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Diacylglycerol, when simplicity becomes complex

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Diacylglycerol (DAG) has unique functions as a basic component of membranes, an intermediate in lipid metabolism and a key element in lipid-mediated signaling. In eukaryotes, for example, impaired DAG generation and/or consumption have severe effects on organ development and cell growth associated with diseases such as cancer, diabetes, immune system disorders and Alzheimer's disease. Although DAG has been studied intensively as a signaling lipid, early models of its function are no longer adequate to explain its numerous roles. The interplay between enzymes that control DAG levels, the identification of families of DAG-regulated proteins, and the overlap among DAG metabolic and signaling processes are providing new interpretations of DAG function. Recent discoveries are also delineating the complex and strategic role of DAG in regulating biochemical networks.

Complexity of DAG-dependent processes

Diacylglycerol (DAG) is a simple lipid consisting of a glycerol molecule linked through ester bonds to two fatty acids in positions 1 and 2 (Figure 1). Its small size and simple composition confer exceptional properties on DAG as a lipid intermediate in metabolism, as a component of biological membranes (Box 1), and as a second messenger. Although in this review we separate DAG-dependent metabolism and signaling functions for reasons of simplicity, we cannot forget that the relationship between these processes is essential for the correct maintenance of homeostasis during cell growth and development. This interconnection has probably fostered the appearance of the numerous mechanisms that control DAG levels in eukaryotic cells, especially in mammals, that we mention throughout this review.

The precise control of DAG production and clearance is necessary to facilitate the correct function of its target proteins, which have also increased in diversity and number throughout evolution. Indeed, since members of the protein kinase C (PKC) family were described as the main effectors of cellular DAG in the 1980s, six additional families have been reported, greatly increasing the number of proteins known to be modulated by direct interaction with this lipid [1,2]. All are characterized by the presence in their sequences of at least one conserved 1 (C1) domain, with different specificities and affinities for DAG. This range of specificities and affinities augments the complexity of DAG-dependent responses and facilitates discrimination by target proteins of the appropriate DAG pool among the numerous reservoirs of this lipid in the cell.

The principal effect of DAG on responsive proteins is considered to be protein translocation to membranes, mediated by its direct binding to the C1 domain [1]. The latest discoveries have nonetheless broadened this concept to include modulation of protein activity and localization of proteins to specific cell membrane subdomains as C1-mediated functions of DAG [3].

In this review we analyze the remarkable complexity of DAG-dependent processes by examining the many pathways that control DAG generation and consumption in addition to the versatility of DAG-regulated responses. We highlight animal models that demonstrate how these characteristics have been exploited by multicellular organisms to gain complexity, and we also discuss the potential therapeutic value of designing new approaches for better and more effective manipulation of DAG-regulated functions.

DAG: a key molecule in lipid metabolism

Diacylglycerol functions not only as a perfect module to which new components can be added for the synthesis of more complex lipids, but also as a source of free fatty acids. Bacteria, yeast, plants and animals all have the ability to metabolize DAG, a crucial function that makes DAG essential for cell growth and development. In recent years, great advances have been made in our understanding of DAG metabolism at the molecular level. One of the most striking discoveries has been the characterization of several enzyme isoforms catalyzing the same chemical reactions involving DAG, such as lipid phosphate phosphatases (LPPs), phospholipase C (PLC) and PLD, and diacylglycerol kinases (DGKs), suggesting that DAG has distinct and previously unknown functional roles in the corresponding metabolic pathways.

De novo DAG synthesis

There are two main pathways of DAG synthesis in yeast and mammals [4]: in one, DAG is synthesized from glycerol-3-phosphate (as a result of triacylglycerol mobilization); in the other, it is generated from dihydroxyacetone-3-phosphate (a glycolysis intermediate). These two precursors undergo several modifications including two acylation steps that give rise first to lysophosphatidic acid

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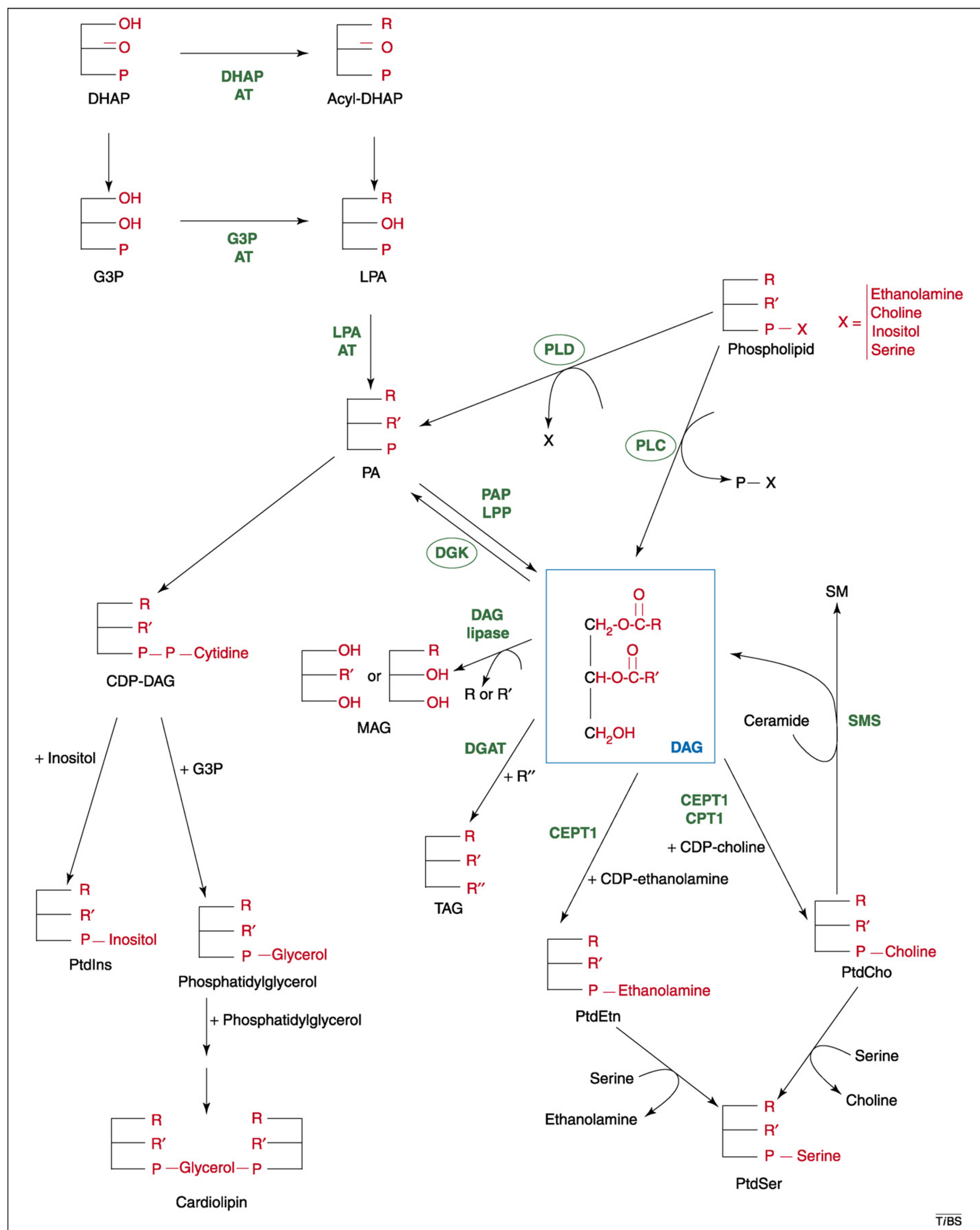

Figure 1. Main pathways implicated in generation and consumption of DAG. Shown are the pathways leading to the production of DAG (blue box) and the metabolites generated from this lipid. The principal enzymes involved in DAG production and degradation (circled enzymes are dependent on extracellular signaling) are shown in green (other enzymes have been omitted for simplicity). Groups that change during the reactions are shown in red (OH is hydroxyl; R, R′ and R″ are fatty acids; P is a phospho group; X is choline, ethanolamine, inositol or serine). The three-carbon invariable chain is in black. AT, acyltransferase; CDP, cytidine diphosphate; CEPT1, choline/

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Box 1. Role of DAG in the structure and dynamics of biological membranes

Biological membranes are composed mainly of glycerophospholipids, sphingolipids and sterols, which confer specific characteristics on distinct cell membranes when combined in different proportions [63]. Owing to their amphipathic nature, these lipids form bilayers in which lipid apolar regions are shielded from the extracellular medium and polar headgroups face the external milieu. The nature of DAG endows this molecule with specialized functions in the membrane.

Membrane curvature

The space requirement of the polar and non-polar regions determines the geometric 'form' of the outline of a lipid, which affects the final curvature of lipid membranes [64]. The concept of spontaneous curvature (ζ) defines the shape of a lipid monolayer made up of single lipid species. When the space occupied by the polar headgroups and apolar tails is similar, the molecule is considered to be cylindrical with a spontaneous curvature equal to 0, which generates a lamellar membrane structure (Figure Ia). The cone shape of lipids with small polar headgroups and large tails ($\zeta < 0$) causes negative curvature of the monolayer, giving rise to inverted micelles (Figure Ib). By contrast, the inverted cone shape of lipids with large polar heads and small tails ($\zeta > 0$) induces positive curvature, giving rise to micelles (Figure Ic). The spontaneous curvature of a lipid depends on the length and saturation of its fatty acids [65], in addition to medium conditions such as pH and/or ion concentration [66]. Notwithstanding, DAG is the lipid with the most negative ζ, because its polar headgroup, comprising only a glycerol moiety, is very small in comparison to that of other lipids [67]. In the opposite range would be lipids such as LPA, which, with only one fatty acid, would have a highly positive ζ (Figure I).

Membrane fusion

When concentrated in small membrane areas, the characteristic negative curvature of DAG and its lack of charge induce unstable, asymmetric regions in membrane bilayers. Intermediates with increased curvature subsequently form to minimize this tension [67]. These intermediates are essential for membrane fusion and fission processes, because they facilitate generation of both the membrane constrictions necessary for fission and the transition states needed for membrane fusion [68].

Membrane interaction and protein activity

Membrane alterations induced by accumulation of DAG also include the generation of small areas in which the apolar regions of neighboring lipids are partially exposed [69]. By improving the hydrophobic interaction of proteins with these membrane areas, DAG affects the activity of some proteins that are integrated in [70] or interact with [71,72] cell membranes.

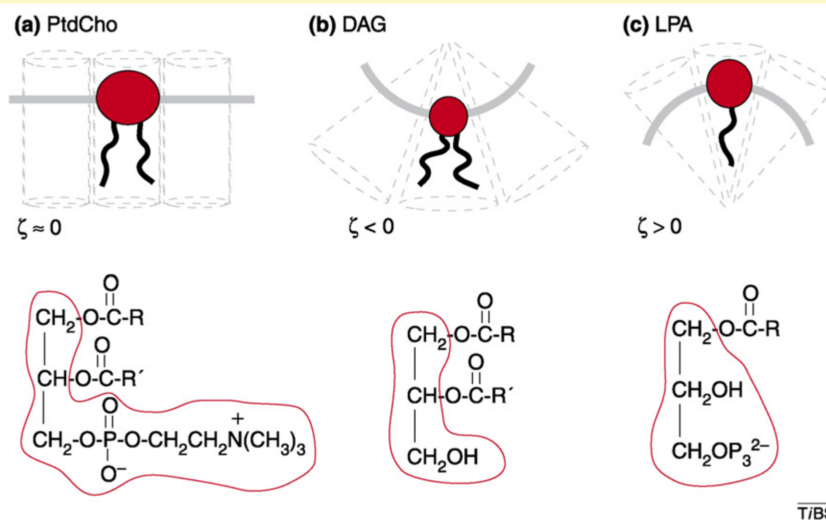

Figure I. Lipids and membrane curvature. **(a)** PtdCho gives rise to a lamellar membrane structure. **(b)** DAG generates inverted micelles. **(c)** LPA generates micelles. Polar regions are shown in red; non-polar regions are shown in black. The geometric 'form' of a lipid is indicated by the dotted gray line. The shape of the lipid monolayer is indicated by a solid gray line.

(LPA) and then to phosphatidic acid (PA); the latter is subsequently transformed into DAG through the action of PA phosphohydrolases (PAPs; Figure 1) [5].

In these two pathways, acylation at the first position of the DAG chain (R) takes place in different subcellular localizations [4]: only saturated fatty acids are added in the mitochondria and peroxisome, whereas both saturated and unsaturated fatty acids are added in the endoplasmic reticulum (ER). The second acylation (R′) occurs principally in the ER membrane, where two proteins with acyltransferase activity are located [4]. Although both proteins catalyze the same reaction, their specificity differs and they incorporate distinct fatty acids into LPA. The subcellular site of the first acylation, together with the distinctly different specificities of the LPA acyltransferases, facilitates the generation of PA with different fatty acid compositions.

Once PA is generated, the action of PAP will metabolize it to DAG. Two PAP activities, PAP1 and PAP2, have been described that differ in enzymatic activity and subcellular localization [4,5]. PAP1 requires Mg^{2+} for catalysis and its only substrate is PA. It is located in the cytosol, from where it translocates to internal membranes such as that of the ER. PAP2, which localizes at membranes, does not require Mg^{2+} for catalysis and has many substrates.

Recent detailed studies have concluded that the enzymatic activity corresponding to PAP2 is exerted by a family of enzymes with broad substrate specificity (e.g. PA, LPA, sphingosine-1-phosphate and choline-1-phosphate, among others) [5]. Some of these enzymes, which are now known

ethanolamine phosphotransferase 1; CPT1, choline phosphotransferase 1; DGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone-3-phosphate; G3P, glycerol-3-phosphate; MAG, monoacylglycerol; PtdSer, phosphatidylserine; SM, sphingomyelin; SMS, sphingomyelin synthase; TAG, triacylglycerol.

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as LPPs, have been characterized including LPP1, LPP2, LPP3, sphingosine-1-phosphate phosphatase, and LPA phosphatase. Studies of their subcellular localization are controversial and LPP enzymes have been reported in both internal and plasma membranes. A PAP activity (originally known as PAP1) has been recently cloned in yeast [6]. Sequence information has been used to search for mammalian orthologs, revealing that the previously characterized protein Lipin1 is a mammalian enzyme with PAP activity [6].

Alternative pathways of DAG synthesis

In addition to *de novo* synthesis, three alternative pathways can generate DAG through the action of sphingomyelin synthase, PLC and PLD. In the last two pathways, DAG generation is highly dependent on extracellular stimulation, and DAG generated by these mechanisms is not usually consumed for metabolic purposes.

Sphingomyelin synthase activity is responsible for sphingomyelin synthesis from phosphatidylcholine (PtdCho) by catalyzing replacement of a glycerol molecule by ceramide, resulting in a reaction that releases DAG (Figure 1). Two sphingomyelin synthases have been recently cloned, termed SMS1 and SMS2, which catalyze sphingomyelin synthesis from PtdCho in the Golgi lumen and in the plasma membrane, respectively [7].

The role of DAG as a lipid precursor

Diacylglycerol can act as a precursor of phosphatidylethanolamine (PtdEtn) and PtdCho (Figure 1). Two mammalian enzymes, choline/ethanolamine phosphotransferase (CEPT1) and choline phosphotransferase (CPT1), catalyze the incorporation of activated alcohols into DAG [8]. CEPT1 is located in the ER and the external nuclear membrane, whereas CPT1 is a Golgi enzyme. Phosphatidylserine is synthesized from PtdEtn or PtdCho by the action of two transferases that catalyze exchange of the ethanolamine or choline group, respectively, for a serine in a reaction that takes place in the ER or the Golgi apparatus (Figure 1).

In addition, DAG can be metabolized into triacylglycerol by esterification of a new fatty acid in the free position of the glycerol moiety (R″; Figure 1). This activity is catalyzed by diacylglycerol acyltransferases in the ER or the plasma membrane [9]. Triacylglycerol is the main energy store and, through a lipase-catalyzed reaction, can be reconverted to DAG as a precursor for the synthesis of complex lipids.

Diacylglycerol can also act as a substrate for diacylglycerol lipases that hydrolyze the fatty acid in position 1 or 2, generating monoacylglycerol (Figure 1). DAG lipases are also strongly linked to signaling functions: in platelets, in response to thrombin their combined action with PLC facilitates the release of arachidonic acid, an intermediate in thromboxane and prostaglandin synthesis [10]; in neurons, this activity is necessary for the generation of 2-arachidonoyl-glycerol, an endocannabinoid, during retrograde synaptic transmission [11].

In addition, DGK activity phosphorylates DAG, transforming it into PA, which is essential for the production of phosphatidylinositol (PtdIns) and cardiolipin (Figure 1). In bacteria, a single transmembrane protein is responsible for this reaction; by contrast, DGK activity has not been cloned in yeast. In multicellular organisms that originated as early as *Dictyostelium discoideum* and *Caenorhabditis elegans*, a family of cytosolic proteins is responsible for phosphorylation of DAG [12], which indicates that higher organisms evolved this highly conserved function to include additional mechanisms that enable enzymes to reach the membrane. This specific regulation positions DGK family enzymes as a perfect link between signaling and metabolism.

DAG levels are strictly regulated

Considering the numerous metabolic pathways in which DAG is implicated, cells must rigorously control the production and clearance of DAG to guarantee a permanent reservoir of this lipid. Indeed, many mechanisms have developed throughout evolution to maintain correct DAG levels during cell growth.

In *Saccharomyces cerevisiae*, the PtdIns carrier Sec14p controls the rate of PtdCho synthesis [13]. When expression of Sec14p is disrupted, the cytidine diphosphate/choline pathway leads to increased synthesis of PtdCho and, as a consequence, increased use of DAG. The subsequent reduction in DAG levels alters Golgi secretory functions and affects cell viability [13]. This function is conserved in more complex organisms, as described for Nir2, a functional equivalent of Sec14p in mammals [14]. Indeed, a *nir2* knockout mouse model shows embryonic lethality [15], and ablation of the *nir2* ortholog in *D. melanogaster* (*rdgB*) induces retinal degeneration [16].

Enzymes with PAP activity are also implicated in mechanisms related to DAG control. In *S. cerevisiae*, ScPAP1 is necessary for correct cell growth and cytokinesis [17]. In *D. melanogaster*, the mammalian LPP orthologs Wunen and Wunen2 are essential for correct germinal cell migration through the mesoderm [18], and another recently described LPP, encoded by *lazaro*, is also linked to phototransduction [19].

These examples in fly and mouse models show how DAG, its precursors and its derived metabolites have increased their functions throughout evolution, with roles in cell growth as suppliers of membrane components and complex lipids, as in yeast, but also roles in the control of organism growth and in organ development and function, as in higher eukaryotes.

DAG: a lipid second messenger

Although adequate levels of DAG must be maintained for metabolic purposes, some external signals result in the immediate generation of DAG. To achieve the appropriate response, cells take advantage of many proteins involved in the three levels responsible for DAG-dependent signaling: namely, production, clearance and response. As a consequence, extracellular stimulation results in rapid, transient changes in specific pools of DAG located in discrete cellular locations with different kinetics. Modification of membrane DAG, in combination with other intracellular responses, is a determining factor in specifying the nature, intensity and duration of a given signal.

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DAG production in response to stimulus: PLC and PLD

PLC enzymes generate DAG through glycerophospholipid hydrolysis, releasing their phosphorylated polar head-group (Figure 1). Although PtdCho-specific PLC activity has been characterized in bacteria and there is evidence of its presence in eukaryotic cells [20,21], a corresponding gene has not been cloned. By contrast, PtdIns-specific PLC enzymes, which hydrolyze phosphatidylinositol-4,

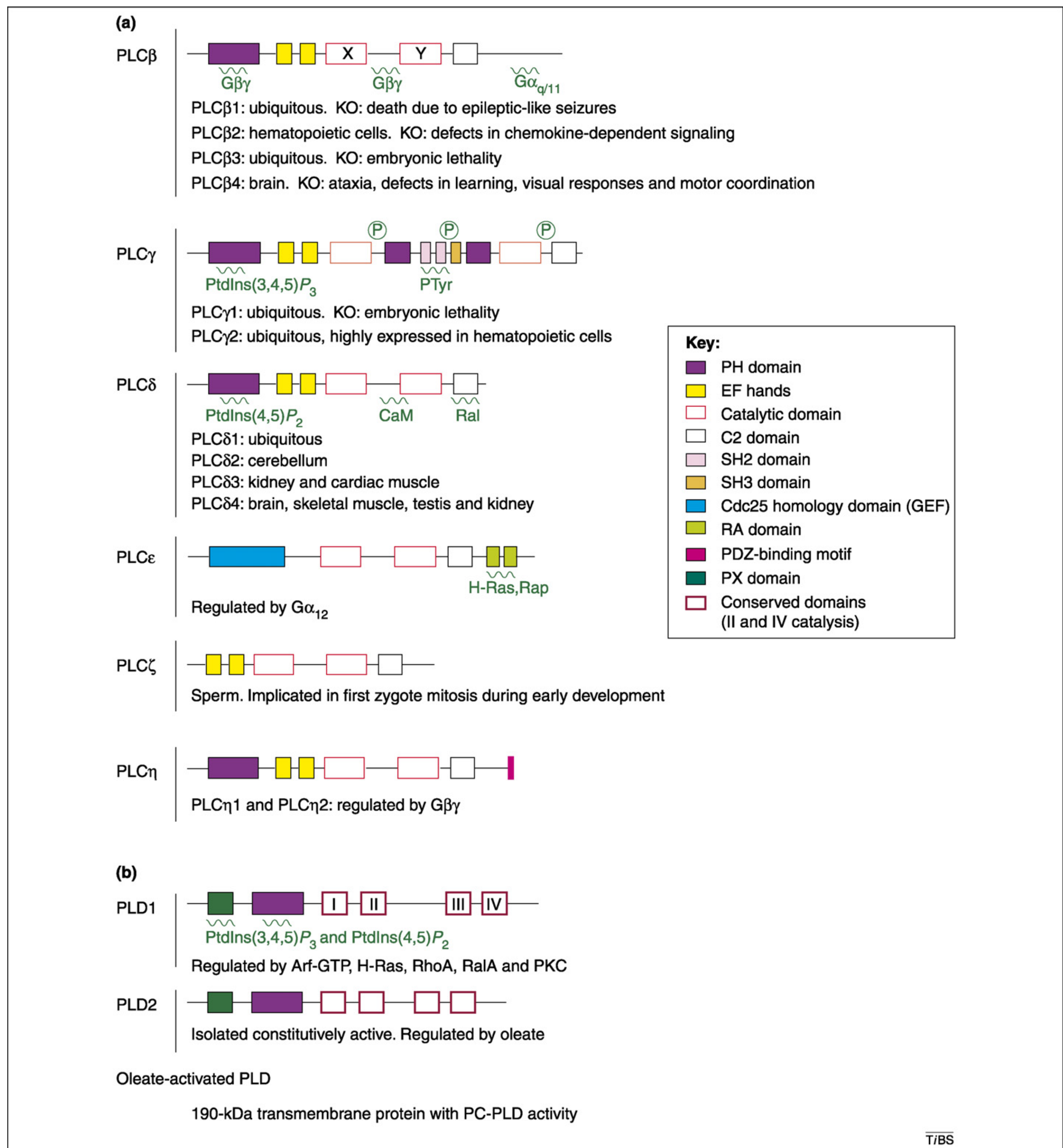

Figure 2. The mammalian PLC and PLD families. Shown are the principal domains and regulatory mechanisms, tissue expression and knockout mouse phenotype of the PLC and PLD families. **(a)** PLC enzymes are characterized by a conserved catalytic domain that is divided into two regions, known as boxes X and Y, which contain the conserved residues necessary for catalysis. Conserved domain 2 (C2), which is present in all PtdIns-specific PLC enzymes, binds Ca^{2+} and phosphatidylserine, and is needed for correct localization of the protein at the membrane. **(b)** The two cloned mammalian PLD enzymes share four conserved domains, two of which contain the phosphatidyltransferase motif (HKD) involved in catalysis. PH and PX domains have also been detected, although they differ from the classical ones and their functions are not well established. The domain structure of a third, oleate-regulated PLD enzyme with PtdCho-specific PLD (PC-PLD) activity has not been characterized as yet. Interacting proteins and lipids are shown in green; protein–lipid contact regions are shown as wavy lines. GEF, guanine exchange factor; P, position of phosphorylatable residues; KO, knockout mouse.

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5-biphosphate, have been studied thoroughly [22]. There are 14 mammalian PtdIns-specific PLC genes, which are grouped into six subfamilies according to their primary sequence and activation mechanisms [23] (Figure 2).

PtdIns-specific PLC enzymes are fundamental components of receptor signal transduction, because their activation not only increases membrane DAG levels, but also produces the inositol-3,4,5-triphosphate essential for Ca^{2+} exit from the ER. The combined action of the two second messengers generated by PLC activation regulates several signaling cascades, making PtdIns-specific PLC essential for signaling in processes such as cell proliferation, differentiation, immune response and/or cell migration [23]. Indeed, some mice deficient in PtdIns-specific PLC show a phenotype that is clearly related to lack of function of isozymes of this family (Figure 2).

Glycerophospholipids (mainly PtdCho, but also PtdIns, PtdEtn and glycerophosphatidylinositol) are metabolized by PLD and either PAP or LPP to generate DAG (Figure 1). Two mammalian PtdCho-specific PLD enzymes, PLD1 and PLD2, have been cloned; a third, higher molecular weight enzyme with oleate-dependent activity has been purified, but not characterized at the molecular level [24] (Figure 2). It is generally considered that the fatty acid composition of

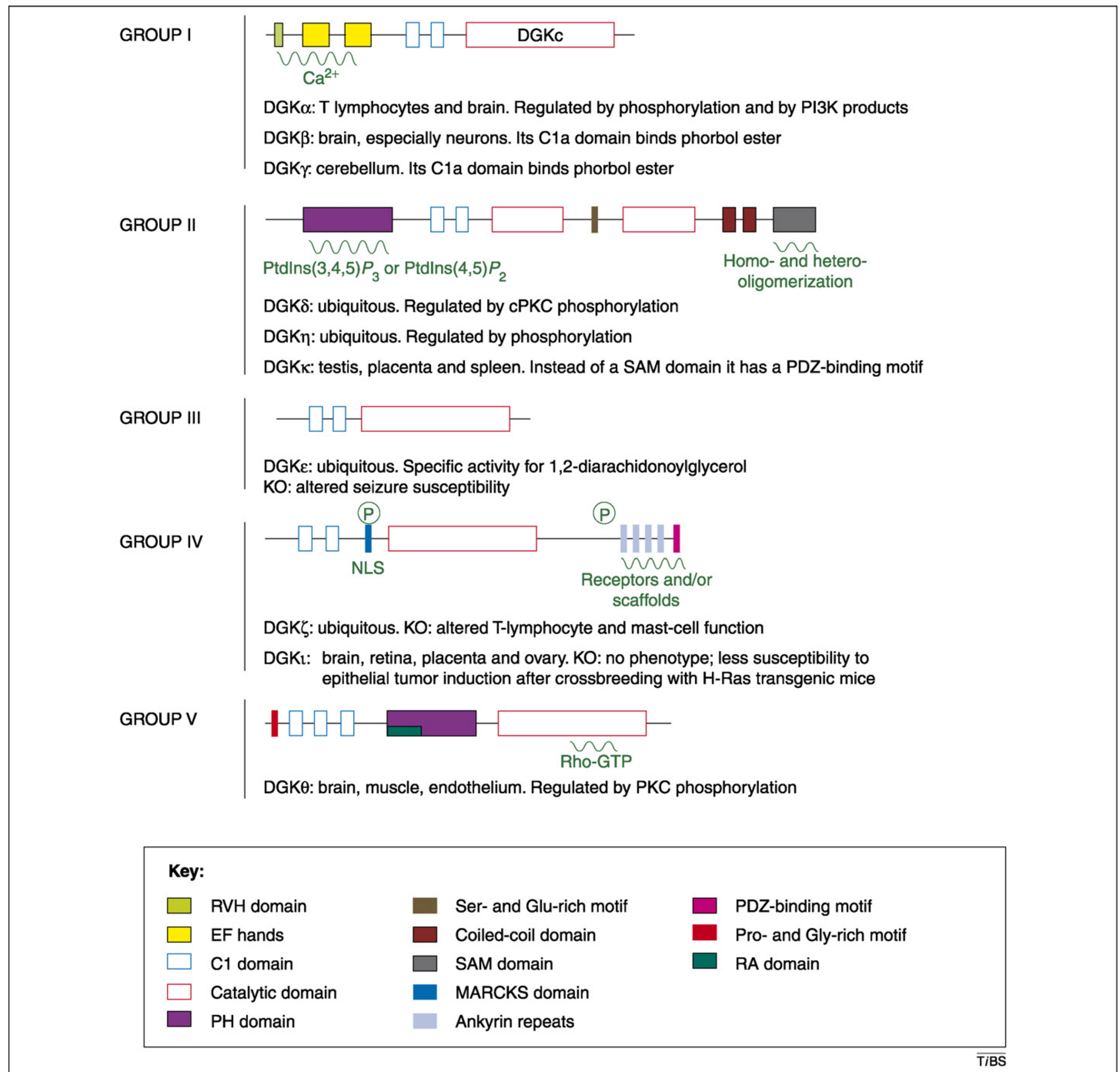

Figure 3. The mammalian DGK family. Shown are the principal domains and regulatory mechanisms, tissue expression and knockout mouse phenotype description of mammalian DGKs. Ten DGKs have been cloned in mammals; these enzymes are characterized by a common catalytic region (DGKc) and have been grouped into five subtypes, depending on the presence of different regulatory motifs in their primary sequences. Interacting proteins, lipids and ions are shown in green; protein–lipid–ion contact regions are shown as wavy lines. cPKC, classical PKC; P, position of phosphorylatable residues; KO, knockout mouse; NLS, nuclear localization signal.

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DAG generated by the combined action of PLD and PAP differs from that of the molecule resulting from hydrolysis by PtdIns-specific PLC [25]. PtdIns usually has a stearic acid in position 1 of the glycerol molecule and arachidonic acid in position 2, whereas PtdCho, which is much more abundant than PtdIns (comprising 40% of total cell lipids versus 5–8% for PtdIns), has mainly saturated or monounsaturated fatty acids in position 2.

PtdCho-specific PLD activity has been linked to vesicular trafficking mechanisms, but has also been proposed to have a role in regulating cell growth and proliferation through effectors such as Raf and mTOR [25].

DAG clearance after stimulation: the DGK family

Members of the DGK family have been linked to the attenuation of DAG levels after cell stimulation. There are ten mammalian DGKs, and phenotypic analysis of DGK knockout mice has confirmed that these enzymes are negative regulators of DAG-responsive proteins (Figure 3). This DGK function is conserved in other organisms such as *D. melanogaster* and *C. elegans*.

In *Drosophila*, disruption of *rdgA*, which encodes the ortholog of mammalian DGKι, prevents termination of TRP channel activity [26]; as a consequence, a continuous Ca^{2+} flux is maintained, inducing retinal degeneration [27,28]. In *C. elegans*, ablation of *dgk-1*, which encodes the ortholog of mammalian DGKθ [29], affects unc13 and PKC presynaptic function [30]; as a result, serotonin-dependent synaptic transmission is increased, inducing defective behaviors in locomotion, egg laying and feeding [30].

DAG response: the C1 domain

All proteins that bind DAG directly, and thus respond to its presence, have at least one C1 domain, which consists of a conserved sequence of 50 amino acids bearing the $HX_{11-12}CX_2CX_{12-14}CX_2CX_4HX_2CX_{6-7}C$ motif [1] (Figure 4a). C1 domains were initially described as domains that bind phorbol esters; their capacity to bind DAG and other related compounds such as bryostatins, indolactanes and merezeins was confirmed later [31].

Studies of the residues necessary for interaction with phorbol esters led to the description of two types of C1

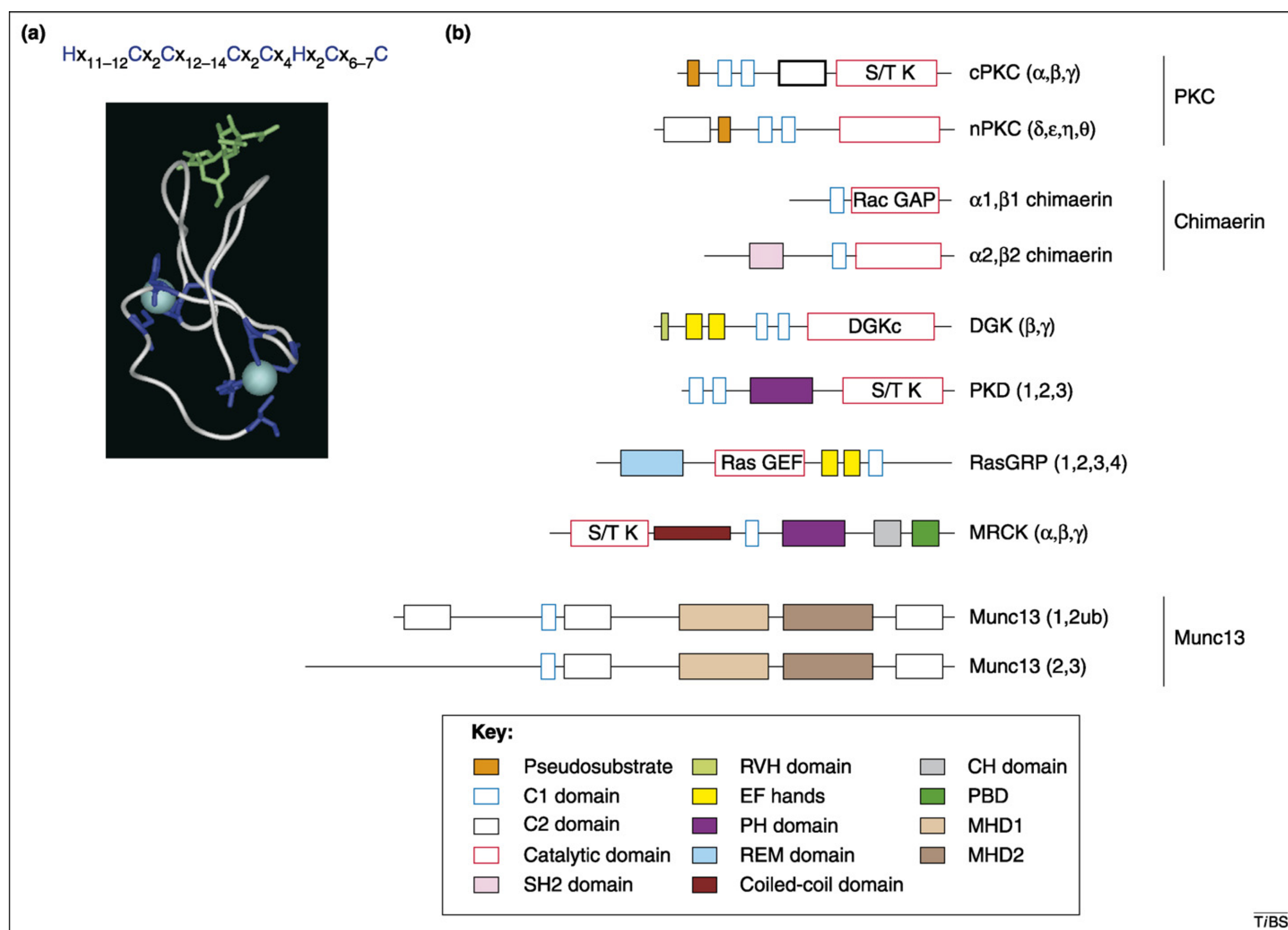

Figure 4. C1 domain structure and C1-domain-containing proteins. **(a)** Structure of the C1 domain (PDB accession code 1PTR) and consensus motif. The cysteine and histidine residues (blue) generate two cavities, in each of which a Zn^{2+} ion (cyan) is coordinated. Each cavity comprises three non-consecutive cysteine residues and one histidine, which maintain close proximity in the ternary structure with the collaboration of other hydrophobic and highly conserved C1 domain residues [61,62]. Coordination of the two Zn^{2+} ions generates a very stable base for membrane interaction that supports two flexible loops, forming the walls of a groove in which DAG or phorbol ester bind; in this case, phorbol-13-acetate (green). This interaction is mediated by hydrogen bonds and causes displacement of the water molecules in this position, allowing greater membrane penetration [62]. **(b)** Domain structures of C1-domain-containing protein families. The catalytic activities are indicated. DGKc, catalytic region of DGK; nPKC, novel PKC; RacGAP, Rac GTPase-activating protein; RasGEF, Ras guanine exchange factor; S/T K, Ser/Thr kinase.

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domain, termed typical and atypical domains [32]. The function of atypical C1 domains has not been fully established, although some studies suggest that they have a role in protein and/or membrane interaction [33]. DGK is the largest family of proteins with two atypical C1 domains; other proteins with atypical C1 domains are Vav, Raf, ROCK, CRIK, C1-TEN, NORE and Lfc.

Proteins with a typical C1 domain are candidates for DAG modulation. In addition to the classical and novel PKC, there are six families of C1-domain-containing proteins [1–3]: namely, the chimaerin, DGK (β and γ), PKD, mammalian unc13 (Munc13), Ras guanine-releasing protein (RasGRP) and myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK) families (Figure 4b). Except for members of the Munc13 family, which have scaffolding functions, the activities of the different DAG receptor families vary from lipid and protein kinases to a Ras guanine exchange factor and a Rac GTPase-activating protein (Figure 4b). Importantly, the identification of non-kinase receptors of DAG has uncovered new and strategic functions of DAG in regulating Ras-dependent responses or Rac-mediated cytoskeletal remodeling.

Although it has been thought that the role of the C1 domain is to guide translocation of DAG-regulated proteins from the cytoplasm to the plasma membrane, this concept is changing with the publication of new reports. Indeed, the recent description of the β2 chimaerin crystal structure [34], the first structure of a complete C1-domain-containing protein, has helped to clarify the function of the C1 domain. This study has shown that, in the inactive conformation of the protein, both the catalytic and the C1 domains are buried. This specific folding is stabilized by intramolecular interactions that cover hydrophobic residues in the C1 domain and the DAG-binding groove, yielding a protein that is unable to sense DAG. According to these data, β2 chimaerin requires an initial signal to expose the catalytic and C1 domains and subsequently to allow DAG binding. Such a mechanism would promote closer contact with DAG-enriched membranes, favoring protein activity (Box 1). This model concurs with the earliest reports, subsequently forgotten, on the C1 domain of PKC that proposed that DAG binding to the C1 domain functioned as an activator of protein activity [35].

These results reconcile all previous data showing that the C1 domains of PKC [36] and PKD [37] are not exposed in the absence of stimulus; however, they cannot explain how DAG-regulated proteins reach the membrane. Numerous PKC-interacting proteins [38] have been described as scaffolding proteins important for the translocation of PKC family members. In terms of other DAG-regulated proteins, similar functions could be ascribed to the few interacting proteins that have been so far reported: for example, Tmp21 for β2 chimaerin [39], actin for RasGRP1 and RasGRP2 [40], heterotrimeric GTPase for PKD [41], and synaptobrevin for Munc13 [42].

Membrane specificity is important for defining the downstream effectors of some DAG-regulated proteins, as has been demonstrated for PKD [43] and RasGRP [44,45]. Owing to their C1 domain specificity, these proteins can localize to internal membranes [46,47], where DAG fatty acids are mostly saturated [8,48]. This observation implies that some C1 domains recognize and bind to DAG species that were previously considered to be part of the metabolic pool, indicating that these species are competent for signaling. Protein localization to distinct cell membranes is thus probably achieved as a result of a combination of binding to scaffolding proteins, C1 domain recognition of different DAG species, and the presence of other regulatory domains in the protein sequence [33]. In addition, DAG specificity and phorbol ester binding can be modified by the number of C1 domains in the sequence [49] and by phosphorylation of nearby residues, respectively [50]. Other recently described functions of the C1 domain, including binding to proteins [33] or other lipids such as phosphatidylserine [51], also contribute to membrane specificity.

The C1 domain thus emerges as a DAG-dependent regulatory module that controls protein activation and determines specific subcellular sites where the protein must remain activated until DAG returns to basal levels. For more precise control of DAG-dependent signaling, C1-domain-containing proteins can also coordinate their actions in the same pathway, as in the case of PKD [52] and RasGRP [53], which are both targets of phosphorylation by PKC.

The function of the C1 domain in defining specific protein localization is extremely important, as seen in Unc13 and Munc13, a protein family with no known catalytic domain (Figure 4b). Here, the role of the C1 domain seems to be to aid assembly of the exocytosis machinery [42] at a membrane site where DAG enrichment promotes membrane instability and fusion (Box 1); this function facilitates the secretion of neurotransmitters in neurons [54] and insulin in pancreatic cells [55]. As a consequence, *C. elegans* Unc13 and mouse Munc13 knockout models show defective neurotransmitter release, which provokes severe alterations in motor coordination [56,57].

Integration of DAG metabolic and signaling functions

Although we have intentionally separated the roles of DAG in metabolism and signaling, clearly no such segregation takes place in living organisms, where these functions converge to control cell homeostasis. The oldest known role of DAG, as a basic membrane component and metabolic intermediate, is highly conserved throughout evolution. In eukaryotes, numerous proteins have evolved the ability to bind to DAG and are thus activated by DAG-dependent signaling, creating additional levels of control to meet the complex needs of multicellular organisms. Alterations in the mechanisms that govern DAG generation and consumption are translated into aberrant localization and/or activation of DAG-regulated proteins, ultimately resulting in pathological conditions. Targeting metabolic enzymes that contribute directly or indirectly to DAG homeostasis presents new opportunities for the successful manipulation of cell mechanisms, which could ameliorate disease states.

Dysregulation of DAG metabolism has been linked to the pathophysiology of several human diseases such as diabetes and malignant transformation. In vascular cells of a rat model of diabetes, for example, the principal metabolic alteration detected is an imbalance in

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glycerophospholipid synthesis owing to reduced DGK activity. This imbalance results in activation of DAG-modulated effectors such as PKC, whose phosphorylation rate is used as a prognostic marker in diabetes [58]. Recently, the correlation between high intracellular levels of DAG and defects in insulin resistance has been further confirmed in mice lacking mitochondrial glycerol phosphate acyl-CoA transferase, which show reduced fat-induced hepatic insulin resistance [59]. These models suggest a mechanism by which increased intracellular DAG could activate serine/threonine kinase cascades, leading to inhibition of insulin receptor signaling. This finding has important ramifications for disease management and highlights the relevance of primary metabolic defects that occur early in disease.

Regarding the role of metabolic enzymes in malignant transformation, one of the enzymes responsible for LPA synthesis, LPA acyltransferase-β, has been described as a prognostic marker in ovarian cancer. Its inhibition reduces tumor formation in mouse ovary, an effect that it is not due to reduced LPA levels, but to the blockade of DAG synthesis, which is translated into reduced activity of its effectors [60].

Future directions

Diacylglycerol is a simple lipid that has been present throughout evolution, but its functions have become increasingly complex as many families of DAG-binding proteins have evolved for signal transduction. The intracellular signaling cascades initiated by DAG intersect with the key functions of this lipid as a membrane component, revealing a sophisticated, highly specialized mechanism of action that we are just beginning to understand. The intricacy and variety of DAG functions are achieved thanks to the concerted action of numerous mechanisms, some of which we have tried to summarize here. As an example, we have described the novel functions of the C1 domain that, although originally identified as a mere binding platform, is now recognized to be an extremely specialized, versatile domain with functions much more complex than those originally attributed to it.

The DAG signaling network holds much promise as a target for the treatment of conditions such as autoimmune disorders, cancer, diabetes and/or neurological diseases. Furthermore, the use of metabolic enzymes as prognostic and/or diagnostic markers will lead to the design of more rational, personalized therapies. Recent progress in our understanding of DAG-regulated processes emphasizes the need for studies to evaluate the potential therapeutic manipulation of DAG generation and clearance.

Update

Since the writing of this article, the phenotypes of mice deficient for DGKα and DGKδ have been reported:

Olenchock, B.A. *et al.* (2006) Disruption of diacylglycerol metabolism impairs the induction of T cell anergy. *Nat. Immunol.* 7, 1174–1181;

Crotty, T. *et al.* (2006). Diacylglycerol kinase δ regulates protein kinase C and epidermal growth factor receptor signaling. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15485–15490

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References

1 Yang, C. and Kazanietz, M.G. (2003) Divergence and complexities in DAG signaling: looking beyond PKC. *Trends Pharmacol. Sci.* 24, 602–608
2 Brose, N. *et al.* (2004) Divergent and convergent signaling by the diacylglycerol second messenger pathway in mammals. *Curr. Opin. Neurobiol.* 14, 328–340
3 Hall, C. *et al.* (2005) C1, see them all. *Trends Biochem. Sci.* 30, 169–171
4 Athenstaedt, K. and Daum, G. (1999) Phosphatidic acid, a key intermediate in lipid metabolism. *Eur. J. Biochem.* 266, 1–16
5 Nanjundan, M. and Possmayer, F. (2003) Pulmonary phosphatidic acid phosphatase and lipid phosphate phosphohydrolase. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 284, L1–L23
6 Han, G.S. *et al.* (2006) The *Saccharomyces cerevisiae* Lipin homolog is a Mg^{2+}-dependent phosphatidate phosphatase enzyme. *J. Biol. Chem.* 281, 9210–9218
7 Huitema, K. *et al.* (2004) Identification of a family of animal sphingomyelin synthases. *EMBO J.* 23, 33–44
8 Henneberry, A.L. *et al.* (2002) The major sites of cellular phospholipid synthesis and molecular determinants of fatty acid and lipid head group specificity. *Mol. Biol. Cell* 13, 3148–3161
9 Coleman, R.A. and Lee, D.P. (2004) Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res.* 43, 134–176
10 Smith, W.L. *et al.* (1991) Prostaglandin and thromboxane biosynthesis. *Pharmacol. Ther.* 49, 153–179
11 Yoshida, T. *et al.* (2006) Localization of diacylglycerol lipase-α around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J. Neurosci.* 26, 4740–4751
12 Kanoh, H. *et al.* (2002) Diacylglycerol kinases: emerging downstream regulators in cell signaling systems. *J. Biochem. (Tokyo)* 131, 629–633
13 Mousley, C.J. *et al.* (2006) Sec14p-like proteins regulate phosphoinositide homoeostasis and intracellular protein and lipid trafficking in yeast. *Biochem. Soc. Trans.* 34, 346–350
14 Litvak, V. *et al.* (2005) Maintenance of the diacylglycerol level in the Golgi apparatus by the Nir2 protein is critical for Golgi secretory function. *Nat. Cell Biol.* 7, 225–234
15 Lu, C. *et al.* (2001) The mammalian retinal degeneration B2 gene is not required for photoreceptor function and survival. *Neuroscience* 107, 35–41
16 Milligan, S.C. *et al.* (1997) The phosphatidylinositol transfer protein domain of *Drosophila* retinal degeneration B protein is essential for photoreceptor cell survival and recovery from light stimulation. *J. Cell Biol.* 139, 351–363
17 Katagiri, T. and Shinozaki, K. (1998) Disruption of a gene encoding phosphatidic acid phosphatase causes abnormal phenotypes in cell growth and abnormal cytokinesis in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 248, 87–92
18 Santos, A.C. and Lehmann, R. (2004) Germ cell specification and migration in *Drosophila* and beyond. *Curr. Biol.* 14, R578–R589
19 Garcia-Murillas, I. *et al.* (2006) lazaro encodes a lipid phosphate phosphohydrolase that regulates phosphatidylinositol turnover during *Drosophila* phototransduction. *Neuron* 49, 533–546
20 Marini, N.J. *et al.* (1996) A pathway in the yeast cell division cycle linking protein kinase C (Pkc1) to activation of Cdc28 at START. *EMBO J.* 15, 3040–3052
21 Exton, J.H. (1994) Phosphatidylcholine breakdown and signal transduction. *Biochim. Biophys. Acta* 1212, 25–42
22 Rebecchi, M.J. and Pentyala, S.N. (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol. Rev.* 80, 1291–1335
23 Harden, T.K. and Sondek, J. (2006) Regulation of phospholipase C isozymes by Ras superfamily GTPases. *Annu. Rev. Pharmacol. Toxicol.* 46, 355–379

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24 Jenkins, G.M. and Frohman, M.A. (2005) Phospholipase D: a lipid centric review. *Cell. Mol. Life Sci.* 62, 2305–2316
25 Hodgkin, M.N. *et al.* (1998) Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? *Trends Biochem. Sci.* 23, 200–204
26 Raghu, P. *et al.* (2000) Constitutive activity of the light-sensitive channels TRP and TRPL in the *Drosophila* diacylglycerol kinase mutant, rdgA. *Neuron* 26, 169–179
27 Hardie, R.C. *et al.* (2002) Molecular basis of amplification in *Drosophila* phototransduction: roles for G protein, phospholipase C, and diacylglycerol kinase. *Neuron* 36, 689–701
28 Minke, B. and Parnas, M. (2006) Insights on TRP channels from *in vivo* studies in *Drosophila*. *Annu. Rev. Physiol.* 68, 649–684
29 Jose, A.M. and Koelle, M.R. (2005) Domains, amino acid residues, and new isoforms of *Caenorhabditis elegans* diacylglycerol kinase 1 (DGK-1) important for terminating diacylglycerol signaling *in vivo*. *J. Biol. Chem.* 280, 2730–2736
30 Nurrish, S. *et al.* (1999) Serotonin inhibition of synaptic transmission: $G\alpha_0$ decreases the abundance of UNC-13 at release sites. *Neuron* 24, 231–242
31 Kazanietz, M.G. *et al.* (2000) Pharmacology of the receptors for the phorbol ester tumor promoters: multiple receptors with different biochemical properties. *Biochem. Pharmacol.* 60, 1417–1424
32 Hurley, J.H. *et al.* (1997) Taxonomy and function of C1 protein kinase C homology domains. *Protein Sci.* 6, 477–480
33 Colon-Gonzalez, F. and Kazanietz, M.G. (2006) C1 domains exposed: from diacylglycerol binding to protein–protein interactions. *Biochim. Biophys. Acta* 1761, 827–837
34 Canagarajah, B. *et al.* (2004) Structural mechanism for lipid activation of the Rac-specific GAP, β2-chimaerin. *Cell* 119, 407–418
35 Newton, A.C. and Koshland, D.E., Jr (1989) High cooperativity, specificity, and multiplicity in the protein kinase C–lipid interaction. *J. Biol. Chem.* 264, 14909–14915
36 Parekh, D.B. *et al.* (2000) Multiple pathways control protein kinase C phosphorylation. *EMBO J.* 19, 496–503
37 Auer, A. *et al.* (2005) Role of the regulatory domain of protein kinase D2 in phorbol ester binding, catalytic activity, and nucleocytoplasmic shuttling. *Mol. Biol. Cell* 16, 4375–4385
38 Poole, A.W. *et al.* (2004) PKC-interacting proteins: from function to pharmacology. *Trends Pharmacol. Sci.* 25, 528–535
39 Wang, H. and Kazanietz, M.G. (2002) Chimaerins, novel non-protein kinase C phorbol ester receptors, associate with Tmp21-I (p23): evidence for a novel anchoring mechanism involving the chimaerin C1 domain. *J. Biol. Chem.* 277, 4541–4550
40 Caloca, M.J. *et al.* (2004) F-actin-dependent translocation of the Rap1 GDP/GTP exchange factor RasGRP2. *J. Biol. Chem.* 279, 20435–20446
41 Diaz Anel, A.M. and Malhotra, V. (2005) PKCη is required for β1γ2/β3γ2- and PKD-mediated transport to the cell surface and the organization of the Golgi apparatus. *J. Cell Biol.* 169, 83–91
42 Dulubova, I. *et al.* (2005) A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? *EMBO J.* 24, 2839–2850
43 Marklund, U. *et al.* (2003) Intracellular location and cell context-dependent function of protein kinase D. *Immunity* 19, 491–501
44 Perez de Castro, I. *et al.* (2004) Ras activation in Jurkat T cells following low-grade stimulation of the T-cell receptor is specific to N-Ras and occurs only on the Golgi apparatus. *Mol. Cell. Biol.* 24, 3485–3496
45 Sanjuan, M.A. *et al.* (2003) T cell activation *in vivo* targets diacylglycerol kinase α to the membrane: a novel mechanism for Ras attenuation. *J. Immunol.* 170, 2877–2883
46 Maeda, Y. *et al.* (2001) Recruitment of protein kinase D to the trans-Golgi network via the first cysteine-rich domain. *EMBO J.* 20, 5982–5990
47 Bivona, T.G. *et al.* (2003) Phospholipase Cγ activates Ras on the Golgi apparatus by means of RasGRP1. *Nature* 424, 694–698
48 Carrasco, S. and Merida, I. (2004) Diacylglycerol-dependent binding recruits PKCθ and RasGRP1 C1 domains to specific subcellular localizations in living T lymphocytes. *Mol. Biol. Cell* 15, 2932–2942
49 Corbalan-Garcia, S. and Gomez-Fernandez, J.C. (2006) Protein kinase C regulatory domains: the art of decoding many different signals in membranes. *Biochim. Biophys. Acta* 1761, 633–654
50 Thuille, N. *et al.* (2005) Critical role of novel Thr-219 autophosphorylation for the cellular function of PKCθ in T lymphocytes. *EMBO J.* 24, 3869–3880
51 Bittova, L. *et al.* (2001) Roles of ionic residues of the C1 domain in protein kinase C-α activation and the origin of phosphatidylserine specificity. *J. Biol. Chem.* 276, 4218–4226
52 Wang, Q.J. (2006) PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol. Sci.* 27, 317–323
53 Brodie, C. *et al.* (2004) PKCδ associates with and is involved in the phosphorylation of RasGRP3 in response to phorbol esters. *Mol. Pharmacol.* 66, 76–84
54 Betz, A. *et al.* (1998) Munc13-1 is a presynaptic phorbol ester receptor that enhances neurotransmitter release. *Neuron* 21, 123–136
55 Kang, L. *et al.* (2006) Munc13-1 is required for the sustained release of insulin from pancreatic β cells. *Cell Metab.* 3, 463–468
56 Varoqueaux, F. *et al.* (2005) Aberrant morphology and residual transmitter release at the Munc13-deficient mouse neuromuscular synapse. *Mol. Cell. Biol.* 25, 5973–5984
57 Maruyama, H. *et al.* (2001) Synaptic exocytosis and nervous system development impaired in *Caenorhabditis elegans* unc-13 mutants. *Neuroscience* 104, 287–297
58 Verrier, E. *et al.* (2004) PPARγ agonists ameliorate endothelial cell activation via inhibition of diacylglycerol-protein kinase C signaling pathway: role of diacylglycerol kinase. *Circ. Res.* 94, 1515–1522
59 Neschen, S. *et al.* (2005) Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-*sn*-3-phosphate acyltransferase 1 knockout mice. *Cell Metab* 2, 55–65
60 Springett, G.M. *et al.* (2005) Lysophosphatidic acid acyltransferase-β is a prognostic marker and therapeutic target in gynecologic malignancies. *Cancer Res.* 65, 9415–9425
61 Hommel, U. *et al.* (1994) Solution structure of a cysteine rich domain of rat protein kinase C. *Nat. Struct. Biol.* 1, 383–387
62 Zhang, G. *et al.* (1995) Crystal structure of the Cys2 activator-binding domain of protein kinase Cδ in complex with phorbol ester. *Cell* 81, 917–924
63 Sprong, H. *et al.* (2001) How proteins move lipids and lipids move proteins. *Nat. Rev. Mol. Cell Biol.* 2, 504–513
64 Cooke, I.R. and Deserno, M. (2006) Coupling between lipid shape and membrane curvature. *Biophys. J.* 91, 487–495
65 Szule, J.A. *et al.* (2002) The effects of acyl chain length and saturation of diacylglycerols and phosphatidylcholines on membrane monolayer curvature. *Biophys. J.* 83, 977–984
66 Kooijman, E.E. *et al.* (2005) Spontaneous curvature of phosphatidic acid and lysophosphatidic acid. *Biochemistry* 44, 2097–2102
67 Shemesh, T. *et al.* (2003) Prefission constriction of Golgi tubular carriers driven by local lipid metabolism: a theoretical model. *Biophys. J.* 85, 3813–3827
68 Lentz, B.R. *et al.* (2000) Protein machines and lipid assemblies: current views of cell membrane fusion. *Curr. Opin. Struct. Biol.* 10, 607–615
69 Goni, F.M. and Alonso, A. (1999) Structure and functional properties of diacylglycerols in membranes. *Prog. Lipid Res.* 38, 1–48
70 Lee, A.G. (2004) How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta* 1666, 62–87
71 Bigay, J. *et al.* (2005) ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. *EMBO J.* 24, 2244–2253
72 Ahyayauch, H. *et al.* (2005) Modulation of PI-specific phospholipase C by membrane curvature and molecular order. *Biochemistry* 44, 11592–11600

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