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Diacylglycerol kinases in membrane trafficking

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Abbreviations: DAG, diacylglycerol; DGK, diacylglycerol kinase; IP3, inositol 1,4,5-trisphosphate; MICAL-L1, Molecules Interacting with CAs-Like1; PA, phosphatidic acid; PKC, protein kinase C; PKD, protein kinase D; PI, phosphatidylinositol; PIP2, phosphatidylinositol 4,5-bisphosphate; RCP, rab coupling protein; SNX27, sorting nexin 27; TRE, tubular recyling endosome

Diacylglycerol kinases (DGKs) belong to a family of cytosolic kinases that regulate the phosphorylation of diacylglycerol (DAG), converting it into phosphatidic acid (PA). There are 10 known mammalian DGK isoforms, each with a different tissue distribution and substrate specificity. These differences allow regulation of cellular responses by fine-tuning the delicate balance of cellular DAG and PA. DGK isoforms are best characterized as mediators of signal transduction and immune function. However, since recent studies reveal that DAG and PA are also involved in the regulation of endocytic trafficking, it is therefore anticipated that DGKs also plays an important role in membrane trafficking. In this review, we summarize the literature discussing the role of DGK isoforms at different stages of endocytic trafficking, including endocytosis, exocytosis, endocytic recycling, and transport from/to the Golgi apparatus. Overall, these studies contribute to our understanding of the involvement of PA and DAG in endocytic trafficking, an area of research that is drawing increasing attention in recent years.

Introduction

The cytosolic diacylglycerol kinases (DGKs) are a family of kinases that regulate the phosphorylation of diacylglycerol (DAG), thus generating phosphatidic acid (PA). DGKs are widely conserved evolutionarily, and are found in organisms as diverse as bacteria,[1] fungi, *Saccharomyces cerevisiae*, plants,[2] multi-cellular organisms including *Drosophila melanogaster* and *Caenorhabditis elegans*,[3,4] and mammals.[5] DGK from *Escherichia coli* share little structural resemblance to the eukaryotic enzymes,[6] although eukaryote DGKs have a highly conserved catalytic domain. *Drosophila melanogaster* DGK shares homologous amino acid sequences at the carboxyl-terminal domain with porcine DGK.[7] In plants such as *Arabidopsis thaliana*, DGK has evolved into 3 phylogenetic clusters. Cluster I, encoded by AtDGK1 and AtDGK2, resembles the mammalian DGKε, while the other 2 clusters, contain only the conserved kinase domain but lack other accessory motifs, and accordingly are much smaller proteins.[5,8] Intriguingly, the yeast *Saccharomyces cerevisiae* DGK does not possess the signature catalytic domain with an ATP-binding domain, but utilizes CTP. It also has a much simpler and less varied amino-terminal regulatory domain than its ATP-dependent homologs.[9] To date, in mammals, 10 DGK isoforms have been identified.[10-19] All DGK isoforms have a conserved catalytic domain with an ATP-binding site that is required for kinase activity, and cysteine-rich regions that are homologous to the C1A and C1B motifs of protein kinase C (PKC) [20.]

Beyond the conserved catalytic domains, the regulatory domains of mammalian DGK isoforms differ greatly, leading to differential localization and regulation of phospholipids in the cells. Based on these regulatory domains, DGK isoforms can be divided into 5 subtypes. **Figure 1** summarizes the catalytic and diverse regulatory domains in each DGK isoform of each type, and **Table 1** summarizes current knowledge of their subcellular localization. In addition to the conserved domains, type I DGKs (DGKα, β, and γ) contain a calcium-binding EF hand motif [21.] Upon activation, DGKα rapidly translocates from the cytosol to the plasma membrane.[22] Type II DGKs (DGKδ, η and κ) have a unique pleckstrin homology (PH) domain and a sterile α motif (SAM) domain[10] that can bind to the endoplasmic reticulum (ER) and affect anterograde transport.[23] Type III DGK (DGKε) has a unique substrate specificity for arachidonate-DAG.[24,25] This substrate specificity renders DGKε the most important isoform that catalyzes the first step of phosphatidylinositol (PI) re-synthesis. Although there is no evidence supporting a direct role for DGKε in membrane trafficking, it is possible that of the DGK isoforms, only DGKε exerts an additional function mediating membrane trafficking via PtdIns cycling. Type IV DGKs (DGKζ and ι) have a nuclear localization signal in a MARCKS homologous domain,[26] 4 ankyrin repeats, and carboxyl terminal PDZ-binding domains.[27] Type V DGK (DGKθ) has 3 C1 domains, a putative PH domain, and a Ras association (RA) domain. In humans, each subtype of the DGK family isoforms displays a tissue-specific expression profile. For example, DGKα is mostly detected in brain and immunologic organs, such as spleen and thymus.[22] DGKβ is expressed in neurons in caudate-putamen, the nucleus accumbens, and the olfactory tubercle.[12,28] DGKδ is substantially expressed in spleen, ovary and skeletal muscle.[10,29,30] DGKζ is also highly expressed in spleen and thymus, as well as in heart and pancreas.[31]

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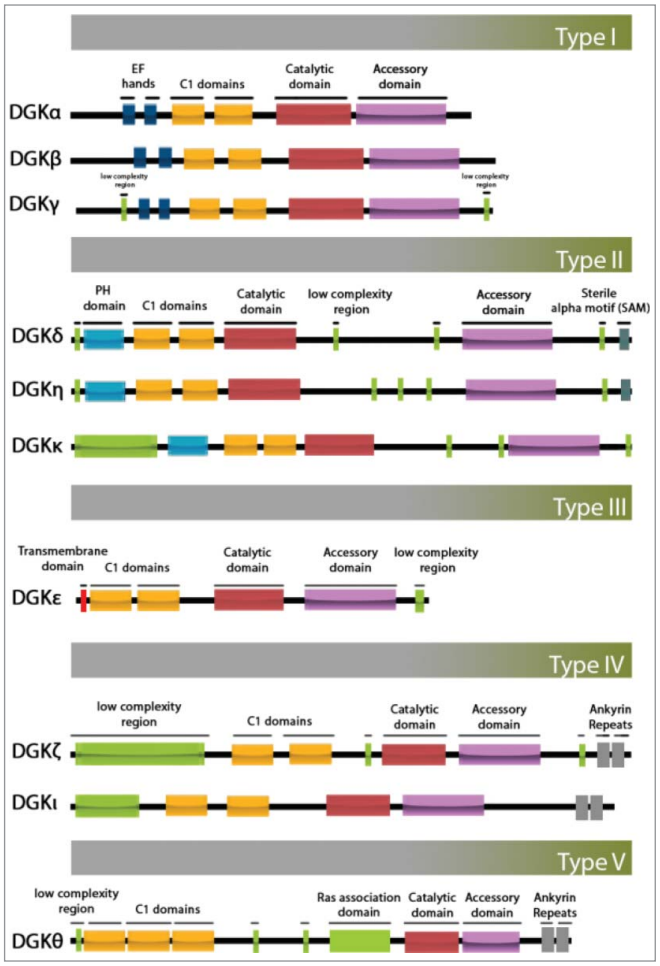

Figure 1. Schematic diagram illustrating the domain architecture of diacylglycerol kinase isoforms. The ten members of the mammalian DGK family are grouped into 5 types according to their regulatory domains. PH, pleckstrin homology domain; SAM, sterile α motif domain.

Studies using DGK knock-out mouse models have revealed the involvement of various DGK isoforms in different diseases. DGKα or DGKζ-null T cells display defects in immune function.[32,33] DGKδ haploinsufficiency causes the development of insulin resistance in skeletal muscle and adipose tissue.[30] In addition, DGKβ/DGKε knockout mice exhibit brain disorders and behavioral abnormalities.[34-36]

Since DGKα was identified in the 1990s, studies on DGK family proteins have primarily focused on their function in regulating signaling pathways. DGK terminates DAG-based signals and accentuates PA signaling, and both DAG and PA serve as important second messengers in the cells. The activation of DGK usually involves the translocation of DGK to a membrane compartment.[37] Upon recruitment to the membrane, DGK can be activated by calcium binding to the EF hand motif. DGK activity is initiated or enhanced by some lipid components including the products of phosphatidylinositol-3-kinase (PI3K) such as phosphatidylinositol-3,4,5-trisphosphate (PIP_3),[38] phosphatidylserine, and sphingosine.[6] It is also activated via Src tyrosine kinase phosphorylation[39] and

Table 1. Known subcellular localizations of different DGK isoforms

Localization	DGK isoforms
Plasma membrane	α,[22, 79] δ,[117] β, γ, ζ [79, 123]
Endoplasmic reticulum	δ,[23] η, κ
Endosome membrane	α,[107] ζ [52]
Nuclear	ζ,[26] ι
Multi-Vesicular Body	α [66]
Trans-Golgi network	α [79]

serine phosphorylation by protein kinase C (PKC).[40] Some upstream players participating in DGK activation include interleukin2 (IL-2) receptor signaling,[41] epidermal growth factor receptor (EGFR),[42] T cell antigen receptor signal transduction,[22] and hepatocyte growth factor receptor (HGFR).[43]

The substrate and product of DGK, DAG and PA respectively, play different roles in endocytic events. These include vesicular trafficking, the secretory pathway regulation, and Golgi apparatus function. The mechanisms by which DAG regulates membrane trafficking are diverse: 1) serving as a second messenger to activate PKC, PKD, and downstream signaling cascades;[44-46] 2) involvement in PI cycling regulation of phosphatidylinositol 4,5-bisphosphate (PIP_2) abundance, inositol 1,4,5-trisphosphate (IP_3)[47] and subsequent Ca^{2+} influx.[48] On the other hand, the local concentration of PA is an important regulator of trafficking, possibly because: 1) PA-enriched membranes with higher curvature tend to undergo fission;[49] 2) it serves as a docking site for recruiting specific proteins such as Rab coupling proteins (RCP),[50] *M*olecules *I*nteracting with *CA*sL-Like*1* (MICAL-L1),[51] and Sorting nexin 27 (SNX27) [52] to the membrane; 3) it is an intermediate for PtdIns re-synthesis.[24] Given the increasing awareness of the relevance of lipid metabolites such as DAG and PA in membrane trafficking, understanding the significance of DGK function in regulating the fine balance of DAG-to-PA levels is becoming an increasingly important research goal.

Exosome Secretion

DAG mediates acrosome fusion with plasma membrane

The acrosome is a secretory granule that is released from mammalian sperm and is essential for fertilization. Acrosomal secretion is a special type of regulated exocytosis, which uses conserved exocytic mechanisms also found in neuronal, endocrinal and other cells.[53] Upon inhibition of DGK in cells with the inhibitor R59022, impaired DGK activity leads to increased levels of DAG. Simultaneously, the level of PA correspondingly decreases in these cells. In this case, considerable stimulation of acrosomal exocytosis is observed.[53,54] Subsequent studies have identified DAG's role in stimulating acrosomal exocytosis through PKC and phospholipase D1 (PLD1) activation, promoting the continued production of PIP_2 and subsequently, IP_3 which is required for the intra-acrosomal calcium efflux during fusion with the plasma membrane of the spermatozoon (See **Figure 2** for the metabolism of DAG and related

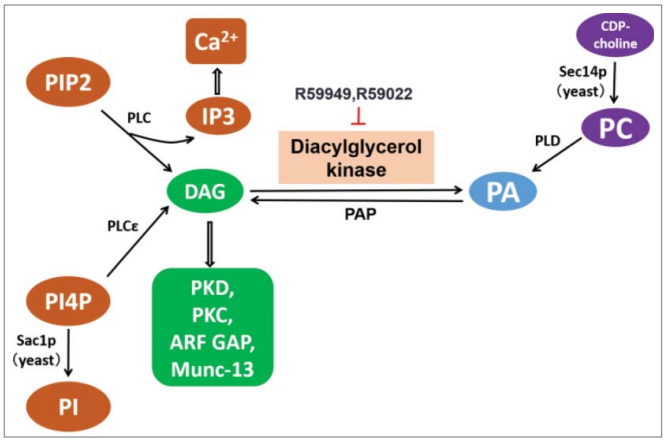

Figure 2. Pathways involved in the metabolism of DAG and PA described in this review. DGK, diacylglycerol kinase. DAG, Diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; PLD, phospholipase D; PLC, phospholipase C; PI4P, Phosphatidylinositol 4-phosphate; PI, phosphatidylinositol; PKD, protein kinase D; PKC, protein kinase C; ARF GAP, ARF GTPase-activating protein. Solid arrows indicate the metabolic pathway, hollow arrows indicate the downstream effector activated by DAG or IP3.

phospholipids). However, PA alone could not trigger exocytosis.[55] Furthermore, it was demonstrated that DAG activates Rab3A leading to the assembly of SNARE complexes and membrane fusion via interaction with Munc-13.[55,56]

PA regulation of the secretory pathway

Phospholipids may serve as an essential part of the machinery driving the fusion and/or fission of membranes, based on their shape and geometric features, and therefore may play a role in the budding and generation of secretory vesicles. Indeed, membrane domains enriched with acidic phospholipids, especially PA, are prone to membrane fusion, in conjunction with Ca^{2+}.[57] However, the shape of PA varies greatly under different Ca^{2+} concentrations. Unsaturated PA has a cylindrical, bilayer-preferring structure under normal cytoplasmic conditions (37°C, pH 7.2, 0.5 mM free Mg^{2+}); but at the mildly acidic intra-Golgi conditions (pH 5.9–6.6, 0.3 mM Ca^{2+}), it displays a conical (type-II) shape,[49] which is prone to form a highly curved membrane facilitating the fission process.

Another documented role for PA in secretion relates to the induction of neutrophil exocytosis from azurophilic granules by anti-neutrophil cytoplasmic antibodies (ANCAs). This likely results from the pathogenesis of endothelial cell damage in small vessel vasculitis, with serine proteases and myeloperoxidase (MPO) released from the activated neutrophils. DGK-generated PA is required for such exocytic activity, since treatment with DGK inhibitors reduces granule release by inhibiting granule fusion at the plasma membrane.[58] In this study, the addition of PA restored the release of MPO in DGK-inhibited cells, whereas supplementing cells with DAG failed to restore exocytosis. These findings collectively lead to the suggestion that ANCA-driven granule exocytosis is mediated by DGK-generated PA. Importantly, PA generated by PLD is not involved in this process.[59,60] PA production is also correlated with the ANCA-induced neutrophil adhesion *in vitro*.[61]

DGK regulates MVB formation and secretion

MVBs are formed by the inward invagination of the limiting membrane of endosomes, giving rise to intraluminal vesicles (ILVs). Although previously considered a mechanism of cargo sorting for lysosomal degradation, MVBs also fuse with the plasma membrane, secreting their ILVs into the extracellular area.[62] Studies show that DGK is involved in multiple processes related to exosome secretion, including the formation and maturation of Multi-Vesicular Bodies (MVBs) (i.e., the number of MVBs per cell and inward vesiculation of MVBs), the budding and release of exosomes from MVBs, and their fusion with the plasma membrane.[63-66] In cytotoxic T lymphocytes, MVBs are responsible for releasing the pro-apoptotic Fas ligand (FasL) at the immunological synapse[67] with DGKα playing an important regulatory role in this process.[63,66] Upon receptor stimulation, FasL and DGKα relocate to FasL-containing MVB structures. DGKα is recruited to MVBs and to exosomes, where it plays a dual role; DGKα kinase activity exerts a negative role in the formation of mature MVBs, as experiments show that treatment with a type I DGK inhibitor, R59949, induces the maturation of CD63-positive/lysobisphosphatidic acid-positive MVBs, and increases the secretion of exosomes.[63,68,69] In contrast, down regulation of DGKα inhibits polarized exosome secretion, and affects degranulation of MVBs at the immune synapse, while the kinase inhibitor increases polarized secretion of exosomes. These studies imply that *DGKα kinase activity* negatively regulates the formation of mature MVBs, while *regions outside the kinase domain* are required for polarization of MVBs and exosome secretion.[69]

The role of DGK on secretion from the Golgi apparatus is mediated via the cellular levels of DAG

The Golgi apparatus is an organelle found in most eukaryotic cells, and it is responsible for the processing, sorting and transporting of proteins and lipids. The formation of Golgi-to-plasma membrane transport carriers is accomplished via budding, elongation, constriction, and finally fission of the Golgi membrane. These events are facilitated by lipid bilayer deformation as well as the concerted efforts of many proteins that act at the various stages of secretion.[70] Previous studies have revealed that DAG plays a dual role in the generation of transport carriers, and thus mediates secretion from the Golgi. It serves in lipid signaling on the *trans*-Golgi network (TGN) for the recruitment and activation of essential proteins onto the TGN membrane. Such proteins include protein kinase D (PKD),[44] protein kinase Cη (PKCη),[45,46] and the ARF GTPase-activating proteins (ARF GAPs) Gcs1p, Age1p and Age2p[71-73] (see **Figure 2** for the downstream effectors of DAG). Reducing cellular DAG levels inhibited recruitment and blocked TGN-to-plasma membrane trafficking.[74] On the other hand, the conical shape of DAG in

the outer leaflet provides negative membrane curvature, which is thought to facilitate membrane fission.[75] Given the importance of DAG levels on TGN-to-plasma membrane transport, the metabolic pathways for the production or consumption of DAG intricately regulate the secretory pathway.[76-78] However, the molecular mechanisms mediating DAG cellular levels during vesicular trafficking under physiological conditions are not well understood.

DGK rapidly reduces the level of DAG, resulting in the inhibition of TGN-to-plasma membrane transport, implicating the negative regulation of DGK on Golgi secretory pathways. Earlier studies established that DGKα translocates to the TGN upon receptor stimulation.[79] Furthermore, work in yeast has revealed that the conversion of DAG to PA by increased DGK expression significantly impairs Golgi function. Sec14p is a phosphatidylinositol (PI)-transfer protein that generates the DAG precursor, phosphatidylcholine (PC). PLD then converts PC into PA, which is dephosphorylated by phosphatidic acid phosphatase (PAP) into DAG (**Fig. 2**). Studies have demonstrated that Sec14p is important in maintaining a favorable lipid environment for TGN-to-plasma membrane trafficking,[80-82] and thus is essential for yeast viability and secretory competence. To date, a large class of loss-of-function mutations in nonessential genes have been identified, the "bypass Sec14p"mutations, that restore cell viability and Golgi secretion with defective Sec14p, indicating that such mutations occur in regulators of TGN transportation downstream of Sec14p. Among the "bypass Sec14p" mutations, Sac1p deficiency functions by inducing accumulation of phosphatidylinositol 4-phosphate (PI4P), a pro-secretory phospholipid in the Golgi. Indeed, yeast DGK expression compromises the ability of Sac1p deficiency to effect "bypass Sec14p," suggesting DGK's negative role in TGN secretory pathway, possibly through reducing the level of PI4P.[83]

It is worth noting that the regulation of the DAG pool is more tightly controlled by Sec14p-dependent PC-PA-DAG conversion than through phosphorylation in the DAG-to-PA conversion pathway.[84] Currently, there is incomplete agreement over the involvement of DGK-generated PA in regulating secretion from the Golgi. On one hand, since PA is a direct product of DGK's activity on DAG and the up-regulation of DAG at the Golgi does not lead to a concomitant PA level increase, this implies that DGK might not be involved in this mechanism,[85] or that PA is rapidly transformed into other phospholipids. On the other hand, a study shows that DGK activity on PA production, rather than the consumption of DAG, regulates nascent vesicle secretion from the TGN.[86]

Studies also show that the level of DAG at the Golgi mediates retrograde transport (Golgi-to-ER), while anterograde transport (ER-to-Golgi) is insensitive to DAG. PA phosphatase mediated DAG production is required for the formation of COPI vesicles and Golgi-to-ER transport.[74,87-89] Interestingly, the level of PA generated via DGK-mediated phosphorylation of DAG affects anterograde transport, which will be described below.

Anterograde transport is mediated by local PA levels

A lipid micro-domain containing interconverting LPA, PA and diacylglycerol has the potential to drive membrane fission through changes in membrane deformation.[49] Several proteins have been identified that may induce fission at the Golgi apparatus: CtBP3/BARS and endophilin facilitate the conversion of lysophosphatidic acid (LPA) to PA,[90,91] PLD mediates generation of PA,[92,93] and protein kinase D binds to DAG.[94] Although no direct evidence shows that DGK is involved in the regulation of membrane fission, since PA is required for the fission of Golgi structures, it is possible that membrane fission and fusion events may require the DGK-generated PA, potentially implicating DGK as a major player in mediating vesicle trafficking.

Although there are 10 isoforms in the DGK family, only DGKα has been localized to the *TGN*,[63] and a portion of cytosolic DGKδ localizes to the ER via its SAM domain.[23] DGKδ expression blocks the formation of COPII-coated structures in the ER and slows ER-to-Golgi transport of Vesicular Stomatitis Virus G Glycoprotein (VSV-G), indicating that the anterograde transport is inhibited by DGKδ. On the other hand, COPI structures are unaffected,[23] suggesting that retrograde transport is independent of DGKδ. Apparently, lipid conversion is not required for anterograde transport, since the overexpression of a DGKδ kinase-dead mutant led to similar inhibition of ER-to-Golgi transport. As described above, the level of DAG affects the formation of COPI endosomes and retrograde transport (Golgi-to-ER), but not anterograde transport (ER-to-Golgi).[74,89] It is possible that the effect of DGK on anterograde transport from the ER to the Golgi is mediated through the production of PA, rather than DAG.

Others

There are some indications that DGK mediates the release of neurotransmitters, possibly through the recruitment of munc-13.[95] Studies on DGKι knock-out mice show that it is involved in presynaptic glutamate release during 3,5-dihydroxyphenylglycine (DHPG)-induced long-term potentiation.[96] DGK1 knockout (the homolog of DGKθ) in Caenorhabditis elegans, led to an increase in acetylcholine release,[97] suggesting that DGK negatively regulates synaptic transmission. However, the molecular mechanism underlying this role of DGK has yet to be established.

Endocytic Recycling

Role of DAG in recycling

DGK affects the DAG/Ras/ERK signaling pathway, either through regulating the DAG levels or directly by mediating the activity of downstream proteins such as PKC, and/or Munc-13.[33,98-101] In either case, PKC serves as an important downstream signaling protein involved in multiple trafficking processes. Classical and novel PKC bind to DAG.[102,103] PKCα localizes to transferrin-positive recycling endosomes in the perinuclear area, and PKCα stimulation by DAG/ phorbol myristate acetate (PMA) accelerates the recycling of transferrin to

the plasma membrane,[104,105] while the sorting of LDLR to the lysosome remains unaffected.[105] Furthermore, perturbation of this DAG gradient through the inhibition of DGK impaired both dynein recruitment and microtubule organizing center (MTOC) polarization.[64]

Role of PA in recycling

Endocytic recycling is mediated by local PA levels

DGKα-derived PA binds and recruits the Rab-coupling protein (RCP) to the tips of invasive pseudopods. Since RCP is required for the recycling of α5β1 integrin during cell migration,[106] DGKα is essential for RCP to drive the recycling of α5β1 integrin.[50]

Another effect of DGK-derived PA in endocytic recycling lies in the regulation of tubular recycling endosomes (TRE) that are decorated by *M*olecules *I*nteracting with *CA*sL-Like*1* (MICAL-L1). MICAL-L1 and Syndapin2 promote the biogenesis of TRE (thus regulating TRE function), and they are both recruited to the TRE membrane through direct interactions with PA.[51] DGKα-derived PA mediates the recycling of Major Histocompatibility Complex Class I (MHC I) without affecting its internalization. In addition, the MICAL-L1-decorated TRE are disrupted upon DGKα-depletion, leading to general defects in endocytic recycling.[107]When the compromised synthesis of PA by DGKα-knock-down is preserved by preventing it catabolism, with the PA phosphatase inhibitor propranolol, the loss of TRE is reversed. This indicates that DGKα regulates endocytic recycling through the level of PA.

PA as a binding partner for SNX27

Sorting nexin 27 (SNX27), a member of the SNX family of proteins involved in intracellular sorting and trafficking[108,109] interacts with DGKζ. This interaction is required for the localization of SNX27 to the sorting endosomes in Jurkat T cells. DGKζ-siRNA accelerated the recycling of transferrin receptor from the endocytic recycling compartment to the plasma membrane in T lymphocytes.[52] Although the reason why DGKζ–knock-down in T lymphocytes has such a dramatically different impact than DGKα-knock-down in other cell types remains unknown, it is possible that compensation by other DGKs and/or other PA-generating pathways in T cells is more robust.

Endocytosis

Dynamin is a key regulator of membrane constriction and fission during endocytosis that binds to anionic lipids (including PA) through its Pleckstrin Homology (PH) domain. The presence of PA increases dynamin's enzymatic activity, and induces its deep penetration into the membrane.[110] Moreover, in experiments where liposomes of different lipid components were co-incubated with dynamin, PA-containing liposomes had the most efficient dynamin-coated tubule formation.[111]

Genome-wide short interfering RNA screening analysis was performed to assess the involvement of different human kinases on endocytosis, measuring the rate of VSV-G entry via clathrin-mediated endocytosis (CME) and Simian virus 40 (SV40) internalization via clathrin-independent endocytosis (CIE).[112] In this study it was predicted that DGKβ negatively influences CIE while DGKγ positively mediates CIE, and DGKδ seems to have a dual effect promoting CIE and inhibiting CME.

By inhibiting type I DGK, total cellular PA levels decreased. Correspondingly, the internalization of epidermal growth factor (EGF) was significantly impaired. However, the uptake of transferrin remained unaffected, suggesting that EGF internalization depends on DGK activity, whereas the uptake of transferrin is independent of the kinase activity. Moreover, upon inhibition of type I DGK, fewer clathrin-coated pits (CCP) formed, indicating a role for DGK activity in CCP formation.[113]

In addition to its kinase activity regulating DAG-PA conversion, DGKs also serve as scaffolding proteins that recruit regulatory proteins required for endocytosis. For example, during clathrin-dependent endocytosis, CCP are formed with the assistance of clathrin and the Adaptor Protein 2 (AP-2) complex. DGKδ co-localizes with these CCP through its interaction with AP-2 via F369DTFRIL and D746PF sequences in the catalytic domain. Furthermore, the uptake of both transferrin and EGF were significantly reduced in the absence of DGKδ. Importantly, the kinase activity is also required for the endocytic process, as the kinase-dead mutant could not reverse the impaired uptake observed upon DGKδ knock-down.[114] This is probably due to the regulatory effect of PA on CME. Although DAG stimulates the internalization of transferrin in some organisms such as *Trypanosomatids* brucei, in human cells DAG levels do not influence transferrin endocytosis.[115] These studies suggest that DGK mediates clathrin-dependent endocytosis either through kinase activity leading to PA production, or by serving as a scaffold protein that recruits AP-2 for CCP formation.

Phagocytosis and macropinocytosis are processes essential for innate immunity and tissue homeostasis, during which cells such as macrophages ingest particulate (phagocytosis) or soluble (macropinocytosis) pathogens into membrane-bound vacuoles. The molecular mechanisms mediating phagocytosis include protein tyrosine kinases, GTP-binding proteins, PKC, actin polymerization and membrane movement.[116] Phosphoinositide metabolism serves as critical regulation of the initiation of both processes. A local phagosomal DAG accumulation is observed by biochemical means during particle ingestion.[117] The phosphorylation by DGK is a critical determinant of DAG at the phagocytic sites.[118] DGK inhibition by R59002 or R59949 increases the DAG-positive phagosomes, and enhances reactive oxygen species (ROS) generation by these phagosomes,[118,119] indicating that DGK terminates the DAG signaling mediating phagosomal ROS production. On the other hand, PA is detected at the plasma membrane in phagocytes. Both DGK and PLD are responsible for the PA production at phagocytic sites.[120,121] Among the 10 isoforms of DGK, DGKβ, DGKγ, and DGKζ are found in the plasma membrane of macrophages, suggesting that multiple DGK isoforms are involved in the regulation of macropinocytosis. The abundance of PA in the plasma membrane correlates with membrane ruffling; accordingly, DGK inhibitor R59002 treatment depresses both the rate and extent of ruffle formation. Upon

DGK inhibition, macropinosome formation and dextran internalization are impaired.[120] In addition to regulating the DAG-PA equilibrium, DGKζ plays a crucial role in phagocytosis and macropinocytosis via the activation of the Rho-GTPase family protein, Rac1.[122,123]

Summary and Conclusion

In addition to the important role DGKs play in cell signaling and immune activity, their effect on endocytic trafficking should not be underestimated. By catalyzing the conversion of DAG to PA, DGKs regulate different stages of endocytic trafficking, including the transport from/to the Golgi apparatus, the formation and secretion of MVBs, endocytosis, and the biosynthesis of recycling endosomes. There are 10 mammalian DGK isoforms, each with different regulatory domains, substrate specificities, and tissue/subcellular distribution, resulting in differential regulation of membrane trafficking. The existence of multiple isoforms suggests that there may be different DGKs at distinct subcellular structures, and that there is localized regulation of DAG/PA levels mediating endocytic trafficking. However, lipid metabolism is a dynamic, bi-directional processes, and DGKs may need to cooperate with other lipid modifiers to create and/or maintain an optimal membrane environment for trafficking. It is also possible that DGKs function in a kinase-independent manner. One example is that DGKδ facilitates CME through its binding to AP-2, but not through its kinase activity in regulating DAG or PA levels.

By mediating membrane trafficking, DGKs control cell morphology, migration, apoptosis, protein biosynthesis, and receptor activation. Although there is no established pathology caused by a malfunctioning DGK isoform, knock-out mouse models reveal significant pathological consequences, including insulin insensitivity, immune function abnormalities and brain disorders, suggesting a potential role for DGKs as therapeutic targets in disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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