

# Lipid signaling in keratinocytes: Lipin-1 plays a PArt<sup>1</sup>

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Although it is well recognized that lipids play an important role in providing the structural barriers that delineate the cell and its various organelles, accumulating evidence also points to the critical involvement of lipids in cell signaling. Unlike some signaling molecules, however, an understanding of lipids as signals must take into account the additional intricacy afforded by the fact that many lipid signals can be interconverted. For example, diacylglycerol (DAG), a lipid known to activate enzymes such as protein kinases and guanine nucleotide exchange factors, can be phosphorylated by diacylglycerol kinase to yield phosphatidic acid (PA), which has its own effector enzymes (Fig. 1). Similarly, PA can be dephosphorylated to produce DAG via PA phosphohydrolases (such as lipin-1). This complexity can also make difficult attempts to understand which lipids are responsible for a particular cell response. However, with the identification and cloning of many of the gene products catalyzing these interconversion reactions, it has become possible to increase our understanding of the complicated lipid signaling mechanisms that underlie many cellular processes.

One such process is the differentiation of keratinocytes that comprise the predominant cell of the epidermis of the skin. The epidermis is a stratified squamous epithelium that delineates and separates an organism from the external environment. Its major function is to serve as a barrier, both to prevent noxious stimuli such as microorganisms from gaining access to the internal milieu and to inhibit the loss of precious body components, such as water, to the outside. Indeed, the water permeability barrier provided by the epidermis is necessary for successful terrestrial existence. Basal keratinocytes sit on the basement membrane at the epidermal junction with the underlying dermal connective tissue compartment and continuously proliferate to replace cells that are damaged, injured, or sloughed to the environment. These cells also express the immature keratins, keratin 5 and 14, that characterize them to be of epidermal origin. Cells from this basal layer move up into the next (spinous) layer where they become growth arrested and begin to express markers of keratinocyte

differentiation, including the mature keratins, keratin 1 and 10 [reviewed in (1)]. As they continue to migrate upwards through the various epidermal layers, the keratinocytes decrease their expression of certain genes/proteins and induce the expression of others in a programmed pattern that contributes to the final mature state of the tissue. Various markers are known to be expressed at different points in this process, including keratins 1 and 10 and involucrin (an early to intermediate differentiation marker), and can be used to assess the differentiation status of the cell. In the last living layer of the epidermis, the granular layer, the keratinocytes secrete lipids that they have synthesized in the form of lamellar bodies and undergo a programmed cell death such that the most superficial cornified layer consists of dead cells, or squames, with a between-squame “filler” of lipids processed from the lamellar bodies [reviewed in (2)]. The squames help to provide the mechanical barrier function of the epidermis whereas the lipids form the water permeability barrier. The signals regulating the intricate process of keratinocyte differentiation have been extensively studied but gaps in our knowledge still exist.

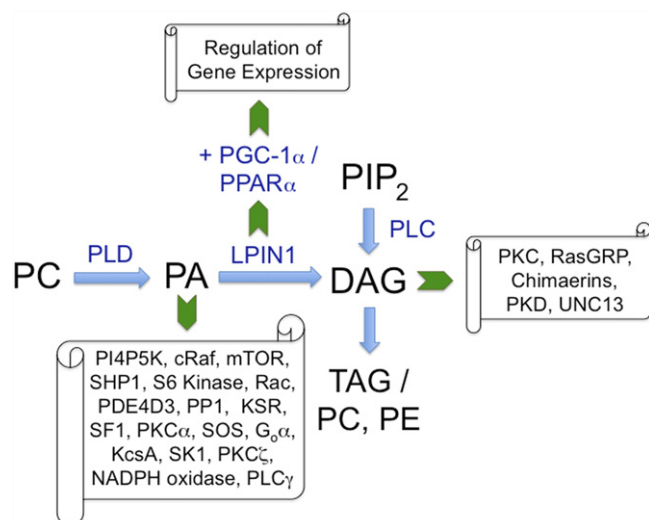
One signaling system that is known to be involved in regulating keratinocyte differentiation is the family of acidic phospholipid-requiring protein kinase C isoenzymes. This family is subdivided into three subtypes: the classical PKCs, PKC- $\alpha$ , - $\beta$ I and - $\beta$ II, and - $\gamma$ , which in addition to their dependence on acidic phospholipids are also activated by DAG and calcium; the novel isoforms that are activated by DAG but are insensitive to calcium (PKC- $\delta$ , - $\epsilon$ , - $\eta$  and - $\theta$ ); and the atypical PKCs that require acidic phospholipid but are not sensitive to either DAG or calcium (PKC- $\lambda$ / $\iota$  and - $\zeta$ ). Various investigators have studied the roles of PKC isoforms in keratinocyte proliferation and differentiation and data suggest an involvement of multiple isoenzymes in these processes [reviewed in (3)]. For example, PKC- $\alpha$  has been shown to be important for growth arrest and early differentiation [reviewed in (4)]; in addition, this isoform is expressed in early spinous keratinocytes where it is active (5). Nevertheless, the mechanism by

W. Bollag is supported by a VA Research Career Scientist Award. The contents of this article do not represent the views of the Department of Veterans Affairs or the United States Government.

DOI 10.1194/jlr.C067074

<sup>1</sup>See referenced article, *J. Lipid Res.* 2016, 57: 563–573.

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**Fig. 1.** The role of Lipin-1 in the regulation of cell processes. Phosphatidic acid (PA) produced as a result of the hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) can be dephosphorylated by lipin-1 (LPIN1) to yield diacylglycerol (DAG). DAG can also be produced by the action of phospholipase C (PLC) on phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) or can be phosphorylated to PA by DAG kinase (not shown). DAG acts as a second messenger to activate several effector enzymes, as shown: classical and novel protein kinase C (PKC) isoenzymes; Ras guanine nucleotide releasing protein (RasGRP), a guanine nucleotide exchange factor for Ras; chimaerins, Rac GTPase-activating proteins; protein kinase D (PKD), a serine/threonine protein kinase with homologies to PKCs and calcium/calmodulin-dependent protein kinases; and the UNC13 proteins, involved in vesicle maturation and exocytosis. The DAG produced by lipin-1 also serves as a precursor for the synthesis of triacylglycerol (TAG) and the phospholipids PC and phosphatidylethanolamine (PE). PA is also a lipid second messenger with effector enzymes as shown: phosphatidylinositol 4-phosphate 5-kinase (PI4P5K), which catalyzes the final step in the synthesis of PIP<sub>2</sub>; cRaf, a mitogen-activated protein kinase kinase; mammalian target of rapamycin (mTOR), a protein kinase; Src homology region 2 domain-containing phosphatase-1 (SHP1), a tyrosine phosphatase; ribosomal p70 S6 kinase, a protein kinase; Rac, a small GTP-binding protein; phosphodiesterase D (PDE4D3), a cAMP-hydrolyzing enzyme; protein phosphatase-1 (PP1), a serine/threonine protein phosphatase; kinase suppressor of ras (KSR), a protein kinase; steroidogenic factor-1 (SF1), a nuclear hormone receptor; PKC- $\alpha$ ; son of sevenless (SOS), a Ras guanine nucleotide exchange factor; G $\alpha$ , the  $\alpha$  subunit of a heterotrimeric G protein; KcsA, a potassium channel; sphingosine kinase-1 (SK1), an enzyme that produces the lipid signal sphingosine 1-phosphate; PKC- $\zeta$ ; NADPH oxidase, a superoxide-generating enzyme; and PLC $\gamma$ , a PIP<sub>2</sub>-hydrolyzing enzyme. Lipin-1 can also act as a coactivator with peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) and peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) to regulate gene expression, particularly of genes involved in lipid metabolism.

which PKC- $\alpha$  activity is regulated is unclear. As a conventional isoform with sensitivity to calcium, presumably changes in cytosolic calcium levels thought to occur in response to a gradient of extracellular calcium in the skin, with lowest levels in the basal layer [reviewed in (6)], could help to stimulate PKC- $\alpha$  activity in the spinous layer. PKC- $\alpha$  activity also requires DAG, which likely arises, at least in part, from the stimulation of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) hydrolysis initiated upon calcium


binding to the keratinocyte calcium-sensing receptor, a G<sub>q</sub>-coupled seven transmembrane domain receptor for which calcium ion serves as ligand. On the other hand, in many cell types, phosphoinositide turnover provides only the early component of DAG produced upon ligand stimulation of a receptor; for these cells subsequent activation of phospholipase D (PLD) substantially contributes to the sustained increase in DAG levels with receptor activation [reviewed in (7)]. PLD catalyzes the hydrolysis of a phospholipid, usually phosphatidylcholine, to generate PA, or phosphorylated DAG.

Lipin-1 is one member of a family of PA phosphohydrolases that hydrolyzes PA to DAG. This reaction is important both because it converts one second messenger with particular responding enzymes into another lipid signal with different effectors and also because it is the first step in the synthesis of triacylglycerol (TAG) and the phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In their article in this issue of the *Journal of Lipid Research*, Chae et al. (8) investigated the role of lipin-1 in keratinocytes and the skin, where the enzyme is found predominantly in basal keratinocytes (with reduced levels in suprabasal layers). Consistent with this in situ protein expression, in cultured keratinocytes, lipin-1 levels are reduced upon confluence- or elevated extracellular calcium-induced differentiation. Further, these authors demonstrate that knockdown of lipin-1 in keratinocytes results in increased PA and decreased DAG levels, as well as reduced PKC- $\alpha$  activity, monitored using Western blotting with antibodies that recognize phosphorylated PKC substrates. Overexpression of lipin-1 has the opposite effect. Lipin-1 knockdown also resulted in an increase in the levels of p21, which could be enhanced with PKC inhibitors, whereas lipin-1 overexpression decreased p21. Consistent with its effect on the levels of p21, a cyclin-dependent kinase inhibitor that promotes growth arrest, lipin-1 knockdown increased the proportion of cells in the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle. In addition to initiating growth arrest, knockdown of lipin-1 decreased the levels of the differentiation markers keratins 1 and 10, again consistent with the reported role of PKC- $\alpha$  in regulating early keratinocyte differentiation (5). Thus, the results reported here suggest an important role of lipin-1 in modulating PKC activity through controlling DAG levels.

There are, however, limitations to the research presented by Chae et al. (8). For example, although many of the effects observed upon lipin-1 knockdown are statistically significant, the differences observed are often relatively minor. The small changes may be the result of incomplete knockdown of lipin-1 by the siRNA treatment (the authors describe an approximate 30–40% decrease in lipin-1 protein levels with RNA interference). In addition, there are other known functions of lipin-1 that were not addressed in this study. Lipin-1 is the first step in the synthesis of TAG and some phospholipids (PC and PE). The neutral lipid TAG has recently been found to play an important role in skin, with defects in certain genes involved in its biosynthesis involved in an ichthyosis skin phenotype [reviewed in (9)]. Similarly, Elder and colleagues (10) have

observed an association between decreased expression of lipid biosynthesis-related genes and psoriasis. Furthermore, the phospholipid PC is the substrate of phospholipase D (PLD), a lipid-metabolizing enzyme that produces the PA that can be acted upon by lipin-1 to generate DAG. PLD has also been shown to be involved in regulating keratinocyte differentiation (11, 12). Finally, lipin-1 has also been demonstrated to act as a transcriptional coactivator together with peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) and peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) to regulate the expression of multiple genes involved in lipid homeostasis [reviewed in (13)]. PPAR activity is known to be important in skin, with PPAR $\alpha$  activation inhibiting inflammation in mouse models of irritant contact dermatitis [reviewed in (14)]. Based on these multiple functions of lipin-1, it seems likely that other mechanisms in addition to changes in PKC- $\alpha$  activity and p21 could contribute to the effects of lipin-1 observed in the skin and other organs. Indeed, a spontaneous null mutation in lipin-1 in the fld mouse produces a lipodystrophy phenotype characterized by fatty liver, peripheral neuropathy, and a lack of adipose tissue depots, including subcutaneous white adipose tissue. According to the Jackson Laboratory, the Lpin1<sup>fld</sup>/Lpin1<sup>fld</sup> mouse also exhibits an abnormal coat, characterized by retarded hair growth and a ruffled appearance (<https://www.jax.org/strain/001592>). In addition, lipin-2 and lipin-3 are also expressed in keratinocytes and epidermis (15), although whether or not these other lipin family members can compensate for the loss of lipin-1 in keratinocytes remains to be determined. Nevertheless, these results suggest a likely important role for lipin-1 in the skin and indicate that additional studies are warranted.

The functions of lipin-1 in catalyzing the first step in TAG and the biosynthesis of certain phospholipids, in modulating the levels of two key lipid messengers, and in regulating the expression of various genes argue that data concerning its action in keratinocytes is important for a comprehensive understanding of skin. Future research examining three-dimensional epidermal constructs derived from human keratinocytes overexpressing or knocked down for lipin-1, as well as mouse models with epidermal-specific overexpression and ablation of lipin-1, should

help in defining the exact role and mechanism of action of this lipid-metabolizing enzyme. 

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