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*JB Review*

# Secreted phospholipase $A_2$ revisited

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**Phospholipase $A_2$ ($PLA_2$) catalyses the hydrolysis of the *sn*-2 position of glycerophospholipids to yield fatty acids and lysophospholipids. So far, more than 30 enzymes that possess $PLA_2$ or related activity have been identified in mammals. About one third of these enzymes belong to the secreted $PLA_2$ ($sPLA_2$) family, which comprises low molecular weight, $Ca^{2+}$ requiring, secreted enzymes with a His/Asp catalytic dyad. Individual $sPLA_2$s display distinct localizations and enzymatic properties, suggesting their specialized biological roles. However, in contrast to intracellular $PLA_2$s, whose roles in signal transduction and membrane homoeostasis have been well documented, the biological roles of $sPLA_2$s *in vivo* have remained obscure until recently. Over the past decade, information fuelled by studies employing knockout and transgenic mice as well as specific inhibitors, in combination with lipidomics, has clarified *when* and *where* the different $sPLA_2$ isoforms are expressed, *which* isoforms are involved in *what* types of pathophysiology, and *how* they exhibit their specific functions. In this review, we highlight recent advances in $PLA_2$ research, focusing mainly on the physiological functions of $sPLA_2$s and their modes of action on 'extracellular' phospholipid targets *versus* lipid mediator production.**

*Abbreviations*: AA, arachidonic acid; ARDS, acute respiratory distress syndrome; $cPLA_2$, cytosolic $PLA_2$; cysLT, cysteinyl LT; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DRG, dorsal root ganglion; EPA, eicopentaenoic acid; HDL, high-density lipoprotein; $iPLA_2$, $Ca^{2+}$-independent $PLA_2$; IRS, inner root sheath; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPS, lipopolysaccharide; LT, leucotriene; LXR, liver X receptor; ORS, outer root sheath; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PS, phosphatidylserine; $PLA_2$, phospholipase $A_2$; PLA2R1, M-type $PLA_2$ receptor; PLAI, $PLA_2$ inhibitory protein; PPAR, peroxisome proliferator-activated receptor; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; $sPLA_2$, secreted $PLA_2$; StAR, steroidogenic acute regulatory protein; VLDL, very low-density lipoprotein.

Phospholipase $A_2$ ($PLA_2$) catalyses the hydrolysis of the *sn*-2 position of glycerophospholipids to yield (mostly unsaturated) fatty acids and lysophospholipids (Fig. 1). In 2002, we published a minireview entitled 'Phospholipase $A_2$' in the *Journal of Biochemistry*, the major topics of which were limited to the classification and enzymatic properties of most if not all $PLA_2$ enzymes, with some functional aspects of arachidonic acid (AA) metabolism, based largely on *in vitro* cell culture studies (*1*). Until then, only a few transgenic/knockout mice for $PLA_2$ enzymes had been available, which included transgenic mice for group IIA secreted $PLA_2$ ($sPLA_2$-IIA) and knockout mice for group IVA cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$) as well as mouse strains in which the gene for $sPLA_2$-IIA (*Pla2g2a*) is naturally disrupted. In 1995, it was found that mouse strains with a frameshift mutation in the *Pla2g2a* gene (so-called 'natural knockout mice') were more susceptible to colorectal cancer than those having the functional enzyme (*2*). However, this finding would have been inevitably affected by the different genetic backgrounds of the mouse strains used as controls, which show distinct susceptibility to diseases. $sPLA_2$-IIA transgenic (*Pla2g2a*-Tg) mice, which were reported in 1996 as the first mouse strain gene-manipulated for $PLA_2$ enzymes, displayed skin abnormality manifested by alopecia without inflammation (*3*), and subsequently resistance to bacterial infection (*4*, *5*) and increased lesions of diet-induced atherosclerosis (*6*). However, except for the anti-bacterial defence, which well reflects the *in vitro* ability of this enzyme to kill Gram-positive bacteria at low concentrations (*7*), the physiological significance of the phenotypes observed in *Pla2g2a*-Tg mice has remained a mystery. $cPLA_2\alpha$ knockout ($Pla2g4a^{-/-}$) mice, which were first reported in 1997, provided a consistent view of the role of this enzyme in the production of eicosanoids [prostaglandins (PGs) and leucotrienes (LTs)] in reproduction, asthma, cancer and other contexts (*8*–*10*). On the basis of these findings, together with the elaborate regulatory system of $cPLA_2\alpha$ by $Ca^{2+}$ and phosphorylation that can account mostly for signal-coupled AA release (*11*, *12*), $cPLA_2\alpha$, rather than $sPLA_2$s, has attracted the attention of the majority of researchers in this field.

Since then, about a decade has passed, during which great advances have been achieved in the field of $PLA_2$ research by generation of a number of transgenic and knockout mouse lines, the development of inhibitors fairly specific for individual $PLA_2$ enzymes, and the identification of human diseases in which particular $PLA_2$ genes are mutated or show polymorphisms. Transgenic and/or knockout mice for the 5 $sPLA_2$ isoforms (groups IB, IIA, III, V and X) have been



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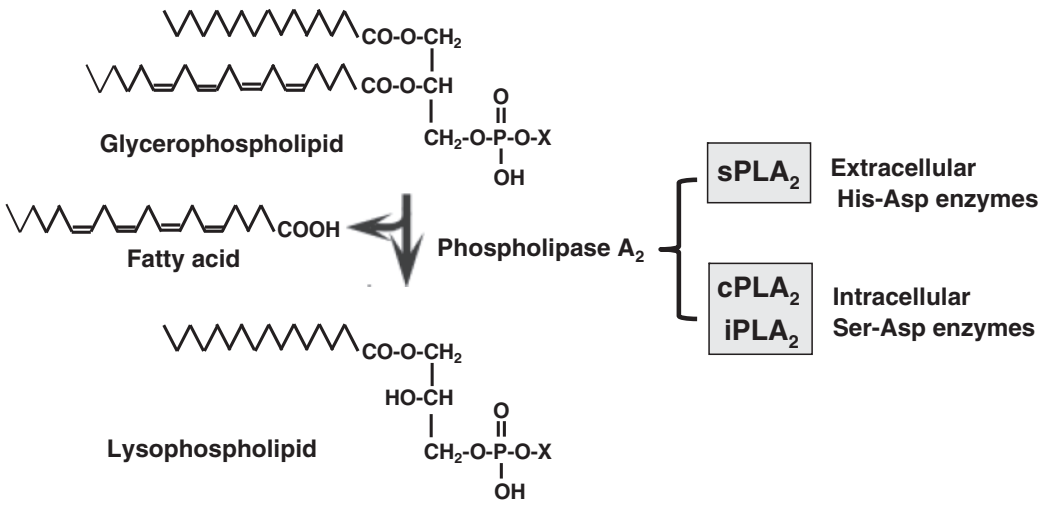


**Fig. 1 $PLA_2$ reaction.** $PLA_2$ enzymes, including extracellular $sPLA_2$s with a His–Asp dyad and intracellular $cPLA_2$s/$iPLA_2$s with a Ser–Asp dyad, hydrolyse the *sn*-2 position of phospholipids to yield fatty acids and lysophospholipids. Several members of the $cPLA_2$ and $iPLA_2$ families also possess $PLA_1$, lysophospholipase, or triglyceride lipase activity, whereas $sPLA_2$s definitively display $PLA_2$ activity.

generated, and it has become evident that individual $sPLA_2$s make a distinct contribution to various biological events such as digestion, inflammation, tissue injury, atherosclerosis, host defence, reproduction and skin homoeostasis (*13–15*). Numerous studies using *Pla2g4a*$^{-/-}$ mice have provided a comprehensive view of the contribution of $cPLA_2\alpha$ to various diseases and homoeostasis. In addition, an overall picture of the regulatory mechanisms leading to activation of $cPLA_2\alpha$, as well as its novel role in membrane trafficking, has emerged (*16–19*). Deficiency and/or mutations of $Ca^{2+}$-independent $PLA_2$ ($iPLA_2$) members have been found to be associated with various disorders such as neurodegeneration and metabolic syndrome in both mice and humans (*20–27*). In this article, we provide an overview of the functions of $sPLA_2$s, focusing mainly on lessons learned from the use of knockout and/or transgenic mice. In fact, it has become obvious that $sPLA_2$s are involved in many biological processes through multiple mechanisms, and as a consequence they have been returning to the forefront of research. The current classification of $sPLA_2$ enzymes, together with the $cPLA_2$ and $iPLA_2$ families that are not described in the main text, is summarized in Table I. For details of the other $PLA_2$ groups, please refer to a number of recent excellent reviews (*28–30*).

## General aspects of the $sPLA_2$ family

To date, 11 $sPLA_2$s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB) have been identified in mammals (Fig. 2). They are subdivided into three major collections, namely conventional group I/II/V/X $sPLA_2$s and atypical groups III and XII $sPLA_2$s (*13–15*). Conventional $sPLA_2$s are 14–19 kDa secreted enzymes with a highly conserved $Ca^{2+}$-binding site and a catalytic His–Asp dyad. In addition to these elements, there are six absolutely conserved disulphide bonds and up to two additional unique disulphide bonds, which contribute to the high degree of stability of these enzymes. $sPLA_2$-IB and -X have an N-terminal propeptide that is proteolytically cleaved off, yielding fully active enzymes. The atypical $sPLA_2$s, $sPLA_2$-III and $sPLA_2$-XIIA/XIIB, have poor homology with the I/II/V/X collection of $sPLA_2$s except for the conserved $Ca^{2+}$-binding loop and catalytic site. For more details of the enzymatic features of individual $sPLA_2$s, please refer to our previous *JB* review in 2002 (*1*).

A database search of $sPLA_2$s in the animal kingdom provides an insight into the evolutional relationships among the isoforms. Of the conventional $sPLA_2$s, $sPLA_2$-IB appears to be the oldest isozyme, whose orthologues are found from nematodes (*e.g. Caenorhabditis elegans*, which has three IB-like genes) to vertebrates, and at a later stage $sPLA_2$-X appeared in amphibians. Beyond the latter stage, evolutionary pressure may have forced the emergence of $sPLA_2$s without an N-terminal propeptide, namely group II $sPLA_2$s: thus, $sPLA_2$-IIA and probably -IID and -IIE, all of which have a standard 'group II' structure, first appeared in snakes, $sPLA_2$-IIC and -IIF, which have a unique extra segment, appeared in birds and $sPLA_2$-V, the simplest and newest isoform, finally appeared in mammals. The group III $sPLA_2$ collection is highly divergent in insects (*e.g.* five genes are found in *Drosophila*), whereas only a single gene for group III is encoded in the vertebrate genome (fish to mammals) and is not present in nematodes. The group XII $sPLA_2$s are found in all vertebrates, but not in invertebrates.

Since $sPLA_2$s are 'secreted' and require mM-order $Ca^{2+}$ for catalysis, their primary target phospholipids should be principally extracellular. $sPLA_2$s can act on cellular membranes in an autocrine or paracrine manner to release not only AA, but also a variety of saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) such as ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are precursors of anti-inflammatory lipid mediators. The counterpart product, namely lysophospholipid such as

**Table I. Classification of the $sPLA_2$, $cPLA_2$ and $iPLA_2$ families.**

| Families | Gene names | Nomenclatures | Alternative names | Characteristics | Mutations or SNPs in human diseases |
|---|---|---|---|---|---|
| $sPLA_2$ | *Pla2g1b* | IB | Pancreatic $sPLA_2$ | Conventional $sPLA_2$ with group I property | Obesity |
| | *Pla2g2a* | IIA | Inflammatory $sPLA_2$ | Conventional $sPLA_2$ with group II property | Colon cancer |
| | *Pla2g2c* | IIC | | Conventional $sPLA_2$ with group II property, absent in humans | |
| | *Pla2g2d* | IID | | Conventional $sPLA_2$ with group II property | COPD with body weight loss |
| | *Pla2g2e* | IIE | | Conventional $sPLA_2$ with group II property | |
| | *Pla2g2f* | IIF | | Conventional $sPLA_2$ with group II property, long C-terminal extension | |
| | *Pla2g5* | V | | Conventional $sPLA_2$ without groups I and II properties | Type 2 diabetes |
| | *Pla2g10* | X | | Conventional $sPLA_2$ with both groups I and II properties | |
| | *Pla2g3* | III | | Atyplical $sPLA_2$, group III collection | Colon cancer, AIDS |
| | *Pla2g12a* | XIIA | | Atypical $sPLA_2$, group XII collection | |
| | *Pla2g12b* | XIIB | | Atypical $sPLA_2$, group XII collection, no activity | |
| $cPLA_2$ | *Pla2g4a* | IVA | $cPLA_2\alpha$ | C2 domain, AA specific, MAPK phosphorylation | Inherited deficiency with platelet dysfunction |
| | *Pla2g4b* | IVB | $cPLA_2\beta$ | C2 domain, JimC domain | |
| | *Pla2g4c* | IVC | $cPLA_2\gamma$ | Prenylation, no C2 domain | |
| | *Pla2g4d* | IVD | $cPLA_2\delta$ | C2 domain | |
| | *Pla2g4e* | IVE | $cPLA_2\varepsilon$ | C2 domain | |
| | *Pla2g4f* | IVF | $cPLA_2\zeta$ | C2 domain | |
| $iPLA_2$ | *Pla2g6* | VIA | $iPLA_2\beta$ | Ankyrin repeat | Neurodegenerative diseases |
| | *Pnpla8* | VIB | $iPLA_2\gamma$ | Mitochondrial and peroxisomal localization signals | |
| | *Pnpla6* | | $iPLA_2\delta$, NTE | Long N-terminal region with a transmembrane domain | Neurodegenerative diseases |
| | *Pnpla7* | | NRE | Long N-terminal region with a transmembrane domain | |
| | *Pnpla2* | | $iPLA_2\zeta$, ATGL | Major triglyceride lipase | Neutral lipid storage disease |
| | *Pnpla3* | | $iPLA_2\varepsilon$, adiponutrin | Transcylase? | Fatty liver |
| | *Pnpla4* | | $iPLA_2\eta$, GS2 | Retinylester hydrolase? absent in mice | |
| | *Pnpla5* | | GS2-like | | |
| | *Pnpla1* | | | Major splicing variant without a catalytic dyad | |

For other $PLA_2$ families, please see ref (*13*).

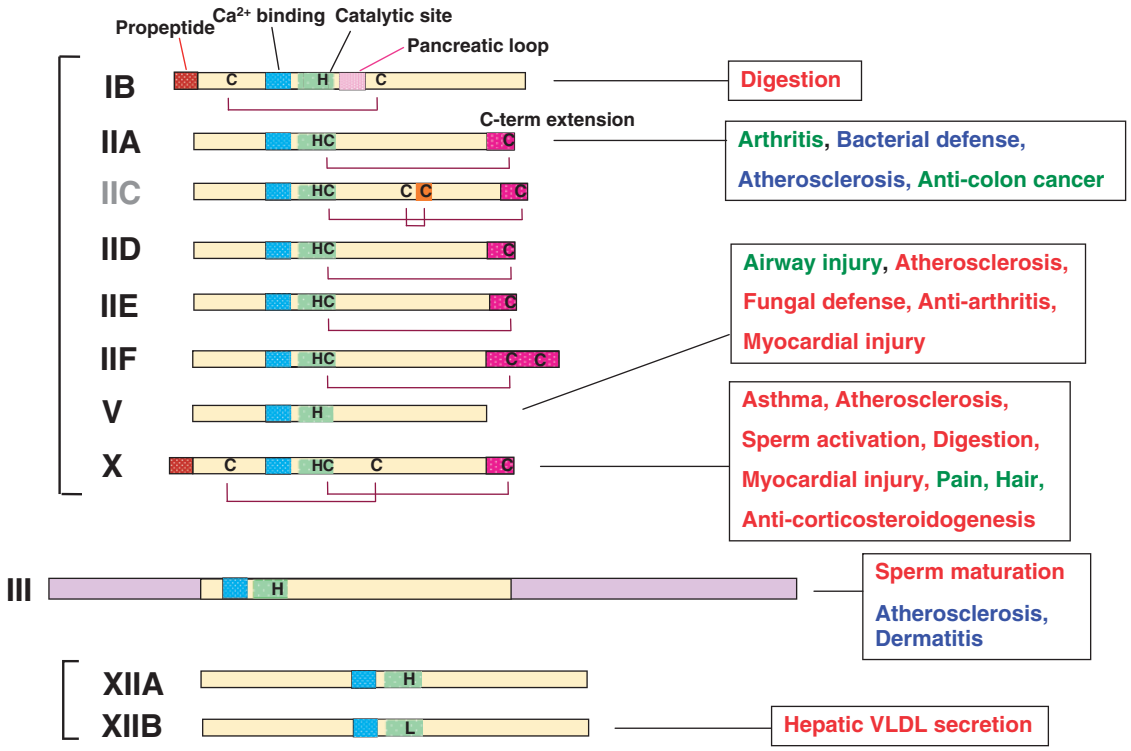


**Fig. 2 The sPLA$_2$ family.** Mammalian sPLA$_2$s comprise 11 isoforms, which are subdivided into three collections, namely group I/II/V/X (conventional sPLA$_2$s), group III and group XII. All enzymes have a conserved catalytic centre with a His–Asp dyad and a $Ca^{2+}$-binding loop. sPLA$_2$-IB, a pancreatic sPLA$_2$, is characterized by an N-terminal propeptide whose proteolytic removal gives rise to a functional enzyme, the presence of a Cys11–Cys77 disulphide bond (group I-specific disulphide), and a unique pancreatic loop. The group II subfamily (IIA, IIC, IID, IIE and IIF) is characterized by absence of the propeptide and the presence of Cys49-Cys within the C-terminal extension (group II-specific disulphide). sPLA$_2$-IIC has a unique insertion with an extra disulphide bond, but this isoform is absent in humans (pseudogene). sPLA$_2$-IIF has a longer C-terminal extension, which is Pro-rich. sPLA$_2$-V is evolutionarily close to the group II subfamily, but lacks the group II-specific disulphide and the C-terminal extension. sPLA$_2$-X has both groups I and II properties since it has an N-terminal propeptide and both groups I- and II-specific disulphides. sPLA$_2$-III is unique in that the central sPLA$_2$ domain, which is more similar to bee venom PLA$_2$ than to group I/II/V/X sPLA$_2$s, is flanked by unique and highly cationic N- and C-terminal domains. The N- and C-terminal domains are removed to produce a mature, sPLA$_2$ domain-only form. The group XII collection contains two isoforms, XIIA and XIIB, whose overall structures (except for the catalytic domain and $Ca^{2+}$-binding site) do not show any homology with other sPLA$_2$s. The catalytic centre His is replaced by Leu in sPLA$_2$-XIIB, indicating that this enzyme is catalytically inactive. Biological functions assigned to individual isoforms, as assessed by knockouts (red), transgenic (blue) or both (green), are shown in the right boxes.

lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA), also has various bioactivities. Moreover, sPLA$_2$s can also act on 'non-cellular' phospholipids, such as those in microvesicles, pulmonary surfactant, lipoproteins, microbial membranes and food substances (Fig. 3). Therefore, the physiological functions of a given sPLA$_2$ could reflect a combination of these multiple actions. In keeping with these general views, we will highlight a variety of biological functions of sPLA$_2$ isoforms *in vivo,* particularly in the context of pathophysiology. In many cases, multiple sPLA$_2$s can participate in a given biological process in distinct, redundant, complementary or counter-regulatory ways.

## sPLA$_2$ and lipid mediators: *in vitro* and *in vivo*

Numerous *in vitro* studies, mostly on the basis of forcible transfection or exogenous addition of recombinant sPLA$_2$s, have shown that several sPLA$_2$s have the ability to liberate AA from cell membranes, leading to augmented production of eicosanoids (PGs and LTs), with a potency order of X > V > III > IIF > IIA > IB > IID > IIE > XIIA (*31–35*). The high capacity of sPLA$_2$-X, -V and -III to release cellular AA correlates with their high capacity to bind phosphatidylcholine (PC), a major phospholipid in the outer leaflet of the plasma membrane (*33–35*). The cellular AA-releasing function of group II sPLA$_2$s requires co-factors such as heparan sulphate proteoglycans, which help to facilitate their residence in the plasma membrane or internalization, and appropriate pro-inflammatory stimuli, which may induce rearrangement of membrane microdomains leading to exposure of their better substrates, phosphatidylethanolamine (PE) and phosphatidylserine (PS) (*34*, *36*). However, since these *in vitro* studies were often performed using super-physiological amounts of exogenous or overexpressed sPLA$_2$s, it remained uncertain whether or not endogenous sPLA$_2$s could play such a role *in vivo*.

Subsequent studies using mice that are transgenic, or more physiologically knocked out, for individual sPLA$_2$s have revealed that they can mobilize lipid mediators *in vivo*. For instance, transgenic overexpression of sPLA$_2$-III (*Pla2g3*-Tg) or sPLA$_2$-X (*Pla2g10*-Tg) in mice leads to increased PGE$_2$ production in the

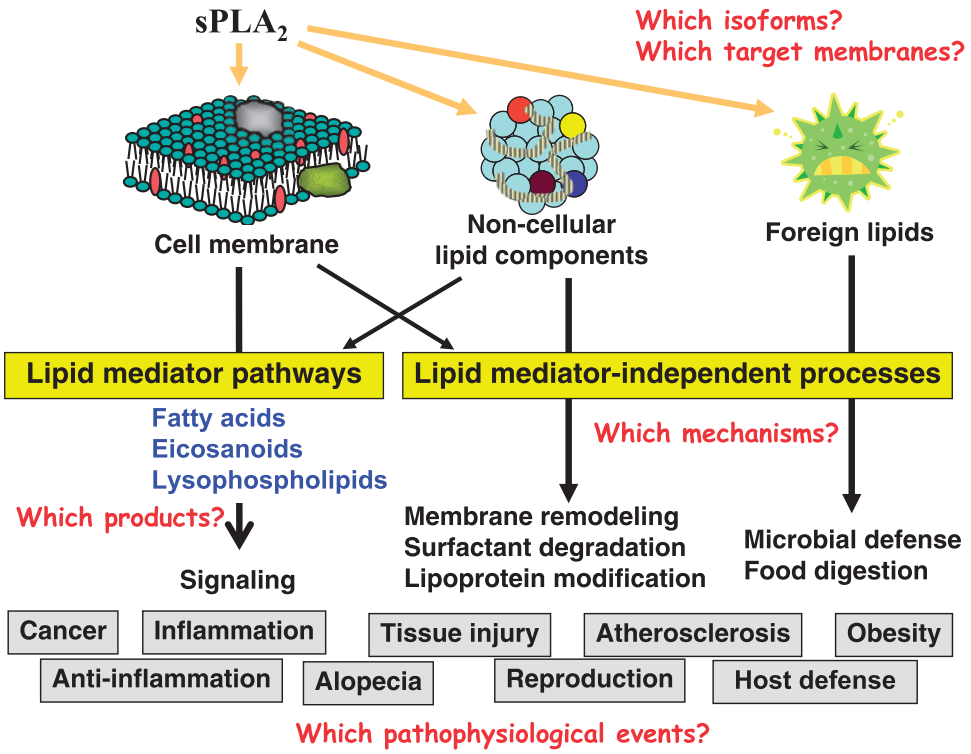


**Fig. 3 Schematic diagram of $sPLA_2$ biology**. Individual $sPLA_2$ enzymes act not only on cellular membranes, but also on non-cellular phospholipids (*e.g.* surfactant and lipoproteins) and foreign phospholipids (*e.g.* bacterial membranes and dietary phospholipids). Some actions of $sPLA_2$s are mediated by lipid mediator–mediated signalling, while others occur independently of lipid mediators. The physiological functions of a particular $sPLA_2$ could reflect a combination of these varied actions. Thus, in order to gain an overall functional view of $sPLA_2$, it is important to clarify (i) the $sPLA_2$ isoforms that act on (ii) specific target membranes, (iii) the lipid mediators that are produced if 'dependent' upon lipid mediators, (iv) the mechanisms that are responsible if 'independent' of lipid mediators and (v) the pathophysiological events in which individual $sPLA_2$s are involved.

skin (*37*, *38*). Macrophage production of cysteinyl LTs (cysLTs) and $PGE_2$ (*39*) and mast cell production of $PGD_2$ (*40*) following zymosan stimulation are partially reduced in mice lacking $sPLA_2$-V (*Pla2g5*$^{-/-}$) compared with replicate control mice. In $sPLA_2$-X-deficient (*Pla2g10*$^{-/-}$) mice, there is a partial reduction of $LTB_4$ synthesis by neutrophils (*41*). Other examples of altered lipid mediator levels in $sPLA_2$ knockout mice in distinct physiological settings will be described below.

It should be noted that, even though the above results imply that $sPLA_2$s can participate in the lipid mediator-biosynthetic pathways *in vivo*, they do not necessarily mean that these enzymes directly hydrolyse membrane phospholipids to provide AA. Indeed, in several *in vivo* situations, the augmented action of $sPLA_2$s on lipid mediator synthesis depends on the coordinated activation of $cPLA_2\alpha$. One idea is that $sPLA_2$-released lysophospholipids (or other bioactive lipids) may amplify the $cPLA_2\alpha$ activation machinery leading to the burst synthesis of lipid mediators (Fig. 4A), as exemplified by $sPLA_2$-mediated LT production by granulocytes (*42*, *43*) and $PGD_2$ production by mast cells (*44*). Alternatively, $sPLA_2$s may regulate the bottleneck step of a given disease, and as such, subsequent propagation of the disease results in explosive activation of $cPLA_2\alpha$ that supplies the bulk of lipid mediators within the affected tissue (Fig. 4B). Such coordinated actions of extracellular and intracellular $PLA_2$s can explain why knockout of either $sPLA_2$ or $cPLA_2\alpha$ culminates in dramatic amelioration of several disease models, such as asthma and arthritis (see below). More importantly, however, many of the phenotypes displayed by $sPLA_2$ gene-manipulated mice described below appear to occur independently of $cPLA_2\alpha$ or lipid mediators (Fig. 4C and D); this again implies complex actions of $sPLA_2$s on distinct phospholipid components outside the cells.

## $sPLA_2$ and inflammation

### *Airway disease*

We found that *Pla2g5*-Tg mice, which were the first $sPLA_2$ Tg mice we generated in 2006, died soon after birth because of acute respiratory distress syndrome (ARDS) resulting from aberrant hydrolysis of lung surfactant phospholipids, including dipalmitoyl-PC and -phosphatidylglycerol (*45*). Soon afterwards, similar fatal airway destruction was also reported in macrophage-specific *Pla2g10*-Tg mice (*46*). Pulmonary surfactant is a lipid–protein complex synthesized by alveolar type II epithelial cells, and plays a role in lowering surface tension along the alveolar epithelium, thereby promoting alveolar stability. Destruction of as little as 10–20% of surfactant lipids can cause loss of alveolar stability and impairment of gas exchange, leading to ARDS. ARDS in

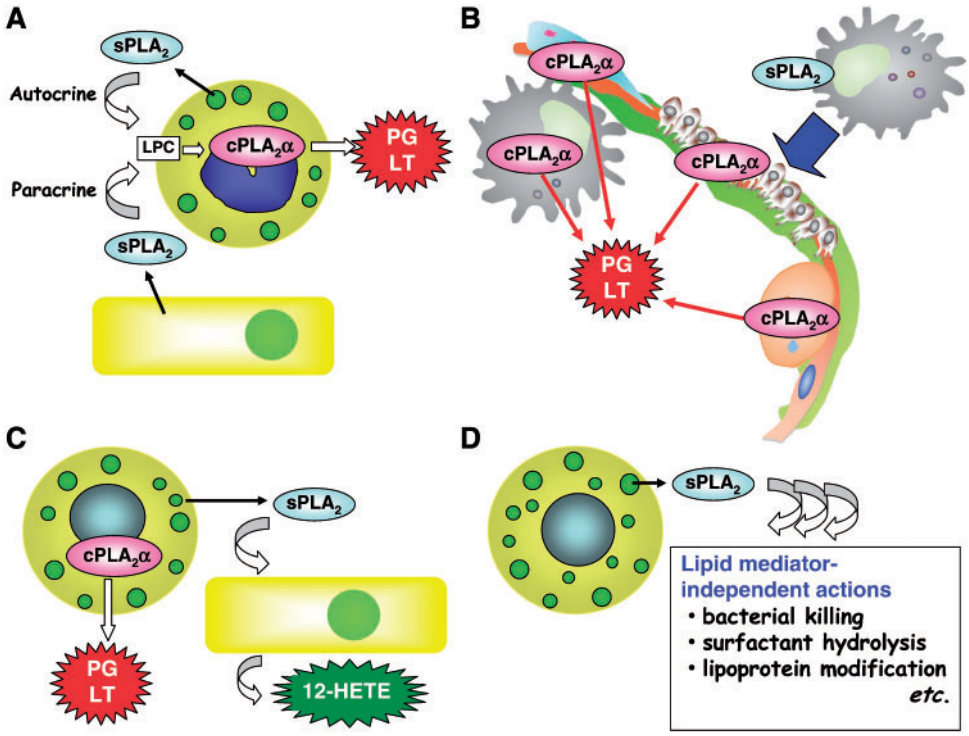


**Fig. 4 Various modes of $sPLA_2$ action.** (A) During proposed sequential $sPLA_2$/$cPLA_2\alpha$ cross-talk, LPC or other products released by $sPLA_2$ in an autocrine or paracrine manner may amplify the $cPLA_2\alpha$ activation pathway in the same cell, leading to full propagation of eicosanoid synthesis. An example is the cooperation of $sPLA_2$/$cPLA_2\alpha$ in eosinophils in bronchial asthma. (B) $sPLA_2$ regulates a certain 'bottleneck' step of the disease in a proximal or distal tissue compartment. Accordingly, suppression of $sPLA_2$ at this step may eventually abrogate subsequent $cPLA_2\alpha$-dependent eicosanoid synthesis linked to the disease. As an example, $sPLA_2$-V in antigen-presenting cells contributes to the sensitization phase (antigen uptake and processing), whereas $cPLA_2\alpha$, which is expressed ubiquitously, contributes to the bulk PG/LT synthesis by various cells in the airway during the effector phase. (C) $cPLA_2\alpha$ and $sPLA_2$ contribute to distinct pools of lipid mediators, probably by acting on different cells. For instance, production of 12-HETE, but not PGs, in the epididymis depends on $sPLA_2$-III. (D) The actions of $sPLA_2$ take place independently of lipid mediators. This includes many examples, *e.g.* anti-bacterial defence by $sPLA_2$-IIA through bacterial membrane degradation, anti-fungal defence by $sPLA_2$-V through macrophage phagocytosis, atherosclerosis by $sPLA_2$-IIA, -III, -V and X through generation of small-dense LDL, alveolar damage by $sPLA_2$-V through surfactant hydrolysis, sperm maturation by $sPLA_2$-III though sperm membrane remodelling, and dietary phospholipid digestion by $sPLA_2$-IB and -X that can be linked to distal adiposity. For details, please see text.

experimental models can be attenuated by administration of a pan-$sPLA_2$ inhibitor, suggesting the contribution of $sPLA_2$(s) to this fatal pulmonary disease (*47*, *48*). Indeed, $sPLA_2$-V is expressed in the bronchial epithelium and alveolar macrophages, and is induced in mouse Th2-dependent asthma models as well as in patients with asthma or severe pneumonia (*49*, *50*). $sPLA_2$-X is also expressed in airway epithelial cells in both mice and humans (*50*, *51*). These two $sPLA_2$s can potently hydrolyse surfactant PC *in vitro* (*52*). Thus, $sPLA_2$-V and -X represent the pathogenic $sPLA_2$s in airway injury, at least in part through the hydrolytic degradation of lung surfactant.

Regardless of the mechanisms involved, the pathological role of these two $sPLA_2$s in airway diseases has rapidly become clearer through subsequent studies using mice with knockout of both enzymes. The first phenotype reported for *Pla2g10*$^{-/-}$ mice in 2007 was remarkable resistance to an antigen-induced asthma model, where the lungs of the mice showing marked attenuation of T cell and eosinophil infiltration, goblet cell metaplasia, thickening of the smooth muscle cell layer, subepithelial fibrosis and Th2 cytokine levels (*53*). In view of the marked reduction of eicosanoid levels in *Pla2g10*$^{-/-}$ lung, this study suggested that the amelioration of the airway phenotype was attributable to reduction of $sPLA_2$-X-mediated eicosanoid synthesis. Considering that mice lacking $cPLA_2\alpha$ (*Pla2g4a*$^{-/-}$) were also protected from this form of induced asthma (*8*), functional cooperation of $sPLA_2$-X and $cPLA_2\alpha$ may be a pathologic feature of asthma. In this scenario, $sPLA_2$-X may be secreted from airway epithelial cells and act in a paracrine manner on recruited leucocytes such as eosinophils, boosting $cPLA_2\alpha$-mediated AA release (Fig. 4A) (*43*). Even if this proves to be the case, it still remains undetermined whether $sPLA_2$-X may play an important role in a specific, as yet unidentified, rate-limiting step for asthma (*e.g.* polarization of alternatively activated macrophages or airway epithelial damage by excessive surfactant hydrolysis), and whether ablation of this regulatory step might subsequently dampen all downstream inflammatory reactions in the airway (Fig. 4B). In fact, the latter mechanism is partly responsible for the pro-asthmatic function of $sPLA_2$-V, as described below.

Soon after the above findings in *Pla2g10*$^{-/-}$ mice, *Pla2g5*$^{-/-}$ mice were also shown to be protected from the same type of antigen-induced asthma (*49*). Likewise, airway hyper-responsiveness in the allergic

mice was partially blocked by intratracheal application of anti-sPLA$_2$-V antibody. Conversely, intratracheal administration of sPLA$_2$-V, but not sPLA$_2$-IIA or a catalytically weak sPLA$_2$-V mutant, increased airway resistance and narrowing along with increased leucocyte infiltration (*49*). Bronchoconstriction elicited by sPLA$_2$-V in mice lacking cPLA$_2\alpha$ (*pla2g4*$^{-/-}$) was comparable to that in wild-type mice, suggesting that sPLA$_2$-V exerts its pro-asthmatic action independently of cPLA$_2\alpha$, and thereby of eicosanoids. Similar approaches were conducted in the LPS-induced airway injury model, and this again showed that *Pla2g5*$^{-/-}$ mice were protected from this ARDS-like inflammation (*54*). These data collectively indicate that sPLA$_2$-V is involved in both chronic and acute airway disorders. The fact that intratracheal treatment with sPLA$_2$-V and its neutralizing antibody recapitulated the phenotypes of *Pla2g5*-Tg and *Pla2g5*$^{-/-}$ mice, respectively, supports the speculation that sPLA$_2$-V causes airway inflammation by hydrolysing pulmonary phospholipids, probably surfactants.

Another regulatory step of Th2-driven allergic pulmonary inflammation in which sPLA$_2$-V plays a regulatory role has been identified very recently. Antigen-presenting cells from *Pla2g5*$^{-/-}$ mice showed a delay in allergen uptake and processing and thereby a partial impairment of allergen-induced maturation (*55*). Accordingly, *Pla2g5*$^{-/-}$ mice displayed a reduction of Th2 polarization, thereby resisting subsequent propagation of asthmatic inflammation. This mechanism fits well with the idea illustrated in Fig. 4B. Collectively, sPLA$_2$-V appears to function in two regulatory steps in asthma: (i) in antigen-presenting cells to regulate antigen processing and maturation and thereby the Th2 immune response, and (ii) in airway-resident cells to facilitate subsequent airway inflammation that may involve surfactant degradation.

### Arthritis

Since the discovery in 1989 of sPLA$_2$-IIA as an inducible enzyme during inflammation (which is why it is often called 'inflammatory' sPLA$_2$) (*56*, *57*), many studies have focused particularly on the pro-inflammatory role of sPLA$_2$-IIA, mostly in the context of eicosanoid generation. In fact, sPLA$_2$-IIA is highly expressed in synovial cells and chondrocytes in the joints of patients with rheumatoid arthritis, but not those of normal subjects (*56*, *58*), leading to the hypothesis that sPLA$_2$-IIA plays an exacerbating role in this autoimmune disease. However, the contribution of sPLA$_2$-IIA to inflammation *in vivo* has remained obscure until very recently, most likely because the C57BL/6 and 129Sv mouse strains, which are generally used in knockout studies, have a natural frameshift mutation in the *Pla2g2a* gene (*2*), which has made it difficult to assess the functions of this enzyme by classical gene targeting strategies. Exploiting the fact that this mutation does not occur in BALB/c mice, the *Pla2g2a* gene has recently been knocked out in BALB/c mice by breeding them with C57BL/6 mice and then backcrossing with BALB/c mice. Using the *Pla2g2a*$^{-/-}$ BALB/c mice, thus obtained, it has been shown that sPLA$_2$-IIA does play a pro-inflammatory role in a model of arthritis induced by K/BxN autoantibody (*59*). Thus, *Pla2g2a*$^{-/-}$ BALB/c mice displayed a reduced degree of arthritic inflammation in comparison with wild-type BALB/c mice (*59*). Conversely, the same K/BxN autoantibody-induced arthritis was exacerbated in *PLA2G2A*-Tg mice. Thus, these observations have provided confirmation of the pro-inflammatory role of sPLA$_2$-IIA in inflammatory arthritis. However, although the pathology of inflammatory arthritis involves cPLA$_2\alpha$ (*60*) as well as lipid mediators, including PGs and LTs (*61–64*), it has not yet been firmly clarified whether sPLA$_2$-IIA would contribute to the synthesis of these eicosanoids or act through other mechanism(s) in inflamed joints.

As opposed to *Pla2g2a*$^{-/-}$ mice, *Pla2g5*$^{-/-}$ mice showed exacerbation of K/BxN autoantibody-induced arthritis, likely because opsonization-dependent clearance of the pathogenic immune complex by macrophages from inflamed joints was attenuated in the latter (*59*). In support of this, systemic administration of recombinant sPLA$_2$-V protein ameliorated the arthritic response by facilitating phagocytic uptake of the immune complex by macrophages. Thus, sPLA$_2$-V may have an anti-inflammatory, rather than pro-inflammatory, role in particular diseases induced by deposition of immune complexes, such as rheumatoid arthritis. The fact that these two particular sPLA$_2$s, sPLA$_2$-IIA and sPLA$_2$-V, exert promoting and protective effects, respectively, on inflammatory arthritis may explain why clinical attempts to treat patients with rheumatoid arthritis with a pan-sPLA$_2$ inhibitor, which blocks both enzymes, have been unsuccessful (*65*). These observations provide a rationale for testing two new therapeutic approaches for treatment of immune complex-mediated inflammation: (i) the use of an sPLA$_2$-IIA-specific inhibitor and (ii) the use of recombinant sPLA$_2$-V.

On the other hand, sPLA$_2$-V apparently plays a pro-inflammatory role in other models. For instance, the early phase of pro-inflammatory cysLT synthesis in a zymosan-induced peritonitis model was partially reduced in *Pla2g5*$^{-/-}$ mice (*39*). In the air pouch model, *Pla2g5*$^{-/-}$ mice showed reduced recruitment of LPS-induced leucocytes through attenuated expression of adhesion molecules (*66*). These observations, together with its pro-asthmatic role as mentioned above, indicate that sPLA$_2$-V has both pro-inflammatory and anti-inflammatory potential depending on the disease model.

### Myocardial injury

Another example of the contributions of two sPLA$_2$s with distinct localizations occurs in the process of myocardial infarction with a similar pro-inflammatory outcome. sPLA$_2$-X is localized in azurophilic granules of neutrophils, but not in cardiomyocytes (*41*, *67*). In a model of myocardial ischaemia/reperfusion, *Pla2g10*$^{-/-}$ mice exhibited a smaller myocardial infarct size and less neutrophil infiltration than did wild-type mice (*41*). This phenotype was recapitulated by adoptive transfer of *Pla2g10*$^{-/-}$ bone marrow cells into wild-type mice, implying the contribution of sPLA$_2$-X in myeloid cells to the myocardial injury. sPLA$_2$-X

is released from infiltrating neutrophils at the infarct myocardium and damages cardiomyocytes, probably through $LTB_4$ production and respiratory burst.

Among various tissues, $sPLA_2$-V shows its highest expression in the heart (*68*), and its expression is elevated in areas of cardiac infarction in human patients (*69*). In the same myocardial ischaemia/reperfusion model, *Pla2g5*$^{-/-}$ mice showed a marked decrease in myocardial infarct size, with lower contents of myocardial $LTB_4$ and $TXA_2$ (*70*), eicosanoids with deleterious effects on ischaemic myocardium (*71*). AA release in response to oxidative damage was reduced in *Pla2g5*$^{-/-}$ cardiomyocytes, further supporting the role of myocardial $sPLA_2$-V in this process, possibly in concert with $cPLA_2\alpha$, although the effect of blood cell-borne $sPLA_2$-V was not addressed in that study. These investigations have revealed the pro-inflammatory role of myocardial $sPLA_2$-V and myeloid $sPLA_2$-X in ischaemic heart damage with a common outcome. Interestingly, administration of a pan-$sPLA_2$ inhibitor capable of blocking both $sPLA_2$-V and -X-reduced myocardial infarct size (*41*), suggesting that $sPLA_2$ inhibition may be an effective strategy for treatment of patients with myocardial infarction.

### *Anti-inflammation*

Since $sPLA_2$s can release ω-3 PUFAs such as EPA and DHA, the potential anti-inflammatory role of $sPLA_2$s should also be taken into consideration. Macrophages transfected with $sPLA_2$-X produce more anti-inflammatory IL-10 and less pro-inflammatory TNF-α than do control cells (*46*), suggesting an anti-inflammatory potential of this enzyme. In fact, the alteration of cytokine profiles in $sPLA_2$-X-transfected macrophages raises the possibility that $sPLA_2$-X might facilitate the polarization of macrophages towards an M2 phenotype, a macrophage subset that plays roles in inflammation resolution and tissue repair. This speculation is substantiated by findings suggesting that PUFAs can activate PPARδ, a key transcription factor for M2 polarization of macrophages (*72*, *73*), and that activation of PPARδ by snake venom $sPLA_2$-released PUFAs can switch on the anti-inflammatory programme in endothelial cells (*74*). This is also concordant with the resistance of *Pla2g10*$^{-/-}$ mice to the asthmatic response (*53*), a typical Th2 response in which IL-4/IL-13-driven M2 macrophages play a pro-asthmatic role (*75*).

$sPLA_2$-IID is the closest homologue of $sPLA_2$-IIA and is constitutively expressed in secondary lymphatic organs such as the spleen and lymph nodes (*76*). A recent genome-wide screening of regulatory T ($T_{reg}$)-specific genes has revealed that $sPLA_2$-IID is highly induced in this cell population and is a potent mediator of $T_{reg}$ function (*77*). $sPLA_2$-IID suppressed the proliferation of $CD4^+$ and $CD8^+$ T cells and promoted the differentiation of $T_{reg}$ cells *in vitro*. Moreover, administration of a $sPLA_2$-IID-Fc fusion protein into mice attenuated experimental models of colitis and multiple sclerosis, suggesting its immunosuppressive function. However, because of the limitation imposed by the use of a fusion protein comprising a small $sPLA_2$ molecule conjugated with a large Fc domain, the physiological relevance of the immunosuppressive function of $sPLA_2$-IID awaits a future study employing *Pla2g2d*$^{-/-}$ mice. Interestingly, a polymorphism (G80S) in the human *PLA2G2D* gene is associated with body weight loss in chronic obstructive pulmonary disease (*78*), suggesting the participation of $sPLA_2$-IID in chronic airway inflammation and/or body weight control.

## $sPLA_2$ and atherosclerosis

The surfaces of lipoprotein particles, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), are surrounded by phospholipids (mainly PC), which serve as a very good 'extracellular' target of several, if not all, $sPLA_2$ isoforms. *In vitro*, the rank order of $sPLA_2$ hydrolytic potency is X > V > III > IIF > IIA > IIE for both LDL and HDL, which appears to roughly correlate with their ability to interact with PC (*79*). Although the activity of $sPLA_2$-IIA on lipoproteins is relatively weak, it can hydrolyze acute phase HDL more efficiently than normal HDL, preferentially attacking PC with oxygenated PUFAs (*80*). Clinically, an elevated plasma $sPLA_2$-IIA level is an independent risk factor for cardiovascular disease (*81*, *82*). LDL particles vary in size and density, and high plasma levels of small-dense LDL particles correlate well with a higher risk of coronary heart disease. A key step for generation of pro-atherogenic small-dense LDL is hypertriglyceridemia, and it is hypothesized that small-dense LDL can penetrate into vessel wall tissue more easily and attach to the extracellular matrix, where the PUFAs in phospholipids on the LDL surface undergo oxidative modification. Several lines of evidence suggest that $sPLA_2$-mediated hydrolysis of lipoproteins also gives rise to a type of small-dense LDL with an increased net negative charge, LPC content and propensity for aggregation, thus contributing to the development of atherosclerosis (*83*, *84*). Indeed, LDL treated with $sPLA_2$-III, -V or -X facilitates the formation of lipid-laden foam cells by macrophages (*79*), a hallmark feature of atherosclerosis. Several $sPLA_2$ isoforms are detectable in atherosclerotic plaques in humans and mice (*79*, *85*).

In 1999, the first experimental evidence for the potential role of $sPLA_2$ in atherosclerosis *in vivo* was obtained from *Pla2g2a*-Tg mice, which developed increased atherosclerotic lesions with slightly reduced plasma HDL levels following intake of a high-cholesterol atherogenic diet (*6*). Adoptive transfer of *Pla2g2a*-Tg bone marrow cells into recipient *Ldlr*$^{-/-}$ mice resulted in a significant increase in atherosclerotic lesions despite the absence of any alteration in lipoprotein composition, suggesting that local LDL modification in the vascular wall is sufficient for macrophage $sPLA_2$-IIA to drive the pro-atherogenic machinery (*86*). Recently, we showed that *Pla2g3*-Tg mice crossed with *ApoE*$^{-/-}$ mice, followed by supplementation with an atherogenic diet, developed more advanced atherosclerotic lesions than did *ApoE*$^{-/-}$ mice, accompanied by marked reductions of LDL and HDL as well as

increases in LPC-rich small-dense LDL and the pro-thrombotic $TXA_2$ (*79*). Interestingly, *Pla2g3*-Tg mice also developed systemic inflammation with increased age (*37*), suggesting that the elevated inflammatory state in the vascular wall may have an additional impact on the promotion of atherosclerosis in these mice. In addition, robust hydrolysis of plasma LDL and HDL was observed in *Pla2g10*-Tg mice (*87*).

Definitive evidence for the contribution of $sPLA_2$s to the development of atherosclerosis has been provided by studies using mice null for $sPLA_2$-V (*88*) and for $sPLA_2$-X (*89*). $Ldlr^{-/-}$ mice subjected to adoptive transfer of *Pla2g5*-transfected bone marrow cells showed increased atherosclerotic lesions with a concomitant elevation of regional collagen deposition, whereas replicate $Ldlr^{-/-}$ mice that underwent adoptive transfer of $Pla2g5^{-/-}$ bone marrow cells showed reduced atherosclerosis (*88*). This result indicates that $sPLA_2$-V in hematopoietic cells (most likely macrophages) does contribute to propagation of atherosclerosis. $sPLA_2$-X deficiency on an $ApoE^{-/-}$ background significantly reduced the incidence of angiotensin II-induced abdominal aortic aneurysm and atherosclerosis, accompanied by a reduction of pro-inflammatory mediators (*89*). Moreover, another study using $Pla2g10^{-/-}$ macrophages has provided another insight into the action of $sPLA_2$-X as a negative regulator of cholesterol efflux from macrophages through alteration of liver X receptor (LXR)-dependent expression of ABC transporters (*90*). Finally, in $ApoE^{-/-}$ mice fed a high-cholesterol diet, a pan-$sPLA_2$ inhibitor that dampens conventional $sPLA_2$s dramatically decreased atherosclerotic lesions, with an increase in plasma HDL (*91*). A phase II double-blind, placebo-controlled trial has demonstrated the efficacy of this inhibitor in patients with cardiovascular disease (*92*). Thus, the use of a $sPLA_2$ inhibitor elicits a promising reduction of biomarkers and effects on surrogate endpoints, encouraging further investigation of whether it can reduce cardiovascular disease events without any off-target toxicity.

## $sPLA_2$ and metabolic syndrome

In contrast to the established roles of $sPLA_2$s in cardiovascular diseases as described above, their roles in metabolic syndrome including obesity, type 2 diabetes and insulin resistance have not yet been fully clarified. A recent tagging single-nucleotide polymorphism analysis has revealed an association of the human *PLA2G5*, but not *PLA2G2A*, gene haplotype with LDL and oxidized LDL levels in patients with type 2 diabetes (*93*). Susceptibility of very low-density lipoprotein (VLDL) and LDL to $sPLA_2$-V-promoted lipolysis has been shown to be higher in individuals with type 2 diabetes than in healthy controls (*94*). Thus, although the release of free fatty acids and LPC from diabetic LDL by $sPLA_2$-V may increase the risk of cardiovascular diseases, the above observations suggest that the $sPLA_2$-V-mediated hydrolysis of fat-overloaded, diabetic LDL might have another effect on systemic metabolic disorders by altering the hyperlipidemia state.

Systemic metabolic status is affected by glucocorticoid produced by the adrenal glands, in which $sPLA_2$-X is expressed. $Pla2g10^{-/-}$ mice have significantly higher plasma corticosterone levels than do wild-type mice (*95*). In contrast, overexpression of $sPLA_2$-X significantly reduces steroid production in cultured adrenal cells in a catalytic activity-dependent manner. Expression of steroidogenic acute regulatory protein (StAR), a rate-limiting protein in corticosteroid production, was higher in the adrenals of $Pla2g10^{-/-}$ mice than in wild-type mice, and LXR-dependent StAR promoter activation was attenuated by $sPLA_2$-X-released PUFAs. Thus, adrenal $sPLA_2$-X functions as a negative regulator of corticosteroid synthesis, likely through repression of the LXR–StAR axis. This series of studies also showed that $Pla2g10^{-/-}$ mice had increased obesity, which may be due to inhibition of the LXR-dependent lipogenic programme by $sPLA_2$-X in adipose tissue (*96*).

## $sPLA_2$ and digestion

Obesity and associated metabolic syndrome are influenced by various factors, among which gastrointestinal lipid digestion/absorption is a causal factor that can be indirectly linked to adiposity. Phospholipids entering the digestive tract via food and bile comprise the second most abundant lipid class found in the intestinal lumen (*97*). Hydrolysis of phosphoplipids on the surface of lipid emulsion is required prior to digestion of triglycerides, a major lipid nutrient (*98*). The reduced lipid absorption efficiency culminates in protection against diet-induced obesity (*99*). For a long time, $sPLA_2$-IB, a 'pancreatic' $sPLA_2$, has been thought to play a role in digestion of dietary phospholipids. Indeed, under a high-fat/carbohydrate diabetogenic diet, the intestinal production and absorption of LPC, a hydrolytic product of dietary and biliary PC, was markedly reduced in $Pla2g1b^{-/-}$ mice. Accordingly, $Pla2g1b^{-/-}$ mice were protected from diet-induced obesity and hepatic glucose intolerance (*100*, *101*). Moreover, the *PLA2G1B* gene is mapped to an obesity susceptibility locus in humans (*102*). However, on a chow diet, intestinal phospholipid digestion in $Pla2g1b^{-/-}$ mice remained intact (*103*), suggesting that additional lipolytic enzyme(s) in the gut can compensate for the lack of $sPLA_2$-IB.

$sPLA_2$-X is expressed most abundantly in the gastrointestinal tract and reproductive organs (*104*). Consistent with its location, we recently found that gut $sPLA_2$-X represents the second 'digestive' $sPLA_2$ (*104*). Accordingly, reduced gastrointestinal phospholipid digestion in $Pla2g10^{-/-}$ mice receiving a chow diet was eventually linked to delayed onset of a lean phenotype with reduced adiposity, decreased plasma leptin and improved muscle insulin tolerance. Thus, $sPLA_2$-IB and $sPLA_2$-X, the two particular $sPLA_2$s that can be activated through proteolytic removal of the N-terminal propeptide (*1*), may play a compensatory role in phospholipid digestion in the gastroenteric lumen. Since $sPLA_2$-IB is abundantly secreted into the duodenum from the pancreas (*1*), while $sPLA_2$-X is constitutively expressed throughout the

gastrointestinal mucosa (*104*), these two 'digestive' $sPLA_2$s may spatiotemporally control the hydrolysis of dietary and biliary phospholipids and thereby absorption of their hydrolytic products, depending on the quantity and quality of dietary and biliary fat input. It is interesting to note that oral application of a pan-$sPLA_2$ inhibitor effectively suppressed diet-induced obesity and diabetes in mice, most probably by preventing the intestinal digestion of dietary and biliary PC by $sPLA_2$-IB and -X (*105*). This result raises the intriguing possibility that pharmacological inhibition of gastrointestinal $sPLA_2$s may be a potentially effective oral therapeutic option for treatment of diet-induced obesity and diabetes.

## $sPLA_2$ and anti-microbial defence

The best-known physiologic function of $sPLA_2$-IIA is defence against bacterial infection through hydrolytic degradation of bacterial membrane phospholipids (*106*). Bacterial membranes are rich in PE and phosphatidylglycerol, which are excellent substrates for $sPLA_2$-IIA (*107*). The rank order of potency among $sPLA_2$s against Gram-positive bacteria is IIA > X > V > XIIA > IIE > IB, IIF (*7*). Thus, except for $sPLA_2$-X, which is an acidic enzyme, the bactericidal activity of $sPLA_2$s correlates with positive charges on their molecular surfaces. In fact, $sPLA_2$-IIA effectively kills Gram-positive bacteria and to a lesser extent Gram-negative bacteria *in vitro*, and importantly, *Pla2g2a*-Tg mice show decreased mortality following bacterial infection, associated with improved clearance of bacteria from their organs (*4*, *5*, *108*, *109*).

$sPLA_2$-V is localized in the Golgi and recycling endosomes in quiescent mouse peritoneal macrophages and is recruited to phagosomes upon ingestion of zymosan from the yeast cell wall (*110*). Peritoneal macrophages from *Pla2g5*$^{-/-}$ mice showed marked attenuation of zymosan phagocytosis, an event that was restored by adenoviral expression of $sPLA_2$-V. *Pla2g5*$^{-/-}$ macrophages showed a delay in phagocytosis, phagosome maturation and *Candida albicans* killing, independently of eicosanoid generation (*111*). Moreover, in a mouse model of systemic candidiasis, *Pla2g5*$^{-/-}$ mice showed increased mortality due to an increased fungal burden in multiple organs. Thus, $sPLA_2$-V contributes to the innate immune response against fungi by facilitating phagocytosis and killing. Although the molecular mechanism underlying the $sPLA_2$-V-mediated regulation of phagocytosis remains unknown, it may produce membrane-fusogenic lysophospholipids in membrane microdomains where fusion between the plasma membrane and the phagosome membrane proceeds.

Collectively, $sPLA_2$-IIA is an 'anti-bacterial' $sPLA_2$ that kills invading bacteria by directly degrading bacterial membranes, whereas $sPLA_2$-V works as an 'anti-fungal' $sPLA_2$ by facilitating macrophage phagocytosis of invading fungi. Since bacterial infection can occur anywhere in the body, marked induction of $sPLA_2$-IIA in the circulation as well as in local areas of inflammation or injury can be regarded as an innate defence response for clearance of invading bacteria. Since $sPLA_2$-V is expressed in alveolar macrophages and bronchial epithelial cells in the airways and urinary ducts of the kidney, where fungal infection frequently occurs in immunocompromised patients, the enzyme may contribute to the first line of innate immunity against fungi. In addition, at least *in vitro*, $sPLA_2$-III, -V and -X are capable of suppressing virus entry into host cells in a manner dependent upon the production of LPC from host cell membranes (*112*, *113*). Interestingly, *PLA2G3* gene polymorphisms are strongly associated with development of AIDS (*114*). Several $sPLA_2$s also elicit an anti-malaria action *in vitro*, which depends on hydrolysis of serum lipid components (probably lipoproteins) rather than direct interaction of the $sPLA_2$ with the infected erythrocyte membrane (*115*). Future work using knockout mice will clarify the anti-viral and anti-parasitic roles of $sPLA_2$s *in vivo*.

## $sPLA_2$ and cancer

Natural *Pla2g2a*$^{-/-}$ mouse strains (due to a frameshift mutation in the *Pla2g2a* gene; see above) were susceptible to colorectal tumourigenesis (*2*), while introduction of the *Pla2g2a* transgene into C57BL/6 mice strongly suppressed it (*116*), leading to the proposal that $sPLA_2$-IIA has an anti-tumour role. Also in humans, $sPLA_2$-IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis (*117*). These results suggest that $sPLA_2$-IIA confers resistance to gastrointestinal cancer in both mice and humans. There is a positive correlation between *PLA2G2A* expression and the oncogenic Wnt/β-catenin pathway (*118*). Mechanistically, gastrointestinal $sPLA_2$-IIA may alter enterobacterial flora through its anti-bacterial activity (see above), thereby negatively affecting tumour development. Conversely, $sPLA_2$-IIA may play a pro-tumourigenic role in other cancers such as prostate cancer, in which it may serve as a marker of highly proliferative carcinoma cells (*119*, *120*). Moreover, skin-specific *Pla2g2a*-Tg mice showed increased sensitivity to chemical carcinogenesis (*121*). Therefore, the pro- and anti-tumourigenic effects of $sPLA_2$-IIA appear to be tissue specific.

In several types of human cancer, $sPLA_2$-III is distributed in microvascular endothelial cells and tumour cells (*122*). Larger tumour xenografts were formed in nude mice implanted with $sPLA_2$-III-transfected colorectal adenocarcinoma cells than with mock-transfected control cells (*122*). Moreover, $sPLA_2$-III may be a good candidate biomarker for human colon cancer (*123*), and a *PLA2G3* haplotype is correlated with a higher risk of human colorectal cancer (*124*). The pathological relevance of the participation of $sPLA_2$-III in tumourigenesis should be addressed using *Pla2g3*$^{-/-}$ mice in the near future.

## $sPLA_2$ and skin

The first phenotype reported in *Pla2g2a*-Tg mice was alopecia with no sign of inflammation (*3*). Although the physiological relevance of this finding was unclear

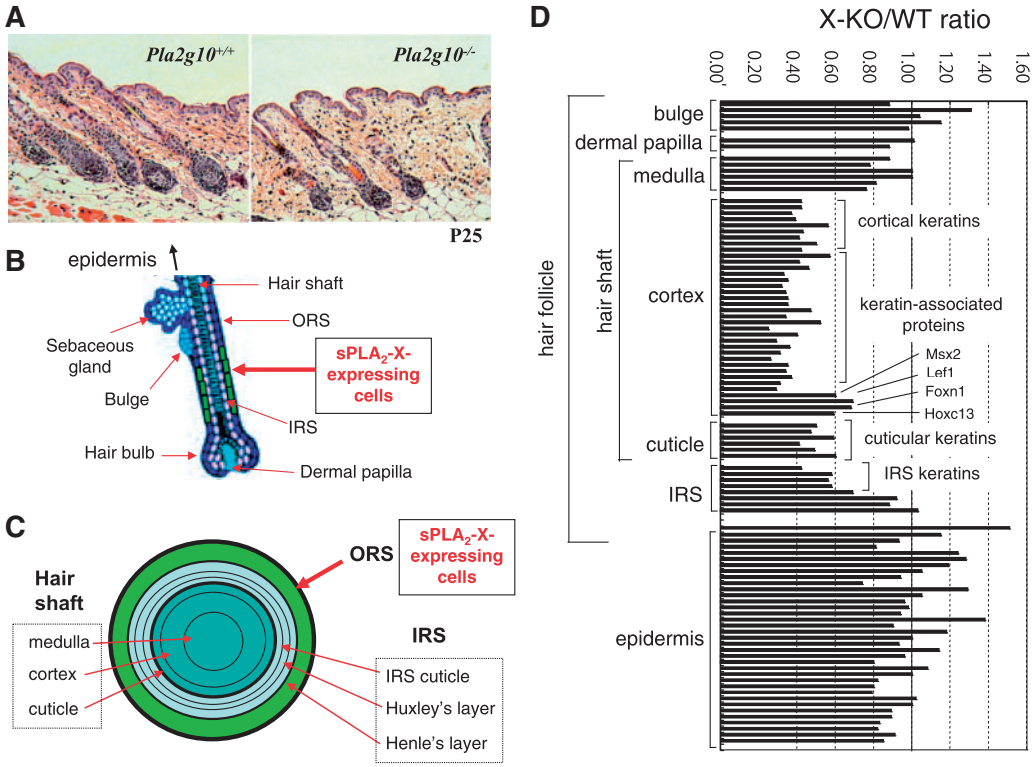


**Fig. 5 Hair regulation by sPLA$_2$-X.** (A) Histological examination of skin sections from *Pla2g10*$^{+/+}$ and *Pla2g10*$^{-/-}$ mice at P25 revealed a propensity for small hair follicles in the null mice, whereas other skin appendages including epidermis, sebaceous gland and dermis appeared normal. (B) Schematic illustration of a longitudinal section of a hair follicle. Endogenous sPLA$_2$-X is localized in the ORS of hair follicles (shown in green). ORS, outer root sheath; IRS, inner root sheath. (C) Schematic illustration of a cross-section of a hair follicle, which consists of seven layers of cells involving a single uppermost ORS layer, three layers (Henle, Huxley and IRS cuticle) of IRS, and the three innermost layers (cuticle, cortex and medulla) of the hair shaft. sPLA$_2$-X is located in the ORS in the anagen (growing) phase of the hair follicle. (D) Microarray gene profiling of skins from *Pla2g10*$^{+/+}$ and *Pla2g10*$^{-/-}$ mice at P25. Genes associated with hairs, but not those with bulge, dermal papilla and epidermis, are reduced by nearly half in *Pla2g10*$^{-/-}$ mice compared with *Pla2g10*$^{+/+}$ mice, suggesting that the null mice have a hair growth defect.

at that time since endogenous sPLA$_2$-IIA does not exist in mouse skin (*2*), it was suggested that there might be a certain endogenous sPLA$_2$ isoform that participates in regulation of hair growth. Hair follicle morphogenesis is governed by interactions between epidermal keratinocytes committed to hair follicle differentiation and dermal fibroblasts committed to forming the dermal papilla of the developing hair follicles (*125*). These epithelial–mesenchymal interactions culminate in the formation of the hair shaft, which is surrounded by the multi-layered inner root sheath (IRS) and outer root sheath (ORS), the latter being the outermost concentric layer of epithelial cells. Hair follicles undergo repeated cycles of growth (anagen), regression (catagen) and rest (telogen) during their life span. Distinct signalling pathways, including lipids, control hair follicle morphogenesis, postnatal hair growth and hair cycling in a coordinated manner.

We have recently found that sPLA$_2$-X was expressed in the ORS of hair follicles in synchrony with the anagen phase of hair cycling (*126*). *Pla2g10*-Tg mice, like *Pla2g2a*-Tg mice, displayed alopecia, along with epidermal hyperplasia and hair follicle distortion accompanied by reduced expression of genes related to hair development. Proteolytically activated sPLA$_2$-X in *Pla2g10*-Tg mouse skin showed preferential hydrolysis of PE with PUFAs, with concomitant production of $PGE_2$. Importantly, the skin of *Pla2g10*$^{-/-}$ mice had abnormal hair follicles in which a subset of hair genes was noticeably reduced, the ORS was hypoplasic, and melanin granules in the hair shaft medulla were decreased (Fig. 5). Unexpectedly, endogenous sPLA$_2$-X was not coupled with $PGE_2$ synthesis in hair follicles, but rather its deficiency resulted in aberrant cyclooxygenase-2 expression, leading to increased hair follicular $PGE_2$ synthesis and thereby to hair abnormality (*126*, *127*). These results reveal a novel role of sPLA$_2$-X in hair homoeostasis within a highly restricted and specialized skin compartment, the hair follicle.

## sPLA$_2$ and reproduction

Various sPLA$_2$s are expressed in male reproductive organs at relatively high levels. In both mouse and human testes, sPLA$_2$-X and -IID are localized in the head and midpiece of elongating spermatids, respectively, and both sPLA$_2$-IIF and -V are distributed in interstitial Leydig cells (*128*). A high level of sPLA$_2$-IIA is detected in human seminal plasma (*129*), where the enzyme originates from the prostate epithelium and may act as an anti-microbial factor to protect this organ and spermatozoa against microbial invasion (*130*). sPLA$_2$-III is localized in testicular Sertoli cells

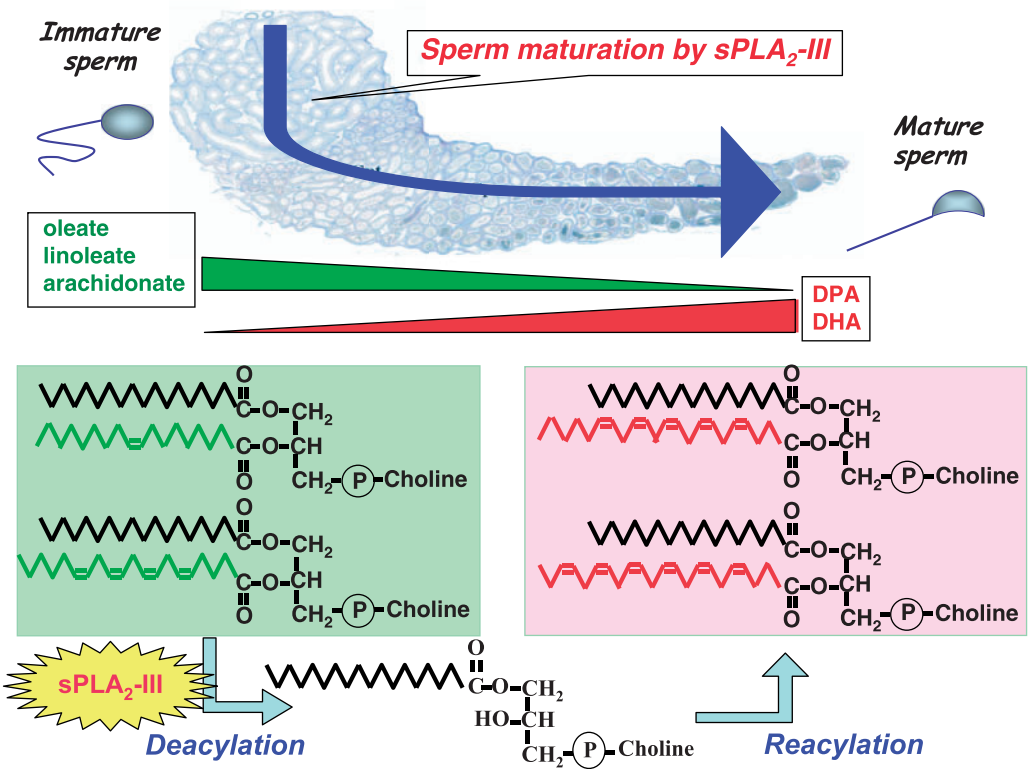


**Fig. 6 The role of sPLA$_2$-III in epididymal sperm maturation**. During their passage through the epididymis, immature sperm cells exiting from the testis undergo proper maturation towards mature motile spermatozoa. sPLA$_2$-III is expressed in the luminal epithelium in the caput (head) epididymidis and is secreted into the lumen, where it encounters immature sperm passing through the duct. During the sperm maturation process, PC in the wild-type sperm membrane undergoes a dramatic shift in its acyl groups from oleic acid, linoleic acid and AA to DPA and DHA, whereas this membrane lipid remodelling event is compromised in *Pla2g3*$^{-/-}$ sperm. Thus, sPLA$_2$-III may participate in the hydrolysis of PC with oleic acid, linoleic acid and AA in the sperm membrane during epididymal transit, and this event may be followed by reacylation of LPC with DHA and DPA, leading to an increase of PC with DPA/DHA in mature spermatozoa. In the *Pla2g3*$^{-/-}$ epididymis, impairment of the deacylation step may eventually perturb the subsequent reconstitution of DPA/DHA in the sperm membrane, culminating in the astheno-zoospermia (sperm with low motility and abnormal morphology) phenotype.

and caput epididymal epithelial cells (*131*). These locations suggest that individual sPLA$_2$ isoforms play distinct roles in the gonadal microenvironments. Studies using sPLA$_2$ knockout mice have revealed that two particular sPLA$_2$s, sPLA$_2$-III and sPLA$_2$-X, play non-redundant roles in post-testicular sperm maturation and functions in the mouse male genital tract (*131*, *132*).

We have recently found that *Pla2g3*$^{-/-}$ mice displayed a striking defect in male, but not female, reproduction due to impaired sperm motility (*131*). This was because sperm maturation in the epididymis, but not spermatogenesis in the testis, was hampered in the null mice. During epididymal transit, PC in the sperm membrane undergoes a dramatic shift in its acyl groups from oleic acid, linoleic acid and AA to docosapentaenoic acid (DPA) and DHA, and this increased proportion of DHA consequently contributes to an increase of sperm membrane fluidity, and thereby motility (*133–137*). In *Pla2g3*$^{-/-}$ mice, this sperm membrane remodelling in the epididymis was severely compromised, and as a result, cauda epididymal spermatozoa from *Pla2g3*$^{-/-}$ mice had aberrant acrosomes and flagella with an abnormal axoneme configuration, and displayed hypomotility and reduced ability to fertilize intact eggs (*131*). This indicates that sPLA$_2$-III may facilitate the hydrolysis of PC with oleic acid, linoleic acid and AA in the sperm membrane during epididymal transit and that this event may be followed by reacylation of LPC with DHA and DPA, leading to an increase of PC with DPA/DHA in mature sperm (Fig. 6). In the *Pla2g3*$^{-/-}$ epididymis, preturbation of the deacylation step may alter the subsequent incorporation of DPA/DHA in the sperm membrane, resulting in the asthenozoospermia (spermatozoa with low motility and abnormal morphology) phenotype. Additionally, the gonads of *Pla2g3*$^{-/-}$ mice contained less 12/15-lipoxygenase metabolites than those of wild-type mice, implying preferential functional coupling of the gonadal sPLA$_2$-III with a specific arm (the 12/15-lipoxygenase pathway) of the PUFA-metabolic pathway (*131*).

It had been proposed that a certain germ cell-associated sPLA$_2$ might play a role in the sperm acrosome reaction by producing LPC (*138–140*). We found that sPLA$_2$-X was stored in acrosomes of mature spermatozoa and was released upon exocytosis (so-called 'acrosome reaction'), a key step essential for fertilization with oocytes (*132*). Although cauda epididymal spermatozoa from *Pla2g10*$^{-/-}$ mice showed normal motility, they exhibited a lower acrosome reaction that was associated with a decreased outcome of

*in vitro* fertilization, and accordingly, the litter size of male *Pla2g10*$^{-/-}$ mice was significantly smaller than that of wild-type mice (*132*). In agreement, the acrosome reaction of mouse spermatozoa was suppressed by addition of a pan-sPLA$_2$ inhibitor or an anti-sPLA$_2$-X antibody, an event that was reversed by addition of recombinant sPLA$_2$-X or its product LPC. Although a recent *in vitro* study showed that exogenous sPLA$_2$-X reduces sperm motility (*141*), the physiological relevance of this observation remains uncertain, since the motility of *Pla2g10*$^{-/-}$ sperm is identical to that of wild-type sperm (*104*).

Taken together, these two particular sPLA$_2$s (sPLA$_2$-III and -X), which are expressed in different locations within the male reproductive organs, exert non-redundant but interrelated functions in two major steps of male fertility; one during sperm maturation in the epididymis, in which sPLA$_2$-III plays a role in enrichment of DHA/DPA-rich mature sperm, and the other during capacitation and the acrosome reaction, likely after ejaculation into the uterus and oviduct, in which sPLA$_2$-X hydrolyses the DHA/DPA-rich sperm membrane to release LPC, which in turn promotes the acrosome reaction. As an additional note, sPLA$_2$-X is also localized in the endometrial epithelium in female reproductive organs (*104*). Considering that exogenous sPLA$_2$-X can powerfully boost the spontaneous acrosome reaction of spermatozoa (*132*), one may speculate that sPLA$_2$-X secreted from the endometrial epithelium into the lumen may assist, in a paracrine manner, the spontaneous acrosome reaction of sperm cells passing through the uterine duct. As such, sPLA$_2$-X boosts the premature acrosome reaction of a likely phospholipid-damaged sperm subpopulation to eliminate them from the fertilization race.

## sPLA$_2$ and neurons

sPLA$_2$-X augments neuritogenesis through production of LPC in neuronal cell culture (*142*) suggesting its neuronal role. *In vivo*, sPLA$_2$-X is localized in ganglia or neuronal fibres in peripheral tissues of both mouse and human, and is found in both A-fibre and C-fibre neurons in mouse dorsal root ganglion (DRG) (*104*). *Ex vivo* neuritogenesis of DRG explants was slightly reduced in *Pla2g10*$^{-/-}$ mice and enhanced in *Pla2g10*-Tg mice relative to the respective controls. DRG isolated from E12.5 *Pla2g10*$^{-/-}$ foetus revealed reduced expression of several neuron-associated genes. Furthermore, the late phase of pain nociception, as assessed by the acetic acid writhing test, was significantly reduced in *Pla2g10*$^{-/-}$ mice and conversely augmented in *Pla2g10*-Tg mice, an event that appeared to be independent of the spinal levels of nociceptive PGs (*104*). Collectively, sPLA$_2$-X in certain neuronal microdomains may have a spatiotemporal effect on neuritogenesis and/or neuronal transmission leading to persistent pain nociception.

It is tempting to speculate that the neuronal location of sPLA$_2$-X in several peripheral tissues may underlie an alternative mechanism for the regulation of tissue-specific homoeostasis by this enzyme. For instance, localization of sPLA$_2$-X in neuronal fibres in male genital organs suggests that the enzyme might be involved in the autonomic nervous response for ejaculation. The expression of sPLA$_2$-X in ganglion cells of the myenteric plexus between smooth muscle cell fibres in the gastrointestinal tract (*142*, *143*) might be a reflection of its potential role in the peristaltic reflex controlled by the enteric nervous system, which could exert further influence on gastrointestinal lipid digestion and absorption. Finally, besides the reported negative regulatory role of sPLA$_2$-X in LXR-dependent metabolic processes in the adrenal glands and adipose tissues (*95*, *96*), the neuronal location of sPLA$_2$-X in adipose tissue might be related to the neuroendocrine regulation of adiposity.

sPLA$_2$-III is also expressed in neurons (*144*). In cultured neuronal cells, overexpression of sPLA$_2$-III facilitated neurite growth and survival, while knockdown of endogenous sPLA$_2$-III by siRNA suppressed these processes. The neuritogenic action of sPLA$_2$-III was correlated with the cellular level of LPC. Moreover, among the sPLA$_2$ members, sPLA$_2$-III showed the highest expression in mouse DRG, and *Pla2g3* siRNA-silenced DRG explants showed decreased axonal branching (*144*). The precise role of sPLA$_2$-III in neurons *in vivo* should be clarified using *Pla2g3*$^{-/-}$ mice.

## More about sPLA$_2$s

The physiological roles of other group II subfamily sPLA$_2$ isoforms (IIC, IID, IIE and IIF) remain elusive, since mice with knockout, or those that are transgenic, for these enzymes have not yet been reported. sPLA$_2$-IIC is expressed in rodent testis, but is not expressed as a functional protein in humans (*145*). As mentioned above, sPLA$_2$-IID has been proposed to have an immunosuppressive function (*77*), but this awaits confirmation using *Pla2g2d*$^{-/-}$ mice. sPLA$_2$-IIE is expressed constitutively in several tissues at low levels, but its role is entirely unknown (*146*). sPLA$_2$-IIF has a unique C-terminal extension that contains an additional Cys residue, which might contribute to formation of a homodimer or a heterodimer with a second protein (*147*, *148*). This enzyme is expressed abundantly in the skin, suggesting its role in the epidermal barrier (unpublished results).

Group XII sPLA$_2$s (sPLA$_2$-XIIA and -XIIB) represent a unique collection of the sPLA$_2$ family. High expression of sPLA$_2$-XIIA, relative to other sPLA$_2$s, is found in many tissues (*149–151*). Interestingly, despite its weak enzymatic activity in a standard PLA$_2$ assay, sPLA$_2$-XIIA kills Gram-negative bacteria efficiently at least *in vitro* (*152*). A study using *Xenopus* suggests the role of this enzyme in early neuronal development (*153*). sPLA$_2$-XIIB is structurally related to sPLA$_2$-XIIA and is expressed in liver and intestine (*151*). A recent study has demonstrated that the expression of sPLA$_2$-XIIB was up-regulated by the transcription factor HNF-4$\alpha$ and its co-activator PGC-1$\alpha$, and deletion of the *Pla2g12b* gene resulted in increased fat accumulation in the liver leading to steatohepatitis, a phenotype similar to that seen in *Hnf4a*$^{-/-}$ mice (*154*).

The aberrant fat accumulation in *Pla2g12b*$^{-/-}$ liver was due to impaired hepatic secretion of VLDL. However, since sPLA$_2$-XIIB lacks the catalytic activity due to replacement of the catalytic centre His with Leu (*151*), the molecular mechanism whereby this catalytically inactive sPLA$_2$ isoform regulates VLDL secretion remains unknown.

## sPLA$_2$ receptor

Finally, we would like to offer some comments on the catalysis-independent functions of sPLA$_2$s. Over the last two decades, a number of membrane receptors and soluble binding proteins interacting with sPLA$_2$s have been described (*15*), although the non-enzymatic actions of sPLA$_2$s largely lack physiological evidence *in vivo*. Nevertheless, the presence of catalytically inactive or very weak sPLA$_2$s may indicate that some functions of sPLA$_2$s depend on their ligand-like action. Such examples include the expression of the catalytically weak or inactive group XII sPLA$_2$s at high levels in various tissues (see above), the activation of synovial cells by a catalytically inactive sPLA$_2$-IIA mutant (*155*, *156*), and the anti-inflammatory effect of a catalytically inactive sPLA$_2$-IID mutant when injected into mice (*77*). In addition, otoconin-90/95, another catalytically inactive sPLA$_2$-like protein that is most similar to sPLA$_2$-IB, acts as a structural component of otoconia (*157*). Knockout studies have shown that otoconin-90/95 is essential for the formation of the organic matrix of otoconia by specifically recruiting other matrix components, given that its deletion led to imbalance but normal hearing (*158*, *159*).

Clearly, the M-type sPLA$_2$ receptor (PLA2R1) is the best-known binding partner of various conventional sPLA$_2$s with different affinities (*160*, *161*). However, the biological roles of PLA2R1 are rather controversial and need more careful evaluation. In essence, PLA2R1 is a mammalian homologue of type α PLA$_2$ inhibitory protein (PLAI) in snakes. Both PLA2R1 and PLAI are made up of multiple C-type lectin domains, and their interaction with sPLA$_2$ leads to inhibition of enzymatic activity or biological effects (*162*, *163*). Furthermore, PLA2R1 internalizes the bound sPLA$_2$ into phagolysosomes, in which the enzyme is rapidly degraded (*164*, *165*). On the basis of these properties, it is likely that PLA2R1 plays a role in sequestration or clearance of sPLA$_2$s from biological fluids, and thereby termination of sPLA$_2$ actions. In contrast, PLA2R1 has also been proposed to participate in sPLA$_2$-evoked signalling including cell proliferation and/or pro-inflammatory effects (*166*). Such a pro-inflammatory role is supported by evidence that *Pla2r1*$^{-/-}$ mice are resistant to endotoxin shock (*167*). Alternatively, *Pla2r1*$^{-/-}$ mice might be protected from inflammation because of a defect in clearance of an immunosuppressive sPLA$_2$.

However, several features of PLA2R1 hold some questions regarding the signalling role of this protein. First, although various mouse sPLA$_2$s bind to mouse PLA2R1 with high to moderate affinity, this ligand specificity is not conserved in other animal species, including humans (*163*). This seems odd, since a ligand–receptor pair, as seen for a variety of cytokines, hormones, growth factors and neurotransmitters, is generally highly specific and well conserved among animal species. Exceptions include several classes of pattern recognition receptors, which broadly recognize microbial and host components (*168–170*). Second, a signalling receptor generally has a long cytoplasmic region bearing one or more signalling motifs, which are essential for interaction with downstream kinases or adaptor molecules. However, PLA2R1 possesses only a short stretch in the cytoplasmic tail, which does not contain any known signalling module except for a motif for endocytosis (*164*). With this structural property, it is difficult to envisage that PLA2R1 itself could act as a signalling receptor. The α chain of cytokine receptors with a short cytoplasmic region transmits a cytokine signal by forming a complex with β and/or γ receptor chains bearing a long cytoplasmic region containing signalling motifs (*171*). By analogy, the presence of the second, as yet unknown signal-transmitting subunit that could form a functional complex with PLA2R1 should be taken into consideration. Third, the most commonly observed signalling event associated with PLA2R1 is the activation of MAP kinases and NF-κB. However, the same signalling pathways are readily activated if the recombinant sPLA$_2$ preparations (mostly *Escherichia coli*-derived) are contaminated with even a tiny amount of bacterial components such as LPS or peptidoglycan, which activate Toll-like receptors (TLRs) (*168*, *169*). Hence, some previous studies might have erroneously reported the responses to contaminating LPS rather than to sPLA$_2$. Nonetheless, it is interesting to note that some C-type lectins, upon binding with their ligands, can act cooperatively with TLRs (*168–170*). For instance, mannose-binding lectin enhances TLR2/TLR6 signalling from the phagosome (*172*), dectin-1, another C-type lectin that recognizes the fungal wall, can collaborate with TLR2 to induce inflammatory responses (*173*), and CD36, another class of pattern recognition receptor that binds to polycationic ligands of both pathogen and self origin, promotes sterile inflammation through assembly of a TLR4/TLR6 heterodimer (*174*). By analogy, therefore, the sPLA$_2$–PLA2R1 interaction might be functionally linked to TLR signalling pathways in innate or adaptive immunity, leading to activation of MAP kinases and NF-κB and thereby to cell proliferation and inflammatory responses.

Recently, two novel roles of PLA$_2$R1 have been proposed (*175*, *176*). First, PLA2R1 has been found to control cellular senescence in human fibroblasts (*175*). PLA2R1 expression is increased during cellular senescence, and overexpression of the receptor promotes senescence while its knockdown prevents the onset of senescence. Interestingly, the expression of sPLA$_2$-IIA is also increased during senescence, and this enzyme appears to play a role in cellular senescence via PLA2R1. These findings suggest a novel role of the receptor as a tumour suppressor gene. Second, PLA$_2$R1 represents the main antigen target in idiopathic membranous nephropathy, an autoimmune human kidney disease (*176*). A majority of

**Table II. Functions of $sPLA_2$s *in vivo* as revealed by gene-manipulated mice.**

| *In vivo* models | $sPLA_2$s | Localizations | Functions | Underlying mechanisms |
|---|---|---|---|---|
| Airway diseases | $sPLA_2$-V | Bronchial epithelium, DC/macrophages | Promotion | Antigen processing by DC, surfactant hydrolysis |
| | $sPLA_2$-X | Alveolar epithelium | Promotion | Production of cysLTs, surfactant hydrolysis? |
| Arthritis | $sPLA_2$-IIA | Synovial cells, chondrocytes | Promotion | Production of $PGE_2$? |
| | $sPLA_2$-V | Macrophages | Protection | Clearance of pathogenic immune complex |
| Myocardial ischemia | $sPLA_2$-V | Myocardiocytes | Promotion | Production of $LTB_4$ and $TXA_2$ |
| | $sPLA_2$-X | Neutrophils | Promotion | Production of $LTB_4$, respiratory burst |
| Atherosclerosis | $sPLA_2$-V | Atherosclerotic lesion | Promotion | Production of atherogenic small-dense LDL |
| | $sPLA_2$-X | Atherosclerotic lesion | Promotion | Production of atherogenic small-dense LDL |
| | $sPLA_2$-IIA | Atherosclerotic lesion | Promotion | Production of atherogenic small-dense LDL |
| | $sPLA_2$-III | Atherosclerotic lesion | Promotion | Production of atherogenic small-dense LDL |
| Obesity | $sPLA_2$-X | Adipose tissue, adrenal gland | Protection | PUFA-dependent LXR repression |
| | | Gastrointestinal tract | Promotion | Gastrointestinal phospholipid digestion |
| | $sPLA_2$-IB | Pancreas | Promotion | Gastrointestinal phospholipid digestion |
| Cancer | $sPLA_2$-IIA | Intestinal Paneth cells, gastric gland | Protection | Maintenance of intestinal microflora? |
| Microbial defence | $sPLA_2$-IIA | Various tissues after infection | Protection | Degradation of bacterial membranes |
| | $sPLA_2$-V | Macrophages | Protection | Phagocytotic killing of fungi |
| Alopecia | $sPLA_2$-X | Hair follicular ORS | Promotion | ORS cell growth |
| Reproduction | $sPLA_2$-III | Epididymal epithelium | Promotion | Sperm maturation by membrane remodeling |
| | $sPLA_2$-X | Sperm acrosome | Promotion | Sperm acrosome reaction by LPC |
| Pain | $sPLA_2$-X | DRG neurons | Promotion | Neuritogenesis by LPC |
| Steatohepatitis | $sPLA_2$-XIIB | Liver, intestine | Protection | VLDL secretion |

DC, dendritic cells.

patients with idiopathic membranous nephropathy have serum antibodies against a conformation-dependent epitope in $PLA_2R1$. The receptor is present in glomerular podocytes in human adult kidney and is likely to be the target of human $IgG_4$ immunoglobulin that specifically recognizes the receptor. The binding of the autoantibodies to $PLA_2R1$ triggers an unknown cellular signalling cascade that eventually leads to podocyte dysfunction.

## Concluding remarks

By the 1980s, research on $sPLA_2$ had become central to the field of lipid biochemistry. After the discovery of $cPLA_2\alpha$ in the early 1990s, however, the main interest of researchers shifted to the regulatory functions of this enzyme, as well as other newly identified intracellular $PLA_2$s, whereas $sPLA_2$s no longer represented the mainstream of $PLA_2$ research. Despite this, efforts by several groups including ours over the past decade have prompted reassessment of the biological functions of $sPLA_2$s and their target substrates using transgenic/knockout mice and specific inhibitors, in combination with the newly developed, highly sensitive, lipidomics technology. It has become apparent that individual $sPLA_2$s play important and diverse roles in various biological events, often acting through 'classical' mechanisms involving lipid mediators, and in other cases probably through 'alternative' mechanisms that are dependent upon their unique actions on extracellular phospholipid targets.

We are currently on the threshold of a new era of $sPLA_2$ biology. Although a growing body of evidence from transgenic or knockout studies has delineated unique roles of individual $sPLA_2$s in various pathophysiological events, in many cases the mechanistic insights are still speculative and incomplete. Continued efforts to analyse $sPLA_2$ gene-manipulated mice in the context of pathology, histology, molecular biology and biochemistry, in combination with lipidomics techniques and informatics, will shed further light on the true functional aspects of $sPLA_2$s including their target substrates and products associated with particular biological events. It is interesting that two or more $sPLA_2$s can function in a given pathophysiological event in a cooperative, complementary or counter-regulatory manner (Table II). Therefore, it seems logical that the control of particular $sPLA_2$s, alone or through a combination of multiple isoforms, would be advantageous for inhibition of selective lipid pathways in the treatment of various diseases. Currently, the pan-$sPLA_2$ inhibitor varespladib, which inhibits the conventional class of $sPLA_2$s, can markedly reduce the area of atherosclerotic lesions in experimental animals, and even in humans in early-phase clinical studies (*92*). These facts point to the $sPLA_2$ family as a potential therapeutic target for atherosclerosis, and probably other diseases in which one or more $sPLA_2$s are involved, such as asthma, arthritis and metabolic syndrome among others. Hopefully, given all these basic and clinical standpoints, the upcoming full elucidation of the $sPLA_2$ networks underlying each pathophysiology will ensure that $sPLA_2$s return to the centre stage of biomedical science in the next decade.

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### Conflict of interest

None declared.

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