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A Thematic Series on Lipid Signaling: Prologue

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In 1925, Gorter and Grendel proposed the first structural model of the biological membrane as a rigid lipid bilayer sandwiched between protein sheets. Since then, various modified models have emerged, leading to the fluid mosaic model based on the principal concept that a membrane is an oriented, two-dimensional viscous solution of amphipathic proteins and lipids in instantaneous thermodynamic equilibrium. Emphasizing the dynamic structure, this theory has provided potential clues for better understanding of a variety of cellular phenomena. Membrane phospholipids play multiple roles in cells by establishing a hydrophobic barrier, by providing a matrix for the many catalytic processes, and by influencing the functional properties of membrane-associated processes. It is also well documented that lipids in membranes are directly or indirectly implicated in signal transduction in response to various extracellular stimuli. The initial discovery that hormones affect phosphoinositide metabolism was made by Hokin & Hokin (1955), who found that stimulation of pancreas and brain cortex slices by acetylcholine resulted in increased incorporation of ^{32}P orthophosphate into phosphoinositide and phosphatidic acid, but not other phospholipids ("phosphatidylinositol cycle"). In the early 1980s, hydrolysis of phosphatidylinositol(4,5)bisphosphate (PIP_2) by phospholipase C (PLC) was established as a major signaling pathway for Ca^{2+}-mobilizing agonists. PLC was first recognized as one of the *lipid signaling enzymes*. The discovery of the second messenger roles of inositol(1,4,5)trisphosphate (IP_3) and diacylglycerol (DG) derived from PIP_2 has accelerated the progress in signal transduction research. Arachidonic acid (AA), a hydrolysis product of phospholipase A_2 (PLA_2), can also function as a second messenger and the precursor of eicosanoids, potent mediators of inflammation as signal transduction. The PLA_2 superfamily consists of a broad range of enzymes with distinct catalytic properties for substrate phospholipids. Cytosolic PLA_2 ($cPLA_2$), which utilizes a nucleophilic serine for hydrolysis, plays a pivotal role in various cellular responses as the signaling PLA_2. Notably, PLA_2 can also act to down-regulate the cell signals. A typical example is platelet-activating factor acetylhydrolase (PAF-AH), which catalyzes conversion of the bioactive phospholipid PAF to an inactive form that acts as an intercellular lipid mediator chemoattractant. Thus the PAF-mediated signaling is attenuated or cut off by activation of PAF-AH. Phosphatidylcholine (PC) as a source of lipid second messengers can be hydrolyzed by PLA_2 and phospholipase D (PLD). PLD catalyzes hydrolysis of PC to generate phosphatidic acid (PA) and choline. It also catalyzes phosphatidylation in which primary alcohols serve as the nucleophilic acceptor in place of H_2O. PA is rapidly converted to the second messenger DG through the action of phosphatidate phosphohydrolase (PAP). The other route of PA metabolism is the generation of lyso PA (LPA) by specific PLA_2, which is now recognized as an active second agonist that undergoes the signal transduction through the Edg (LPA) receptor. Some recent studies show the direct generation of DG from PC *via* the action of PC-PLC, but they yet largely rely on a putative specific inhibitor, D609. Thus far, the mammalian PC-PLC has not yet been characterized at the molecular level, although some bacterial PC-PLCs have been characterized in terms of amino-acid sequence and cDNAs. Therefore, the exact roles of PC-PLC in signal transduction remain to be defined.

In addition to these glycerolipid-derived messengers, sphingolipid-derived messengers have recently been documented to play a crucial role in the signaling pathway. Sphingomyelin (SM) is a potent precursor for several lipid second messengers (or mediators). SM produces ceramide by SMase (acidic or neutral), which is further metabolized to sphingosine and then to sphingosine-1-phosphate (S1P) *via* the sequential actions of ceramidase and sphingosine kinase (SPHK). Recently accumulating evidence indicates the involvement of ceramide in induction of apoptosis in various cell types. Although the action of S1P as an intercellular or intracellular messenger is still in debate, the majority of reports favor the former. S1P as a second agonist stimulates the Edg (endothelial differentiation gene) receptor and triggers a number of signal transduction pathways. Of much interest is the intimate cross-talk that exists between the signal transduction pathways involving the metabolism of glycerolipids and sphingolipids.

Besides SPHK, which is attracting increasing attention, PI 4-kinase (PIK)/PI 4-P 5-kinase (PIPK), PI3-kinase (P13K), and DG kinase (DGK) belong to the category of *lipid kinases*. The successive actions of PIK and PIPK lead to production of $PI(4,5)P_2$, which is the precursor for the second messengers IP_3 and DG and also acts directly to modify effectors such as actin-binding proteins. PIP_2 is also an essential co-factor for the activity of PLDs (1a, 1b, 2). PI3Ks are a subfamily of lipid kinases that catalyze the specific incorporation of a phosphate molecule into the 3-position of the inositol ring of phosphoinositide. This enzyme is a dual-specificity kinase that can phosphorylate serine/threonine residues in addition of phosphoinositides. PI3K-mediated signaling involves independent pathways

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that lead to MAPK or AKT activation. The major product of PI3K, PI(3,4,5)P_3, and its metabolites, PI(3,4)P_2 and PI(3,5)P_2, the so-called phoxy lipids, bind to PX domains in a variety of proteins involved in cell signaling pathways (PLD, PI3K) and membrane trafficking. DG, a principal second messenger derived from PIP_2 *via* PI-PLC, PC *via* PC-PLC or PLD/PAP, is an endogenous protein kinase C (PKC) activator, and so its intracellular level should be strictly regulated. The enzyme involved in such homeostasis is DG kinase, which catalyzes phosphorylation of DG to produce PA, which also acts as a putative second messenger or protein modulator. Thus, DGK catalyzes a reaction that attenuates the DG level and down-regulates the PKC-mediated signaling.

As with protein phosphatases, *lipid phosphatases* are involved in regulating the signal transduction. They dephosphorylate the lipid second messengers or mediators, *e.g.*, PA and PI(3,4)P_2/PI(3,4,5)P_3. Such enzymes are PAP, PTEN, SH1P-1, and PI(3,4)P_2 4-phosphatase. PAP catalyzes dephosphorylation of PA to generate DG. Accordingly, the homeostatic balance in the levels of PA and DG is largely controlled by the actions of PAP and DGK, although DG lipase is also involved. PTEN (phosphatase and tensin homolog deleted on chromosome ten), a tumor suppressor, has homology to dual-specificity phosphatases: it can function not only as a protein phosphatase but also as a lipid phosphatase. PTEN dephosphorylates focal adhesion kinase (FAK) and also the by-products of PI3K. PTEN specifically catalyzes the dephosphorylation of the 3-position of PI(3,4)P_2 and PI(3,4,5)P_3, leading to down-regulation of the PI3K/Akt pathway. SHIP-1 and PI(3,4)P_2 4-phosphatase act to dephosphorylate PI(3,4,5)P_3 and PI(3,4)P_2 at the 5- and 4-positions, respectively. PI(3)P is the ultimate product of the sequential dephosphorylation steps by these two enzymes.

In addition to a variety of protein or peptide receptors, there are also many *lipid receptors* that play important roles in the cell signal responses. S1P and LPA are the ligands for the Edg receptors. S1P produced by the action of SPHK stimulates Edg 1, 3, 5, 6, 8 receptors, and LPA stimulates Edg 2, 4, 7 receptors. PAF receptor is also a phospholipid (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) receptor. The knockout of the PAF receptors has revealed PAF as the key mediator in allergy and endotoxin shock. There are three receptors for arachidonic acid–related ligands: prostaglandins (PG), leukotrienes (LT), and 2-arachidonoylglycerol (2-AG)/*N*-arachidonoylethanolamine (annandamide).

TABLE I. **Lipid signaling system.**

Lipid signaling enzymes
Phospholipases
Phospholipase A_2 (PLA_2)
Phosphoinositide-specific phospholipase C (PI-PLC)
Phosphatidylcholine-phospholipase C (PC-PLC)
Phospholipase D (PLD)
Sphingomyelinase (SMase)
Platelet-activating factor-acetylhydrolase (PAF-AH)
Lysophospholipase (LPL)
Lipid kinases
Phosphoinositide kinases
Diacylglycerol kinases (DGK)
Sphingosine kinases (SPHK)
Lipid phosphatases
Phosphatidate phosphohydrolase (PAP)
Phosphatidylinositol(3,4,5)P_3/phosphatidylinositol(3,4)P_2 3-phosphatase (PTEN)
Phosphatidylinositol(3,4,5)P_3 5-phosphatase (SHIP)/ Phosphatidylinositol(3,4)P_2 4-phosphatase
Lipid receptors
Edg receptors
Platelet-activating factor (PAF) receptors
Prostaglandin (PG) receptors
Leukotriene (LT) receptors
Cannabinoid (CB) receptors
Intranuclear signaling

Substantial evidence points to the existence of a nuclear phosphoinositide cycle resembling that in the plasma membrane. Moreover, various signaling enzymes, such as PI-PLC, PIPK, DGK, PLA_2, PLD, and SMase are present in nuclei. However, despite much work, we are still far from an adequate explanation of the *intranuclear signaling* events.

The table lists topics closely related to lipid signaling, most of which will be described in this mini-review series (Table I). However, because of limited space, some topics more broadly related to lipid signaling, such as lipid modification of signaling protein molecules (GPI-anchoring, myristoylation/palmitoylation, isoprenylation), are omitted.