activated ROS did not further enhance the light activation of this phospholipase (70). In addition, it was observed that GDPβS inhibits the light-activated but not the basal activity of PLA2, which is consistent with a role for a stimulatory G-protein in the light activation of PLA2 (101). Experiments in which Gt was removed from DROS with repeated washings and Gt-depleted ROSs were analyzed for phospholipase activity in the presence and absence of light demonstrating a loss of both light- and GTPγS-induced activation of PLA2. Gt removal was not accompanied by a loss of PLA2 activity but rather PLA2 activation by light was abolished. When exogenous Gt was re-added to Gt-poor ROS in the presence or absence of light, light activation of PLA2 in dark-adapted ROSs was restored. Experiments in which the isolated purifed subunits of Gt were added to Gt-poor ROS membranes in the presence or absence of light demonstrated that Gtβγ subunits stimulate PLA2 activity (101). Furthermore, it has been recently reported that calcium-independent PLA2 group VIA may play an important role in the regulation of retinal pigment epithelium phagocytosis of photoreceptor OSs and that it may also be involved in the regulation of photoreceptor cell renewal (104).

It has been demonstrated that: *i*) ROSs exhibit high acyltransferase (AT) activity, lysophosphatidylcholine being the preferred substrate; *ii*) PKC activation produces a significant increase in PE and PI acylation with oleate; *iii*) PKC inhibits PC acylation; and *iv*) PS and PA acylation remain unchanged. It was also observed that ROS PKA activation increases oleate incorporation into PS and PI whereas PC, PE, and PA acylation undergo no changes. These findings suggest that changes in ROS protein phosphorylation produce, in turn, specific changes in AT activity depending on the phospholipid substrate. The effect of light on AT activity in ROS membranes shows that acylation in these membranes undergoes no changes independently of the illumination conditions used (105).

PLD

PLD plays an important role in signal transduction of a variety of cells. PLD hydrolyses PC in order to produce PA and choline (106, 107). PA is a biologically active molecule and can be hydrolyzed by LPP to yield DAG (108). PLD hydrolytic activity can be triggered by a wide variety of agonists such as hormones, neurotransmitters and growth factors (109–111). The use of primary alcohols abolishes PA production catalyzed by PLD and eventually disrupts a number of cell events. Based on the transphosphatidylation reaction, PA has been shown to function as an important lipid second messenger in a wide variety of cell functions such as membrane trafficking, endocytosis, exocytosis, cell growth, differentiation, and actin cytoskeleton reorganization (106, 107).

Two separate genes, encoding mammalian PLD1 and PLD2, and several splice variants have been cloned and described (106, 112). PLD1 is a 120 kDa protein localized in the perinuclear region, has a low basal activity, and is synergistically activated by PKCα and by small G-proteins such as ARF and Rho (112, 113). PLD1 contains two catalytic domains (HKD motifs) that interact through a phospho-histidine intermediate for catalyzing PC hydrolysis. The N-terminal region contains a PKCα binding domain, PX and PH domains, whereas the C-terminal region contains a Rho binding domain. PLD2, whose molecular weight is 100 kDa, contains two HKD domains and requires $PI(4,5)P_2$ binding for activation (114). PLD2 displays a detectable basal activity and is mainly localized at the plasma membrane. It has been reported that PLD expression in the central nervous system is ontogenetically regulated. In rodents, PLD1 and PLD2 mRNA were found to increase from the embryonic to the postnatal period (115). The pattern of PLD expression in the postnatal period is in accordance with synapto- and myelinogenesis in rat brain. In contrast, PLD2 is highly expressed in astrocytes and ependimal cells (115, 116).

Immunohistochemistry studies, particularly on the retina, have demonstrated the presence of PLD in several cellular layers (117). Expression patterns in the developing rat retina showed that PLD1 immunoreactivity appears rarely in neuroblasts of the mantle zone of the primitive retina by embryonic day 13 (118). In the postnatal period (at day 7) when newly-formed segments of the photoreceptor cells appeared, PLD1 was also present (118). The simultaneous detection of PLD1 with glutamine synthetase in the retina occurred first in differentiating neurons after birth and in Müller cells at postnatal day 8; the pattern was conserved thereafter (118). The expression profile of PLD1 during retina development suggested that PLD1 could play important roles in neurons whose differentiation is associated with glutamate, and in glutamate-mediated cellular signaling in Müller cells. Moreover, Western blot analysis using monoclonal anti-PLD1 antibody demonstrated that PLD1 is present in purified bovine ROSs (82). In accordance with these findings, immunohistochemistry of rat and pig retina also demonstrated that ROS layer shows high levels of PLD1 expression (115, 118, 119). PLD2 expression in retina has not been reported to date. Biochemical characterization has demonstrated the presence of a native PLD activity in ROS from bovine retina (120). ROS PLD kinetic parameters are similar to those reported for brain synaptosomal PLD (121).

A wide variety of intracellular regulators of PLD activity have been reported (111, 122). They include phosphoinositides, conventional PKC, ARF and Rho family small GTPases, and tyrosine phosphorylation. ARF family small GTPases, composed of six isoforms, ARF1–6, were identified as PLD activators in bovine brain cytosol and HL-60 cells (123, 124) before the discovery of PLD isozyme variants. ARFs were recognized as activators of PLD1 and they

also activate PLD2, though to a lesser extent. PI(4,5)P2 is an essential cofactor in ARF-dependent activation of PLD1 (112, 123, 125). In vitro activation of PLD2 enzyme by $PI(4,5)P_2$ was much higher than the activation measured for PLD1 (126). PLD2 has been demonstrated to directly interact with and to be activated by the lipid kinase phosphatidylinositol 4-phosphate 5-kinase (PIP5K) through its product $PI(4,5)P_2$ in an in vivo setting, showing that PLD2 activity can be regulated solely by the level of $PI(4,5)P_2$ (127). Rho family small GTPases, RhoA, Rac1, and Cdc42, have also been identified as PLD1 activators (106, 128, 129). It is generally accepted that Gα12 and Gα13-induced responses are exclusively mediated by the small G protein Rho. Nevertheless, Gα12 and Gα13 activate divergent cellular responses including PLC-ε and PLD activation (130). It has been shown that PLD1 activation by Rho family small GTPases is synergistic with ARF, this being consistent with the notion that the binding site of PLD1 for ARF is different from that for the Rho family (131).

The distribution of Rho GTPase family proteins (Rac1, Cdc42, RhoA, and RhoB) in chick retina was demonstrated by immunohistochemistry (132). Moreover, the presence of Rac has been reported in purified ROS (133). Previous findings from Wieland's laboratory (134)demonstrate that soluble fractions obtained from ROS may be ADP-ribosylated by C3 toxin. Additionally, it has been reported that exogenous recombinant RhoA added to ROS binds to activated rhodopsin (135). Furthermore, the presence of monomeric G-protein RhoA in purified ROS has been reported (82). It has also been demonstrated that light exposure of bovine retinas regulates RhoA distribution in photoreceptor cells. RhoA-enrichment in DROS was 2.5- to 4-fold higher than LROS-enrichment. The association pattern of RhoA with ROS membranes is contrary to that previously reported for other proteins such as PI3K, DAGK, and c-Src (10, 45, 73, 81).

As mentioned earlier, the presence of $PI(4,5)P_2$ is a strict requirement for PLD activation by small G-proteins such as ARF and RhoA. $PI(4,5)P_2$-dependent PLD activity is stimulated by cytosolic fractions (where inactive small G-proteins reside) in the presence of nonhydrolizable analogs of GTP (123, 136, 137). Previous experiments on ROS from bovine retina demonstrated that this acidic phospholipid greatly increases PLD activity. The specificity of this effect was evidenced by the ineffectiveness of other acidic phospholipids (PI, PS, PA) in the stimulation of enzyme activity (82). $PI(4,5)P_2$-dependent ROS PLD activity was stimulated in the presence of GTPγS and retinal cytosolic fractions, showing that the enzyme's regulation is similar to that reported for PLD1 in other experimental systems (82, 123).

Experiments carried out on DROS and on LROS demonstrated that light participates in ROS PLD modulation. DROS showed higher $PI(4,5)P_2$-dependent PLD activity levels than those measured in LROS. The increase in $PI(4,5)P_2$-PLD activity observed in DROS is in accordance with previous data that demonstraste PLC activation by light and the consequent diminution of $PI(4,5)P_2$ levels. In this connection, $PI(4,5)P_2$ levels in DROS could act as cofactors for the stimulation of $PI(4,5)P_2$-dependent PLD activity. To date, there is no evidence of oleate-dependent PLD activity in isolated ROS (82, 120). The activation of PLD activity reported in DROS was also coincident with increased RhoA levels derived from immunoblotting studies (82). The involvement of RhoA in light-induced PLD regulation was demonstrated by using ADP-ribosylation catalyzed by pertussis toxin (PTx) and Botulinum C3 toxin. PTx ADP-ribosylates the Gtα subunit of the heterotrimeric Gt retained in this G-protein in its inactive state (GDP bound) whereas C3 toxin specifically ADP-ribosylates Rho family proteins, inhibiting all Rho-dependent cell functions (138, 139). In DROS, where the highest RhoA levels were found, the presence of C3 toxin inhibited PLD activity whereas PTx had no effect on enzyme activity. Recombinant constitutively active RhoA restored LROS PLD activity levels to those found in DROS. LROS in the presence of the active form of RhoA showed PLD activity values similar to those observed in DROS, indicating that RhoA is in its active form and is able to stimulate PLD activity to maximal values in isolated ROS from dark-adapted retinas (82). This is in accordance with previous findings reporting the absence of RhoA GDP dissociation inhibitor and the exclusive membrane localization of Rac1 in purified ROS (132). The highest PLD activity levels and the highest RhoA levels in DROS argue in favor of the involvement of light-induced RhoA translocation in PLD modulation (82). These findings strongly suggest that light not only inhibits PLD activity but also regulates the distribution of RhoA in photoreceptor cells.

It is well known that several PKC isoforms participate in PLD1 activation by RhoA (140). The presence of a variety of kinases in ROS, such as RK, PKC, and PKA, has been previously reported (93, 141–143). It was demonstrated that ROS PLD is activated by PKC (82). It has also been demonstrated that PLD1 phosphorylation by PKA inhibits the interaction between PLD and RhoA (144). In ROS, the presence of cAMP was found to inhibit PLD activity. The regulation of ROS PLD activity by PKC and PKA matches the activation mechanism reported by RhoA-stimulated PLD isoforms (82). The experimental evidence currently available strongly suggests a role for light in the regulation of PLD activity in vertebrates but does not involve the participation of the enzyme in the phototransduction process. A role for PLD gene has been reported in *Drosophila melanogaster*, proving that unlike in vertebrates, this enzyme participates in the phototransduction process (145). The fruit fly phototransduction pathway involves the activation of PLC and depends on subsequent production of DAG and downstream metabolites (49). In the past ten years, different lipid mediators have been found to be involved in this process. In this respect, previous research indicates that PLD null mutant flies exhibit decreased light sensitivity and increased susceptibility to light-induced retinal degeneration (145). Additionally, PLD overexpression rescues null fly PLC from light-induced degeneration. PLD overexpression also restores visual signaling in flies lacking PI transfer protein, which is a key player in the replenishment of $PI(4,5)P_2$ substrate for the

PLC pathway (145). The findings of LaLonde et al. (145) demonstrated that an overexpression of PLD in *Drosophila* facilitates phototransduction by increasing PA levels with the consequent stimulation of PIPK. This PA-stimulated PIPK generates adequate levels of PI(4,5)P_2 and protects the visual system from light degeneration (145). Furthermore, mutations in laza (a gene that codified phosphatidate phosphatase) have been found to reduce light response and to accelerate termination kinetics (146). Loss of laza suppresses the severity of the phenotype caused by mutation of DAGK (RDGA), thus indicating that Laza functions conversely to RDGA (146). It was also shown that retinal degeneration resulting from overexpression of PLD is suppressed by elimination of Laza (146). On the other hand, it has been recently reported that elevated PA levels interrupt membrane transport during rhabdomere biogenesis (51). These data demonstrate the involvement of the PLD pathway not only in light response but also in rhabdomere formation in *Drosophila melanogaster*.

LPP

LPP isoforms, termed LPP1 (PAP2a), LPP2 (PAP2c), and LPP3 (PAP2b) have been cloned. Lipid phosphate monoesters, including PA, lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), and ceramide 1-phosphate (C1P), are dephosphorylated by LPP. They are intermediaries in phospho- and sphyngolipid biosynthesis and also play important roles in intra- and extracellular signaling. Dephosphorylation of these lipids eliminates their signaling activity and generates products with additional biological activities or metabolic fates (108). The key enzymes responsible for the dephosphorylation of these lipid phosphate substrates are called lipid phosphate phosphatases. The different isoforms are distributed between endomembrane compartments and the plasma membrane. The role of LPPs in intracellular lipid metabolism and in the regulation of both intra- and extracellular signaling pathways that control different cellular functions has been described (147–152). PA, LPA, S1P, and C1P are mutually competing substrates of LPPs. LPP1, LPP2, and LPP3 show the maximal catalytic efficiency for LPA, PA, and S1P, respectively (153, 154). PA and its dephosphorylated product, DAG, have important functions in signaling and PA itself emerges as a regulator of pleiotropic signaling responses (155).

As previously described for PLD, LPP activities were evaluated under different illumination conditions using two different ROS populations: DROS and LROS (156). When the effect of light on LPP activity was evaluated using only [2-^{3}H]PA as substrate, a marked inhibition of DAG and MAG production was observed in LROS with respect to DROS (156). The effect of light on LPP activities in ROS obtained from DROS has been previously demonstrated (76). The significant inhibition of LPP activity caused by light and the absence of differences in LPP3 levels between DROS and LROS could be related either to the absence or the presence of a specific protein affected by light-driven translocation (156). The results of studies using PTx and cholera toxin (CTx) coincide with these observations. In PTx-ADP-ribosylated DROS, LPP activity was higher than that found under control light conditions. ADP-ribosylation by CTx inhibited LPP activity with respect to dark control conditions, mimicking the effect of light. All these findings demonstrate that light inhibition of LPP activity in ROS is a Gt-mediated mechanism (76).

As in the case of LPP, PLD activity is inhibited by light (82) whereas the effect of light on DAGK (73) is the opposite. This could indicate that PA and DAG levels are of physiological relevance in ROS under illumination; i.e., under light conditions, an increase in DAGK activity promotes higher PA availability whereas under dark conditions an increase in PLD/LPP activities yields higher DAG availability (156). Experimental conditions favoring PKC phosphorylation inhibit LPP in purified ROS (78). Light stimulates DAG generation by PI(4,5)P_2 -PLC activity (69) and may thus activate ROS PKC. This could be at least one of the light-induced mechanisms involved in LPP modulation. On the other hand, downstream signals by the action of LPP may play a key role in light photoreceptor desensitization/adaptation (156).

The functional significance of light-modulated LPP activities in vertebrate photoreceptors has not been fully elucidated to date. However, a role for LPP in *Drosophila melanogaster* phototransduction has been reported. In *Drosophila* photoreceptors, photoisomerized rhodopsin activates a Gq protein releasing the α subunit, which in turn, activates PLC (157). In *Drosophila*, DAG and PA levels are regulated by the synergistic activity of DAGK and by the recently described LPP gene called laza (158).

LPA and S1P, two alternative substrates for LPP, significantly decrease DAG production from [2-^{3}H]PA in a concentration-dependent manner in DROS. It was also observed that C1P exerts an inhibitory effect on DAG formation only at concentrations higher than 100 μM (156). In LROS, a decrease in DAG formation was observed at 100 μM of LPA and at 20 μM of S1P whereas at 100 μM of S1P, DAG returned to basal levels. C1P diminished DAG production at all the concentrations assayed (10–100 μM). S1P and LPA in DROS and LPA and C1P in LROS produced the highest competitive effect on PA hydrolysis (156).

Immunoblot analysis using Arg-Gly-Asp domain anti-LPP3 revealed that LPP3 is present in ROS (156), a finding reinforced by results observed in the presence of S1P (156). LPP3 has been localized with PLD in caveolin-enriched DRMs where LPP3 metabolizes PLD2-derived PA (159). The presence of PLD activity (120) and the presence of DRMs (29) in purified ROS underscore a physiological role for LPP3 in this system. It was previously demonstrated that LPP from ROS is mainly located in the disc membrane (160). LPP3 protein regulates cell-cell interaction and acts as an ectoenzyme with the catalitic site facing the extracellular space. For this reason, ecto-LPP activity seems to provide lipid messengers in the extracellular medium inside ROS discs.

Low ionic buffer treatment renders a ROS membrane preparation depleted of soluble and peripheral proteins

(depleted ROS) as was observed by SDS-PAGE analysis (156). This treatment demonstrated that LPP3 was only present in membrane fractions. No light-dark differences in LPP3 protein levels were observed either in intact ROS or in depleted ROS (156) but the same LPP activities and similar dark/light differences were observed both in intact ROS and in depleted ROS. On the contrary, it has been reported that DAGK activity in ROS depleted of soluble and peripheral proteins differs from that in intact ROS (74). No enzyme activity was detected in the soluble fraction obtained from depleted DROS and LROS (156), thus corroborating the fact that LPPs are membrane-associated proteins (160). DAG generation from [^{3}H]PA was lower in depleted DROS and LROS in the presence of LPA, S1P, or C1P than in their absence. The effect of alternative LPP substrates obtained in depleted ROS with respect to those observed in intact ROS seems to indicate the involvement of soluble and/or peripheral proteins in the regulation of LPP (156).

In order to determine whether the effect of S1P and C1P on DAG production is due to their competitive characteristics alone or to these and sphingosine (Sph) and Cer, the respective dephosphorylation products of S1P or C1P by LPP, Sph, or Cer were included in PA hydrolysis assays. In intact DROS, Sph and Cer were found to inhibit DAG production to a similar degree at all the concentrations assayed. In intact LROS, Sph (100 μM) and Cer (50,100, and 300 μM) significantly inhibited DAG production. On the other hand, in DROS and LROS depleted of soluble and peripheral proteins, Sph and Cer inhibited DAG formation (156). The pattern observed in the presence of S1P, mainly in DROS, could either be a consequence of LPP competing with PA or due to the formation of Sph from S1P, which may inhibit PA hydrolysis. The last possibility was corroborated by observations of the effect of Sph on DAG generation, where a similar decrease was observed in the presence of S1P and Sph (156). In this respect, it has been reported that Sph not only inhibits DAG formation but also stimulates PA formation, inhibiting LPP on the one hand and stimulating PLD and DAGK on the other (161, 162).

C1P is a potent inhibitor of protein phosphatases, and protein phosphatases have been involved in the inhibition of LPP in isolated ROS (78, 163, 164). It has also been reported that C1P is required for the activation and translocation of other enzymes involved in lipid metabolism such as cytosolic PLA2 (165). Cer-induced inhibition of DAG production from PA has been observed. Additionally, Cer has been found to be involved in the activation of photoreceptor apoptosis (166). Furthermore, it has been suggested that LPP2 and LPP3 play an important role in apoptotic processes. This is supported by the fact that DAG and Sph, the products of LPP, are involved in apoptosis induction (151) whereas S1P and LPA have anti-apoptotic roles (167).

The effects of alternative LPP substrates observed in depleted ROS with respect to those observed in intact ROS appear to indicate the involvement of soluble and/or peripheral proteins in the regulation of LPP. The presence of Sph and Cer strongly inhibits LPP activitiy in depleted DROS, where the level is similar to that found in depleted LROS (in the absence of Sph or Cer), indicating that Sph and Cer are involved in the inhibitory effect of light on LPP. These findings indicate that the competition between PA and LPA or S1P or C1P by the active site of LPP is modulated by the ROS illumination state and by ROS protein association/dissociation. LPP and the products generated by these enzymes could control the cell-death decisions in photoreceptor cells.

DAGL

DAG formed by LPP activity is partially hydrolyzed by DAGL, yielding MAG. DAGL is coupled to LPP and it would appear that these enzyme activities occur in a sort of enzyme complex in which DAG generation and its partial degradation by DAGL immediately occurs. On this premise, DAGL activity has been described in isolated vertebrate ROS (76–78, 160). DAGL is also strongly linked to the following signaling functions: *a*) in platelets, in response to thrombin their combined action with PLC facilitates the release of arachidonic acid (168); and *b*) in neurons, this activity is necessary for the generation of endocannabinoid 2-arachidonoyl-glycerol during retrograde synaptic transmission (169). The hydrolysis of DAG in ROS was observed at a very low substrate concentration (10 nM). This is indicative not only of the physiological importance of the enzyme in ROS but also of the fact that LPP and DAGL work in a coupled form (77, 170). There are three possible pathways of MAG formation using PA as substrate: *i*) LPP/DAGL, *ii*) by the action of PLA/LPP, and *iii*) by the action of PLA/LPA phospholipase. The role of *ii* and *iii* in MAG generation can be discarded because PLA in ROS shows its maximal activity at pH 9.0 with negligible activity at pH 6.5 (70), the pH at which DAGL activity was assayed; furthermore, PLA/LPA phospholipase activity has not been described in ROS to date. It can therefore be concluded that MAG generation in ROS occurs exclusively via the LPP/DAGL pathway (170). Studies on light effects and reconstitution experiments in which depleted membranes were combined with their respective soluble fractions demonstrated that: *1*) DAGL activity is inhibited under light conditions and in depleted DROS, *2*) the activity in depleted DROS reaches similar values to those under light conditions, and *3*) DAGL activity is recuperated when depleted DROS are combined with their respective soluble fractions (170). These results suggest that any soluble or peripheral protein detached from ROS or protein redistribution produced by bleaching stimulates DAGL activity, or that the bleaching process induces the detachment of the DAGL enzyme (170). Both possibilities are feasible because light-adapted ROS are insensitive to protein depletion. The functional significance of light modulation in DAGL activity in vertebrate photoreceptors has not been fully elucidated to date. However, evidence of the role of DAGL in *Drosophila* phototransduction has been reported (171, 172).

LPA, S1P, and C1P have been found to inhibit MAG production in a dose-dependent manner in DROS. In

LROS, it was observed that MAG generation increases at low concentrations of LPA, diminishing until reaching the control value. A decrease of 50% in MAG formation was observed at 100 μM of C1P. In depleted DROS, it was observed that MAG generation decreases in the presence of LPA, S1P, and C1P compared with in their absence (170). In depleted LROS, on the other hand, MAG production diminished by 40% in the presence of C1P (170).

In intact DROS, DAGL substrate (DAG) decreased in the presence of either LPA or S1P (156) and MAG production was inhibited by one percentage point higher. In depleted DROS, LROS, and depleted LROS, DAG was found to decrease in the presence of either LPA or S1P (156), whereas MAG either decreased slightly, was stimulated, or underwent no changes in each of the membrane preparations. A stimulatory effect on DAGL activity therefore occurred in the presence of LPA and S1P.

In summation, DAGL activity was inhibited in the presence of LPA and S1P in intact DROS and was stimulated in depleted DROS and in entire and depleted LROS. The fact that S1P and LPA diminish DAGL activity in DROS and that they produce a stimulatory effect on DAGL in ROS membranes where protein redistribution occurs (LROS) or where soluble or peripheral proteins are detached (depleted DROS) would appear to indicate that S1P and LPA produce their effects either by modulating or interacting with a protein involved in the phototransduction cascade that modulates DAGL activity (170). The effects observed in the presence of LPA could be a consequence either of its detergent-like properties or its role as a lipid mediator with growth factor-like activity (173). The latter can only be corroborated in the presence of LPA-receptor in photoreceptor cells, which has not been reported to date. Sph or Cer generated from S1P and C1P by LPP may modify DAGL activity, as corroborated by the observations of the effect of Sph and Cer on MAG generation in intact DROS. The fact that S1P and C1P in depleted DROS and in intact and depleted LROS have the opposite effect to Sph and Cer suggests that these lipids act independently on DAGL activity (170). In addition, DAGLs from DROS were found to diminish in the presence of Cer, reaching similar values to those of LROS. Cer appeared to induce either protein translocation or detachment of DAGL enzyme from ROS membrane.

The concentration of DAG in small membrane areas, its characteristic negative curvature, and its lack of charge induce unstable asymmetric regions in membrane bilayers. Intermediaries with increased curvature minimize this tension and are essential for membrane fusion and fission processes (174). Consequently, DAG may affect physiological processes by altering the membrane structures and fluidity and may favor the shedding of membranous discs. DAG is a precursor for the cannabinoid receptor CB-1 (175) and a MAG by-product of the termination of protein kinase C/DAG-mediated intracellular signaling (176).

In light of these findings on LPP and DAGL, it can be concluded that the pathway involving LPP/DAGL has an important role in controling PA/DAG/MAG levels. Taken together, the results also suggest that the metabolism of PA/DAG/MAG following light-mediated ROS stimulation plays an active role in organizing signaling responses following the initial light stimulus.

DAGK

DAG was discovered as a lipid second messenger through its involvement in PKC activation (177, 178). In vertebrate ROS, PKC phosphorylates Gtα and Gtβ (179), γ subunits of cyclic GMP-phosphodiesterase (180), rod cyclic GMP-gated channel (181), arrestin (182), RGS9-1, a GTPase-accelerating protein (183, 184), and rhodopsin (185–188). The major proteins phosphorylated in vitro were rhodopsin, RK, phosducin, and a 130 kDa cytosolic protein; however, only rhodopsin was the protein kinase C (PKC) substrate in vivo (188). In vitro experiments indicate that rhodopsin phosphorylation and rhodopsin deactivation can be catalyzed by a substrate-regulated kinase, RK, or a second messenger-regulated kinase, PKC (91). They are present in the ROS and phosphorylate rhodopsin's C-terminal residues equally well in vitro (189). However, in RK ($^{-/-}$) mice, the light-dependent phosphorylation of rhodopsin was eliminated and an abnormal photoresponse was found, revealing that RK is required for normal rhodopsin deactivation (190). In addition, light triggering rapid priming phosphorylations of rhodopsin by RK in Ser(338) followed by a slower phosphorylation in Ser(334), which is regulated by PKC, was suggested on the basis of phosphospecific antibodies against each of these two sites (191). PKC activity in ROS is regulated by Ca^{2+} and lipids so this isozyme(s) belong(s) to the subfamily of conventional PKCs (91).

There is increasing evidence of DAG participation in numerous signaling events. Thus, the strict regulation of DAG levels is crucial for cellular fate. One pathway for DAG metabolization is phosphorylation of the free hydroxyl group to produce PA. DAG is phosphorylated by DAGKs to produce PA, lowering the level of DAG and consequently downregulating PKC activation. Direct interaction between DAGKs and PKCs, DAGKγ association with PKCγ (192), DAGKθ association with PKCε (193), and DAGKζ association with PKCα (194) have also been reported.

DAGK activity was first observed in rat brain by Hokin and Hokin (195) and its activity has been detected in various subcellular compartments in a wide range of tissues and cells. Ten mammalian DAGK isoforms classified into five types have been identified to date (196). The heterogeneity of this gene family is similar to that of PKC and PLC families. Apart from the C1 and catalytic domains, DAGK has other regulatory domains that form the basis for their division into five subtypes. Type I DAGKs have calcium-binding (EF hand motifs) and are more active in the presence of calcium (197–199). Four DAGK isozymes have been cloned using cDNAs from a rat brain library (α, β, γ, and ζ). Interestingly, the mRNA levels of each isozyme are expressed following a different pattern in the central nervous system: DAGKα is expressed in oligodendrocytes; DAGKβ is expressed in caudate-putamen neurons; DAGKγ is expressed in cerebellar Purkinje cells, retina, and ROS (73, 200); and DAGKζ is expressed in cerebellar and cerebral cortices. The molecular diversity together with the differential expression

patterns of DAGK suggest a physiological importance for the enzyme in the neurotransmission (201).

In addition to PKC, DAG has other molecular targets, such as Ras guanylnucleotide-releasing protein, chimerins, and Unc-13 (202). Thus, DAG participates in various cellular responses, including proliferation, differentiation, and cytoskeletal organization (203, 204) through activation of its target proteins. It has been suggested that when DAG signaling is turned off, DAGK subsequently activates a PA signaling event. PA per se has signaling properties and can stimulate vesicle trafficking and bind and regulate the activity of numerous enzymes, including the recruitment of Raf to the Ras signaling complex, Ras-GTPase activating protein (GAP), PKCζ, p21 activated kinases, and protein phosphatase 1 (200). PA binds and activates the mammalian target of rapamycin, a master regulator of cell growth (205). PA generation through DAG phosphorylation can also be the first step in PI resynthesis and DAGK from *Drosophila* retina (rdgA) exemplifies its complex role not only as a key regulator of DAG levels, but also as a pivotal component of the PI cycle (206). PA also activates PI5K 8- to 14-fold (207, 208). Although evidence suggests that PLD associates with and activates PI5K by generating PA (209, 210), it was demonstrated that DAGKζ colocalizes and coimmunoprecipitates with PI5K type Iα and enhances its activity by generating PA (200). Consistent with this, Tolias et al. (211) found that DAGK activity is associated with a complex of proteins that include PI5K, Rac, Rho, Cdc42, and Rho-GDI.

Vertebrate retinal photoreceptor cells have an active phosphoinositide metabolism and several steps in the PI cycle are stimulated by light (10, 46, 48, 68, 69, 72, 74, 79, 83, 84). The two pathways that deplete the PI(4,5)P_2 normally present in membranes (PLC- and PI3K- mediated pathways) are light-activated in vertebrate ROS, although the physiological consequences of their activities are not yet fully clear. A neuroprotective role associated with PI3K pathway activation was suggested (212). As was previously mentioned (see above), LROS were found to have higher PLCγ1 activity than DROS and a higher PLCγ1 was associated with LROS (69). These results are in accordance with results from Ilincheta de Boschero and Giusto (74) who found that membranes obtained from DROS washed in dark with low ionic strength buffer (5Dm), show higher polyphosphoinositide [PI(4)P and PI(4,5)P_2] phosphorylation with respect to that found in membranes obtained from DROS exposed to light and washed in light with low ionic strength buffer (5Lm). This fact suggests that bleaching increases the binding of PLC to the membranes. It has also been reported that DAGK activity in DROS increases their association to membrane in response to light, based on the increased DAGK activity found in 5Lm preparations (74).

LROSs provide a more physiological model for evaluating the light modulation of ROS enzymes. In these experiments, DAGK activity, identified as a DAGKγ isozyme, was reported to be photoassociated and activated by light. However, the possibility that the kinase is also activated in vivo by light, as a result of its association with an activator, was not discarded (73).

DROSs treated with moderate ionic strength buffer after dark or bright light exposure (100Dm and 100Lm) provided a membrane fraction that was differentially enriched in photoassociated peripheral proteins (74, 213). The level of DAGK activity in ROS membranes kept in the dark and washed under physiological ionic conditions was found to be approximately 77% of that measured in nontreated ROS. These data are consistent with previous results, thus suggesting that DAGK is mainly a membrane-bound enzyme (10). The cytosolic fraction of the enzyme (approximately 30% of total activity), which is removed by moderate ionic strength from isolated DROS in dark (100Dm) or after light exposure (100Lm), was not modified by light and was extractable from ROS, either under light conditions or in the dark. The low DAGK activity (23% of the original) found in nonbleached ROS membranes extracted at low ionic strength (5Dm) suggests that this enzyme is predominantly a membrane-associated protein, mostly peripheral, that becomes soluble at low ionic strength.

The finding that bleached membranes extracted at low ionic strength (5Lm) exhibited higher PA synthesis than nonbleached membranes (5Dm) and that the activity of the former was similar to that of membranes treated at nearly physiological ionic strength (100Dm and 100Lm), strongly suggests that light modifies a DAGK activity associated to the membrane. On the other hand, it was observed that light exposure at moderate ionic strength does not modify PA synthesis with respect to dark. This is an indication that in isolated DROS there is no soluble photo-associable enzyme. Data derived from the reassociation of membrane components in the dark are also indicative of a peripheral DAGK and also reveal that light has the ability to modify PA synthesis by changing the association and the activity of the enzyme to the membrane (74).

GTP and its analog, GTPγS, provide a powerful tool for investigating the possible involvement of Gt in modulating DAGK. At moderate ionic strength and with a membrane-bound heterotrimer (GDP bound state), it was observed that the association of soluble proteins induced by light did not modify enzyme activity. However, Gtα seems to be responsible for the inhibition of PA synthesis inasmuch as its selective extraction increases PA synthesis, as could be observed when membranes obtained from DROS treated with GTPγS in moderate ionic strength buffer (100D+GTPγS)m were assayed (74). A similar increased PA synthesis was found in membranes obtained from light-exposed DROS treated with GTP in moderate ionic strength buffer (100L+GTP)m. The possible stimulatory role of Gtβγ cannot be discarded. Using moderate ionic strength extraction, all subunits remained in the membrane. It therefore seems likely that either Gtβγ per se or their interaction with α subunit suppress the inhibitory action of the latter on DAGK activity (74).

All these data could be interpreted in the dynamic context of photoreceptor cells. It has been recently suggested that the directionality of protein translocation can be achieved if the protein is trapped by a binding site (''sink'') localized in a specific cellular compartment. The translo-

cating protein, in turn, relocalizes in the direction of its immobile-binding partner along the concentration gradient created by interaction. In the case of transducin upon light-induced dissociation into Gtα and Gtβγ, these subunits detach from the membrane because each entity has only one lipid anchor. The dissociated state can also be induced in the dark by a myristoylated Gβγ-binding synthetic peptide (MSIRK), which induces separation of the subunits and causes them to dissociate and redistribute throughout the cell. This occurs even without GTP binding (214). If Gt is locked into the activated state by GTPγS binding (214), it cannot return to the OS even in darkness. Interestingly, Gtβγ travels from the OS to the IS as the complex with phosducin (34). Diffusion of the larger complex should be slower than that of free monomeric Gtα (39 kDa), which could at least partially explain why Gtβγ lags behind Gtα while migrating from the OS (33).

In light of these findings, the exposure of bovine retinas to light and subsequent ROS isolation provide a model in which light-related protein translocation occurs. It can be hypothesized that Gtα depletion of membranes by light-induced translocation to the IS increases DAGK activity (unpublished observations).

The functional significance of light-activated DAGK in photoreceptors is not clear. However, downstream signals originating with the activation of PI(4,5)P_2-PLC appear to play a role in light adaptation and desensitization. The activation of PKC and subsequent phosphorylation of several proteins, as described above, takes place. Phosphorylation of rhodopsin seems to prevent further activation of heterotrimeric Gt and thus attenuates visual excitation. Although RK was found to be an absolute requirement for phosphorylating rhodopsin, a slower phosphorylation of Ser334 takes place, regulated by PKC (191). PKC-dependent phosphorylation of PDEγ has been shown to increase binding to PDEαβ (180), which would deactivate the enzyme and reduce cyclic GMP hydrolysis (215). Cyclic GMP levels could also be elevated by PKC phosphorylation of guanylyl cyclase (216). The net result of activation of DAG-dependent PKC would be to regulate visual transduction by decreasing the photoresponse.

Light-induced binding of soluble DAGK (identified as DAGKγ) to ROS membranes (73) and/or increased activity of a peripherally associated DAGK, upregulated by Gt subunits dissociation (74), revert these effects and return sensitivity to ROS by converting DAG to PA.

Membrane recruitment or a transducin-dependent activation of DAGK and PI(4,5)P_2-PLC in vertebrate photoreceptor cells may also represent light-dependent mechanisms for regulating enzymes in the phosphoinositide cycle.

Interestingly, light also stimulates PI synthetase (79). In addition, several ROS proteins undergo light-dependent tyrosine phosphorylation, such as PI3K (80) and PIPKIIα, in bovine ROS (217). It was suggested that Tyr(P) residues generated in a light-dependent manner provide a module for protein-protein interaction between cytosolic photoreceptor proteins and integral membrane/cytoskeletal components of ROS, thus providing docking sites for the transport of cytosolic proteins between the inner and outer segment either through association with the cytoskeleton or an integral ROS protein (45). All these events of importance for maintaining the structural integrity of photoreceptor cells could also form part of visual transduction regulation.

SUMMARY AND CONCLUDING REMARKS

Dark-light differences in the translocation of proteins involved in vertebrate phototransduction (Gtαβγ) and in addition, RhoA, are shown in **Fig. 2**. Other proteins (arrestin, recoverin, phosphatase 2A, and PDE) redistributed under light/dark conditions and involved in phototransduction are not shown. The lipid messengers PA/DAG/

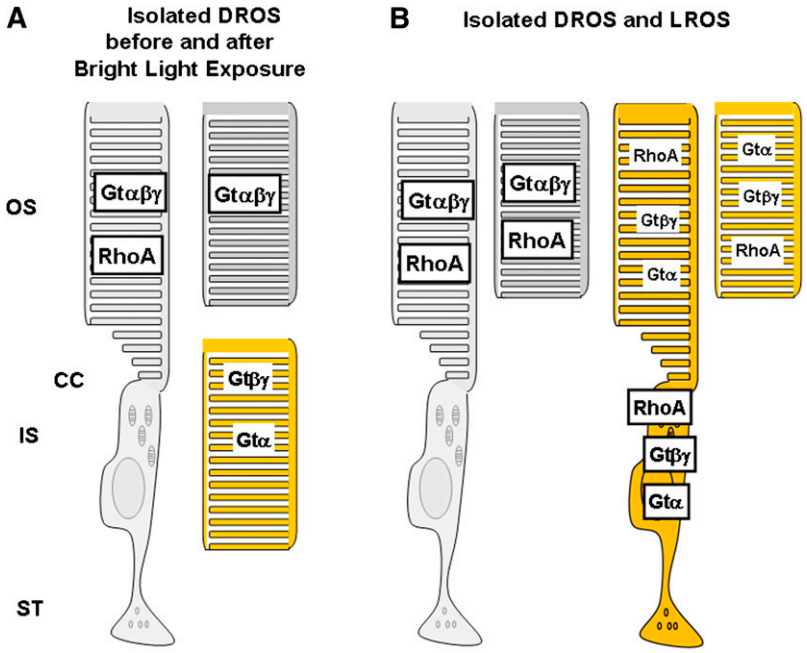

Fig. 2. Schematic representation of transducin and Rho A in two experimental models of isolated rod outer segments from vertebrate retinas under dark and light conditions. Association to ROS membrane of heterotrimeric Gtαβγ and RhoA under dark conditions are shown as black framed squares (A and B models). The membrane dissociation state and translocation of Gtα, Gtβγ, and RhoA under light conditions are shown as unframed squares (in small letters). In A, ROS were obtained from dark-adapted retinas and assayed after a period of dark or bright light exposure. In B, DROS or LROS were obtained from dark-adapted or light-adapted retinas, respectively. Other proteins redistributed under light/dark conditions and involved in phototransduction are not shown. CC, connecting cilium; IS, inner segment; OS, outer segment; ST, synaptic terminal.

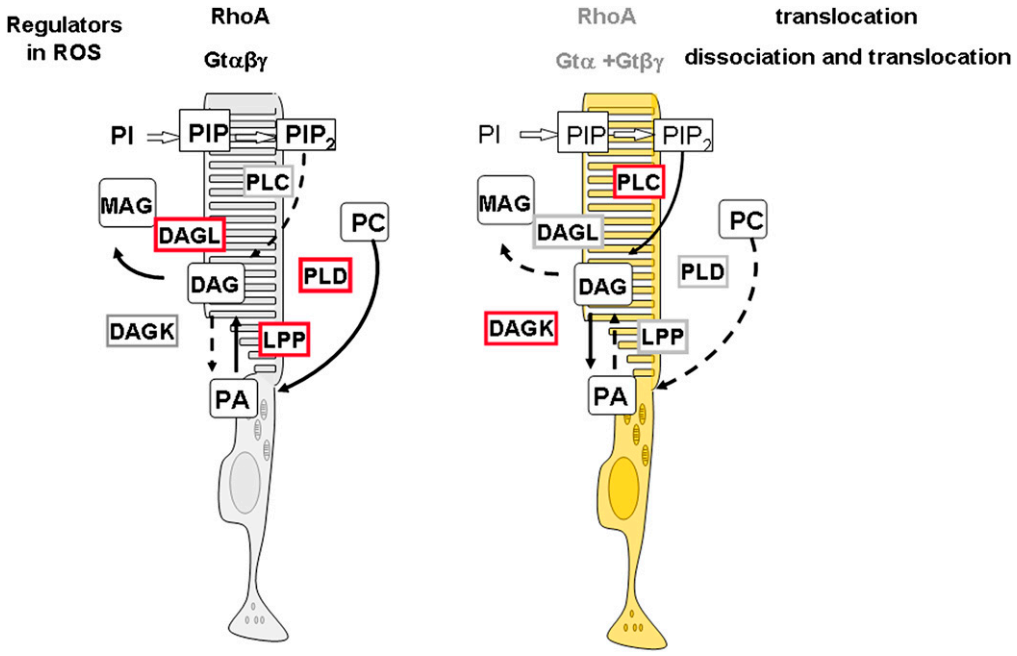

Fig. 3. Dark and light conditions modulate the pathways for PA, DAG, and MAG generation in vertebrate rod outer segments. A model for the involvement of RhoA and transducin in the regulation of these pathways. Stimulated (red squares) and inhibited (gray squares) enzyme activities are shown under dark or light conditions. As shown in Fig. 2, RhoA and transducin in its heterotrimeric form, both present in DROS, positively modulate PLD, LPP, and DAGL. Under light conditions, RhoA translocation decreased PLD activity. Transducin dissociation and its translocation stimulates PIP_2-PLC and DAGK activities. This hypothesis is based on the experiments carried out in the models proposed in Fig. 2 (A and B).

MAG and dark-light differences in their related enzymes, PLD/DAGK/LPP/DAGL, are shown in **Fig. 3**. These figures summarize findings previously reported by Dr. Giusto and her coworkers.

Two experimental approaches for ROS preparation from vertebrate retinas under dark/light conditions (Fig. 2) were followed. In one of these approaches, dark adapted retinas were used for ROS preparation, after which ROS were either maintained in the dark or exposed to bright light (Fig. 2A). In the other approach, DROS and LROS were obtained either from dark-adapted retinas or from retinas exposed to bright light (Fig. 2B).

These models showed that RhoA and Gt [in its trimeric state (Gtαβγ)], which are present in ROS in the dark (Fig. 2A and B), positively modulate PLD, LPP, and DAGL, respectively (Fig. 3A). Gt dissociation (Gtα+Gtβγ) and its translocation to the inner segment (Fig. 2B) could be stimulating PI(4,5)P_2-PLC and DAGK activities (Fig. 3B) whereas PLD, LPP, and DAGL activities are decreased. Rho A translocation to the inner segment in LROS decreased PLD activity. PA-DAG-MAG generation from PC through PLD, LPP, and DAGL are turned on under dark conditions whereas DAG and PA generation from PI(4,5) P_2-PLC and DAGK, respectively, are turned off. Under light conditions this is reversed.

The metabolic pathways described here are regulated by membrane association-dissociation and/or by protein-protein interactions of phototransduction components. Phosphorylation-dephosphorylation mechanisms in the regulation of these pathways were also previously reported (3). This fine regulation in the generation of second lipid messengers appears to be a key component in vertebrate ROS physiology.

The interaction among enzymes controling PA/DAG levels, the identification of PA/DAG-regulated proteins, the eventual participation of these messengers in phototransduction protein translocation, and the overlap between PA/DAG metabolism and the signaling process all constitute interesting topics and open questions for future research aimed at elucidating the physiological role of PA, DAG, and MAG in vertebrate photoreceptor cells.

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