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Spatiotemporal dynamics of lipid signaling: protein kinase C as a paradigm

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Summary

The lipid second messenger diacylglycerol (DAG) acutely controls the rate, amplitude, duration, and location of protein kinase C (PKC) activity in the cell. There are three classes of PKC isozymes and, of these, the conventional and novel isozymes are acutely controlled by DAG. The kinetics of DAG production at various intracellular membranes, the intrinsic affinity of specific isoforms for DAG-containing membranes, the coordinated use of additional membrane-binding modules, the intramolecular regulation of DAG sensitivity, and the competition from other DAG-responsive proteins together result in a unique, context-dependent activation signature for each isoform. This review focuses on the spatiotemporal dynamics of PKC activation and how it is controlled by lipid second messengers.

Introduction

Lipids acutely control the structure, function, and localization of an abundance of signaling proteins. Under unstimulated conditions, the enrichment of specific lipids in distinct subcellular organelles lays the framework for poising signaling proteins to efficiently respond to second-messenger signaling. A generally accepted model places phosphoinositide-4,5-bisphosphate (PIP_2) enrichment at the plasma membrane, phosphoinositide-4-phosphate at the Golgi, and phosphoinositide-3-phosphate at endosomes (reviewed in [1]); recent reports have placed basal diacylglycerol (DAG) enrichment at the Golgi [2–4]and phosphatidylserine (PS) enrichment at the cytoplasmic side of both the plasma membrane and endosomes [5]. Within this framework of organization, cells have a variety of mechanisms to stimulate and regulate production of relatively short-lived lipid second messengers which can promote activating conformations and direct signaling proteins to new locations. Signaling proteins simultaneously sense the cellular address of the appropriate lipid ‘zip codes’ and respond to stimulated production of specific lipid second messengers through one or more specialized membrane-targeting domains. For example, modules such as PKC conserved region 1(C1) domains target proteins to DAG; PKC conserved region 2(C2)domains to specific phospholipids; pleckstrin homology (PH)and Epsin N-terminal homology(ENTH)domains to specific phosphoinositides; and Bin/Amphiphysin/Rvs(BAR)domains to highly-curved lipid structures [6,7]. Such lipid signaling modules control the spatiotemporal dynamics of one of the archetypal mediators of lipid signaling, protein kinase C(PKC).

Protein kinase C

The PKC family of Ser/Thr kinases comprises 10 members in the AGC kinase branch of the kinome[8]. PKC was initially discovered as a lipid-independent proteolytic product by

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Nishizuka and colleagues in 1977 [9]. Subsequently, it was found that the full-length enzyme is stimulated by the products downstream of phospholipase C (PLC)-catalyzed hydrolysis of PIP_2, DAG and Ca^{2+} [10,11]; this milestone finding recently celebrated its 25th anniversary [12]. Following on the heels of this seminal discovery, the connection was made that PKC was the long sought-after receptor for the tumor-promoting high-affinity DAG analogs, phorbol esters [13], a finding which catapulted the enzyme to the forefront of signaling research. All 10 mammalian PKC isoforms contain a highly-conserved kinase core at the carboxyl terminus and an amino-terminal autoinhibitory pseudosubstrate peptide, but differ by, and are classified according to, their divergent amino-terminal regulatory regions (Figure 1). Conventional PKC (cPKC) isoforms (α, alternative splice variants βI and βII, and γ) contain a Ca^{2+}-sensitive C2 domain and two tandem DAG-sensitive C1 domains; novel PKC (nPKC) isoforms (δ, θ, ε, and η) contain a Ca^{2+}-insensitive C2 domain and two tandem DAG-sensitive C1 domains; and atypical PKC (aPKC) isoforms (ζ and ι(human)/λ(mouse)) contain a singleC1 domain that is insensitive to DAG (reviewed in [8,14]).

PKC isoforms are processed by a series of ordered phosphorylation events to mature into the catalytically-competent species (reviewed in [8]). Maturation is initiated by phosphorylation at PKC's activation loop threonine by the upstream kinase, phosphoinositide-dependent kinase-1(PDK-1), and then requires mammalian target of rapamycin complex 2 (mTORC2) and its own intrinsic kinase activity for phosphorylation/autophosphorylation at two carboxyl-terminal sites, the hydrophobic and turn motifs (reviewed in [8,15,16]). In most systems, the maturation of cPKC and nPKC isoforms is constitutive, such that monitoring the phosphorylation state of PKC is not indicative of acute activation. Once catalytically competent, PKC resides in an inactive state in the cytosol with the inhibitory peptide lodged into its active site. For cPKC and nPKC isoforms, high-affinity Ca^{2+}-and/or DAG-mediated membrane binding provides the energy required to expel the inhibitory peptide from the active site and allow PKC to phosphorylate downstream substrates. For aPKC isoforms, acute activation in response to growth factor signaling has been demonstrated, but the exact mechanism remains ambiguous [17]; regulation by protein-protein interactions may be more important [18].

Spatiotemporal dynamics of PKC signaling

1. Lipid second messengers control the dynamics of PKC signaling

The crux of PKC activation occurs at the level of lipid second messenger production and turnover. For conventional isozymes, the most common pathway for lipid second messenger production involves a G_q-coupled receptor triggering PIP_2 hydrolysis by PLCβ isoforms. This produces DAG and IP_3, which transiently elevates intracellular Ca^{2+}. PLCδ is acutely activated downstream of Ca^{2+} release, which may also contribute to PKC signaling mediated by G_q (reviewed in [19,20]). PLCγ and ε activated downstream of receptor tyrosine kinase and G_{12} signaling, respectively, are potential activators of PKC; PLCε is a particularly interesting potential activator of PKC since it contains intrinsic feed-forward mechanisms to hydrolyze phosphoinositides in a prolonged manner (reviewed in [20]). Phosphatidic acid phosphatase (PAP) may also play a role in generating basal DAG by dephosphorylating phosphatidic acid (PA) [2], although it remains to be tested whether this pathway contributes to acute, receptor-mediated DAG production. Termination of signaling occurs when receptors are internalized and degraded to halt signaling to PLC, and DAG is converted into PA by the action of DAG kinases (reviewed in [21]).

The effects of lipid second messenger production and turnover on signaling occur on a time scale that is amenable to live cell imaging. Thus, many live cell imaging studies have monitored the agonist-evoked membrane translocation of fluorescently-tagged PKC isoforms [22]. Additional studies using fluorescence resonance energy transfer (FRET)-based reporters for

DAG production (Diacylglycerol Reporter, DAGR) and PKC activity (CKinase Activity Reporter, CKAR; Figure 2) have revealed that stimulated changes in second messenger levels correlate with PKC activity. For example, plasma membrane-targeted CKAR revealed phase-locked oscillations of Ca^{2+} and endogenous PKC activity in Hela cells upon stimulation of endogenous histamine receptors [23,24]. Furthermore, organelle-targeted versions of CKAR have unveiled the dynamics of signaling at defined intracellular locations[25]. Specifically, in COS7 cells, UTP stimulation of endogenous P2Y receptors results in an acute activation of endogenous PKC at the plasma membrane, followed by sustained activity at the Golgi (Figure 3). The duration of PKC activity at the two locations is driven by persistence in localized DAG production, as revealed in independent experiments by plasma membrane-and Golgi-targeted DAGR. These studies and others demonstrate that localized regulation of second messenger production and turnover largely determine the extent of stimulated PKC signaling at a given cellular location.

2. Ligand affinity tunes cellular outcome of PKC activation

Lipid binding modules of the same class often bind ligand with different intrinsic affinities, which influences the cellular concentration required to activate or localize a particular signaling protein. Differential affinities of the C1 domains of novel vs. conventional PKC isoforms for DAG-containing membranes serve as a case in point. Specifically, nPKC isoforms bind DAG-containing membranes with two orders of magnitude higher affinity than do cPKC isoforms [26]. This higher intrinsic affinity of the C1 domain allows nPKC isoforms to be activated by cellular concentrations of DAG alone, while cPKC isoforms require both Ca^{2+} (via C2) and DAG (via C1) to achieve the high-affinity membrane binding necessary to expel the inhibitory pseudosubstrate peptide from the active site [27]. Interestingly, a single amino acid substitution in the C1b domain (Tyr123 in human PKCβ isoforms, Trp252 in human PKCδ) underlies the difference in DAG sensitivity between cPKC and nPKC isoforms [3]. Thus, the residue at this position (which we refer to as position 22 in the C1b domain)serves as a switch between low vs. high-affinity DAG binding for the C1b domains of PKC isoforms.

High-affinity DAG binding influences not only the selective activation of nPKC isozymes, but also their cellular localization. As first shown with nPKCθ in Jurkat T cells [2], reducing basal DAG production by inhibiting either PLC or PAP blocked localization of this isoform to the Golgi. Similarly, converting the C1b domain of nPKCδ to a low-affinity DAG binding domain (by mutating Trp22 to Tyr) blocked basal Golgi localization of the isolated C1 domain as well as full-length protein in COS7 cells. Conversely, changing Tyr22 to Trp in the C1b domain of cPKCβ, converting it to a high-affinity DAG binding domain, was sufficient to localize this conventional C1 domain to the Golgi. Note that introduction of the same mutation into the C1b domain of full-length cPKCβII did not target it to Golgi, although it partially precluded the requirement of Ca^{2+} for activation *in vitro* [3]. This result suggests that determinants outside the C1 domain (likely the C2 domain) govern cellular targeting of cPKC isoforms. Also, while the Golgi is a major site of nPKC activity[2,26], it is entirely possible that stimulated DAG production at other regions of the cell can temporarily draw nPKC isoforms away from the Golgi (e.g. to the plasma membrane[28]).

Ca^{2+}-dependent interactions with anionic phospholipids via the C2 domains of cPKC isoforms also occur with variable affinities; however, these differences are fairly subtle. PKCα, β, and γ have similar intrinsic affinities for Ca^{2+} (PKCα=PKCγ>PKCβ), and their Ca^{2+}-dependent membrane affinities (PKCβ>PKCα=PKCγ) are also similar (both measurements within a two-fold difference) [29–31]. Collectively, the differences in Ca^{2+} affinity and Ca^{2+}-dependent membrane affinity do not affect the on-rate of the isolated C2 domains in response to stimulation of purinergic receptors in PC12 cells, but they somehow affect the residence time at the membrane, with the C2 domain from PKCα persisting longer at the membrane (200 s)

than that of PKCγ (72 s) or β (48 s) [31]. This effect appears to be governed additionally by the anchoring of PIP_2 at a site in the C2 domain distal to the Ca^{2+}-dependent membrane binding site, even though the presence of PIP_2 in binding assays does not dramatically augment the two-fold range in binding affinities amongst the C2 domains *in vitro* [31]. Certainly, further cellular consequences of these fine binding differences remain to be determined. Nevertheless, in cells, lipid-dependent membrane binding affinity has been proven to affect isoform-specific PKC signaling both by setting the threshold for activation and determining localization.

3. Coincident selectivity for multiple ligands within the same domain refines localized PKC responses

Lipid signaling domains may have selectivity for more than one ligand. For example, while the C2 domains of cPKC isoforms bind anionic phospholipids non-specifically in the presence of Ca^{2+}, the preferential recognition of PIP_2 with a basic patch on the C2 domain distal to the Ca^{2+}- dependent binding site [32]directs cPKC isoforms to the PIP_2-rich plasma membrane [33]. In an elegant series of experiments in which PIP_2 production was artificially and selectively increased at the plasma membrane by an inducible Rapamycin-FK506-binding protein system, the residency time of cPKC isoforms at the plasma membrane was increased even though generation of DAG was normal under these conditions [34]. It would be interesting to test whether PIP_2 was the sole mediator of plasma membrane targeting by selectively targeting PIP_2 production to a different site (e.g. at the Golgi) and determining if this is sufficient to bring cPKC isoforms to a non-traditional location. Additionally, it remains to be tested whether the interaction with PIP_2 serves solely as a mechanism to refine targeting, or if it can provide a contribution toward the high-affinity binding necessary to release the pseudosubstrate peptide from the active site.

It is well established that the C1 domains of the DAG-responsive mammalian PKC isoforms also have selectivity for PS over other anionic phospholipids [35]. Although some studies have suggested a role for PS in disrupting interactions between the C1 and C2 domains to confer conformational flexibility and greater accessibility to DAG[36], there is a stereo-specific enhancement in DAG-dependent membrane binding to the isolated C1b domain of PKCβ by L-phosphatidyl serine, indicating that the PS-responsive determinant for PKC activation is intrinsic to isolated C1 domains [35]. While it remains to be tested whether acute or localized differences in PS influence PKC activity on a cellular level, the recent generation of a specific fluorescent tool for visualizing cellular PS should greatly assist in determining how cellular PKC activity is affected by this lipid cofactor[5].

4. The presence of multiple signaling domains in the same protein enhances PKC signaling

In the context of the full-length protein, the binding of lipid second messengers to a domain can be enhanced by the presence of other modules in the protein. The C1 domain, in full-length cPKC isoforms, is assisted in its search for DAG by the Ca^{2+}-dependent membrane binding of the C2 domain [27]. The C2 domain pre-targets the enzyme to the plasma membrane, allowing the C1 domain to search in two dimensions for DAG. While the C2 domain of nPKCs is unresponsive to Ca^{2+}, it does assist with protein-protein interactions that can further refine the localization of DAG-dependent signaling. Specifically, the C2 domains of both cPKC and nPKC isoforms contain sequences that interact with the receptor for activated C kinase (RACK) family of protein scaffolds (reviewed in [37]). Additionally, the C2 domain of nPKCδ (and possibly that of nPKCθ) has the ability to bind phospho-tyrosine, but the biological consequence of this has yet to be determined[38]. However, unlike cPKC isoforms, engagement of the novel C2 domain is not required to provide energy to release the pseudosubstrate peptide to acutely activate nPKC isoforms; DAG-dependent membrane binding of the novel C1 domain is sufficient for activation [26].

Other protein interaction modules exist in specific PKC isoforms. For example, cPKCα contains a post-synaptic density-95/discs large/zonula occludens 1 (PDZ) ligand at its extreme carboxyl terminus[39]. While cPKC homologs in some other eukaryotes also encode a C-terminal PDZ ligand (e.g. the Drosophila eye-PKC PDZ ligand binds the inactivation-no-after-potential D(INAD)PDZ scaffold [40]), this feature is unique to PKCα amongst mammalian cPKC isoforms. This PDZ ligand binds protein interacting with C kinase1 (PICK1), an interaction that is critical for PKCα's role in cerebellar long-term depression[41]. The inclusion of PKCα's PDZ ligand in a proteome-wide PDZ interaction screen uncovered an interaction with, among other proteins, the membrane-associated guanylate kinase (MAGUK)family of protein scaffolds[42]; our preliminary data suggests a role for MAGUK protein scaffolds in localizing PKC activity in Caco-2 cells (unpublished data). Thus, in full-length PKC, other domains can assist and refine the response of C1 domain to DAG.

5. Lipid binding modules can be selectively revealed in the full-length PKC

While the presence of other domains in the full-length protein can heighten and refine the response to lipid second messengers, lipid binding domains can also be masked in the context of the full-length protein. While this is much more of a paradigm for another C1 domain-containing protein, β-chaemerin[43], it has been proposed that the C1 domains of specific cPKC isoforms are masked under certain cellular conditions. This phenomenon was first described for cPKCγ, when Oancea and Meyer showed that the isolated C1 domain translocated to cellular membranes with exogenous DAG far more quickly than did the full-length PKCγ, while, in response to Ca^{2+}, the isolated C2 domain translocated to membranes with the same kinetics as the full-length protein[44]. They postulated that the region N-terminal to the C1 domain encompassing the pseudosubstrate acts as a clamp to keep the C1 domain inaccessible to DAG; the clamp is released when the C2 domain engages membranes. For cPKCα, it was recently shown that acidic residues in the C-terminal tail bind basic residues in the C2 domain, and that this interaction maintains PKCα in a closed conformation that masks the C1a domain; the C1a domain is presumably revealed when the basic patch on the C2 domain engages PIP_2 in the membrane. [45]. This model is strengthened by the evidence that, while mutation of residues in either charged region to Ala residues sensitizes the enzyme to exogenous DAG, swapping the residues in the basic and acidic patches restores DAG insensitivity. For PKCα, another inter-domain interaction between the C1 and C2 domains has been demonstrated [36,46]and is thought to regulate accessibility of the C1 domain to DAG. Ultimately, C1 domain masking appears to occur by isoform-specific mechanisms, or not at all in the case of cPKCβII [35], and it is still unclear whether inter-domain interactions affect cPKC isoforms on a cellular level. Nevertheless, the ability of cPKC isoforms to selectively hide their C1 domains could have cellular consequences. One possible outcome is that this orders the response to Ca^{2+} and DAG, such that the C2 domain must respond to Ca^{2+} before the C1 domain can sense DAG. This effect would allow the C2 domain selectivity to more efficiently govern the localization of the cPKC response: since the C2 domain prefers the PIP_2-rich plasma membrane[33], ordering the response could help override the slight basal preference of the tandem C1 domains for internal membranes (unpublished data).

While the mechanism of domain masking in cPKC isoforms may be isozyme-specific, there appears to be a consensus that the C2 domain of nPKC isoforms is an autoinhibitory module that impedes DAG-dependent membrane binding. Both nPKCε and nPKCδ respond faster to C1 ligands when the C2 domain is physically removed [26,47]. In cells, unmasking the C1 domain of nPKCε (and the Aplysia homolog, Apl nPKCII) appears to depend upon phosphatidic acid (PA) [48,49]. Specifically, blocking the formation of PA desensitizes nPKCε to receptor-mediated translocation[49]. Recently, it was shown that PA binds the C1b domain of Apl nPKCII to both relieve suppression from the C2 domain and synergize with DAG to recruit Apl nPKCII to cellular membranes, an effect mediated by a single Arg residue

at position 273 in the full-length Apl nPKCII [50]. Because this critical determinant is conserved in the C1b domain of mammalian nPKCε, the mechanism is likely conserved. Thus, the PKC family provides examples of how inter-domain interactions in the context of a full-length protein can restrain the response of isolated domains and provide a further element of regulation in cells.

6. Competition from other proteins for the same ligand results in context-dependent PKC responses

Another factor that can govern signaling on a cellular level is the expression of several different proteins in a cell that are responsive to a common ligand. It is possible that the expression of a protein with affinity for a particular ligand will out-compete proteins responding to the same ligand in the same cell. This effect has been demonstrated for PKC isoforms during receptor-mediated stimulation. That is, while nPKC isoforms bind DAG with higher affinity than do cPKC isoforms, nPKCε is prevented from translocation to plasma membrane in response to DAG by the faster, Ca^{2+}-assisted response of cPKCα in histamine-stimulated HEK cells [28]. However, when Ca^{2+} is chelated, nPKCε can now respond at the plasma membrane. Furthermore, when exogenous DAG is added to the same cells, nPKCε alone translocates to the plasma membrane; but, upon photolysis of caged Ca^{2+}, cPKCα displaces nPKCε. Titration of cPKCα expression showed a concentration-dependent suppression of nPKCε translocation in response to receptor-mediated stimulation, further supporting the idea of competition between the two isoforms for DAG-dependent membrane binding. In addition to competing with each other, isoforms in the PKC family also have competition from the growing list of proteins that contain a C1 domain and respond to cellular DAG, including protein kinase D, RasGRP, β-chaemerin, and Munc18 (reviewed in [51]). While the importance of this competition for the same ligand remains to be fully investigated, it is interesting to speculate that the unique protein expression profile of different cell types could lead to the ability of a protein to respond to DAG production at a given location in one cell type, but be out-competed and respond differently in another cell type.

Conclusions

The spatiotemporal dynamics of PKC signaling are chronically and acutely controlled by lipid binding to their membrane-targeting modules, the C1 and C2 domains. Differences in intrinsic affinities and selectivities of these two membrane-targeting modules control the rate, magnitude, duration, and localization of signaling by PKC isozymes (see Figure 4). In general, cPKC isoforms respond rapidly at the plasma membrane with the same kinetics as Ca^{2+} formation, driven by rapid recruitment via the C2 domain to the PIP_2-rich plasma membrane, which in turn allows efficient binding of the membrane-embedded ligand DAG via the C1 domain; nPKC isoforms respond more slowly at Golgi, driven by direct recruitment via their C1 domain to Golgi-bound DAG. Conventional PKC activity terminates more rapidly because of faster turnover of DAG at the plasma membrane, whereas nPKC activity is sustained because of prolonged DAG accumulation at the Golgi. Fine-tuning of these responses by protein scaffold interactions, allosteric mechanisms, and competition with other proteins for common lipid second messengers gives a unique, cell type-dependent activation signature to each isozyme.

Abbreviations

DAG
: Diacylglycerol

PKC
: protein kinase C

PIP_2
phosphoinositide-4,5-bisphosphate

PS
Phosphatidylserine

C1
PKC conserved region 1

C2
PKC conserved region 2

PH
pleckstrin homology

ENTH
Epsin N-terminal homology

BAR
Bin/Amphiphysin/Rvs

PLC
phospholipase C

cPKC
Conventional PKC

nPKC
novel PKC

aPKC
atypical PKC

PDK-1
phosphoinositide-dependent kinase-1

mTORC2
mammalian target of rapamycin complex 2

PAP
phosphatidic acid phosphatase

PA
phosphatidic acid

FRET
fluorescence resonance energy transfer

DAGR
Diacylglycerol Reporter

CKAR
C Kinase Activity Reporter

RACK
receptor for activated C kinase

PDZ
post-synaptic density-95/discs large/zonula occludens 1

PICK1
protein interacting with C kinase 1

MAGUK
membrane-associated guanylate kinase

PB-1
phox and bem-1

CFP
cyan fluorescent protein

YFP
yellow fluorescent protein

DBD
DAG binding domain

FHA
forkhead-associated

PM-CKAR
plasma membrane-targeted CKAR

PM-DAGR
plasma membrane-targeted DAGR

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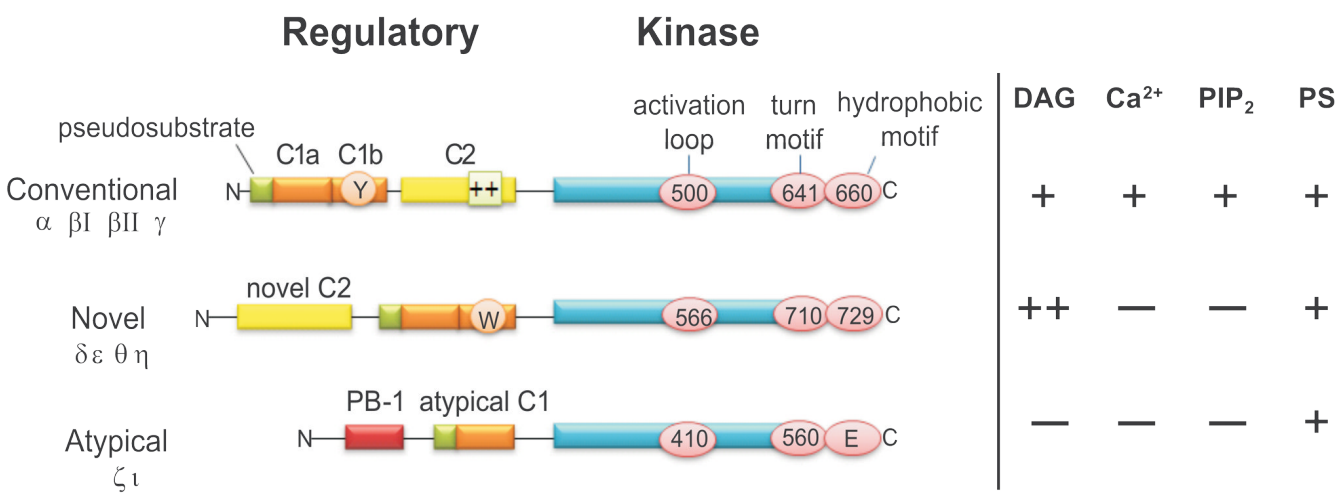

Figure 1.

Primary structure of protein kinase C (PKC) family members. All PKC isoforms contain an autoinhibitory pseudosubstrate peptide (green) and a carboxy-terminal kinase core (blue). The kinase core contains three phosphorylation/autophosphorylation sites (pink) critical for maturation: the activation loop, turn motif, and hydrophobic motif (numbering shown for cPKCβ, nPKCδ, and aPKCζ; note that the hydrophobic motif site is a Glu in aPKC isoforms). PKC isoforms are grouped according to their regulatory domains: cPKC isoforms contain two tandem DAG-sensitive C1 domains (orange) and a Ca^{2+}-sensitive C2 domain (yellow); nPKC isoforms contain two tandem DAG-sensitive C1 domains and a novel, Ca^{2+}-insensitive C2 domain; and aPKC isoforms contain a PB-1 (phox and bem-1) protein-protein interaction module (red) and an atypical, DAG-insensitive C1 domain. The C1 domains of all PKC isoforms interact with PS, and the C2 domains of cPKC isoforms interact non-specifically with anionic phospholipids, including PS. Critical features underlying responsiveness to second messengers are highlighted: Tyr (Y) at position 22 in the C1b domain of cPKC isoforms confers weak DAG responsiveness; Trp (W) at position 22 in the C1b domain of nPKC isoforms confers strong DAG responsiveness; and a basic patch (++) in the C2 domain of cPKC isoforms (located distal to the Ca^{2+}-dependent lipid binding site) specifically recognizes PIP_2.

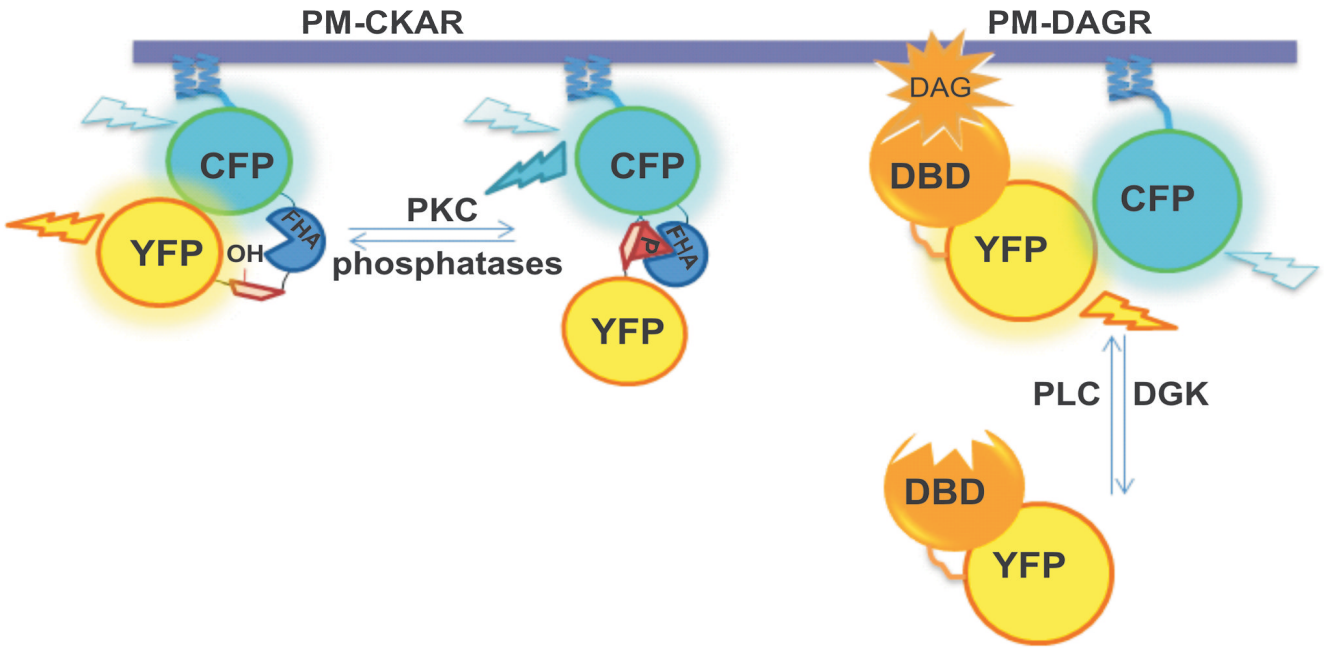

Figure 2.
Reversible, genetically-encoded, FRET-based reporters for visualizing PKCactivity (CKinase Activity Reporter, CKAR) and DAG accumulation (Diacylglycerol Reporter, DAGR). For CKAR, phosphorylation of the PKC-specific substrate peptide allows the forkhead-associated (FHA) domain to interact with it, which decreases FRET between donor cyan fluorescent protein (CFP) and acceptor yellow fluorescent protein (YFP) molecules. For DAGR, the CFP donor is tethered to a membrane and the YFP acceptor is linked to a DAG binding domain (DBD); FRET increases with DAG production as the DBD translocates to the membrane. Shown are plasma membrane-targeted versions of the reporters, PM-CKAR and PM-DAGR.

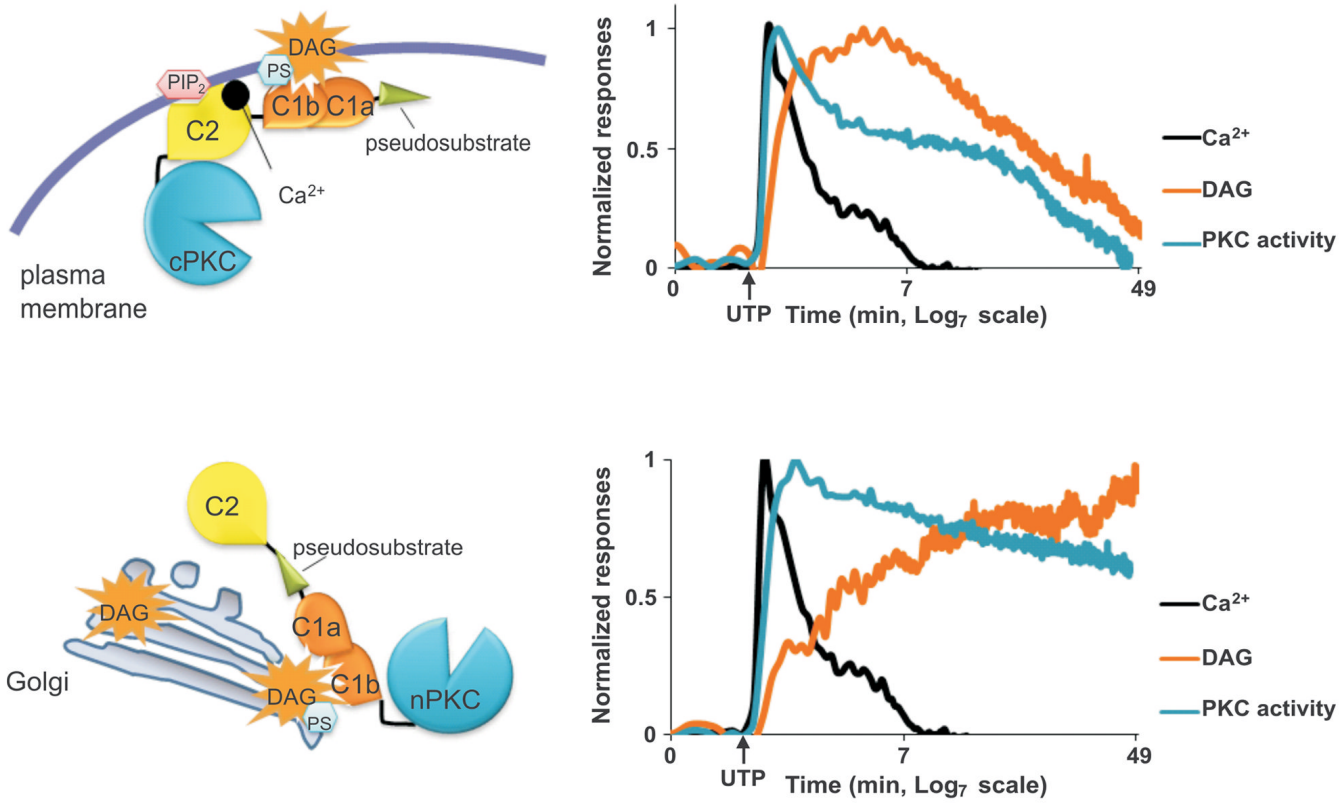

Figure 3.

Differential activation signatures of PKC at the plasma membrane and the Golgi, as read out using targeted FRET-based reporters. In response to UTP, the early phase of PKC signaling at the plasma membrane is acutely coupled to Ca^{2+} release, while the duration of signaling is controlled by the rate of DAG turnover. Compared to the plasma membrane, PKC signaling at the Golgi overlaps less with Ca^{2+} and is sustained by prolonged DAG accumulation. These profiles of PKC signaling are consistent with a role for Ca^{2+}-responsive cPKC isoforms at the plasma membrane and high-affinity DAG-binding nPKC isoforms at the Golgi.

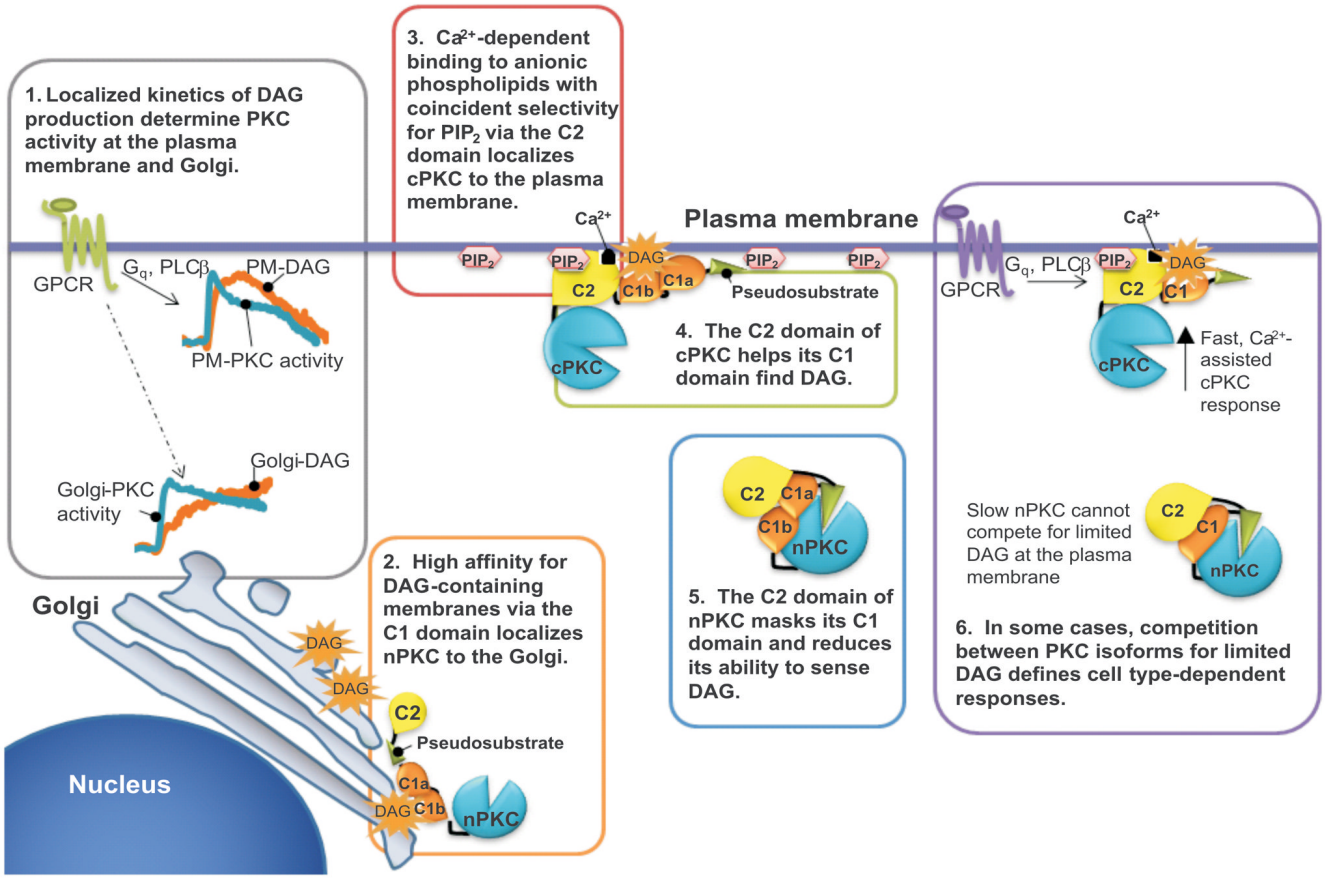

Figure 4.

Examples of PKC regulation at the cellular level in response to lipid second messenger signaling. 1) Localized kinetics of DAG production determine PKC activity at the plasma membrane and Golgi. 2) High affinity for DAG-containing membranes via the C1 domain localizes nPKC to the Golgi. 3) Ca^{2+}-dependent binding to anionic phospholipids with coincident selectivity for PIP_2 via the C2 domain localizes cPKC to the plasma membrane. 4) The C2 domain of cPKC helps its C1 domain find DAG. 5) The C2 domain of nPKC masks its C1 domain and reduces its ability to sense DAG. 6) In some cases, competition between PKC isoforms for limited DAG defines cell type-dependent responses.