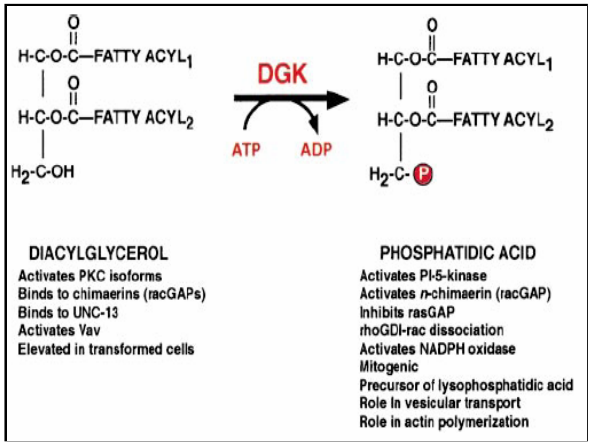
1. DIACYLGLYCEROL KINASES [1,2,3]

1.1. Diacylglycerol and phosphatidic acid

Diacylglycerol kinases (Dgks) phosphorylate diacylglycerol (DAG) to produce phosphatidic acid (PA). Diacylglycerol (DAG) is a membrane lipid that activates numerous proteins, including conventional and novel protein kinase C (PKC) isoforms, Ras guanyl nucleotide-releasing proteins (RasGRPs) and some transient receptor potential channels, and recruits a number of proteins to membrane compartments, including the chimaerins, protein kinase D and the Munc13 proteins. Because of its broad effects, the availability of DAG needs to be tightly regulated. The conversion of DAG to PA, operated by Dgks, represents one of the metabolic routes to the control of DAG levels, together with the hydrolysis of a fatty acyl chain by diacylglycerol lipase, which generates a monoacylglycerol and a free fatty acid, and the addition of CDP-choline or-ethanolamine to form phosphatidylcholine or phosphatidylethanolamine. For this reasons, Dgks have mostly been regarded as negative regulators of DAG-mediated signaling. Notwithstanding, the product of the reaction catalyzed by Dgks, PA, has important signaling roles. PA can stimulate DNA synthesis and is potentially mitogenic; it helps recruit Raf and sphingosine kinase 1 to the plasma membrane; it is also involved in vesicle trafficking and modulates the activity of several enzymes, including PI 5-kinases, PAK1, Ras-GAP, PKCζ, protein phosphatase, mTOR [4], p190RhoGAP [5], RhoGDI [7,8]. Although the bulk of PA signaling is thought to be generated via phospholipase D (PLD) enzymes, Dgks likely also contribute to its intracellular concentration.

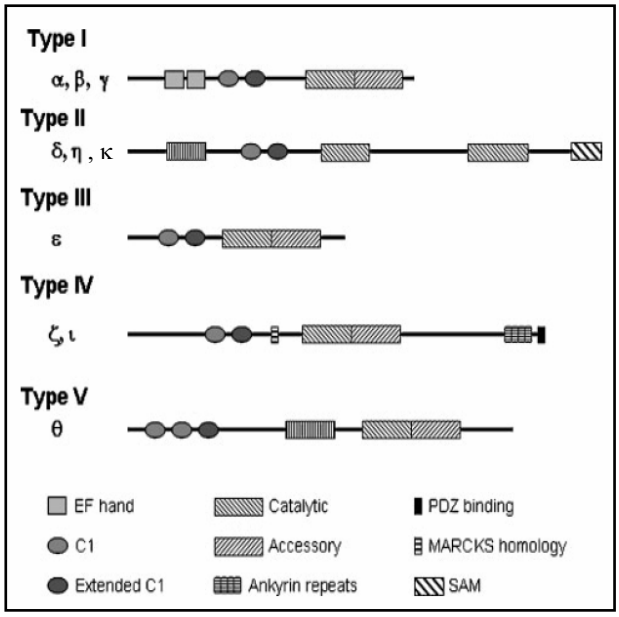


DAG and PA roles.
From Topham and Prescott, J. Biol. Chem. 1999 (Ref 2).

1.2. Diacylglycerol Kinases family: structure and tissue distribution

Ten isoforms of Dgks have been cloned to date in mammals. In contrast to mammals, one or only a few Dgk isoforms have been identified in organisms such as Caenorhabditis elegans, Drosophila melanogaster, Dictyostelium discoideum and Arabidopsis thaliana. No *DGK* gene has been identified in yeast, and there is one bacterial diacylglycerol kinase that has little similarity to eukaryotic Dgks. The structural diversity of Dgks in mammals suggests that they have evolved to perform roles in processes specific to higher vertebrates. All of the eukaryotic Dgk isoforms identified to date are similar in having a kinase domain consisting of catalytic and accessory domains. According to the presence of specific domains and to their structure, Dgks family have been subdivided into five coherent groups (I to V). Each catalytic domain has a consensus ATP binding site with the sequence Gly-X-Gly-X-X-Gly, where X is any amino acid. The Dgk catalytic domains likely function similarly to those of protein kinases by presenting ATP as phosphate donor. However, structural differences between the Dgk and protein kinase catalytic domains suggest that Dgk catalytic domains may have access to DAG in lipid bilayers, while protein kinase catalytic domains, in general, do not require such access. In addition to their kinase domains, all eukaryotic Dgks have at least two cysteine-rich regions homologous to the C1A and C1B motifs of PKCs. Whereas structural predictions suggest that these domains likely bind DAG, no Dgk C1 domain has been conclusively shown to bind diacylglycerol. It is interesting to note that the C1 domain in Dgks that is closest to the catalytic domain has an extended motif of 15 amino acids that is highly conserved between Dgks and not present in other C1 domains. The conserved residues in this extended motif are critical for DAG kinase activity, suggesting that this extended C1 domain might have a critical function in binding DAG. Type I Dgks have calcium binding EF hand motifs, making these isoforms more active in the presence of calcium. Dgks with pleckstrin homology (PH) domains at their amino termini are grouped as type II. Dgkδ also has at its C-terminus a sterile alpha motif (SAM) that is necessary to localize Dgkδ to the endoplasmic reticulum where it participates in vesicle trafficking between there and the golgi apparatus. The SAM domain might also mediate oligomerization of type II Dgks. The type III Dgkε has no identifiable regulatory domains, but it does have an unusual specificity toward acyl chains of DAG, strongly preferring an arachidonoyl group at the *sn*-2 position. This

preference suggests that Dgkε might be a component of the biochemical pathway that accounts for the enrichment of PI species with arachidonate. Type IV Dgks have a region homologous to the phosphorylation domain of the MARCKS protein that acts as a nuclear localization signal. These Dgks also have four ankyrin repeats at their C-termini. The type V DGKθ has three C1 domains and a region with weak homology to a PH domain. There is additional complexity in the mammalian Dgk family due to alternative splicing that occurs with Dgks β, γ, δ, ζ, ι, η, and probably others. Their diversity indicates that the mammalian Dgks are an important family of enzymes that regulate a variety of signaling events.



Mammalian Dgk family. Modified from Topham, J. Cell. Biochem. 2006 (Ref. 1).

Except for Dgks δ and ε, all mammalian Dgk isoforms are expressed in the brain at levels equivalent or higher that in other tissues. They have been detected in a number of different regions of the brain, including the hippocampus (β, γ, ε, ζ, ι, θ), cerebellum (γ, ε, ζ, ι, θ), and olfactory bulb (β, γ, ζ, θ), and in the retina (ε, γ, ι). Their prominent expression in these structures indicates that Dgks are an integral part of central nervous system and visual function. Several Dgks (α, ε, ζ, η) are also expressed in the lung, with Dgks ζ and α being expressed in alveolar type II cells. Dgks are also highly expressed in muscle, with Dgks δ and ζ expressed in striated muscle and Dgks β and ε being expressed in cardiac muscle. Finally, Dgks are also highly expressed in the spleen (α, ζ, δ, η), thymus (α, ζ) and various cultured white blood cells (α, δ, ζ). It is interesting to note that several Dgks can be expressed in

the same tissue and even in the same cell. Further, coexpressed Dgks are usually from different subfamilies, strongly suggesting that each Dgk subfamily has a specific function.

1.3. Subcellular localization: clues to function

Dgks gain access to DAG by translocating to a membrane compartments where DAG is generated, and then their activity is further modified by phosphorylations and by binding to cofactors and other proteins. This complexity permits tissue- or cell-specific regulation of each Dgk isotype, depending on the availability of cofactors and the type of stimulus that the cell receives. Moreover, the overlapping distribution of the Dgks suggests that each Dgk isotype has distinct tissue- or cell-specific roles. The subcellular location where Dgks are active in different tissues strongly suggest that Dgks finely tune DAG- and PA-mediated signals in a strictly spatial-regulated manner.

Several Dgks localize to the cytoskeleton. Indirect evidence suggests that they may participate in regulating cytoskeleton dynamics. This is not surprising because lipid mediators are known to bind to numerous proteins that associate with the cytoskeleton and dramatically affect their activities. For example, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) binds to actin capping proteins, which then dissociate from the actin complex, allowing rapid polymerization to occur. PI 5-kinases, that generate $PI(4,5)P_2$, are positively regulated by PA. Although evidence suggests that it is the PLDs that associate with and activate PI 5-kinases by generating PA, also DGKζ colocalize and co-immunoprecipitated with PI 5-kinase type Iα and enhanced its activity by generating PA. Consistent with this, also a Dgk activity was associated with a complex of proteins that included a PI 5-kinase, Rac, Rho, Cdc42, and RhoGDI. Several Dgk isotypes co-immunoprecipitate with Rho family proteins when overexpressed in cells. However, physiologic significance of these interactions is not clear. Moreover, evidence in human platelets demonstrate that Dgks have a prominent role in normal cytoskeleton responses. This is not surprising because changes in the platelet cytoskeleton are partly mediated by DAG, which rapidly accumulates in platelets treated with thrombin. Platelet Dgks are primarily responsible for metabolizing DAG, attenuating downstream signaling and allowing a return to the basal state. But, while their activity in platelets is crucial, no

one has conclusively identified which Dgk isoforms are present or rigorously examined their roles. Altogether, however, this data suggest that Dgks likely have a prominent role in regulating the cytoskeleton.

In addition to the cytoskeleton, Dgks also appear to prominently function in the nucleus, where there is a PI cycle that is regulated separately from its extranuclear counterpart. DAG produced in the nucleus appears to function separately from extranuclear DAG. Its nuclear signaling function has received little attention, but data clearly indicate that in most cases DAG acts to promote cell growth. In fact, DAG peaks shortly before S phase, suggesting that it may participate in the G1/S transition. Accordingly, overexpressed Dgkζ caused cells to accumulate at the G0/G1 transition. The effect required both Dgk activity and a functional nuclear import signal, suggesting that the effect was caused when Dgkζ metabolized nuclear DAG. Within the nucleus, DAG signaling, like its extranuclear counterpart, appears to be compartmentalized. And it appears that DAG signaling is not confined to the nuclear envelope, as one would expect, but also occurs at distinct locations within the body of the nucleus. Nuclear Dgks appear to have prominent and specific roles. Dgks α, ζ, and ι translocate to the nucleus, while a fraction of cellular DGKθ is constitutively nuclear. And different Dgk subtypes are confined to specific, separate compartments within the nucleus. For example, Dgkα associates with the nuclear envelope, while Dgks θ and ζ are found in multiple regions in the body of the nucleus. It appears that Dgks affect nuclear signaling either by terminating DAG signals or by generating PA. For example, in T-lymphocytes, PA produced by nuclear Dgkα appeared to be necessary for IL-2-mediated progression to S phase of the cell cycle. Conversely, as noted above, nuclear Dgkζ inhibited progression from G1 to S phase of the cell cycle, likely by metabolizing DAG. And nuclear Dgkθ appeared to metabolize nuclear DAG induced by thrombin and nerve growth factor. These data suggest unique roles for nuclear Dgks α, ζ, and θ indicating both the complexity and importance of lipid signaling and Dgk function in the nucleus.