NEW INSIGHTS INTO SECRETORY PHOSPHOLIPASE A₂

Secretory Phospholipase A₂ Releases Arachidonic Acid during Secretion  As described in the review by Murakami and Kudo in this issue, it has been proposed that secretory phospholipase A₂ (sPLA₂) enzymes can release arachidonic acid (AA) from cells through two pathways; the external plasma membrane pathway and the heparan sulfate proteoglycan (HSPG)-dependent pathway. However, each of these mechanisms requires the prior secretion of sPLA₂. Recently, a new model for sPLA₂ action in which the de novo synthesized sPLA₂s can act intracellularly without the requirement for prior secretion has been demonstrated.¹) This model can explain why exogenously added sPLA₂s are orders of magnitude less efficient at phospholipid hydrolysis than those produced within the cell; the concentration of sPLA₂s (and even of the phospholipid substrates) within the secretory compartments may be orders of magnitude higher than those secreted and dispersed into the extracellular medium.

Lessons from sPLA₂ Knockout Mice  Conclusions regarding the contribution of sPLA₂ enzymes to eicosanoid generation have relied on data obtained from transfected cells or the use of inhibitors that fail to discriminate between individual members of the large family of mammalian sPLA₂ enzymes. Also, even though group IIA sPLA₂ transgenic mice provide some insights into the in vivo role of this enzyme in antibacterial defense, atherosclerosis and skin carcinogenesis (see the review by Murakami and Kudo in this issue), abnormal overexpression of group IIA sPLA₂ in a mouse strain in which its gene is intrinsically disrupted does not reveal the true functions of this enzyme. Recently, two gene targeting studies have provided unequivocal evidence for the in vivo functions of two sPLA₂s, group IB and V.²³) Because of high expression of group IB sPLA₂ in the pancreas, it has been presumed that this enzyme plays a role in the digestion of dietary lipids. Gene targeting of group IB sPLA₂ has revealed that, although the knockout mice show no abnormalities in dietary lipid absorption when consuming a chow diet, following a high-fat diet these mice are resistant to high-fat diet-induced obesity.²³) The observed weight difference is due to decreased adiposity present in the knockout mice. Furthermore, compared with wild-type mice, group IB sPLA₂-null mice have lower plasma insulin and leptin levels after high-fat diet feeding. The knockout mice do not exhibit impaired glucose tolerance associated with the development of obesity-related insulin resistance, as observed in wild-type mice. These results suggest a novel role for group IB sPLA₂ in the protection against diet-induced obesity and obesity-related insulin resistance, thereby offering a new target for the treatment of obesity and diabetes.

In macrophages isolated from group V sPLA₂-deficient mice, zymosan-induced generation of leukotriene C₄ and prostaglandin E₂ (PGE₂) is attenuated by one-half compared with that in wild-type macrophages.³) The early phase of plasma exudation in response to intraperitoneal injection of zymosan and the accompanying in vivo generation of cysteinyl leukotrienes are markedly attenuated in group V sPLA₂-null mice compared with wild-type controls. These data provide clear evidence for a role for group V sPLA₂ in regulating eicosanoid generation in response to an acute inflammatory stimulus of the immune response in vivo, suggesting a role for this enzyme in innate immunity.

Novel sPLA₂s and Atherosclerosis  Since group V and X sPLA₂s are able to hydrolyze phosphatidylcholine (PC) in the lipoprotein particles far more efficiently than group IIA sPLA₂, the contribution of these two PC-hydrolyzing sPLA₂s to the development of atherosclerosis has been predicted (see the review by Murakami and Kudo in this issue), although the evidence for the presence of these enzymes in the atherosclerosis plaque was lacking. Recently, it has been confirmed that group V and X sPLA₂s are present in atherosclerosis lesions in vivo. Group V sPLA₂ is detected in human atherosclerotic aortic lesions and aortic root sections from apolipoprotein E-deficient mice.⁴) Similarly, group X sPLA₂ is detected in foam cell lesions in the arterial intima of high
fat-fed apolipoprotein E-deficient mice and of Watanabe heritable hyperlipidemic (WHHL) rabbits.\(^5,6\) Thus, modification of plasma lipoproteins by sPLA\(_2\)-V and -X may be more relevant than that by sPLA\(_2\)-IIA to the pathogenesis of atherosclerosis.

NEW INSIGHTS INTO CYTOSOLIC PLA\(_2\)\(\alpha\)

**The C2 Domain-Mediated Translocation of Cytosolic PLA\(_2\)\(\alpha\)** The C2 domain-directed, Ca\(^{2+}\)-dependent translocation of cytosolic PLA\(_2\) (cPLA\(_2\)\(\alpha\)) from the cytosol to the perinuclear membrane has been thought to be essential for the initiation of AA release and subsequent production of lipid mediators in agonist-stimulated cells (see the review by Hirabayashi et al. in this issue). However, the question of how and why cPLA\(_2\)\(\alpha\) preferentially targets the perinuclear membrane has remained unanswered. The C2 domain of cPLA\(_2\)\(\alpha\) binds to charge-neutral PC in preference to anionic phosphatidylserine (PS), a property that is in contrast to the C2 domain of protein kinase C\(\alpha\) (PKC\(\alpha\)), which binds to PS and moves preferentially to the plasma membrane. Interestingly, a cPLA\(_2\)\(\alpha\) mutant, in which the C2 domain is replaced with that of PKC\(\alpha\), translocates to the plasma membrane rather than the perinuclear membrane, whereas a PKC\(\alpha\) mutant harboring the cPLA\(_2\)\(\alpha\) C2 domain translocates to the perinuclear membrane.\(^7\) The plasma membrane-locating cPLA\(_2\)\(\alpha\) mutant exhibits poor PGE\(_2\)-biosynthetic ability, indicating that the perinuclear translocation of cPLA\(_2\)\(\alpha\) is important, even if not obligatory, for its functional coupling with cyclooxygenase (COX) enzymes.\(^8\) The cPLA\(_2\)\(\alpha\) C2 domain has specificity for the nuclear membrane mimic over the plasma membrane mimic due to the high PC content in the former, and aromatic and hydrophobic residues in the Ca\(^{2+}\) binding loops of the cPLA\(_2\)\(\alpha\) C2 domain are important for its lipid specificity.\(^7\) This argues that the biophysical principles that govern the in vitro membrane binding of C2 domain can account for most of its subcellular targeting properties. In addition, it has recently been shown that ceramide-1-phosphate (Cer-1-P) interacts directly with the C2 domain of cPLA\(_2\)\(\alpha\) in a lipid- and Ca\(^{2+}\)-dependent manner.\(^9\) Thus, stimulus-coupled production of Cer-1-P at the Golgi/perinuclear regions may also influence the localization of cPLA\(_2\)\(\alpha\).

Targeting of cPLA\(_2\)\(\alpha\) to other membrane compartments has also been shown. In macrophages, cPLA\(_2\)\(\alpha\) translocates to phagosomes during phagocytosis of zymozan.\(^10\) cPLA\(_2\)\(\alpha\)-generated AA has been shown to be an essential requirement for the activation of NADPH oxidase in phagocytes, and upon stimulation cPLA\(_2\)\(\alpha\) is transiently recruited to the plasma membranes, prior to perinuclear translocation, via direct association with NADPH oxidase.\(^11\) The association of cPLA\(_2\)\(\alpha\) with NADPH oxidase in the plasma membranes correlates with the kinetic burst of superoxide production. Thus, the ability of cPLA\(_2\)\(\alpha\) to regulate two different functions in the same cells (superoxide generation and eicosanoid production) is temporally achieved by a dual subcellular localization of cPLA\(_2\)\(\alpha\) to different targets. In mouse primary hippocampal neuron cultures, cPLA\(_2\)\(\alpha\) is localized in caveolae, and the scaffolding domain of caveolin-1 directly binds to cPLA\(_2\)\(\alpha\), leading to inhibition of its enzymatic activity.\(^12\) This raises the possibility that caveolin-1, via the inhibition of cPLA\(_2\)\(\alpha\), may interfere with synaptic facilitation and long-term potentiation formation in the hippocampus. In monocytes stimulated with M-CSF, cPLA\(_2\)\(\alpha\) can be localized within the nucleus through physical interaction with B-Myb.\(^13\) Both the N- and C-termini of cPLA\(_2\)\(\alpha\) interact with B-Myb, and this interaction in the nucleus negatively regulates c-Myc expression.

**The Catalytic Domain of cPLA\(_2\)\(\alpha\) is Required for Membrane Residence** Whereas the C2 domain is essential for the initial association of cPLA\(_2\)\(\alpha\) with the membrane as noted above, the catalytic domain remains associated with the membrane even after a decrease in [Ca\(^{2+}\)]\(_i\), levels, indicating the contribution of the catalytic domain to membrane residence of cPLA\(_2\)\(\alpha\). This Ca\(^{2+}\)-independent membrane association is greatly reduced by mutation of Trp\(^{644}\), a residue located at the membrane-exposed face of the catalytic domain.\(^14\) Phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) binds with high affinity and specificity to cPLA\(_2\)\(\alpha\), facilitating membrane binding and activity even in the absence of Ca\(^{2+}\).\(^15\) A study using cPLA\(_2\)\(\alpha\) point mutants revealed that the electrostatic repulsion between anionic residues located on or near the active-site lid and the anionic membrane surface in the catalytic domain triggers the opening of the active site and that a cluster of cationic residues (Lys\(^{491}\), Lys\(^{493}\), Lys\(^{494}\), and Arg\(^{496}\)) forms a specific binding site for PIP\(_2\).\(^16\) Furthermore, three hydrophobic residues at the rim of the active site Ile\(^{999}\), Leu\(^{400}\), and Leu\(^{552}\) partially penetrate the membrane, thereby promoting membrane binding and activation of cPLA\(_2\)\(\alpha\). Thus, the interfacial activation mechanism for cPLA\(_2\)\(\alpha\) involves the removal of the active site lid by nonspecific electrostatic repulsion, the interdomain hinge movement induced by specific PIP\(_2\) binding, and the partial membrane penetration by catalytic domain hydrophobic residues.

**Role of Ser\(^{505}\) Phosphorylation in cPLA\(_2\)\(\alpha\) Activation** Beyond the regulatory role of Ca\(^{2+}\), the maximal activation of cPLA\(_2\)\(\alpha\) in cells requires sustained phosphorylation of Ser\(^{505}\) by mitogen-activated protein kinases (see the review by Hirabayashi et al. in this issue). The main role of Ser\(^{505}\) phosphorylation is to promote membrane penetration of hydrophobic residues in the active-site rim (see above) by inducing a conformational change in the protein, and these enhanced hydrophobic interactions allow the sustained membrane interaction of cPLA\(_2\)\(\alpha\) in response to transient Ca\(^{2+}\) increase.\(^17\)

**Unique Posttranslational Modification of cPLA\(_2\)\(\gamma\)** cPLA\(_2\)\(\gamma\) is unique among the cPLA\(_2\) enzymes in that it is a Ca\(^{2+}\)-independent, membrane-associated enzyme that possesses a C-terminal prenylation motif. At this motif, cPLA\(_2\)\(\gamma\) undergoes at least three sequential posttranslational modifications, including farnesylation at Cys\(^{538}\), cleavage of the Cys\(^{538}\)–Cys\(^{339}\) bond, and carboxymethylation of the resultant C-terminal prenylated cysteine.\(^18\) These modifications eventually allow this enzyme to be constitutively associated with the membrane. Although the physiologic functions of cPLA\(_2\)\(\gamma\) (as well as those of cPLA\(_2\)\(\beta\) and cPLA\(_2\)\(\delta\)) are unknown, overexpression of cPLA\(_2\)\(\gamma\) in mammalian cells results in the increase in fatty acid release under certain conditions.\(^19\) Furthermore, cPLA\(_2\)\(\gamma\) is located in the endoplasmic reticulum (ER) and Golgi and alters the fatty acid composition of phosphatidylethanolamine, suggesting its potential...
role in phospholipid remodeling in the ER membrane.

NEW INSIGHTS INTO Ca\(^{2+}\)-INDEPENDENT PLA\(_2\)

**Ca\(^{2+}\)-Independent PLA\(_2\)\(\beta\) as an Executor of Apoptosis**

The involvement of group VIA Ca\(^{2+}\)-independent PLA\(_2\) (iPLA\(_2\)), or iPLA\(_2\)\(\beta\), in apoptosis was first demonstrated by the findings that the fatty acid release during Fas-induced apoptosis of U937 cells is accompanied by iPLA\(_2\)\(\beta\) activation by caspase-3-directed cleavage at Asp\(^{183}\) and that some features of the apoptosis, such as PS externalization, is suppressed by the iPLA\(_2\) inhibitor bromoenol lactone (BEL).\(^{20}\)

Recently, evidence that supports the role of iPLA\(_2\)\(\beta\) in various phases of apoptosis has emerged from several studies. Apoptosis of insulin-secreting cells induced by ER stress is amplified by overexpression of iPLA\(_2\)\(\beta\) and is suppressed by BEL.\(^{21}\) Apoptosis is associated with the stimulation of iPLA\(_2\)\(\beta\) activity, perinuclear accumulation of iPLA\(_2\)\(\beta\) protein and activity, and caspase-3-catalyzed cleavage of full-length 84-kDa iPLA\(_2\)\(\beta\) at (probably) Asp\(^{183}\) to a 62-kDa product that associates with the nuclei.

Efficient engulfment of the intact cell corpse is a critical end point of apoptosis, required to prevent secondary necrosis and inflammation. The presentation of “eat-me” signals on the dying cell is an important part of this process of recognition and engulfment by professional phagocytes. Lysophosphatidylcholine (LPC) secreted from apoptotic cells acts as a chemoattractant for monocytes, and the production of LPC occurs through the caspase-3-mediated activation of iPLA\(_2\)\(\beta\).\(^{22}\) Following this cleavage, iPLA\(_2\)\(\beta\) is processed to a smaller and more active 26-kDa fragment. Additionally, the exposure of membrane LPC following iPLA\(_2\)\(\beta\) activation in apoptotic T cells facilitates cell-surface binding of IgM and complements, which eventually leads to clearance of apoptotic cells.\(^{23}\)

Hypoxic cell death is independent of caspases and is associated with nuclear shrinkage. Hypoxia causes elevation of iPLA\(_2\)\(\beta\) activity and translocation of iPLA\(_2\)\(\beta\) to the nucleus, and nuclear shrinkage and cell death are suppressed by BEL or by siRNA-based knockdown of iPLA\(_2\)\(\beta\).\(^{24}\) These results indicate that iPLA\(_2\)\(\beta\) is also crucial for a caspase-independent cell death-signaling pathway leading to nuclear shrinkage.

**Ca\(^{2+}\) Entry and iPLA\(_2\)\(\beta\)**

It has been reported that iPLA\(_2\)\(\beta\) is activated by the depletion of intracellular Ca\(^{2+}\) stores and that iPLA\(_2\)\(\beta\) activity is blocked by calmodulin (see the review by Akiba and Sato in this issue). However, the biological relevance of these events has remained unclear. Recent evidence has suggested that these regulatory events for iPLA\(_2\)\(\beta\) are operative in the activation of the store-operated cation (SOC) channels and store-operated Ca\(^{2+}\) entry pathway. Depletion of cellular Ca\(^{2+}\) by thapsigargin induces activation of iPLA\(_2\)\(\beta\) and SOC channels, and antisense suppression of iPLA\(_2\)\(\beta\) prevents these processes.\(^{25}\) SOC channels can be activated by calcium influx factor (CIF) that is produced upon depletion of calcium stores, and CIF displaces inhibitory calmodulin from iPLA\(_2\)\(\beta\), resulting in the activation of iPLA\(_2\)\(\beta\) and generation of lysophospholipids that in turn activate SOC channels and capacitative Ca\(^{2+}\) influx.\(^{26}\) Upon refilling of the stores and termination of CIF production, calmodulin rebinds to iPLA\(_2\)\(\beta\), inhibits it, and the activity of SOC channels and capacitative calcium influx is terminated. The finding that iPLA\(_2\)\(\beta\) is a crucial molecular determinant in the regulation of cellular Ca\(^{2+}\) homeostasis raises the intriguing possibility that iPLA\(_2\)\(\beta\) can modulate the activation of Ca\(^{2+}\)-dependent cPLA\(_2\)\(\alpha\) indirectly and may thus provide an answer for controversial reports for the participation of cPLA\(_2\)\(\alpha\) versus iPLA\(_2\)\(\beta\) on lipid mediator production.

**Conditional Transgenic Overexpression of iPLA\(_2\)\(\beta\) in Hearts**

Recently, heart-specific iPLA\(_2\)\(\beta\) transgenic mice have been generated.\(^{27}\) Coronary artery occlusion in Langendorff perfused hearts from transgenic mice results in a marked increase in fatty acids released into the venous effluent, a 4-fold increase in LPC mass in ischemic zones, and malignant ventricular tachyarrhythmias within minutes of ischemia. Pretreatment of Langendorff perfused transgenic hearts with BEL just minutes prior to induction of ischemia completely ablates fatty acid release and lysophospholipid accumulation and rescues the hearts from malignant ventricular tachyarrhythmias. These results demonstrate that ischemia activates iPLA\(_2\)\(\beta\) in intact myocardium and that iPLA\(_2\)\(\beta\)-mediated hydrolysis of membrane phospholipids can induce lethal malignant ventricular tachyarrhythmias during acute cardiac ischemia.

**iPLA\(_2\)\(\gamma\), a Peroxisomal PLA\(_2\)**

Group VIIIB iPLA\(_2\), or iPLA\(_2\)\(\gamma\), is expressed as several variants with distinct translation initiation sites, resulting in 88-, 77-, 74- and 63-kDa protein products, and has a C-terminal peroxisomal localization signal.\(^{28}\) In rat liver, iPLA\(_2\)\(\gamma\) exists as a 63-kDa protein and is localized in the peroxisome.\(^{29}\) Since the peroxisome membrane is enriched in AA-containing phospholipids, the localization of iPLA\(_2\)\(\gamma\) in this organelle suggests the possibility that this enzyme can contribute to eicosanoid generation.

REFERENCES