Phosphoinositide metabolism is an important intracellular signaling system that regulates a variety of cellular functions. Phospholipase C (PLC) is a key enzyme in this system. Recent studies on genetically manipulated mice have clarified the functions of PLC in vivo. This review focuses on the roles of PLC in organogenesis and embryonic development.

Phosphoinositide metabolism is an important intracellular signaling system involved in a variety of cellular functions, including secretion of hormones, transduction of neurotransmitters, growth factor signaling, membrane trafficking, and regulation of the cytoskeleton (12, 38, 73). Phosphoinositide-specific phospholipase C (PLC) is a key enzyme in this system; this enzyme hydrolyzes phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) to generate two second messengers, inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG), in response to activation of receptors by hormones, neurotransmitters, growth factors, and other molecules. DAG mediates the activation of protein kinase C (PKC), and IP₃ triggers the release of calcium ion (Ca²⁺) from intracellular stores (2, 66). In addition to the role of PtdIns(4,5)P₂ as a substrate for PLC and PI 3-kinase, PtdIns(4,5)P₂ also directly regulates a variety of cell functions, including cytoskeletal reorganization, cytokinesis, membrane dynamics, nuclear events, and channel activity (11, 13, 16, 21). Therefore, strict regulation of PtdIns(4,5)P₂ levels by PLC or other converting enzymes is necessary for homeostasis.

To date, 13 PLC isozymes have been cloned from mammalian species and categorized into the following six classes on the basis of structure and regulatory activation mechanisms: PLC-1 (1, 3, and 4), PLC-2 (1-4), PLC-γ (1 and 2), PLC-δ, PLC-ε, and the recently discovered class, PLC-ζ (1 and 2), on the basis of structure and regulatory activation mechanisms (20, 29, 61, 71, 102). Each isozyme is composed of subtype-specific and conserved domains (Figure 1). All PLC isozymes contain catalytic X and Y domains as well as various regulatory domains, including the C2 domain, EF-hand motif, and pleckstrin homology (PH) domain. Subtype-specific domains contribute to the specific regulatory mechanisms and include the src homology (SH) domain in PLC-γ (71) and the Ras-associated domain and Ras-GTPase exchange factor-like domain in PLC-ε (43, 79). The activation mechanisms of some PLCs have been clarified. PLC-γ isozymes are activated by a variety of stimuli downstream of receptors that contain seven transmembrane-spanning segments. PLC-γ isozymes are activated downstream of receptors with intrinsic or associated tyrosine kinase activity through their SH domains (8, 42), whereas PLC-ε is activated by Gα12/13, Ras and Hbo (6, 92). Recently, Hicks et al. found that a portion of the X-Y linker occludes the active site of PLC-β2 and that deletion of an X-Y linker constitutively activates PLC-β2, indicating that PLC-β2 has autoinhibitory regulation (26). Similar deletions in PLC-β1, -β1, and -ε also resulted in activation of these isozymes, suggesting that autoinhibition of PLC isozymes by an X-Y linker is a generalized process. This finding led to the proposition that the recruitment of PLCs to the plasma membrane leaves the active site free, since the X-Y linker region is repelled by negatively charged membranes.

Regarding physiological functions of PLCs, surprising new insights have been gained from new genetically modified mice in which specific PLCs have been deleted or introduced (Table 1). These studies revealed fundamental and specific roles of PLCs in organogenesis and embryonic development, presumably including cell migration, proliferation, and differentiation. Although this review focuses on the roles of PLC isozymes in organogenesis and embryonic development, PLCs also regulate activation and functions of many mature cells and tissues, which are reviewed elsewhere (69, 80).

Fertilization and Egg Activation

Fertilization and egg activation are the very first steps of embryonic development, and several studies have demonstrated that PLC-β4 and PLC-ζ are critically involved in this phenomenon (Figure 2). PLC-β4 knockout (KO) mice exhibit male infertility, and in vitro fertilization studies have shown that sperm from these animals induces activation of fewer eggs and does not elicit calcium oscillations (22). Since oscillating increase in calcium is critical for initiation of egg activation, these results indicate that PLC-β4 in sperm is an essential factor for induction of calcium oscillation and egg activation. The acrosome reaction is an essential step for fertilization because only sperm that have completed the acrosome reaction can penetrate the extracellular matrix of the egg, i.e., the zona pellucida (ZP) (86, 87). In some reactions,Solubilized reaction in wild-type reaction efficiency of intracellular PLCs has a primary role (4, 23). These findings have led to the conclusion that PLCs are essential components of fertilization and egg activation.
pellucida (ZP), and fuse with the egg plasma membrane (86, 87). In the mammalian sperm, the acrosome reaction is initiated by binding to the ZP. Solubilized mouse ZP induces the acrosome reaction in wild-type (WT) sperm but cannot induce this reaction efficiently in PLC-4 KO sperm. The elevation of intracellular calcium concentration ([Ca2+]i) has a primary role in the execution of the acrosome reaction (4, 14). WT sperm treated with ZP have been found to exhibit a continuous [Ca2+]i increase, whereas ZP induced only a small increase in [Ca2+]i in PLC-4 KO sperm, suggesting a role for PLC-4 in the Ca2+ response during the ZP-induced acrosome reaction (23).

Mammalian sperm appear to deliver a soluble factor into the egg to initiate [Ca2+]i oscillation; this soluble factor has been assumed to be PLC-ζ protein. PLC-ζ is a sperm-specific enzyme, and recombinant PLC-ζ protein has been shown to induce calcium oscillation and activation of the egg in mice (47, 74). Some studies suggest that the X- Y linker of PLC-ζ is an important regulatory region for its activity. PLC-ζ has a predicted nuclear localization signal (NLS) in the X- Y linker region, and nuclear localization of PLC-ζ is observed concomitantly with cessation of [Ca2+]i oscillation. PLC-ζ with a mutated NLS fails to localize to the pronuclei, and this PLC-ζ mutant causes continuation of [Ca2+]i oscillations beyond the time of pronuclei

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<td>KO</td>
<td>Defect in B-cell development; impaired development of peripheral lymph nodes; osteoporosis and decreased numbers of osteoclasts; defective separation of blood and lymphatic vessels; impaired cytotoxic function in NK cells; FcR-mediated Ca2+ flux and degranulation of mast cells; defective platelet aggregation to collagen</td>
<td>7, 10, 24, 25, 27, 28, 30, 36, 53, 56, 60, 68, 70, 82, 84, 89, 91</td>
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<td>PLCε</td>
<td>KO (catalytically inactivated)</td>
<td>Congenital malformations of both the aortic and pulmonary valves, leading to a moderate-to-severe degree of regurgitation with mild stenosis; resistance to skin tumorigenesis</td>
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<td>PLCζ</td>
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<td>PLCζ</td>
<td>TG (CAG)</td>
<td>Parthenogenetic development; benign ovarian teratomas</td>
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Table 1. Physiological functions in genetically modified mice

"through their SH activated by phospholipase C (PLC), Hicks et al. (23) shows that PLC-ζ, similar to PLC-4, inhibits activation of intracellular calcium oscillations. Solubilized mouse ZP induces the acrosome reaction in wild-type (WT) sperm but cannot induce this reaction efficiently in PLC-4 KO sperm. The elevation of intracellular calcium concentration ([Ca2+]i) has a primary role in the execution of the acrosome reaction (4, 14). WT sperm treated with ZP have been found to exhibit a continuous [Ca2+]i increase, whereas ZP induced only a small increase in [Ca2+]i in PLC-4 KO sperm, suggesting a role for PLC-4 in the Ca2+ response during the ZP-induced acrosome reaction (23)."
formation. This suggests that NLS-dependent pronuclei localization of PLC-ζ plays a role in terminating [Ca\(^{2+}\)]i oscillations at fertilization in mouse eggs (33, 52). Surprisingly, some sperm fractions without full-length PLC-ζ also showed [Ca\(^{2+}\)]i oscillation-inducing and PLC-ζ-like PLC activity (48). This activity seems to have been caused by PLC-ζ fragments generated by proteolytic cleavage at the X-Y linker region (49). Therefore, the X-Y linker region of PLC-ζ has putative NLS and a proteolytic cleavage site and is likely to have important regulatory functions during mammalian fertilization.

Several animal models have been developed to study the functions of PLC-ζ. A transgenic RNAi approach exploiting an shRNA under control of the BNA polymerase III-driven Irb promoter was used to reduce production of sperm-specific PLC-ζ during spermatogenesis in mice (45). Sperm derived from these transgenic mice induced a normal frequency of Ca\(^{2+}\) oscillations in fertilized eggs. However, [Ca\(^{2+}\)]i oscillations in eggs fertilized with sperm from these transgenic mice was sustained for substantially shorter periods and was terminated prematurely. These data demonstrate that PLC-ζ is required for persistent [Ca\(^{2+}\)]i oscillations following fertilization in vitro and also suggest a continued role for PLC-ζ beyond initiating [Ca\(^{2+}\)]i oscillations (46). Oscillations consistent with these abnormal patterns of [Ca\(^{2+}\)]i oscillations in vitro, mating of transgenic founder males to females results in lower rates of egg activation and no transgenic offspring. Another animal model is a transgenic mouse that expresses truncated PLC-ζ, lacking the NH\(_2\)-terminal 58 amino acid residues including the proteolytic cleavage site and is likely to have important roles during egg activation and sterility and that abnormal PLC-ζ expression underlies this functional defect.

### Development of the Circulatory System

The circulatory system is the first and most essential organ system to begin functioning during embryonic development. Several PLC isozymes have roles in the development of the circulatory system. Two distinct morphogenic mechanisms, vasculogenesis and angiogenesis, establish the embryonic vascular pattern. Vasculogenesis represents formation of primary vessels from endothelial cell progenitors in situ, whereas angiogenesis is the formation of vessels from the existing ones. Liu et al. reported that vasculogenesis is impaired in PLC-ζ/− KO embryos (55). PLC-ζ/+/− KO embryos undergo severe growth retardation, resulting in lethality at approximately embryonic day 9.0 (39). Correct vascular patterning is essential for embryonic development, and therefore defects in vasculogenesis may be responsible for embryonic lethality of PLC-ζ/− KO mice (Figure 3A). Migration and proliferation of endothelial cells is essential to angiogenesis. Knockdown of PLC-ζ in endothelial cells causes impaired migration and enhanced proliferation on VEGF, suggesting that PLC-ζ plays some roles in angiogenesis (3). Vascular development in the placenta is also essential for embryonic development. The placenta consists of maternal and embryonic parts, with the latter being composed of three distinct trophoblast cell layers, namely, the labyrinth layer, the spongiotrophoblast layer, and the giant trophoblast layer, moving from the embryo side to the maternal side. Of these, the labyrinth layer contains a large number of maternal and embryonic vessels and is the site of oxygen, nutrient, and waste exchange between the mother and the embryo. The PLC-ζ/−/- DKO mice died at around embryonic day 11.5-13.5, and their placenta has an avascularized layer. This abnormal vascularization leads to insufficient exchange of gas, nutrients, and waste and is therefore likely to be the cause of the midgestational embryonic lethality in PLC-ζ/−/- DKO mice (38). In fact, PLC-ζ/−/- DKO embryos survived by embryonic day 14.5, providing further evidence that embryonic lethality observed in PLC-ζ/−/- DKO mice is caused mainly by placental vascular defects (63). During later development, the lymphatic vasculature separates from the blood vasculature and acquires specialized structures. PLC also appears to be involved in the formation of lymphatic vessels (Figure 3B). Mouse mutants and lymphatic developmental defects and transgenic mice lacking the NH\(_2\)-terminal 58 amino acid residues show abnormal vascularization in initiating the heart and lymphatic vascular plexus (11). These results indicate that the inability of human sperm to initiate [Ca\(^{2+}\)]i oscillations leads to failure of egg activation and sterility and that abnormal PLC-ζ expression underlies this functional defect.

**FIGURE 1.** Domain structure of each type PLC

| Catalytic and regulatory domains and their interacting molecules are shown. PH, pleckstrin homology domain; EF, EF-hand domain; X and Y, X and Y domains, respectively; C2, C2 domain; SH, src homology domain; RaxGEF, Rax GTPase exchange factor-like domain; RA, Rax associating domain. |
most essential role in the development of embryonic stem cells (38). PLC-γ1 is essential for normal development of the heart. Heart organogenesis is important for proper function of heart. Heart valve development involves heart valve cells that subsequently give rise to the septa of the heart. Regarding heart organogenesis, normal valvulogenesis is important for proper function of heart. Heart valves develop from endocardial cushions, which form when endothelial cells in the atrioventricular and outflow tract areas undergo an epithelial-to-mesenchymal transformation and invade the adjacent acellular matrix. The cells then proliferate to form heart valve cushions that subsequently give rise to the septa of the four-chambered heart as well as the cardiac valves (17). Heart valvulogenesis also appears to be regulated by PLC (FIGURE 2D). Tadano et al. developed mice whose PLC-γ1 was catalytically inactivated by gene targeting (81). The hearts of these mice developed with WT pla-

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The process of fertilization. 1) The sperm approaches the egg. 2) The sperm binds to the zona pellucida and the acrosomal enzymes digest the ZP. 3) The sperm penetrates the ZP. 4) The plasma membrane of sperm and egg fuse. Mammalian sperm appear to deliver a soluble factor, PLC-γ1, into the egg to initiate 

1. Development of the Hematopoietic and Immune Systems

Hematopoiesis is essential in embryonic development. The PLC-γ1 KO mice died around embryonic day 9.0. Hematopoiesis of PLC-γ1-null mice is impaired, and it is likely that this impairment may, at least in part, be the cause of death in these mice (55). Analysis of tissue contribution of PLC-γ1 KO ES-derived cells in the chimeric PLC-γ1 KO mice revealed that the PLC-γ1 KO ES-derived cells could not contribute to the lymphoid and hematopoietic organs, suggesting that PLC-γ1 is essential for the development of hematopoietic stem cells (76). Consistent with this tissue contribution assay, an in vitro system also showed that PLC-γ1 KO ES cells differentiate into erythrocytes and monocytes/macrophages to a lesser extent compared with control WT ES cells in vitro system. These results indicate that PLC-γ1 play essential roles in hematopoiesis (FIGURE 4).

Although proper development of the immune system is dispensable for embryonic development, development of the system is critical in the postnatal life of the animals. The functions of PLC-γ1 and PLC-γ2 in the immune system have been studied extensively. PLC-γ1 and PLC-γ2 play important roles in the activation and normal function of innate and adaptive immune cells, including B cells, T cells, natural killer cells, and macrophages (7, 32, 83, 88, 91). In addition to immune cells, including B cells, T cells, natural killer cells, and macrophages (7, 32, 83, 88, 91). In addition
to these functional roles in immune cells, PLC-γ1 and PLC-γ2 isozymes have important roles in development of B cells (25, 84). Kunisaki generated IFN-γ-inducible PLC-γ2 KO mice and found that these mice had defects in B-cell development in the bone marrow at the pre-B cell stage (25). In the bone marrow of these mice, the transition of B220+CD43– pre-B cells from B220+CD43– pro-B cells was abnormal, which in turn limited differentiation into B220+IgM+ mature B cells. Further study revealed that PLC-γ2 deficiency impeded early B-cell development, resulting in accumulation of early pre-B cells (89). Since B-cell maturation was further impaired compared with wild type, it seems to play a critical role (90). The popu-

FIGURE 3. Roles of PLC in circulatory system
Vascular precursor cells called angioblasts coalesce and differentiate to form blood vessels. This process is called vasculogenesis. Angiogenesis is a physiological process involving the growth of new blood vessels from preexisting vessels. PLC-γ1 KO mice show impaired vasculogenesis. PLC-γ3 may play some roles in angiogenesis. Vascular development in the placenta is essential for embryonic development. The placenta consists of maternal and embryonic vessels, with the latter being composed of the trophoblast cell layers, namely, the labyrinth layer, the spongiotrophoblast layer, and the giant trophoblast layer. The labyrinth layer contains a large number of maternal and embryonic vessels and is the site of oxygen, nutrient, and waste exchange between the mother and the embryo. PLC-γ1 and PLC-γ3 are essential for normal structure of the labyrinth layer. During development, the lymphatic vasculature originates from the vein and separates from the blood vasculature. Lymphatic vessels develop from lymphatic precursors that arise from developing veins (arrow). PLC-γ2 has an essential role in initiating and maintaining the separation of the blood and lymphatic vasculature. Heart valves develop from endocardial cushions, which form when endocardial cells in the atrioventricular and outflow tract areas undergo an epithelial-to-mesenchymal transformation and invade the adjacent aortic wall. The cells then proliferate to form cushions that subsequently give rise to the septum of the four-chambered heart as well as the cardiac valves. Proliferation of the heart valve cells was reported to be negatively controlled by ErbB1-mediated suppression of Smad1/5/8. PLC-ε has a crucial role downstream of the epidermal growth factor receptor in the control of semilunar valvulogenesis by its action of inhibiting Smad1/5/8 activation.
was further impaired in PLC-γ1−/− PLC-γ2 KO mice compared with that of PLC-γ2 KO mice. PLC-γ1 also seems to play important roles in B-cell development (90). The population of B220+ cells in the bone marrow was shown to be decreased in PLC-γ2 KO relative to WT mice and was further markedly decreased in PLC-γ1−/− PLC-γ2 KO relative to PLC-γ2 KO mice. The mechanisms by which PLC-γ2 regulates B-cell development have been studied extensively. Signals propagated by the B-cell receptor (BCR) and its precursor, pre-BCR, are critical for the development and activation of B lymphocytes (50, 65). One of the major downstream-signaling pathways triggered by BCR cross-linking is the Ca2+-signaling pathway in B lymphocytes. BCR-induced Ca2+ mobilization requires the assembly of a BCR signalosome that comprises the spleen tyrosine kinase (Syk), Bruton’s tyrosine kinase (Btk), the adaptor protein B-cell linker (BLNK), and PLC-γ2 (19, 51). It has been reported that a PLC-γ2 SH2 mutant, which inhibits coupling between BCR and PLC-γ2, failed to restore B-cell maturation, implying that a defect in BCR signaling led to the reduced number of mature B cells in PLC-γ2 KO mice (28). Signaling through the BCR is also required for the development and maintenance of mature splenic B cells. In fact, mature B cells are markedly decreased in the spleen of PLC-γ2 KO mice (25).

In addition to B-cell development, PLC-γ2 also regulates development of the second lymphoid tissue. PLC-γ2 KO mice show impaired development of peripheral lymph nodes (10). Mice that are deficient in receptor activator for nuclear factor-κB ligand (RANKL), or its receptor RANK, show similar lymph node agenesis (46), suggesting that PLC-γ2 regulates lymph node organogenesis downstream of RANKL. In addition to lymph node organogenesis, RANKL signaling is also known to be essential in osteoclastogenesis. In fact, PLC-γ2 deficiency has been shown to cause failure of PLC-γ2-deficient bone marrow osteoclast precursors to differentiate into osteoclasts after RANKL stimulation in vitro (46, 56). In addition, PLC-γ2 KO mice are osteopetrotic and have decreased numbers of osteoclasts due to defective upregulation of nuclear factor of activated T cells (NFAT) 2, which is a critical transcription factor activated by RANKL that controls osteoclast differentiation (57). Taken together, these findings indicate that PLC-γ2 regulates osteoclastogenesis as a downstream effector of RANKL in mice (FIGURE 4). Interestingly, re-introduction of
PLC-γ2 but not PLC-γ1 restores RANKL-mediated osteoclast differentiation of PLC-γ2-deficient bone marrow-derived osteoclast precursors, suggesting that PLC-γ2 has an important and unique role that cannot be replaced by the highly homologous PLC-γ1 in RANKL-mediated biological functions.

**Development of Skin**

The skin forms an effective barrier between the organism and the environment and prevents the invasion of pathogens and repels chemical and physical assaults as well as the unregulated loss of water and solutes. Proper skin organogenesis is therefore important to the health of animals. The skin is composed of the epidermis, dermis, hypodermis, and many mini-organs, including hair follicles and nails and mammary, sebaceous, and sweat glands. The epidermis is a stratified epithelium mainly composed of keratinocytes. The histochomistry and function of the epidermis depends on a finely tuned balance between keratinocyte proliferation and differentiation. The skin is therefore an excellent model for studying cellular differentiation and organogenesis. Several PLCs have important roles in skin organogenesis. The mammalian epidermis displays a characteristic calcium gradient, with low calcium levels in the lower, basal layers and progressively increasing calcium levels toward the outer layers ([59]). Extracellular calcium causes differentiation of keratinocytes in vitro, and this phenomenon recapitulates in vivo differentiation of keratinocytes in many aspects. PLC-γ is likely to regulate keratinocyte differentiation, because downstream signals of PLC, such as increased \([\text{Ca}^{2+}]\)i and PKC activation, are known to regulate keratinocyte differentiation ([5], [54]). Some PLC isozymes show dynamic expression patterns during keratinocyte differentiation. PLC-γ1 and PLC-δ1 protein are upregulated during calcium-induced differentiation of keratinocytes in vitro ([67]). PLC-δ1 and PLC-δ2 are expressed in the suprabasal epidermis in vivo (FIGURE 5) ([1], [62]). Of these PLC isozymes, the role of PLC-γ1 in keratinocyte differentiation has been extensively studied. Antisense PLC-γ1 reduces levels of the differentiation markers involucrin and transglutaminase in response to extracellular calcium elevation ([95]). Antisense PLC-γ1 also reduces elevation of \([\text{Ca}^{2+}]\)i in response to extracellular calcium elevation. The mechanism whereby PLC-γ1 is activated on elevation of extracellular calcium is suggested ([96, 97]). The elevation in extracellular calcium causes complex formation of adherence junction proteins and recruitment of PI 3-kinase to the complex. Activated PI 3-kinase then generates phosphatidylinositol 3,4,5-triphosphate, which activates PLC-γ1. To date, the in vivo functions of PLC-γ1 in epidermal keratinocytes have not been studied in PLC-γ1-null mice since these mice undergo early embryonic lethality. PLC-δ1 is another PLC isozyme upregulated during differentiation of keratinocytes. Primary keratinocytes from PLC-δ1 KO mice cannot sustain elevations in \([\text{Ca}^{2+}]\)i and calcineurin-NFAT activation in response to an elevation in extracellular calcium. In addition to impaired calcium signaling, PKC activation is also decreased in the epidermis of PLC-δ1 KO mice ([62]). Hair shafts are made by the hair follicle, a complex organ with hair shafts of both mice being bent and failing to penetrate the epidermis. In nude mice, the gene encoding the Foxn1 seems to be replaced by the highly homologous PLC-γ1 since these mice show no hair keratin expression ([58]). Several downregulation of PLC-γ1 expression is marked in Foxn1 KO mice. PLC-δ1 expression was reported to be upregulated in mice with alopecia areata ([60]). The influence of PLC isozymes on hair follicle and organogenesis has been discussed in detail but not yet been studied in detail.

**Regulated differentiation and organogenesis of animals.** Since organogenesis require interactions of genes and proteins, it is important to understand the genetic and environmental factors that affect skin organogenesis. The skin is composed of the epidermis, dermis, hypodermis, and many mini-organs, including hair follicles and nails and mammary, sebaceous, and sweat glands. The epidermis is a stratified epithelium mainly composed of keratinocytes. The histochomistry and function of the epidermis depends on a finely tuned balance between keratinocyte proliferation and differentiation. The skin is therefore an excellent model for studying cellular differentiation and organogenesis. Several PLCs have important roles in skin organogenesis. The mammalian epidermis displays a characteristic calcium gradient, with low calcium levels in the lower, basal layers and progressively increasing calcium levels toward the outer layers. Extracellular calcium causes differentiation of keratinocytes in vitro, and this phenomenon recapitulates in vivo differentiation of keratinocytes in many aspects. PLC-γ is likely to regulate keratinocyte differentiation, because downstream signals of PLC, such as increased \([\text{Ca}^{2+}]\)i and PKC activation, are known to regulate keratinocyte differentiation. Some PLC isozymes show dynamic expression patterns during keratinocyte differentiation. PLC-γ1 and PLC-δ1 protein are upregulated during calcium-induced differentiation of keratinocytes in vitro. PLC-δ1 and PLC-δ2 are expressed in the suprabasal epidermis in vivo. PLC-γ1 and PLC-δ1 are essential for normal keratinocyte differentiation. Conclusively, the role of PLC in keratinocyte differentiation is important for the development of skin.
encoding the transcription factor Foxn1 is spontaneously mutated; this mutation leads to insufficient hair keratin expression and abnormal hair shaft structures (54). Since enormous expression of Foxn1 results in upregulation of PLC-δ1 and PLC-ε1 expression is markedly decreased in skin of nude mice, Foxn1 seems to function as an upstream regulator for PLC-δ1 expression in hair follicles (64). Recently, PLC-δ1 was reported as the gene responsible for hair defects in mice with a recessive spontaneous mutation oligoH9254. Analysis of the genomic structure revealed that the δ1 mutation was a deletion on the distal end of chromosome 9 including the PLC-δ1 gene. In fact, PLC-δ1 expression cannot be detected in the dorsal skin of oligoH9254/oligoH9253 double heterozygotes exhibit hair defects observed in oligoH9254 mice. Taken together, PLC-δ1 has essential roles in normal formation of hair follicle and hair shaft. However, the detailed mechanisms by which a lack of PLC-δ1 causes hair defects remain to be clarified.

Concluding Remarks

Since organogenesis and embryonic development require interactions of various cells, genetic manipulations in mice have provided a powerful tool for investigating gene function in these processes. Studies with genetically manipulated mice have revealed that a lack of some PLC isoforms causes defects in fertilization and development of the circulatory, hematopoietic, immune, and skin systems. Downstream molecules of PLC, Ca2+, elevation, and PKC activation play important roles in these systems and may be the reason why PLC has essential roles in the development of these processes. Given there are 13 PLC isoforms and that they have significantly overlapping tissue and intracellular distributions, it is very interesting that each PLC isoform has unique and distinct roles in organogenesis and embryonic development. The structural complexity of PLC isoforms may correlate with the variety of biological functions of PLC. Simple-structured PLCs such as PLC-δ and PLC-ε are critical in fertilization, whereas development of an adaptive immune system is regulated by PLCs with relatively complex structures. It is possible that simple-structured PLCs have roles in basic biological systems, whereas complex-structured PLCs regulate more sophisticated biological functions. PLC and its downstream signals play important roles in various biological events from less understanding of calci-


