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Phospholipase A_2

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Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the *sn*-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA), a precursor of eicosanoids including prostaglandins (PGs) and leukotrienes (LTs). The same reaction also produces lysophosholipids, which represent another class of lipid mediators. So far, at least 19 enzymes that possess PLA_2 activity have been identified in mammals. The secretory PLA_2 ($sPLA_2$) family, in which 10 isozymes have been identified, consists of low-molecular-weight, Ca^{2+}-requiring, secretory enzymes that have been implicated in a number of biological processes, such as modification of eicosanoid generation, inflammation, host defense, and atherosclerosis. The cytosolic PLA_2 ($cPLA_2$) family consists of 3 enzymes, among which $cPLA_2\alpha$ plays an essential role in the initiation of AA metabolism. Intracellular activation of $cPLA_2\alpha$ is tightly regulated by Ca^{2+} and phosphorylation. The Ca^{2+}-independent PLA_2 ($iPLA_2$) family contains 2 enzymes and may play a major role in membrane phospholipid remodeling. The platelet-activating factor (PAF) acetylhydrolase (PAF-AH) family represents a unique group of PLA_2 that contains 4 enzymes exhibiting unusual substrate specificity toward PAF and/or oxidized phospholipids. In this review, we will overview current understanding of the properties and functions of each enzyme belonging to the $sPLA_2$, $cPLA_2$, and $iPLA_2$ families, which have been implicated in signal transduction.

Key words: arachidonic acid, eicosanoid, leukotriene, phospholipase A_2, prostaglandin.

Classification of PLA_2s

Historically, only one mammalian PLA_2 enzyme, which is abundantly present in pancreatic juice and is now called group IB PLA_2, was known before 1986 (*1*). The second secretory PLA_2 ($sPLA_2$) or group IIA PLA_2, which is stored in secretory granules of immune cells and is markedly induced at various inflamed sites, was cloned in 1989 (*2*). A new period of $sPLA_2$ was opened by the cloning of two novel isozymes, group IIC and V, in 1994 (*3, 4*). The subsequent search for novel $sPLA_2$s by screening public nucleic databases led to the cloning of the novel mammalian $sPLA_2$s: group X (*5*), IID (*6*), IIE (*7*), IIF (*8*), III (*9*), and XII (*10*) in historical order of identification. Now, 10 mammalian $sPLA_2$s are known, which have a highly conserved catalytic site, Ca^{2+}-binding loop, and common molecular mass of 14–19 kDa (except for group III enzyme, see below).

A novel, high-molecular-weight (85 kDa) PLA_2, now called cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$), or group IVA PLA_2, was purified and cloned in 1991 (*11*). $cPLA_2\alpha$ shows a marked preference for AA over other fatty acids and is activated by submicromolar Ca^{2+} and by phosphorylation (*11, 12*). Later, a database search strategy revealed two novel $cPLA_2$ paralogs, called $cPLA_2\beta$ (group IVB) and $cPLA_2\gamma$ (group IVC) (*13, 14*). These three enzymes have conserved catalytic sites containing a catalytic serine.

Another cytosolic form of PLA_2, which does not require Ca^{2+} for catalysis, was purified and cloned in 1996 (*15*). This Ca^{2+}-independent PLA_2, referred to as $iPLA_2$, is a high-molecular-weight (85 kDa) enzyme possessing a catalytic serine and occurs in multiple alternative splicing variants (*16*). Another Ca^{2+}-independent membrane-bound PLA_2 whose catalytic site is homologous with $iPLA_2$ was identified by searching nucleic acid databases in 2000 (*17*). Thus, the first and second $iPLA_2$s are now designated as group VIA and VIB, respectively. The $iPLA_2$ family has been considered to play an important role in membrane phospholipid remodeling (*18*).

Another subgroup of the Ca^{2+}-independent PLA_2 family, members of which were purified and cloned in the mid 1990s, comprises the enzymes of 30–40 kDa that show unique substrate preference for specialized phospholipids. Because of their common property to hydrolyze, and thereby inactivate, the lipid mediator PAF, they are called PAF-acetylhydrolases (PAF-AHs) (*19–22*). Along with the PLA_2 nomenclature, PAF-AHs are classified into group VII and VIII PLA_2s.

As the main theme of this minireview series is signal transduction, we focus on the lipid mediator production by $sPLA_2$, $cPLA_2$, and $iPLA_2$ in this article. PAF-AH will be described in detail in another minireview in this series.

$sPLA_2$s

General aspects. The structures of 10 mammalian

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Abbreviations: AA, arachidonic acid; PG, prostaglandin; LT, leukotriene; PAF, platelet-activating factor; PLA_2, phospholipase A_2; $sPLA_2$, secretory PLA_2; $cPLA_2$, cytosolic PLA_2; $iPLA_2$, Ca^{2+}-independent PLA_2; PAF-AH, PAF acetylhydrolase; COX, cyclooxygenase; LOX, lipoxygenase; HSPG, heparan sulfate proteoglycan; MAPK, mitogen-activated protein kinase.

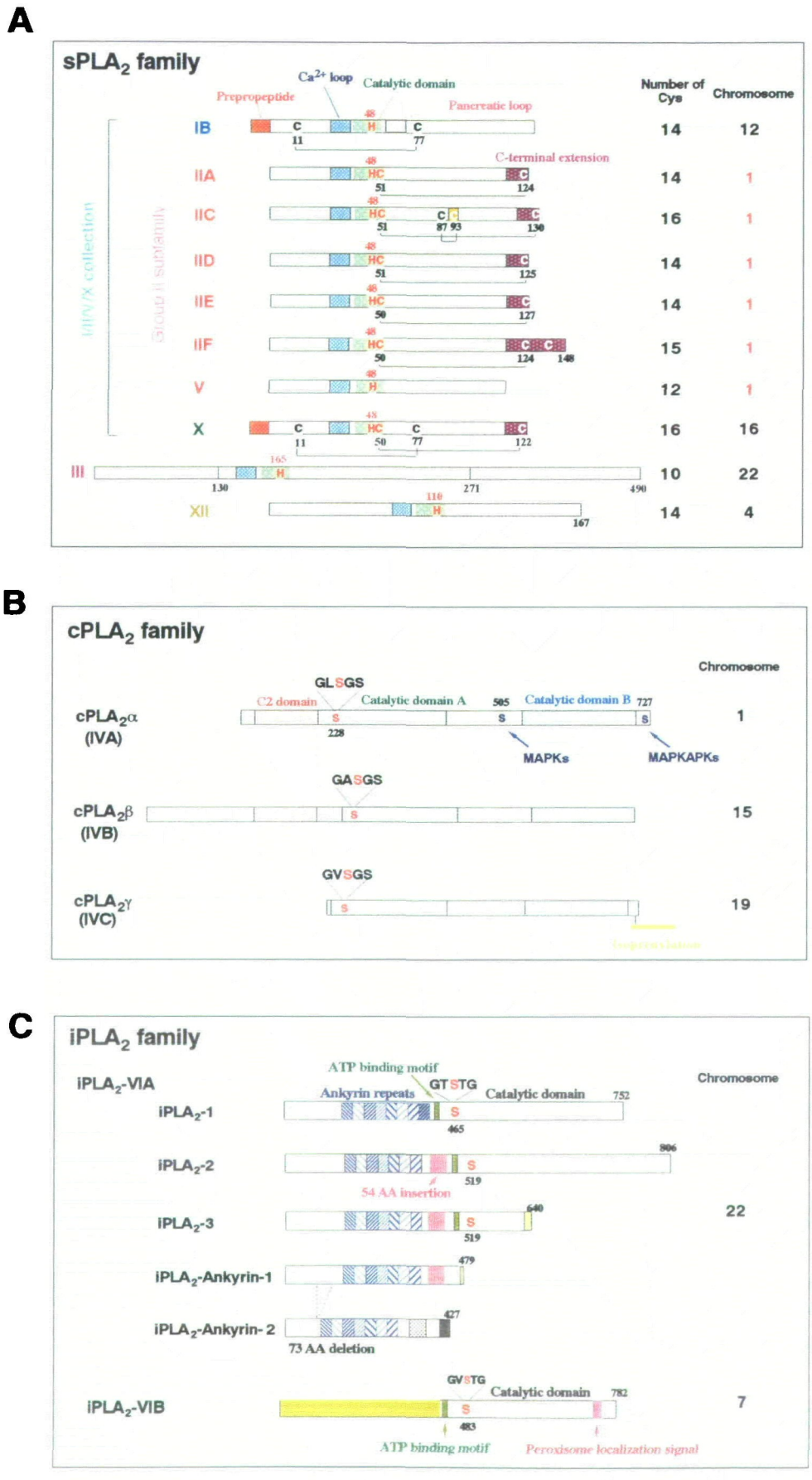

Fig. 1. **Structure of PLA_2s.** (A) $sPLA_2$s. The catalytic domain around the catalytic histidine and the Ca^{2+}-binding loop are well conserved in all $sPLA_2$s. Structural characteristics of each $sPLA_2$ are indicated. (B) $cPLA_2$s. Two separate catalytic domains and residues required for enzyme catalysis are conserved in all $cPLA_2$s. The C2 domain is present in $cPLA_2\alpha$ and $cPLA_2\beta$ but not $cPLA_2\gamma$, which is C-terminally isoprenylated. Two serine residues phosphorylated by MAPKs and MAPK-activated protein kinases (MAPKAPKs) exist only in $cPLA_2\alpha$. (C) $iPLA_2$s. $iPLA_2$-VIA occurs in at least 5 splice variants, among which VIA-1 and VIA-2 have a complete catalytic domain. The other 3 variants lack the catalytic domain and instead have unique C-terminal short stretches. $iPLA_2$-VIB has the $iPLA_2$ catalytic domain but lacks the ankyrin repeats. There is a peroxisome localization sequence near the C-terminus of $iPLA_2$-VIB. See details in the text.

$sPLA_2$s are illustrated in Fig. 1A. $sPLA_2$s belonging to the group I/II/V/X collection are closely related, secreted enzymes of 14–19 kDa with a highly conserved Ca^{2+}-binding loop (XCGXGG) and a catalytic site (DXCCXXHD). Beside these elements, there are 6 absolutely conserved disulfide bonds and up to 2 additional unique disulfide bonds, which contribute to the high degree of stability of these enzymes. Substrate hydrolysis proceeds through the activation and orientation of a water molecule by hydrogen bonding to the active site histidine. Adjacent to this histidine, there is a conserved aspartate residue, which, together with the Ca^{2+}-binding loop, acts as a ligand cage for Ca^{2+}. As the genes for $sPLA_2$-IIA, -IIC, -IID, -IIE, -IIF, and -V are clustered on the same chromosome locus, they are often referred to as the group II subfamily $sPLA_2$s. $sPLA_2$-III and $sPLA_2$-XII share homology with the I/II/V/X collection of $sPLA_2$s only in the Ca^{2+}-binding loop and catalytic site, thereby representing distinct group III and XII collections, respectively.

$sPLA_2$s hydrolyze the ester bond at the *sn*-2 position of glycerophospholipids in the presence of mM concentrations of Ca^{2+} with no strict fatty acid selectivity. Most group II subfamily $sPLA_2$s act on anionic phospholipids such as phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylserine in marked preference to charge-neutral phosphatidylcholine (PC); $sPLA_2$-V and -X hydrolyze both anionic phospholipids and PC efficiently, and sPLA2-IB is intermediate. These head group preferences are due mainly to the differences in their ability to bind to lipid vesicle interface. Oxidative modification of phospholipids alters the physiological state of the membrane, which in turn affects the susceptibility of oxygenated and non-oxygenated fatty acid residues toward $sPLA_2$ attack in a multifaceted way.

Some of the group II subfamily of $sPLA_2$s are highly cationic and bind tightly to anionic heparanoids such as heparin and heparan sulfate. The clusters of basic amino acids near the C-termini of $sPLA_2$-IIA, -IID, and -V are essential for their heparanoid binding, with other basic residues diffused throughout the molecules playing a supporting role (*23–25*). The phospholipid head group specificity and heparan sulfate proteoglycan (HSPG)–binding property affect the cellular functions of each $sPLA_2$.

Properties of each $sPLA_2$ isozyme. $sPLA_2$-IB has a unique 5-amino-acid extension termed the pancreatic loop in the middle part of the molecule and a group I–specific disulfide between Cys^{11} and Cys^{77} (*1*). It is synthesized in the pancreatic acinar cells, and after secretion into the pancreatic juice, an N-terminal heptapeptide of the inactive zymogen is cleaved by trypsin to yield an active enzyme in the duodenum. The digestion of dietary phospholipids appears to be a primary function of pancreatic $sPLA_2$-IB. It is also expressed in trace amounts in several non-digestive organs such as lung, kidney and spleen (*26*). $sPLA_2$-IB, but not its zymogen, is a high-affinity ligand for the M-type $sPLA_2$ receptor, and the receptor-mediated signaling may lead to activation of $cPLA_2\alpha$ (*27*). Mice deficient in the M-type $sPLA_2$ receptor show resistance to endotoxin shock (*28*).

$sPLA_2$-IIA has a group II–specific disulfide linking Cys^{50} with Cys at the C-terminus and a C-terminal extension of 7 amino acids in length. The levels of $sPLA_2$-IIA in sera or exudating fluids are well correlated with the severity of inflammatory diseases (*2*). The expression of $sPLA_2$-IIA is markedly induced by proinflammatory stimuli (*29*) and downregulated by anti-inflammatory cytokines and glucocorticoids (*30*) in a wide variety of cells and tissues. Consistent with this inducibility, the promoter region of the *$sPLA_2$-IIA* gene contains TATA and CAAT boxes as well as several elements required for binding of transcription factors such as AP-1, C/EBPs, CREB, NF-κB, STAT, and PPARγ (*31, 32*). In several cells, $sPLA_2$-IIA induction is under the control of the $cPLA_2\alpha$-12/15-lipoxygenase (LOX) pathway (*32, 33*). In the mouse, the expression of $sPLA_2$-IIA is restricted to the intestine or not observed at all (*34*), revealing a notable species difference.

Although $sPLA_2$-IIA can act poorly on the surface of quiescent cells because of its weak binding capacity to the PC-rich external leaflet of the plasma membrane, in activated cells it can be sorted into the caveolin-rich vesicular and perinuclear compartments through binding to glypican, a glycosylphosohatidylinositol-anchored HSPG (*24, 25*). After proper sorting, $sPLA_2$-IIA releases AA from membrane microdomains, in which cell activation–associated membrane rearrangements, such as altered asymmetry, accelerated oxidation, and increased fluidity, occur (*33, 35, 36*). This route, called *the HSPG-shuttling pathway*, allows this enzyme to be concentrated into particular membrane compartments, where the perinuclear AA-metabolizing enzymes, such as cyclooxygenases (COXs) and 5-LOX, also exist (*24, 25*) (Fig. 2A).

Besides its proinflammatory effects, the anti-bacterial and atherosclerotic properties of $sPLA_2$-IIA have been studied using $sPLA_2$-IIA transgenic mice (*37, 38*). In activated mast cells, $sPLA_2$-IIA is redistributed from secretory granules into the opening granular membranes and augments degranulation by producing fusogenic lysophospholipids *in situ*, which facilitate the fusion between granular and plasma membranes (*39*). Some mouse strains, in which the *$sPLA_2$-IIA* gene is intrinsically disrupted due to a frameshift mutation, exhibit resistance to colorectal tumorigenesis, although the reason is unknown (*34*).

$sPLA_2$-IIC has an additional disulfide between Cys^{87} and Cys^{93} in an extended loop region and is expressed in rodent testis (*3*). In contrast, the absence of a portion of one exon in the human genome indicates that it is a pseudogene and not expressed as a functional protein in the human.

$sPLA_2$-IID, which is structurally similar to $sPLA_2$-IIA, is constitutively expressed in the immune and digestive organs and is upregulated by proinflammatory stimuli in some restricted tissues (*6*). Its expression is markedly reduced in lymphotoxin α–deficient mice (*40*). $sPLA_2$-IID augments stimulus-induced cellular AA release through the HSPG-shuttling mechanism, as does $sPLA_2$-IIA (*25*).

$sPLA_2$-IIE, another $sPLA_2$-IIA–related enzyme, is expressed constitutively in several tissues at low levels and is also upregulated by proinflammatory stimuli (*7*). Although the affinity of $sPLA_2$-IIE for heparanoid is weaker than that of $sPLA_2$-IIA and -IID, it is still capable of augmenting stimulus–induced cellular AA release through the HSPG-shuttling mechanism (Murakami, M. *et al.*, submitted).

$sPLA_2$-IIF possesses a unique 30-amino-acid C-terminal extension that contains an additional cysteine residue (*8, 41*), which may contribute to formation of a homodimer or a heterodimer with a second protein. Its expression is limited to the testis of adult mice, whereas it is detected in various human tissues at low levels. It is expressed at high level in mouse embryo, implying that its expression is developmen-

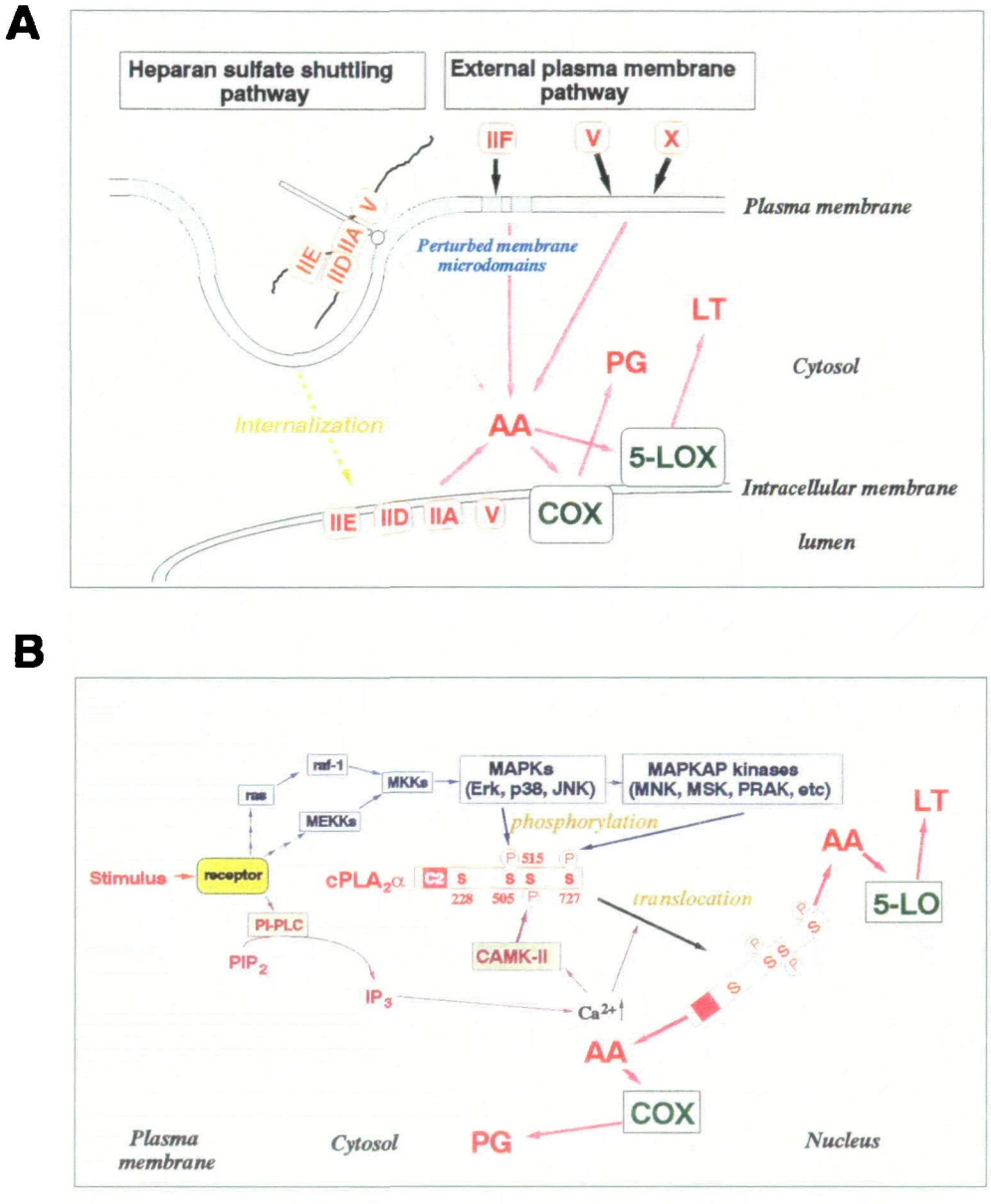

Fig. **2. Regulatory mechanisms for eicosanoid generation by PLA_2s.** (A) $sPLA_2$s. The heparin-binding group II enzymes, $sPLA_2$-IIA, -IID, –IIE, and -V, are sorted into the caveolin-rich vesicular domains *via* binding to the HSPG glypican, and internalized into the lumen side of the perinuclear membrane. The AA released by these $sPLA_2$s may be efficiently supplied to the adjacent COX and 5-LOX. The PC-hydrolyzing enzymes, $sPLA_2$-X and -V, act on the PC-rich outer plasma membrane to release AA. $sPLA_2$-IIF binds to the perturbed plasma membrane microdomain *via* its unique C-terminal region and releases AA. The AA released from the plasma membrane may diffuse or be transported across the cytosol to the perinuclear COX and 5-LOX. (B) $cPLA_2\alpha$. In response to an increase in $[Ca^{2+}]_i$, $cPLA_2\alpha$ translocates from the cytosol to the perinuclear membrane, where COX and 5-LO exist, in a C2 domain–dependent fashion. Dual phosphorylation by MAPKs and MAPKAPKs or phosphorylation by Ca^{2+}/calmodulin kinase II (CaMKII) is required for full activation of $cPLA_2\alpha$. See details in the text.

tally regulated. Although $sPLA_2$-IIF does not bind heparanoids, it has the ability to increase cellular AA release probably through acting on the perturbed microdomain on the plasma membrane (Fig. 2A). The uniquely long C-terminal extension, which prolongs the duration of the interaction between the enzyme and the plasma membrane, is essential for cellular AA-releasing function of $sPLA_2$-IIF (Murakami, M. *et al.*, submitted).

$sPLA_2$-V does not have the group I– and group II–specific disulfides and the group II-specific C-terminal extension (*4*), although it shows a higher level of identity with group II $sPLA_2$s than does $sPLA_2$-IB. $sPLA_2$-V is the primary $sPLA_2$ in the mouse, where it is expressed at higher levels than the other group II subfamily $sPLA_2$s in various tissues and immune cells (*42*), and it is also distributed widely in human tissues, among which the highest expression is found in the heart (*4*). Its expression is also markedly induced by proinflammatory stimuli (*42*). Distinct subcellular localization and kinetic induction of $sPLA_2$-IIA and -V in some cells suggest their segregated functions in such a situation (*43*). $sPLA_2$-V shows high affinity for both heparanoids and PC, and releases cellular AA by two distinct mechanisms (*23–25, 44*). As in the case of $sPLA_2$-IIA, high heparanoid affinity allows $sPLA_2$-V to utilize the HSPG-shuttling pathway (*23–25*). $sPLA_2$-V is also capable acting on the plasma membrane surface independently of HSPG (*25, 44*). The latter action, called *the external plasma membrane pathway*, depends primarily on its ability to bind PC in the outer plasma membrane. Since this pathway does not require membrane rearrangements, $sPLA_2$-V as well as $sPLA_2$-X (see below) can release AA from various types of unstimulated cells far more efficiently than do $sPLA_2$-IB and other group II subfamily $sPLA_2$s.

$sPLA_2$-X has both the group I– and II–specific disulfides, the group II–specific C-terminal extension, and the group I-specific propeptide (*5*). Like $sPLA_2$-IB, $sPLA_2$-X is synthesized as a zymogen, and removal of the N-terminal propeptide produces an active mature enzyme (*45*). The mature

enzyme interacts with the $sPLA_2$ receptor with high affinity (*46*). $sPLA_2$-X is expressed in the immune and digestive organs and testis (*5*). Due to its high activity toward PC and lack of affinity for heparanoids, $sPLA_2$-X releases cellular AA predominantly through the external plasma membrane pathway (*25, 35, 45*). The AA released by $sPLA_2$-V or -X from the external cell surface can diffuse across the cytosol and reach the perinuclear COX and 5-LOX enzymes for conversion to eicosanoids (Fig. 2A). Notably, $sPLA_2$-X is highly expressed in some colon carcinomas (*47*), suggesting its possible participation in the COX-2–dependent tumorigenesis.

$sPLA_2$-III is an unusually large protein (55 kDa) among members of the $sPLA_2$ family. It consists of three domains, of which the central $sPLA_2$ domain displays all of the features of group III bee venom $sPLA_2$s, including 10 cysteines and the key residues of the Ca^{2+} loop and catalytic site, and is flanked by large and unique N- and C-terminal regions (*9*). It is expressed in the kidney, heart, liver and skeletal muscle. The $sPLA_2$ domain alone is sufficient to elicit AA release (Murakami, M., unpublished results).

$sPLA_2$-XII is a 19-kDa enzyme containing a central catalytic domain with a His/Asp catalytic dyad, yet the location of cysteines outside the catalytic domain is distinct from that of other $sPLA_2$s (*10*). Furthermore, in the consensus segment of the Ca^{2+}-binding loop ($X_1CG_1X_2G_2$), the G_2 residue is replaced by proline in $sPLA_2$-XII. Strong expression of $sPLA_2$-XII is found in the heart, skeletal muscle, kidney, and pancreas and weaker expression in various tissues in the human. In the mouse, there are two alternative spliced forms with distinct subcellular localizations, one of which is expressed in T_{H2} cells (*48*). The cellular function of this enzyme remains unresolved.

$cPLA_2$s

Structures. $cPLA_2$s consist of 3 isozymes, $cPLA_2\alpha$, $cPLA_2\beta$, and $cPLA_2\gamma$, which are classified into group IVA, ICB, and IVC, respectively (*11, 13, 14*). Since the latter group naming is less familiar, the former naming, which is common and well accepted, is used in this review. These 3 isoforms contain two catalytic domains, A and B, interspaced with isoform-specific sequences (Fig. 1B). The lipase consensus sequence, GXSGS, is located in the catalytic domain A. $cPLA_2\alpha$ and $cPLA_2\beta$ have an N-terminal C2 domain, which is critical for Ca^{2+}-dependent association with phopholipid membranes (*11*). $cPLA_2\gamma$ lacks the C2 domain, but it contains an isoprenylation site at the C-terminus and is membrane-bound (*13*). As described below, activation of $cPLA_2\alpha$ is regulated by cytoplasmic Ca^{2+} levels and by phosphorylation following various stimuli (Fig. 2B).

The C2 domain of $cPLA_2\alpha$, which traps 2 Ca^{2+} ions, is topologically similar to that of phospholipase Cδ (*49*) and binds to PC in preference to other phospholipids (*50*). The $cPLA_2\alpha$ catalytic domain contains a short stretch that is homologous with phospholipase Bs and lysophospholipases and that includes Gly^{197}/Gly^{198} of the oxyanion hole, Arg^{200} and the lipase motif, which contains the catalytic center Ser^{228}. While the nucleophilic Ser^{228} attacks at the *sn*-2 ester bond of phospholipids, Asp^{549} contributes to activation of this catalytic center. Ser^{505} and Ser^{727}, which represent two critical phosphorylation sites for $cPLA_2\alpha$ activation, are located three dimensionally near the interdomain linker region.

$cPLA_2\alpha$ is ubiquitously and constitutively expressed in most cells and tissues, and is modestly increased following certain stimuli (*51*). The promoter region of the *$cPLA_2\alpha$* gene contains potential binding sites for AP-1, AP-2, NF-κB, C/EBP, PEA3, OCT, and GRE, but no TATA box or SP1-binding site, thus being distinct from typical housekeeping genes (*52*). Among the human tissues examined, $cPLA_2\beta$ is abundantly expressed in the pancreas, brain, heart and liver, and $cPLA_2\gamma$ in the skeletal muscle (*13, 14*).

$cPLA_2\alpha$ shows a remarkable selectivity toward phospholipids bearing AA at the *sn*-2 position (*11*). $cPLA_2\alpha$ also possesses *sn*-1 lysophospholipase and transacylase activities (*53*), although the biological significance of these activities is unclear. The *sn*-1 *versus sn*-2 specificity and the *sn*-2 fatty acid selectivity of $cPLA_2\beta$ and $cPLA_2\gamma$ are less strict (*i.e.* they have phospholipase A_1 activity as well) (*13, 14*). Since cellular functions of $cPLA_2\beta$ and $cPLA_2\gamma$ are poorly understood, we focus on the activation mechanisms of $cPLA_2\alpha$ in the following section.

Regulatory functions. The C2 domain–directed, Ca^{2+}-dependent translocation of $cPLA_2\alpha$ from the cytosol to perinuclear membranes (Golgi, endoplasmic reticulum, and nuclear envelope) is an essential step for the initiation of stimulus-coupled AA release (*54, 55*). Duration of $[Ca^{2+}]_i$ stabilizes the association of $cPLA_2\alpha$ with the perinuclear membrane. This spatiotemporal localization permits efficient functional coupling between $cPLA_2\alpha$ and downstream eicosanoid-biosynthetic enzymes, COXs and 5-LOX.

The maximal activation of $cPLA_2\alpha$ requires sustained dual phosphorylation of Ser^{505} and Ser^{727} by mitogen-activated protein kinases (MAPKs) and by MAPK-activated protein kinases, respectively (*12, 56*). Ca^{2+}/calmodulin kinase–II has also been found to bind $cPLA_2\alpha$ and phosphorylate it on Ser^{515}, resulting in its activation independently of the MAPK pathway (57). Phosphorylated $cPLA_2\alpha$ generally fails to release AA without an increase in $[Ca^{2+}]_i$, indicating that phosphorylation *per se* is insufficient for, but rather plays an augmentative role in, $cPLA_2\alpha$ activation.

Several proteins have been identified as regulatory molecules of $cPLA_2\alpha$ function in either a positive or negative way. Vimentin, a component of the intermediate filaments that is enriched in the perinuclear region, binds to the C2 domain of $cPLA_2\alpha$ in a Ca^{2+}-dependent manner and facilitates $cPLA_2\alpha$-mediated AA release (*58*). The calpactin light chain, p11, interacts with the C-terminal portion of $cPLA_2\alpha$ and inhibits its function (*59*). Annexin I binds to the C2 domain and negatively regulates $cPLA_2\alpha$ (*60*). The nuclear $cPLA_2\alpha$-interacting protein, which is a splice variant of Tip60, interacts with the N-terminal region of $cPLA_2\alpha$ and activates the enzyme in the nucleus (*61*). The physiological relevance of these $cPLA_2\alpha$-interacting proteins awaits further elucidation.

$cPLA_2\alpha$ is cleaved by caspase-3 at Asp^{522} during apoptosis (*62, 63*). This cleavage results in disruption of the $cPLA_2\alpha$ catalytic dyad, Ser^{228} and Asp^{549}, leading to inactivation of the enzyme. The resultant truncated $cPLA_2\alpha$ fragment inhibits the AA-releasing function of intact (*i.e.* uncleaved) $cPLA_2\alpha$, probably by competing with the perinuclear binding site (*63*). Thus, the caspase-truncated form of $cPLA_2\alpha$ behaves like a naturally occurring dominant-negative molecule for stimulus-induced AA release, rendering apoptotic cells no longer able to produce lipid mediators.

Macrophages and mast cells obtained from $cPLA_2\alpha$

knockout mice produce minimal PGs, LTs, and PAF in response to stimuli (*64, 65*). The airway anaphylactic response, adult respiratory distress syndrome, and ischemic brain injury are markedly reduced in $cPLA_2\alpha$ knockout mice compared with wild-type mice (*64–66*). The delayed onset of labor in female $cPLA_2\alpha$ knockout mice suggests that the enzyme is an important component in the signaling cascade of parturition (*64, 65*). $cPLA_2\alpha$ knockout in Apc^{mun} mice markedly decreases the size of the small intestinal polyps (*67*), implying its participation in the COX-2-directed colon cancer development. The intestinal epithelium in $cPLA_2\alpha$ knockout mice has numerous small ulcerative lesions (*67*), indicating the role of $cPLA_2\alpha$ in the production of cytoprotective PGE_2 in the gastrointestinal tracts.

It should be noted that the phenotypes of $cPLA_2\alpha$ knockout mice are not entirely identical to those of mice with disrupted genes for COXs, 5-LOX or PG receptors, even though some of them crucially overlap. This again implies the involvement of additional PLA_2 enzymes in these biological events. Making the situation more complex, the functions of several $sPLA_2$s are highly dependent upon $cPLA_2\alpha$ in some circumstances (*23, 33, 36, 68*). This indicates that the blockage of $cPLA_2\alpha$ eventually shuts off the $sPLA_2$-dependent biological responses.

$iPLA_2$s

Structure and expression. The classical $iPLA_2$, $iPLA_2$-VIA, exists in an aggregated form and occurs in several splice variants (*15, 16*). At least 2 enzymatically active forms of the enzyme, termed VIA-1 and VIA-2, have been identified. $iPLA_2$-VIA-1 is an 85-kDa protein that contains 8 ankyrin repeats in the N-terminal region, followed by a catalytic domain containing a consensus lipase motif GXSXG, where Ser^{465} acts as a catalytic center. $iPLA_2$-VIA-2, an 88-kDa isoform, has a primary structure essentially identical to VIA-1, except that the eighth ankyrin repeat is interrupted by an additional 54 amino acids. The ankyrin repeats may enable the oligomerization of the enzyme. There is a nucleotide-binding motif prior to the catalytic Ser in both splice variants. Other splice variants lack the C-terminal catalytic domain and may act as dominant-negative inhibitors for $iPLA_2$-VIA-1 and IVA-2 (*16*).

$iPLA_2$-VIB contains a consensus lipase motif, a glycine-rich nucleotide-binding motif, and a C-terminal peroxisome localization signal (*17*). The positions of the nucleotide-binding motif and the catalytic site in $iPLA_2$-VIB are similar to those in $iPLA_2$-VIA. Several sequences highly conserved between $iPLA_2$-VIA and -VIB are clustered in the C-terminal half, whereas the N-terminal region of $iPLA_2$-VIB lacks the ankyrin repeats.

Both $iPLA_2$-VIA and VIB are ubiquitously expressed in various tissues. They are fully active in the absence of Ca^{2+}, and at least the former enzyme shows no strict *sn*-2 fatty acid and head group specificity of the substrate phospholipids (*16–18*). $iPLA_2$-VIA also exhibits *sn*-1 lysophospholipase activity, transacylase activity, and PAF-AH activity. $iPLA_2$-VIA-2, but not VIA-1, is activated several-fold by ATP (*17*).

Functions. $iPLA_2$-VIA (and probably-VIB) plays a pivotal role in phospholipid remodeling through constitutive deacylation of phospholipids (*18*). Beyond its housekeeping role, *i.e.*, maintenance of phospholipid homeostasis, several lines of evience suggest that $iPLA_2$-VIA is also involved in stimulus-coupled AA release (*23, 69, 70*). For instance, agonist-induced PGE_2 production is attenduated by an antisense oligonucleotide for $iPLA_2$-VIA (*69*), and overexpression of $iPLA_2$-VIA leads to increased AA release in response to A23187 (*23, 70*). The latter observation suggests that even though $iPLA_2$-VIA does not require Ca^{2+} for activity *in vitro*, it may be regulated by Ca^{2+} or Ca^{2+}-dependent factors in cells. In apoptotic cells, $iPLA_2$-VIA is cleaved by caspase-3–like proteases at Asp^{183}, which results in enzyme activation and attendant increase in fatty acid release (*71*). This event may be responsible for membranous changes during apoptosis.

Conclusions

Current understanding of the regulation of eicosanoid synthesis by $sPLA_2$s and $cPLA_2\alpha$ is illustrated in Fig. 2. The control of particular PLA_2s should have advantages over the inhibition of selective lipid mediator pathways or other biological events in the treatment of pathological states. Since several PLA_2s can be involved in the pathology of various diseases, the understanding of the expression, function and regulation of each PLA_2 in specific tissues and disease states is of particular importance. In certain situations, it would be favorable to control the activity of different PLA_2s for the treatment of disorders.

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