The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist

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Eyster KM. The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. Adv Physiol Educ 31: 5–16, 2007; doi:10.1152/advan.00088.2006.—Reviews of signal transduction have often focused on the cascades of protein kinases and protein phosphatases and their cytoplasmic substrates that become activated in response to extracellular signals. Lipids, lipid kinases, and lipid phosphatases have not received the same amount of attention as proteins in studies of signal transduction. However, lipids serve a variety of roles in signal transduction. They act as ligands that activate signal transduction pathways as well as mediators of signaling pathways, and lipids are the substrates of lipid kinases and lipid phosphatases. Cell membranes are the source of the lipids involved in signal transduction, but membranes also constitute lipid barriers that must be traversed by signal transduction pathways. The purpose of this review is to explore the magnitude and diversity of the roles of the cell membrane and lipids in signal transduction and to highlight the interrelatedness of families of lipid mediators in signal transduction.

THE CELLS OF A MULTICELLULAR ORGANISM use chemical messengers to communicate with each other, and single-celled organisms respond to chemical messengers in their environment. Moreover, the intracellular compartments and organelles of a cell must communicate with each other. The chemical messengers used in cellular communication are either water soluble or lipid soluble. Cells, themselves, are defined by the physical presence of the lipid bilayer membrane barrier that separates the inside of a cell from the outside of a cell, and intracellular compartments are defined by lipid membrane barriers as well. Extracellular and intracellular water-soluble chemical messengers cannot cross cellular membranes, so their messages must be transduced across the membrane. Lipid-soluble messengers can cross cell membranes and communicate directly with the contents of the cell by binding to intracellular receptors. Signal transduction is the field of science that seeks to comprehend the mechanisms that cells have developed to interpret the messages carried by extracellular and intracellular chemicals into messages that the cell can understand. Thus, the field of signal transduction is important because of its fundamental role in cellular communication and regulation of cellular responses.

The field of signal transduction is also important because cellular communication often goes awry in pathological situations. Many diseases result from aberrant communication among cells or from problems with the machinery of signaling pathways (6, 27, 28, 54, 67). For example, mutations in components of signal transduction pathways that regulate mitosis can result in tumorigenesis (6, 34, 46, 89, 90).

Cellular physiology requires the presence of a barrier between cells, but the cell membrane is not merely a barrier that must be traversed; rather, the membrane and its constituent lipids are also indispensable participants in many events of signal transduction. Reviews of signal transduction have often focused on the cascades of protein kinases and protein phosphatases and their cytoplasmic substrates that become activated in response to extracellular signals. Lipids, lipid kinases, and lipid phosphatases have not received the same amount of attention as proteins in studies of signal transduction. It is important to not only acknowledge the contribution of the membrane and lipids to signal transduction but also to recognize that this contribution is substantial. For example, membrane lipids participate as components of signal transduction pathways and as docking sites for cytoplasmic signaling proteins, and they give rise to cleavage products that act as ligands or substrates for other signaling molecules. Nonmembrane lipids have a role in signal transduction as well; lipids serve as ligands, and posttranslational lipid modifications provide a means for proteins to associate intimately with the membrane. The magnitude and diversity of the roles of the membrane and lipids in signal transduction can be easily overlooked. Therefore, this review focused on the role of the cell membrane and its constituent lipids in signal transduction.

A look at the big picture sets the stage for a discussion of the details of lipid signal transduction. The first important aspect of the big picture is the interrelatedness among the lipid signaling pathways (Fig. 1). The details of these complex interactions are described in the following text, but several examples can be pointed out here. As shown in Fig. 1, phosphatidylinositol is the parent molecule for a number of lipid messengers. The presence in a given cell of one phospholipase (PL) enzyme versus another determines whether a parent lipid molecule such as phosphatidylinositol gives rise to arachidonic acid (AA), phosphatidic acid (PA), or platelet-activating factor (PAF). The interrelatedness of lipids involved in signal transduction is further illustrated by the fact that the polar head group of sphingomyelin, phosphorylcholine, is the same as the polar head group of phosphatidylinositol. Many other examples of the interrelatedness of lipid signaling pathways are described in the following text.

The second important aspect of the big picture is the significant amount of interaction among the signal transduction pathways activated by lipid mediators (Fig. 2). Many of the
lipid mediators leave the cells in which they originate and bind to G protein-coupled receptors (GPCRs) in the membrane of the same cell or of neighboring cells. Many of the lipid signals converge on PLC, phosphatidylinositols (PIs), or Ca\(^{2+}\) (Fig. 2). As described in the following text, the interrelatedness and interconvertibility of many of the lipid mediators contribute to the complexity of these pathways.

Lipid Components of the Cell Membrane

A discussion of membrane lipids and lipid structures will facilitate this review of lipid signal transduction and will clarify the interrelatedness of lipid mediators. At the most fundamental level, the cell membrane is composed of a lipid bilayer with polar hydrophilic head groups that face the cytoplasmic and extracellular spaces and hydrophobic tails that face each other. Integral membrane proteins are embedded in the lipid bilayer or associated with the membrane by posttranslational attachment of a lipid group to the protein.

Phospholipids comprise the most abundant class of membrane lipids. Phospholipids are composed of two fatty acid tails, glycerol, a phosphate group, and a polar head group (Fig. 3). Of the phospholipids, phosphatidylethanolamine, phosphatidylcholine, and PI are found primarily in the inner leaflet of the membrane, whereas phosphatidylcholine is found primarily in the outer leaflet of the membrane. Phospholipids do not flip flop from one leaflet to the other independently. A class of enzymes called phospholipid scramblases catalyze the movement of phospholipids from one leaflet to the other (77).

Sphingomyelin and related molecules such as ceramide and sphingosine make up another class of compound membrane lipids (Fig. 3). Sphingomyelin is composed of sphingosine, a fatty acid, a phosphate group, and choline. Ceramide and sphingosine are released by the sequential cleavage of sphingomyelin. The properties of sphingomyelin molecules allow them to form hydrogen bonds with each other within the membrane. Cholesterol is also an important component of the cell membrane of eukaryotic cells (Fig. 3). In eukaryotic cells, cholesterol fills the gaps among sphingomyelin molecules. The result is membrane regions with high concentrations of sphingomyelin and cholesterol called lipid rafts (15, 65, 83). Some protein components of signal transduction pathways have an affinity for lipid rafts, whereas others are excluded (15). Extracellular proteins with glycosylphosphatidylinositol (GPI) anchors and myristoylated or palmitoylated intracellular proteins associate with lipid rafts (65). Transmembrane proteins also enter lipid rafts, but the mechanisms that regulate the entry of extracellular ligands are shown in italics.

Fig. 1. The interrelatedness and interconvertibility of the lipid signaling pathways discussed in the text. PLA\(_2\), phospholipase A\(_2\); PLC, phospholipase C; PLD, phospholipase D; LPC, lysophosphatidylcholine; COX, cyclooxygenase; LOX, lipoxygenase; CYP2C, cytochrome P-450 2C; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPETE, hydroxyperoxyeicosatetraenoic acid; EETs, eicosatetraenoic acids; PAF, platelet-activating factor; PA, phosphatidic acid; DAG, diacylglycerol; IP\(_3\), inositol (1,4,5)-trisphosphate; PI(4)P, phosphatidylinositol (4)-phosphate; PI(4,5)P\(_2\), phosphatidylinositol (4,5)-bisphosphate; PI(3,4,5)P\(_3\), phosphatidylinositol (3,4,5)-trisphosphate; PI5K, phosphatidylinositol 5-kinase; PI3K, phosphatidylinositol 3-kinase; C1P, ceramide-1-phosphate; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphorylcholine.

Fig. 2. The interrelatedness of lipid ligands and intracellular lipid mediators. Many lipid mediators [prostaglandins (PG\(_E_2\), PG\(_F_2\), and PG\(_I_2\)], thromboxane A\(_2\) (TXA\(_2\)), leukotrienes (LTs), S1P, PAF, LPC, SPC, and lysophosphatidic acid (LPA)] act as ligands at G protein-coupled receptors (GPCRs), which activate PLC-\(\beta\). In turn, PLC-\(\beta\) cleaves the membrane lipid PI(4,5)P\(_2\), to release DAG and IP\(_3\). IP\(_3\) releases Ca\(^{2+}\) from intracellular stores, and Ca\(^{2+}\) and DAG together activate PKC. Growth factors (GFs) activate receptor protein tyrosine kinases (RTKs) and the same PI(4,5)P\(_2\), breakdown via PLC-\(\gamma\). GFs also activate PI3K as well as the MAPK pathway. Ca\(^{2+}\) and MAPK together activate cytoplasmic PL\(_A_2\) (cPL\(_A_2\)) to convert phosphatidylcholine to LPC plus arachidonic acid (AA). Ca\(^{2+}\) and MAPK also activate lysopAF acetyltransferase (lysoPAF AT) to convert LPC to PAF. MAPK stimulates sphingosine kinase, which converts sphingosine to S1P. PI(4,5)P\(_2\), PKC, and the small G proteins RaL and ARF6 together activate PLD to convert phosphatidylcholine to PA. PA activates PI5K as well as the serine/threonine protein kinase Raf. It can also be converted to DAG or LPA (dotted lines). PI(3,4,5)P\(_3\) forms a binding site for phosphoinositide-dependent protein kinase 1 (PDK1) and the serine/threonine protein kinase Akt/ PKB. PDK1 then phosphorylates Akt/PKB as part of its activation. Factors that serve as extracellular ligands are shown in italics.
association of transmembrane proteins with lipid rafts are poorly understood. There does not appear to be a specific protein domain that targets transmembrane proteins to lipid rafts (65). The presence of sphingomyelin and, therefore, lipid rafts allows sphingomyelin to serve an important structural role in the cell membrane as well as to participate in signal transduction pathways.

A fatty acid is composed of a long-chain aliphatic carboxylic acid. Fatty acids may be saturated (no double bonds in the hydrocarbon chain), monounsaturated, or polyunsaturated (one or more double bonds in the hydrocarbon chain, respectively). The presence of a double bond in a fatty acid adds a kink. Fatty acids that have more double bonds have more kinks in their structure and take up more space. Thus, as the number of double bonds increases, the membrane becomes more fluid because the fatty acids fit less closely together. Similarly, the fewer the number of double bonds (saturated fatty acids), the more tightly the fatty acid tails fit together and the less fluid the membrane.

Physiologically, this means that hydrolysis of a given species of membrane lipid may yield different fatty acids. Also, the identity of a lipid is determined by its parent structure and not by its fatty acids, since the fatty acids may vary.

**Phospholipases**

The family of PL enzymes cleaves membrane phospholipids; each of the PLs acts at a different site on the phospholipid (Fig. 4). Both cell membrane and intracellular membrane phospholipids are substrates for PLs. The products that result from these cleavages are involved in a variety of aspects of signal transduction.

Typically, more is taught and written about PLC than other PLs. PLC is the most familiar of all the PL enzymes involved in signal transduction. PLC cleaves the polar head phosphate from phospholipids, leaving behind a molecule of diacylglycerol (DAG). The polar head phospholipid is released into the cytoplasm, whereas DAG with its fatty acid chains remains an integral component of the membrane.

Two isoforms of PLC are activated in response to extracellular ligands. The β isoform of PLC (PLC-β) is activated via ligand stimulation of GPCRs and Gq isoforms of heterotrimeric G proteins (57) (Fig. 5A). Examples of ligands that activate PLC-β via GPCR/Gq include prostaglandin (PG)F2α, PGE2 (via the EP3 receptor), prostacyclin, and thromboxane (55). In contrast, stimulation of the γ isoform of PLC (PLC-γ) is achieved by ligand-activated enzyme-linked tyrosine kinase receptors (7) (Fig. 5A). Examples of ligands that activate PLC-γ via enzyme-linked tyrosine kinase receptors include EGF, PDGF, FGF, and NGF (7). Two second messengers are released by the PLC cleavage reaction. The polar PI head group [PI (1,4,5)-trisphosphate (IP3)] is liberated upon cleavage by PLC and released into the cytoplasm, where it acts as a second messenger (Fig. 5). IP3 releases Ca2+ from intracellular stores (4) (Fig. 2). Ca2+, in turn, activates its own series of signaling functions (78), including the activation of cytoplasmic PL-A2 (cPL-A2) (8). DAG is the additional second messenger. DAG was the first membrane lipid to be identified as a direct component of a signal transduction pathway (39). The first of the cellular messenger functions of DAG to be identified was the activation of the conventional (α, β, β′, and γ) and novel (ε, η, δ, and θ) isoforms of PKC (50) (the atypical isoforms of PKC, PKC-α, and -ζ, are not activated by DAG). In addition, DAG activates chimaerins, proteins with GTPase-activating protein activity toward Rac (87). DAG functions are confined to the lipid bilayer (Fig. 5A). Interestingly, the conventional and novel isoforms of PKC bind to the membrane phospholipid phosphatidylinerine as well as to DAG.
to achieve activation (82). Since DAG and phosphatidylserine are confined to the membrane, the conventional and novel isoforms of PKC must translocate to the membrane for activation.

The body of literature describing the family of PLD isoforms is much smaller than that for PLC. The two isoforms of PLD (PLD1 and PLD2) are both palmitoylated as a posttranslational modification and both contain pleckstrin homology (PH) and phox homology (PX) lipid binding domains. The palmitoylation and two lipid binding domains all contribute to the association of PLD isoforms with membrane lipids (21). PLD cleaves the polar head group from phospholipids, leaving PA behind. Thus, PA is composed of the fatty acid chains, glycerol, and phosphate group of the initial phospholipid (Fig. 4).

Activation of PLD1 requires association with a complex of proteins and lipids. These include two of the small GTPases, RaLA and ARF6, the conventional PKC isoform PKC-α, and the membrane phospholipid PI(4,5)P2 (introduced above as the substrate for PLC) (25) (Figs. 2 and 6). PLD does not appear to be directly associated with a receptor; that is, PLD is not activated by a GPCR/G protein or by an enzyme-activated receptor as are the PLC isoforms described above. Rather, PLD isoforms are activated by downstream effectors of other signaling pathways. The activation of PKC by PLC-β or PLC-γ, as described above, and activation of guanine nucleotide exchange factors that activate RaLA and ARF6 result in the activation of PLD (36). Therefore, PLD responds to activation by extracellular ligands, but the response is more indirect than direct.

The most important substrate for PLD in regard to signal transduction is phosphatidylcholine, which yields PA and choline upon cleavage by PLD (25) (Figs. 1, 4, and 6). PA is a lipid mediator of PLD and carries out specific signal transduction functions, whereas choline has not been shown to have signaling properties. One function of PA is to activate the enzyme PI-4-phosphate 5-kinase (PI5K), which is responsible for the phosphorylation of PI 4-phosphate to yield PI(4,5)P2 (25). Thus, the PLD1-PA pathway is involved in the synthesis of PI(4,5)P2, which is the substrate for the PLC pathway. Also, because PI(4,5)P2 is required for the activation of PLD1, synthesis of PI(4,5)P2 can be considered a positive feedback step in maintaining the activation of PLD1 (Fig. 2). PA is also involved in the activation of the serine/threonine protein kinases Raf and mammalian target of rapamycin (25). In addition, PA and DAG are interconvertible. PA can be converted to DAG by phosphatidic acid phosphohydrolase and back by DAG kinase (Figs. 1 and 4). DAG has its own set of signaling functions, as described above.

The PLA2 family is much larger than the PLC or PLD families, with multiple forms of both cytosolic and secretory isoforms (53). Secretory PLA2 (sPLA2) isoforms are stored in secretory granules and secreted in response to stimuli (53). sPLA2 isoforms are activated by Ca2++. Since the levels of Ca2+ in the extracellular space are high enough to maintain the activity of sPLA2, the actual regulation must occur at the points of gene transcription (regulation of availability of the gene product) and of protein secretion. Factors that increase the transcription and secretion of sPLA2 include inflammatory...
mediators such as TNF-α and IL-1β and downstream mediators of the 12/15-lipoxygenase (LOX) pathway such as 12(S)- or 15(S)-hydroxyoctadecadienoic acid (HODE) and 12(S)-hydroxyeicosatetraenoic acid (HETE) (44). cPLA2 isoforms are activated by increases in intracellular Ca\(^{2+}\) and by phosphorylation by several MAPKs (48). Therefore, an increase in intracellular Ca\(^{2+}\) by activation of the PLC-IP3 pathway to release intracellular Ca\(^{2+}\) (Fig. 2) or by opening membrane Ca\(^{2+}\) channels to allow Ca\(^{2+}\) entry from the extracellular space activates cPLA2. Also, activation of the MAPK pathway by growth factors (such as EGF, PDGF, FGF, and NGF) (7) activates cPLA2 through phosphorylation by MAPK. Moreover, the literature indicates that multiple levels of interaction exist among the various isoforms of PLA2 in both regulation and function. For example, the activation of cPLA2 increases the concentrations of HETE and HODE, which stimulate the transcription of sPLA2 (44), and cPLA2 and sPLA2 interact in the cleavage of lipids (8).

All of the isoforms of PLA2 cleave membrane phospholipids to yield one free fatty acid plus lysophospholipid (Fig. 4). The most physiologically important substrate for PLA2 is phosphatidylcholine (Figs. 1 and 7). The fatty acids that are incorporated into phosphatidylcholine vary, but the most common...
and most physiologically important fatty acid that can be released from phosphatidylcholine by PLA₂ is AA (eicosatetraenoic acid) (53). The resulting lysophosphatidolipid is lysophosphatidylcholine (LPC) (69). LPC binds as a ligand at specific GPCRs (33) and activates PLC-β to release IP₃ and DAG, with resultant increases in intracellular Ca²⁺ and activation of PKC (91) (Fig. 2). In addition, LPC regulates MAPK in a cell type-dependent manner (91). The activation of MAPK by LPC occurs by a link between the GPCR and phosphatidylinositol 3-kinase (PI3K) (93). The GPCR for LPC is found at highest levels in the spleen and thymus, so it is considered to play an important role in immune system regulation (94). AA released by PLA₂ is the parent molecule for a variety of lipid mediators. The action of COX on AA yields the PG family of mediators. The action of CYP2C on AA gives rise to epoxyeicosatrienoic acids (EETs) (35). EETs are metabolized to dihydroxyepoxyeicosatrienoic acid (DHETE) by soluble epoxide hydrolase (sEH). The LOX isoenzymes give rise to LTs, EET, HETE, HPETE, and lipoxins. LysoPAF AT converts LPC, also released by the action of PLA₂ on phosphatidylcholine, into PAF.

The action of PLD plus PLA₂ on a phospholipid yields lysophosphatidic acid (LPA; Fig. 4), which acts as an extracellular ligand by binding to specific edg/LPA GPCRs (42). LPA stimulates the activity of PLC-β, PLD, PLA₂, the small GTPase Rho, and the nonreceptor protein tyrosine kinase Src (23). Diverse roles have been ascribed to LPA, including cell proliferation, survival, and invasion (23).

**Platelet-Activating Factor**

PAF is a modified phospholipid whose potent biological activities have been recognized for several decades (68). PAF is derived from the membrane phospholipid phosphatidylcholine. cPLA₂ cleaves one fatty acid from phosphatidylcholine to yield LPC. Acetyl-CoA:lysoPAF acetyltransferase (lysoPAF AT) then acetylates LPC to yield PAF (61) (Figs. 4 and 7). Both cPLA₂ and lysoPAF AT are activated by Ca²⁺ and via phosphorylation by MAPK family members such as ERK1, ERK2, and p38. However, specific MAPKs are responsible for phosphorylating the two enzymes in different cellular responses. For example, ERK1/2 phosphorylates cPLA₂, whereas p38 MAPK phosphorylates lysoPAF AT (61). In cellular stress responses, p38 MAPK activates both enzymes in the PAF synthetic pathway (61). PAF can be found in the circulation, but data have suggested that the PAF that remains associated with the membrane of the cell of synthesis is the more bioactive fraction. Membrane-associated PAF acts in a paracrine manner at GPCRs on neighboring cells (12, 68). Receptor binding by PAF activates PLC and cPLA₂. The result of PLC activation is a downstream increase in intracellular Ca²⁺ and the activation of PKC (Fig. 4). The result of PLA₂ activation is increased substrate for the COX, LOX, and CYP2C pathways as well as for the PAF pathway (Fig. 7). The result is a positive feedback effect in that the increased intracellular Ca²⁺ contributes to the activation of both cPLA₂ and lysoPAF AT. This enzyme activation coupled with increased substrate results in greater PAF synthesis (Fig. 2). The functions of PAF include inflammatory responses, stress responses, ovulation, and blastocyst implantation (12, 61, 68).

**Lipid Kinases and Phosphatases**

The focus in signal transduction, both in teaching and experimental design, is usually on protein kinases and protein phosphatases. As the understanding of lipid signaling molecules expands, the functions of lipid kinases and lipid phosphatases are proving to be equally important to those of proteins. The PI3K pathway is the most thoroughly studied of the lipid phosphorylation pathways. PI3K is a downstream effector of mitogens (90). Mutations of this pathway that result in unregulated activation have been implicated in cancer (90); hence, the broad interest in the PI3K pathway. Under normal circumstances, PI3K is activated by extracellular ligands such as insulin (19, 72) and other mitogenic growth factors (90). PI3K activation is increased substrate for the COX, LOX, and CYP2C pathways (Fig. 2). The parent substrate for PI3K is PI, the same phospholipid that is the substrate for the PLC and PLD pathways. There are many isoforms of PI3K; some of them prefer PI(4,5)P₂ as a substrate to yield PI(3,4,5)P₃ [PI(3,4,5)P₃] as the end product (Figs. 5B and 8) (84). Other isoforms of PI3K prefer PI as substrate to yield PI (3-) monophosphate [PI(3)P] as the end product (84). Both PI(3)P and PI(3,4,5)P₃ act as docking sites for proteins that translocate...
to the plasma membrane (Fig. 8). Proteins with FYVE or PX domains (named for the first four proteins identified with the domain: Fab1, YOTB, Vac1p, and EEA) (18, 45) bind to PI(3)P. PI(3,4,5)P3 serves as the binding site for proteins containing PH domains [such as Akt/PKB, phosphoinositide-dependent protein kinase 1 (PKD1), Btk family tyrosine kinases, and PLC-γ1] (17, 45, 70). Thus, the activity of PI3K results in the formation of binding sites for proteins at the intracellular face of the plasma membrane (Fig. 8).

A variety of protein kinases are activated after their binding to PI(3,4,5)P3. For example, Akt/PKB and PKD1 bind to PI(3,4,5)P3 at the cytoplasmic face of the membrane through their PH domains. Binding of these two enzymes to membrane lipids brings them into juxtaposition with each other such that PKD1 phosphorylates Akt/PKB in its activation loop (58, 74) (Fig. 8). Akt/PKB is then phosphorylated on a second site by PKD2. The dually phosphorylated Akt/PKB then dissociates from PI(3,4,5)P3 and carries out its function of phosphorylating cellular proteins.

The family of isoforms of Ca2+- and lipid-dependent protein kinase (PKC) also requires phosphorylation of the activation loop by PKD1 while bound to PI(3,4,5)P3 (58). However, phosphorylation of PKC in the activation loop is a maturation step rather than a direct activator of the enzyme. After proteins are synthesized in the cell, they must undergo processing before they are mature and can carry out their cellular functions. During maturation, PKC isoforms translocate to the cell membrane and bind to PI(3,4,5)P3. This brings them into juxtaposition with PKD1, which phosphorylates PKC in the activation loop. The conventional and novel isoforms of PKC must be phosphorylated in this manner before they can be activated by binding to Ca2+ and/or lipids (11, 58). The atypical isoforms of PKC also undergo phosphorylation in the activation loop while bound to PI(3,4,5)P3. However, the subsequent activational steps for atypical PKC isoforms remain obscure. Some evidence has suggested that phosphorylation of atypical PKC isoforms (PKC-α and PKC-ζ) (50) by PDK1 is sufficient for activation (14, 58), whereas other data have suggested that activation involves phosphatidylinositol 3-kinase (PI3K) protein kinases through both direct (Akt/PKB) and indirect (PKC) models.

In the same manner that protein phosphatases are critical to balance the function of protein kinases (22), lipid phosphatases are critical to balance the function of lipid kinases. For example, phosphatase and tensin homolog deleted on chromosome 10 is a lipid phosphatase that dephosphorylates the three position of PI(3,4,5)P3 (46, 89) and thereby reverses the lipid phosphorylation step of PI3K on PI(3,4,5)P3. Myotubulin dephosphorylates the three position of PI(3)P (89), and the five position of PI(3,4,5)P3 is dephosphorylated by Src homology domain 2 (SH2)-containing inositol phosphate-1 and -2 (6).

**Sphingomyelin, Ceramide, and Lipid Rafts**

Sphingomyelin is an important structural component of the cell membrane and of lipid rafts. Sphingomyelin serves an important functional role as well, as it is the parent compound of several lipid mediators (15) (Fig. 9). The family of sphingomyelinase isoforms cleaves sphingomyelin to yield ceramide and phosphorylcholine (16). There are at least five isoforms of acidic, neutral, and basic sphingomyelinases. The pH designation refers to the optimal pH for association of the substrate with the enzyme rather than the pH for optimal activity of the enzyme (41). Despite extensive studies, details of the location and physiological regulation of sphingomyelinase isoforms have remained incomplete (49). An example of the complexity of lipid signaling is illustrated by our present knowledge about the differential regulation of sphingomyelinsisoforms by TNF. The binding of TNF to its 55-kDa receptor results in the stimulation of inflammatory pathways if neutral sphingomyelinsisoform is activated but results instead in apoptosis if acidic sphingomyelinsisoform is activated (40, 71). In both cases, the mechanism of action is release of ceramide from sphingomyelin. Because ceramide is extremely hydrophobic, it remains in the membrane compartment in which it is formed. Neutral and acidic sphingomyelinases are in separate membrane compartments, so the ceramide they produce stays in those separate compartments. This compartmentalization isolates the inflammatory pathway from the apoptosis pathway. Signaling by Fas ligand also activates sphingomyelinsisoform activity and leads to apoptosis (41).

Similarly to sphingomyelin, ceramide serves both membrane structural roles as well as signal transduction roles. Structurally, ceramide molecules coalesce to form microdomains within the lipid rafts. Thus, the action of sphingomyelinsisoform within the lipid raft gives rise to ceramide microdomains. Proteins with an affinity for lipid rafts or for ceramide are brought into juxtaposition with other components of their respective pathways within the lipid rafts (15). Ceramide serves as a lipid mediator in its own right and also gives rise to other lipid mediators. Ceramide can be phosphorylated by the lipid kinase ceramide kinase (2) to yield ceramide-1-phosphate (C1P; Fig. 9). The regulation of ceramide kinase has not been well defined, although data have suggested that its activity increases in response to Ca2+ (2). Alternatively, ceramide can

![Fig. 8. Activation of the protein kinases PDK1 and Akt/PKB by PI3K. In the absence of stimulation, the protein kinases Akt/PKB and PDK1 are found in the cytoplasm (left). When the lipid kinase PI3K is activated, it phosphorylates PI(4,5)P2 (blue) at the three position to form PI(3,4,5)P3 (red). Akt/PKB and PDK1 translocate to the membrane to bind to the newly formed PI(3,4,5)P3 (right). Binding of the protein PDK1 to the lipid PI(3,4,5)P3 activates PDK1. The juxtaposition of Akt/PKB and PDK1 that occurs when they bind to adjacent molecules of PI(4,5)P2 allows PDK1 to phosphorylate Akt/PKB. The phosphorylation of Akt/PKB activates its kinase activity.](http://advan.physiology.org/)

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be converted to sphingosine by ceramidase. The regulation of ceramidase has not been well characterized (73). Sphingosine kinase, another lipid kinase, then phosphorylates sphingosine to form sphingosine-1-phosphate (S1P; Fig. 9) (33). Sphingosine kinase is partially activated by the ERK family of MAPKs, although additional but unidentified factors also appear to be involved in its activation (2).

Ceramide, C1P, sphingosine, and S1P have all been shown to carry out second messenger functions. Ceramide can directly activate PKC-\(\epsilon\) (38) and other signaling pathway components (30, 40, 71), although the mechanisms of activation have remained in question, whereas sphingosine can inhibit PKC (29). Ceramide is a direct activator of type 1 and 2A protein phosphatases (9), whereas C1P is a potent inhibitor of these serine/threonine protein phosphatases (10). Both ceramide and sphingosine stimulate cell cycle arrest and apoptosis (10). In contrast, both C1P and S1P stimulate cell survival and proliferation and are antiapoptotic (10). Both C1P and S1P are involved in inflammatory reactions but at different points. S1P induces the transcription of COX2, whereas C1P activates cPLA\(_2\) to release AA, the substrate for COX2 (2, 10). C1P appears to have only intracellular messenger functions, whereas S1P has intracellular messenger functions and can also be released from the cell and bind to G protein-linked edg/S1P receptors (42, 43). As an intracellular messenger, S1P has been proposed to release intracellular Ca\(^{2+}\) in a manner similar to but independent of IP\(_3\) (2) (Fig. 2). Since ceramide, C1P, sphingosine, and S1P are interconvertible, the specific array of lipid mediators in this pathway in a given cell depends on which enzymes are available and have been activated in that cell. The biological effects of these lipid mediators are still under investigation, and it is expected that other cellular targets remain to be discovered.

In an alternative pathway, sphingomyelin deacylase converts sphingomyelin to sphingosylphosphorylcholine (Fig. 9) (51). Sphingosylphosphorylcholine has intracellular messenger functions and can also leave the cell to bind to GPCRs (42, 51) (Fig. 2). Sphingosylphosphorylcholine increases the intracellular concentration of Ca\(^{2+}\), activates ERK, and inhibits cell proliferation (51).

Within the sphingomyelin family of lipid mediators, the reactions are reversible and the lipids are interconvertible. The discussion above focuses on the breakdown of a parent lipid to a product lipid that carries out a specific function as is most likely to occur in a rapid signal transduction event. However, the reverse reactions are utilized in the de novo synthesis of the lipid. For example, the de novo synthesis of sphingomyelin proceeds from sphingosine through ceramide to sphingomyelin. An increase of ceramide in a lipid raft may come from sphingomyelin or sphingosine.

**Fatty Acids**

Fatty acids are integral structural elements of many of the lipids that have been discussed herein including phospholipids,
sphingomyelin, ceramide, lysophospholipids, DAG, phosphatidic acid, LPA, PAF, C1P, and sphingosylphosphorylcholine (Figs. 3, 4, and 9). Fatty acids can be released from these lipids by PLA2 and other deacylases. In the past, the focus of signaling research has typically been on the parent compounds rather than on the fatty acids. However, the focus has been shifting to include fatty acids as we learn more about the functions of fatty acids beyond their role in energy metabolism. Fatty acids have been identified as ligands for members of the steroid superfamily of intracellular receptors (37, 47, 94) as well as for membrane GPCRs (43). Fatty acids have also been implicated in the accumulation of ceramide by stimulating de novo synthesis (31) or by increasing neutral sphingomyelinase activity (41). The role of AA as a precursor for multiple lipid mediators is another fatty acid function that is important in signal transduction. It is likely that additional functions of fatty acids in signal transduction have yet to be discovered.

Role of Dietary Lipids in Signal Transduction

The fatty acids that become incorporated into membrane lipids can come from the diet or can be synthesized by cells. A very interesting current topic in both the lay and scientific press is the role of dietary lipids in health and disease. Saturated fatty acids include palmitic and stearic acids (80) and are found in coconut oil, butter, and red meat. Monounsaturated fatty acids include palmitic and oleic acids (80) and are found in olive and peanut oils. Polyunsaturated fatty acids are categorized as ω-6 (linoleic, γ-linolenic, and AA) or ω-3 (α-linolenic, eicosapentaenoic, and docosahexaenoic acid). The ω designation refers to the location of the first double bond in the hydrocarbon chain relative to the methyl end of the molecule (i.e., the sixth carbon or third carbon) (92). The ω-6 polyunsaturated fatty acids are found in many plant oils such as olive, canola, soybean, corn, cottonseed, sunflower, and palm. The primary source of long-chain ω-3 polyunsaturated fatty acids is fatty fish (3, 80) such as salmon and tuna. The sources of saturated and ω-6 polyunsaturated fatty acids are more common in Western diets than those of ω-3 fatty acids. A large body of evidence supports the concept of healthy effects of polyunsaturated ω-3 fatty acids such as those found in fatty fish (28, 60). When ω-3 fatty acids are included in the diet, those fatty acids are involved in cellular functions in competition with the more common ω-6 fatty acids because the same enzymes are used for the processing of ω-3 and ω-6 fatty acids. The ω-3 fatty acids can participate in the structures (i.e., membrane lipids) and functions (i.e., signal transduction) described in this review for fatty acids in general, but the structural differences of ω-3 and ω-6 fatty acids result in functional differences as well. The ω-3 fatty acids are released into the cell when membrane lipids containing them are cleaved by enzymes such as PLA2 or sphingomyelin deacylase. The ω-3 fatty acids are poorer substrates for COX, LOX, and CYP2C enzymes than are ω-6 fatty acids such as AA (37). Since COX and LOX mediate inflammatory pathways, the reduced efficiency of these enzymes with ω-3 fatty acids as substrates allows ω-3 fatty acids to have an anti-inflammatory effect when they have been incorporated into cell membranes (37). As mentioned above, fatty acids have been shown to act as ligands at both intracellular (37, 47, 94) and membrane receptors (43) and can increase the concentration of ceramide in cell membranes (31, 41). Whether the ability of ω-3 fatty acids to regulate the activity of these pathways differs from that of ω-6 fatty acids has not been thoroughly examined, but it is expected that their effects will differ. The ω-3 fatty acids also change the composition of the lipid rafts and the fluidity of the membranes into which they are incorporated (37). Signal transduction proteins that typically associate with lipid rafts may be excluded when the content of ω-3 fatty acids is increased because ω-3 fatty acids make the lipid rafts thicker or because the lipid environment is more unsaturated than when ω-6 fatty acids are high (37). Lipid-anchored proteins may no longer associate with the lipid raft if the lipid attachment is an ω-3 fatty acid instead of an ω-6 fatty acid. For example, the nonreceptor tyrosine kinase Fyn may be unable to associate with lipid rafts when it is anchored with an ω-3 fatty acid instead of an ω-6 fatty acid (37). Thus, many mechanisms by which dietary polyunsaturated fatty acids may affect signal transduction have been proposed. However, the specific mechanisms involved have not yet been rigorously demonstrated.

Lipids as Ligands

The above discussion describes specific lipid mediators that leave the cells of origin and bind to GPCRs in the membranes of neighboring cells or on the cell of origin. These lipid mediators include PGs, leukotrienes, lysophospholipids, sphingosylphosphorylcholine, and PAF (42) (Fig. 2). Another class of lipid-soluble ligands acts as conventional hormones; they travel in the blood to distant sites, where they bind to receptors and carry out their biological activity. The steroid hormones estradiol, progesterone, testosterone, aldosterone, and cortisol are all derived from cholesterol. 1,25-Dihydroxyvitamin D₃ (cholecalciferol) is not a vitamin at all; rather, it too is a hormone derived from cholesterol. In contrast, the thyroid hormones triiodothyronine and tetraiodothyronine are synthesized from the amino acid tyrosine; the biosynthesis of the hormones from tyrosine renders them lipid soluble. These lipid-soluble ligands bind to intracellular receptors in the steroid receptor superfamily. This family of receptors is much larger than the number of ligands known to bind to them (1). Receptors for which no ligands have yet been identified are called orphan receptors. For example, peroxisome proliferator-activated receptors (PPARs) were previously considered as orphan receptors of the steroid receptor superfamily. PPAR-γ was identified as the binding site for the thiazolidinedione class of insulin sensitizers (54) before the endogenous ligands for the receptor were identified. It is now recognized that a variety of lipids/lipid-derived ligands bind to orphan receptors as in the case of PPAR. For example, derivatives of androgens are ligands for the constitutive androstane receptor (24, 76). Fatty acids are ligands for all isoforms of PPAR (37, 47, 94), and PGs have been proposed as ligands for PPARs as well (37, 47, 94). Fatty acids have been proposed as ligands at hepatic nuclear factor-4 and retinoic acid X receptors and as antagonists of the liver X receptor, but the supporting data have not been conclusive (37).

The classical mechanism of action of ligands bound to the steroid superfamily of receptors is to modify DNA transcription of specific genes. The ligand/receptor complex dimerizes and binds to DNA as a transcription factor (1). This is called the genomic mechanism of action and takes time to develop. The steps involved include DNA transcription to mRNA, processing of mRNA, movement of mRNA into the cytoplasm,
transcription of mRNA into protein, and proper folding and posttranslational modification of the protein. Thus, there is a time delay of hours to days from the time of the initiation of the signal until a processed protein that is capable of changing the biological activity of the cell is available. This is in dramatic contrast to ligands that bind to membrane receptors and initiate reversible phosphorylation cascades that result in measurable changes in the biological activity of their target cells within milliseconds to seconds. Rapid responses to steroid family hormones have been reported in addition to the classical delayed genomic responses mentioned here. Indeed, non-genomic effects of lipid-soluble steroid hormones were first reported decades ago (63, 64). However, at the time of their discovery (1960s), the genomic mechanisms for steroid hormones were quite novel and therefore so compelling that the non-genomic mechanisms received little attention. Although the literature on non-genomic effects of steroid and thyroid hormones has grown rapidly (85, 88), there is little consensus on which specific signaling pathways are activated by individual lipid-soluble ligands in a physiological manner. There are reports suggesting activation of nearly every signal transduction pathway known by nearly every steroid/thyroid hormone identified. However, we know that the effects of a given ligand are very specific. Therefore, continued refinement of our understanding of the activation of non-genomic signaling pathways by steroid/thyroid hormones will be required to clarify the mechanisms by which ligand specificity of action is maintained.

Lipid-Modified Proteins in Signal Transduction

Many of the components of signal transduction pathways are modified posttranslationally by the addition of lipid moieties. For example, α-subunits of heterotrimeric G proteins may be myristoylated or palmitoylated (57) and γ-subunits of heterotrimeric G proteins are prenylated (57). Small G proteins such as the Ras, Rab, and Rho/Rac/Cdc42 families undergo palmitoylation, farnesylation, or geranylgeranylation (81), and non-receptor tyrosine kinases of the Src family may be myristoylated or palmitoylated (52). Palmitoylation of signaling proteins may be reversible such that the protein undergoes cycles of lipid attachment and disattachment with concomitant cyclic association with the membrane when attached to the lipid. Gαs is an example of a protein that undergoes reversible palmitoylation (52).

Extracellular ligands for membrane receptors may also bear lipid modifications. For example, ligands for two of the more recently described pathways, Hedgehog and Wnt, are both palmitoylated (34, 59). In addition, Hedgehog enjoys the distinction of being the only known protein to which a cholesterol moiety is attached (34). Other extracellular proteins are held in association with the plasma membrane through GPI-anchoring tails. GPI-anchored proteins are involved in signal transduction and associate with lipid rafts (75). Examples of GPI-anchored proteins involved in signal transduction include PH-20 and GFRα1. PH-20 is a multifunctional protein found in mammalian sperm membranes (13). The signaling function of PH-20, an increase in intracellular Ca2+ levels, is activated by hyaluronic acid (13). The GPI-anchored protein GFRα1 is a coreceptor with the receptor tyrosine kinase Ret. In the absence of the ligand, glial-derived neurotrophic factor (GDNF), GFRα1 is associated with the lipid raft by itself but needs to do so to achieve efficient tyrosine kinase signaling. In the presence of GDNF, GFRα1 recruits Ret to the lipid raft so that it is active (83).

Themes in Lipid-Mediated Signal Transduction

As indicated at the beginning of this review, the cell membrane is a structural barrier that is necessary to cell function, but it has important physiological roles in signal transduction as well. The field of lipid signal transduction has received only a fraction of the attention of that of protein signal transduction, and there are many unanswered questions in this field. Several themes have emerged in this discussion of lipid mediators in signal transduction. The first theme is the relatedness and, often, the interconvertibility of families of lipid mediators (Fig. 1). A second common theme is the relatedness of lipids that act as ligands and the signal transduction pathways that utilize lipid mediators. Lipid mediators that leave the cell of origin and bind to GPCRs in the cell membrane of the same or neighboring cells include PGs (PGF2α, PGE2, prostacyclin, and thromboxane), S1P, PAF, LPC, sphingosylphosphorylcholine, and LPA (Fig. 2). Most of these GPCRs are linked to PLC-β, so they stimulate a lipid-mediated signaling pathway within the cell that leads to increased activity of PKC and to increased concentration of intracellular Ca2+. The central role of Ca2+ in lipid-mediated signaling is another important theme as many of the enzymes involved in the lipid signaling pathways are Ca2+ regulated (Fig. 2). Moreover, the enzyme systems that require Ca2+ for activation and that produce lipid mediators that increase Ca2+ obtain a degree of positive feedback through this interaction. A final important theme is the role of lipid mediators in inflammatory reactions. PGs, leukotrienes, PAF, and lysophosphatidic acids are all lipid mediators in various aspects of inflammatory reactions.

The field of lipid signal transduction is complicated. The difficulty of visualizing lipid biochemistry, the lower profile of lipid signaling compared with protein signaling, and the many “black holes” that remain in our understanding of lipid signaling all contribute to the complicated nature of lipid signal transduction. Certainly, there are many details of lipid signaling that must be worked out. The relatively recent identification of messenger functions of C1P and sphingosylphosphorylcholine support the concept that other lipid mediators remain to be discovered. This review provides a framework in which to place those new details of lipid signaling as they emerge. Moreover, this review illustrates the magnitude and diversity of lipid mediators in signal transduction and highlights the importance of staying current in the roles of lipid mediators in cellular physiology.

GRANTS

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