Lyso phosphatidic acid (LPA) is one of the simplest glycerophospholipids with one fatty acid chain and a phosphate group as a polar head (1–3). LPA exists in a wide range of organisms from prokaryotes to eukaryotes. Previously, it was just considered as a metabolic intermediate in \textit{de novo} lipid synthesis and a component of the plasma membrane. But it is now known as a bioactive lipid mediator that induces many kinds of cellular processes including cell proliferation, prevention of apoptosis, cell migration, cytokine and chemokine secretion, platelet aggregation, smooth muscle contraction, transformation of smooth muscle cells and neurite retraction (1–4). As a lipid mediator, LPA is unique in that it is produced and rapidly degraded by specific routes and that its actions are evoked by six cognate receptors. LPA species include both saturated fatty acids (16:0, 18:0) and unsaturated fatty acids (16:1, 18:1, 18:2, 20:4) (5–10) and these LPA species exhibit differential biological activities (4, 11–13). This implies that that the different LPAs are recognized by different LPA receptors.

The first report of LPA bioactivity was in 1960 when it was discovered that LPA induced the contraction of isolated rabbit duodenum preparations. After 18 years, a vasopressor factor in soybean lecithin was identified as LPA (14). Subsequently, LPA in incubated serum was shown to cause the aggregation of feline and human platelets (15). These findings prompted several more studies of LPA but a crucial question remained: do the effects of LPA come from its reaction with a specific receptor or from its detergent-like physical property. In 1989, LPA was shown to stimulate cell proliferation in a pertussis toxin-sensitive manner (16). This was a significant finding that suggested that LPA acts through its cognate G protein-coupled receptor (GPCR). An answer to this question was obtained in 1996 when an orphan GPCR, vzg-1/Edg2, which is now known as LPA1, was shown to respond to LPA (17). Currently, there are at least six identified GPCRs for LPA, LPA1–6. Three of these receptors, LPA1–LPA3 (also known as vzg-1/Edg2, Edg4 and Edg7, respectively) are members of the endothelial cell differentiation gene (Edg) family (18, 19). Other members of this family include S1P1–S1P5 (also known as Edg1, Edg5, Edg3, Edg6 and Edg8, respectively), which are receptors for another important lysophospholipid, sphingosine-1-phosphate. On the other hand, LPA4 (P2Y9/GPR23), LPA5 (GPR92/93) and LPA6 (P2Y5) were found to be ‘non-Edg’ LPA receptors that belong to the purinergic receptors (P2Y) family (20–23). These two families have different evolutionary process and acquire their function independently.

There are at least two pathways by which LPA is produced (Fig. 1). In the first pathway, LPA is produced from lysophospholipids by a plasma enzyme, autotaxin (ATX/ENPP2) (24, 25), which we describe in Part II. In the second pathway, phosphatidic acid...
Phosphatidic acid (PA)

Lysophosphatidylcholine (LPC)

PA-PLA₁α/β

Lysophosphatidic acid (LPA)

LPA receptor (LPA₁-6)

Fig. 1 Biosynthetic pathways of LPA. There are two pathways of LPA production. In the first pathway, ATX converts lysophospholipids, mainly LPC, to LPA. LPA is also produced from PA by PA-PLA₁α.

(PA) is first generated from phospholipids or diacylglycerol and then deacylated by phospholipase A₁ (PLA₁) or phospholipase A₂ (PLA₂). PLA₁ has two isoforms: PA-selective phospholipase A₁α (PA-PLA₁α, also known as LIPH) and PA-PLA₁β (also known as LIPI) (26, 27). PA-PLA₁β was recently shown to be involved in hair follicle development (28). Little is known about PA-PLA₁β, except that it is highly expressed in the testis and thyroid and in Ewing family tumours (29).

LPA is also downregulated by degradative pathways. One such pathway involves a transmembrane exophosphatase named lipid phosphate phosphatase 1, 3 (LPP1, 3) (30). When LPA was added to LPP-expressing cells, it was degraded with a half-life of 3 min (31). LPP1 mutant mice have increased levels of plasma LPA, and LPA injected intravenously is metabolized at a 4-fold lower rate than in the wild type (32). LPP3-deficient mice are embryonic lethal by embryonic day 9.5 due to abnormal blood vessel formation (33). Interestingly, similar embryonic vascular defects were observed in ATX-deficient mice (34) and LPA₁-deficient mice (35). Vascular defects were also observed in zebrafish embryos in which ATX was downregulated or LPA₁ and LPA₄ were downregulated (36) as described later. Therefore, LPP3 is thought to be involved in the ATX-LPA signalling pathway.

LPA has been implicated in various pathological conditions, and thus is a potential drug target. For example, Ki16425, a potent antagonist for LPA₁/3, is in preclinical development as a drug for fibrous diseases such as lung fibrosis. As an LPA-producing enzyme, ATX is also an attractive drug target, because its level in plasma is altered in some pathological conditions such as chronic liver diseases (38), obesity (39), and cancer (40–44). However, it should be noted that the ATX level is upregulated in some physiological conditions such as pregnancy (45). It is possible that ATX inhibitors may also be developed using the recently determined crystal structure of ATX (46). In this review, we will summarize recent advances in how LPA is produced and how it is involved in both physiological and pathological conditions.

Uncovered Crystal Structure of an LPA-Producing Enzyme, ATX

As mentioned above, ATX is a secreted glycoprotein that acts as a lysophospholipase D (lysoPLD), converting lysophosphatidylcholine (LPC) into LPA (Fig. 1) (24, 25). ATX is one of seven ENPP-type ectophosphodiesterases that contain a central phosphodiesterase (PDE) domain responsible for their catalytic activity (47). In addition, like its closest family members, ENPP1 and ENPP3, ATX has three additional domains, including two somatomedin-B-like (SMB1, 2) domains located at the N-terminus and a nuclease-like domain located at the C-terminus. Although ENPP family proteins have high sequence homology, only ATX exhibits lysoPLD activity (48). So far, five alternative splicing ATX isoforms have been identified: ATXα (ATXm), ATXβ (ATXt), ATXγ (PD-Iz) (49), ATXδ and ATXε (50) (Fig. 2). ATXβ and ATXδ, which are the most abundant and second most abundant isoforms, respectively, share similar biochemical characters.

In 2011, we and another group working together resolved the X-ray crystal structures of mouse ATXδ and rat ATXβ (46). The structures revealed that ATX possesses a hydrophobic lipid-binding pocket that is always accessible to the solvent, unlike conventional lipases which have a hydrophobic pocket that is usually occluded by a ‘lid’ structure, which only opens upon substrate binding. Owing to the central catalytic domain interacting extensively with the two SMB domains on one side, and with the nuclease-like domain on the other, this unique lipase has a stable, robust architecture.

A structural comparison of ATX with a bacterial NPP enzyme from Xanthomonas axonopodis revealed the presence of a 19-amino-acid insertion in X.axonopodis NPP (residues 156–174) at the PDE
domain of ATX. Intriguingly, all ENPP family members except ATX have a similar insertion sequence. The insertion loop narrows the pockets, which would prevent accommodation of acyl chains of lysophospholipids and thus prevent them from having lysoPLD activity.

We solved the crystal structure of ATX in complex with various LPA species (14:0, 16:0, 18:1, 18:3 and 22:6). In all of the complexes, the phosphate group, glycerol moiety and acyl-chain encompassing C1–C12 of LPA were held by ATX in a similar manner. In the ATX–14:0-LPA complex, the lipid tail of 14:0-LPA was accommodated in a hydrophobic pocket with a local conformational change from the lipid free-form. The lipid tail of 16:0-LPA was accommodated in the hydrophobic pocket in a similar manner to 14:0-LPA, although its electron density was relatively poor. The lipid tails of the unsaturated LPA such as 18:1 and 18:3-LPA were elaborately accommodated in the pocket due to their bends at the unsaturated bonds. By contrast, the electron density of 18:0-LPA was not clearly observed. The preference for LPA species may reflect the substrate specificity of ATX, because ATX prefers LPC species with shorter and more unsaturated fatty acid as substrate and the rank order is 14:0 > 16:0 > 18:3 > 18:1 > 18:0. Amino acid substitutions showed that certain residues in the pocket were required for lysoPLD activity and substrate specificity of ATX. These data thus explain the ability of ATX to hydrolyze LPCs having different lengths and saturations and thus produce the corresponding LPAs.

An electron dense region corresponding to LPA was also observed within the hydrophobic channel that is formed by SMB1 and the catalytic domain. This channel was also blocked by the above-mentioned 19-amino-acid insertion loop. A mutant ATX with an insertion loop of ENPP1 could not hydrolyze LPCs to LPA and showed significantly impaired cell motility-stimulating activity. ATX induces the migration of various cell types by producing LPA and the consequent activation of LPA receptors. However, LPA could not be detected in cell culture, indicating that the LPA produced by ATX is efficiently delivered to its destination. It thus seems likely that the hydrophobic channel is a second LPA-binding site that serves as an exit shuttling the LPA products to LPA receptors or the plasma membrane adjacent to LPA receptors. Interestingly, ATX has a flat molecular surface on the side of the channel entrance and is able to bind to β3 integrins at the SMB domain. This flat surface appears to be suitable for an interaction with the plasma membrane where it could regulate LPA signalling at the cell surface.

**Physiological and Pathological Role of LPA**

Studies of gene-manipulated animals have shown that LPA receptors and LPA-producing enzymes have several patho-physiological roles (Table I). Here we describe several examples of such roles.

**Hair follicle development**

Recent genetic studies of human hair disorders have suggested that PA-PLA1α (LIPH) and LPA4 (P2Y5) have roles in hair follicle development (22, 23, 28, 51). Homozygous mutations in the PA-PLA1α and LPA4 genes cause congenital hair disorders termed LAH2 and LAH3, respectively. Patients with LAH2 and LAH3 were characterized by hereditary woolly hair and/or sparse hair. Study of PA-PLA1α-deficient mice revealed that they also have hair disorders

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Table I. Patho-physiological roles of LPA.

<table>
<thead>
<tr>
<th>Patho-physiological roles</th>
<th>Enzymes</th>
<th>Receptors</th>
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<tr>
<td>Hair follicle development</td>
<td>PA-PLA1α</td>
<td>LPA4</td>
<td>(22, 23, 28, 51)</td>
</tr>
<tr>
<td>Vascular development</td>
<td>ATX, LPP3</td>
<td>LPA4,6</td>
<td>(30, 33–26, 32)</td>
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<tr>
<td>Pulmonary/renal fibrosis</td>
<td>unidentified</td>
<td>LPA4</td>
<td>(53, 54)</td>
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<tr>
<td>Neuropathic pain</td>
<td>ATX</td>
<td>LPA4</td>
<td>(55, 56)</td>
</tr>
<tr>
<td>Embryo implantation</td>
<td>ATX, LPP1</td>
<td>LPA4,5</td>
<td>(57–60)</td>
</tr>
<tr>
<td>Spermatogenesis</td>
<td>ATX, LPP1</td>
<td>LPA4,5</td>
<td>(61–64)</td>
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**Fig. 2 Domain architecture of ATX.** Schematic domain organization of ATX. Five alternative splicing isoforms of ATX (ATXα, β, γ, δ and ε) have been identified.
including wavy vibrissa hair, a matted coat and disorganized pelage hair (28). The wavy hair phenotype of PA-PLA1α-deficient mice is also seen in mutant mice of TNFα converting enzyme (TACE/ADAM17), TGFα and epidermal growth factor receptor (EGFR). Accordingly, PA-PLA1α-LPA-LPA6 signalling was shown to regulate a TACE-TGFα-EGFR pathway (EGFR transactivation) (Fig. 3) (28). In this pathway, PA-PLA1α produces 2-acyl-LPA from PA in the developing hair follicles and then 2-acyl-LPA activates LPA6 in an autocrine and/or juxtacrine manner. Subsequently, TACE mediates the shedding of membrane-bound TGFα. Activation of EGFR by soluble TGFα induces development of hair follicles. It is first evidence of the physiological role of GPCR-induced EGFR transactivation in vivo.

**Vascular development**

ATX-deficient mice are embryonic lethal due to severe vascular defects (34). None of the LPA receptor-deficient mice have shown a similar phenotype, although LPA4-deficient mice are partially embryonic lethal with bleeding (35). Therefore, it was unclear which LPA receptors are involved in embryonic vascular development and how ATX regulates it. A recent study with zebrafish resolved this issue. Zebrafish has orthologues of all LPA-related genes including ATX and LPA receptors except for LPA5. Zebrafish is an ideal model for the analysis of vasculature formation because of its simple vascular network, optically clear body, growth outside the mother and convenient gene knockdown technique with morpholino antisense oligonucleotide. In addition, in EGFP-transgenic zebrafish, vasculature formation can be observed in live embryos. Taking advantage of these properties, we demonstrated that ATX knockdown and LPA1/LPA4 double knockdown in zebrafish embryos cause similar severe vascular defects (36). In zebrafish embryos, intersegmental vessels (ISV) sprout bilaterally from the dorsal aorta and grow dorsally following each vertical boundary between the somites. ATX knockdown and LPA1/LPA4 double knockdown stalled the development of ISVs in mid-course and caused them to aberrantly connect to neighbouring ISVs. The mechanisms by which ATX and LPA regulate formation of the vasculature remains to be determined. However, it is likely that LPA stimulates the migration of endothelial cells because ATX mRNA is expressed in the neural tube to which the ISVs appeared to extend. In addition to its abnormal ISV extension, connection between the ISVs and the dorsal aorta is disrupted in LPA1 and LPA4 double knockdown zebrafish embryos. This suggests that LPA maintains the connection of endothelial cells by modulating cell–cell adhesion. LPA1 knockdown was also found to cause defects in thoracic duct formation followed by pericardial and trunk edema in zebrafish embryos (52).

The level of LPA is also regulated by degradative pathways. Three enzymes involved in these pathways are lipid phosphate phosphatases (LPP1-3) (30). LPP3-deficient mice are also embryonic lethal with vascular defects (33). This suggests that an ATX-LPA-LPA1,4-LPP3 axis is involved in embryonic vascular formation and appropriate degradation of LPA is essential for development.

**Pulmonary and renal fibrosis**

Interstitial disorders of the lung and subsequent aberrant wound-healing responses can lead to pulmonary fibrosis. Idiopathic pulmonary fibrosis (IPF) is the most severe form of the disorder and is characterized by progressive development of alveolar inflammation, accumulation and proliferation of fibroblasts and collagen deposition. IPF restricts ventilation by stiffening...
the lungs. A lack of understanding the mechanisms of IPF have hindered the development of pharmacological therapies. Recently, LPA-LPA₁ signalling has been shown to have a critical role in the progression of bleomycin-induced pulmonary fibrosis (53) (Fig. 4). LPA was initially identified as a fibroblast chemoattractant in bronchoalveolar lavage (BAL) fluid from mice fibroblasts. In LPA₁ knockout mice challenged with bleomycin, fibroblast accumulation and vascular leakage were dramatically attenuated, while fibroblast proliferation, expression of matrix components and leukocyte recruitment were not changed. In IPF patients, increased level of LPA was existed in BAL fluids and BAL fluids-induced fibroblast migration was blocked by Ki16425 (a potent LPA₁ and LPA₃ specific antagonist). These results showed that the LPA-LPA₁ axis is critically involved in pulmonary fibrosis in human.

LPA signalling through LPA₁ promotes bronchial and alveolar epithelial cell apoptosis and fibroblast resistance to apoptosis (54). In addition to recruiting fibroblasts and inducing vascular leakage, LPA may contribute to the development of pulmonary fibrosis. Interestingly, LPA induces lung epithelial cell apoptosis through the process of ‘anoikis’, cell apoptosis induced by the detachment from the extracellular matrix.

As in pulmonary fibrosis, chronic renal lesion leads to fibrosis in the tubulo-interstitium (TIF), which, in turn, leads to end-stage renal disease (ESRD). Although the mechanism of renal fibrosis is poorly understood, it may involve an LPA-LPA₁ axis (65). Unilateral ureteral obstruction (UUO)-induced renal TIF, for which a mouse model has been developed, was found to be associated with an increased release of LPA by kidney. Human patients with chronic renal failure also showed high plasma LPA level. In addition, expression of LPA₁ mRNA was significantly increased after UUO. UUO-induced elevations of collagen type III and α-smooth muscle actin mRNA (fibrosis marker) and F4/80 mRNA (inflammatory marker) was significantly attenuated in kidneys from LPA₁ knockout mice. Furthermore, expression of connective tissue growth factor (CTGF) and transforming growth factor β (TGFβ) that are thought to play a crucial role in TIF was also lower in LPA₁ knockout mice than in control mice. These results were also obtained in mice treated with Ki16425. This report (65) also demonstrated that in a mouse epithelial renal cell line (MCT), LPA treatment induced a great increase in CTGF mRNA expression and a weak increase in TGFβ mRNA expression in a Ki16425-sensitive manner. These results suggest that the LPA-LPA₁ axis plays an important role in development of TIF by modifying of CTGF expression, although the kidney cell type that is the target of LPA and the enzyme that is responsible for LPA production in UUO-induced TIF model mice remain to be identified.

Neuropathic pain
Peripheral nerve injury can lead to neuropathic pain that is characterized by allodynia (pain as a result of non-noxious stimuli) and hyperalgesia (an increased response to a normally painful stimuli). Morphological and biochemical abnormalities such as demyelination and upregulation of the γ-isofrom
of protein kinase C (PKCγ) and α2δ1 subunit of the voltage-gated calcium channel (Cav2.2δ1) expression are also associated with neuropathic pain. Intrathecal injection of LPA induced neuropathic pain with the aforementioned abnormalities in BoTXC3 (a Rho inhibitor) and Y-27632 (a ROCK inhibitor) sensitive manner (55). Pharmacological and genetic deletion of LPA1 also completely abolished not only intrathecal LPA-induced neuropathic pain but also partial sciatic nerve ligation (general neuropathic pain model)-induced neuropathic pain. These data suggest that LPA-LPA1 signalling has a crucial role in the initiation of neuropathic pain. Lysophosphatidylcholine (LPC) (lysolecithin) was also found to induce neuropathic pain-like symptoms (56). These behaviours were completely abolished in LPA1 knockout mice and partially rescued in ATX heterozygous mice. ATX heterozygous mice also partially resist partial sciatic nerve ligation-induced abnormalities. Therefore, LPA may be synthesized from LPC by ATX at the site of the nerve injury. These results suggest that inhibition of the ATX-LPA-LPA1 axis is a potential drug target for treatment of the initial phases of neuropathic pain.

**Functions of LPA in reproduction**

Follicular fluids have a high level of lysoPLD activity (probably from ATX) as shown by its ability to produce LPA (66). Seminal fluids also contain high levels of LPA and ATX (67). In addition, LPA receptors, especially LPA1, LPA3, are strongly expressed in female and male genital tracts, including the uterus and testis. As shown in the following section, LPA has roles in both female and male reproductive systems.

**Female reproduction.** LPA has been shown to influence several female reproductive functions. LPA stimulated egg maturation in vitro (68) and ovum transport in ex vivo system (69). However, until now there is no direct in vivo evidence for these LPA actions because none of the mice in which any one of the six LPA receptors had been knocked out showed defects in egg maturation nor ovum transport. It is thus unclear whether LPA works via multiple LPA receptors or an unknown LPA receptor. On the other hand, it is clear that LPA has a critical role in the process of implantation (Fig. 5).

In 2005, Ye et al. found that LPA contributes to embryo implantation via LPA3 (57). LPA3 is highly expressed in endometrial epithelium at peri-implantation in a progesterone–dependent manner (58). LPA3-deficient female mice showed implantation failure phenotypes including delayed implantation and crowded implantation sites. Sharing of one placenta with multiple embryos was frequently observed in LPA3-deficient uteri. Mice deficient in cytosolic phospholipase A2 (cPLA2) or cyclooxygenase 2 (COX-2), both of which are expressed in the implantation sites and have roles in prostaglandin synthesis, showed a similar phenotype. LPA3-deficient uteri showed reduced levels of prostaglandins (PGE2 and PGF2α), and administration of prostaglandins to LPA3-deficient mice rescued the delayed implantation but not embryo crowding (57). This suggests that LPA3 regulates implantation timing and spacing independently. Interestingly, administration of an LPA3-specific agonist into uteri induced myocontraction (59), which is known to be important for regulating embryo spacing. Thus, LPA3 appears to regulate embryo spacing in uteri by inducing myocontraction. Recently, Ye et al. (60) suggested that LPA3 signalling induces dynamic remodelling of ECM in the peri-implantation uterus, which is required for normal implantation.

In recent years, an increasing number of women have undergone infertility treatment, such as blastocyst transplantation into the uterus. However, the success rate of this treatment is only ~30%. Currently, we don’t have any effective approaches to raise the success rate. The studies on LPA3 strongly suggest that LPA3 agonist could improve the success rate of infertility treatments.

**Male reproduction.** Little is known about the physiological roles of LPA in the male reproductive system. However, LPA receptors (LPA1, LPA3) are highly expressed in mouse testis (61), and seminal fluids contained LPA with high amounts of ATX and PLA2, which are involved in synthesizing LPA (62, 63). Other evidence that LPA1, LPA3 have roles in male reproduction is that

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**Fig. 5 LPA-LPA3 axis in embryo implantation.** At implantation sites, LPA is produced in the vicinity of embryos and stimulates LPA3 signalling. LPA3 signalling contributes to implantation by (i) determining the timing of implantation via the COX-2-prostaglandin pathway, (ii) controlling embryo spacing through myocontraction and (iii) inducing the remodeling of extracellular matrixes in the uterus.
LPA₁, LPA₂ and LPA₃ single knockout male mice showed age-dependent loss of spermatogenesis, increased apoptosis and decreased germ cell proliferation (64). However, LPA₁, LPA₂ and LPA₃ single knockout male mice showed milder reproductive abnormalities (61). These results showed that LPA₁, LPA₂ and LPA₃ receptors were redundantly involved in spermatogenesis. Interestingly, a similar defect in spermatogenesis was observed in transgenic mice expressing lipid phosphate phosphatase 1 (LPP1) which degrades LPA (64), suggesting again that LPA contributes to spermatogenesis.

**Conclusion**

In this review, we have discussed recent advances in understanding the role of LPA as a bioactive lipid mediator. Indeed, recent studies have revealed numerous biological roles of LPA both in physiological and pathological conditions through studies of synthetic pathways, receptor and degradative enzymes. The development of pharmacological compounds, such as receptor-specific antagonists/agonists and inhibitors of synthetic enzymes, should not only help to understand the roles of LPA but also lead to novel treatments for various diseases.

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**Conflict of Interest**

None declared.

**References**


Lysophosphatidic acid as a lipid mediator


