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Protein Kinase D Signaling: Multiple Biological Functions in Health and Disease

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Abstract

Protein kinase D (PKD) is an evolutionarily conserved protein kinase family with structural, enzymological, and regulatory properties different from the PKC family members. Signaling through PKD is induced by a remarkable number of stimuli, including G protein-coupled receptor agonists and polypeptide growth factors. PKD1, the most studied member of the family, is increasingly implicated in the regulation of a complex array of fundamental biological processes, including signal transduction, cell proliferation and differentiation, membrane trafficking, secretion, immune regulation, cardiac hypertrophy and contraction, angiogenesis and cancer. PKD mediates such diverse array of normal and abnormal biological functions via dynamic changes in

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[1]The abbreviations used are:

PLC	phospholipase C
DAG	diacylglycerol
PKC	protein kinase C
PKD	protein kinase D
PKD2	protein kinase D2
PKD3	protein kinase D3
CRD	cysteine-rich domain
PH	pleckstrin homology
GPCR	G protein-coupled receptor
CaMKs	Ca^{2+}/calmodulin-dependent protein kinases
PDBu	phorbol 12,13-dibutyrate
PMA	phorbol-12-myristate-13-acetate
siRNA	small interfering RNA

its spatial and temporal localization, combined with its distinct substrate specificity. Studies on PKD thus far indicate a striking diversity of both its signal generation and distribution and its potential for complex regulatory interactions with multiple downstream pathways, often regulating the sub-cellular localization of its targets.

Introduction: regulation of PKD signaling

A large number of external signals involved in inter-cell communication, including hormones, neurotransmitters, growth and developmental factors, cytokines and bioactive lipids bind to receptors that promote the stimulation of the isoforms of the phospholipase C (PLC)[1] family. PLCs, catalyze the hydrolysis of phosphatidylinositol 4,5-biphosphate to produce two second messengers: Ins (1,4,5)P_3, which triggers the release of Ca^{2+} from internal stores and DAG, which elicits cellular responses through classic (α, β, γ) and novel (δ, ε, η, θ) isoforms of PKC [reviewed in (78)]. The mechanisms by which PKC-mediated signals are propagated to critical downstream targets remain incompletely understood. Protein kinase D1 (PKD1)[1], the founding and most studied member of a new protein kinase family within the CAMK group (comprising PKD1, PKD2 and PKD3) and separate from the PKCs [reviewed in (78)], are attracting intense attention. PKD1 (96), formerly called PKCμ (41), has been extensively studied *in vitro*, with regards to identifying the functions of its domains and the effect of cell signaling on its activity and sub-cellular localization. In unstimulated cells, PKD1 is in a state of low catalytic (kinase) activity maintained by autoinhibition mediated by the N-terminal domain, a region containing a repeat of cysteine-rich zinc finger-like motifs and a pleckstrin homology domain [(33, 34, 78, 101); mouse PKD1 domains in **Fig. 1**]. PKD1 could be stimulated by phosphatidylserine micelles containing either DAG or phorbol esters in cell-free preparations (97). These early studies implied that PKD1 represents a novel component of the signal transduction pathways initiated by DAG in target cells.

Subsequent studies, aimed to define PKD1 regulation within intact cells, elucidated a mechanism of PKD1 activation distinct from the direct stimulation of enzyme activity by DAG/phorbol ester plus phospholipids obtained *in vitro*. Specifically, cell treatment with phorbol esters or DAG analogues induced a dramatic inter-conversion of PKD1 from an inactive to an active form, as shown by *in vitro* kinase assays performed in the absence of lipid co-activators. In all these cases, PKD1 activation was potently blocked by prior treatment with PKC inhibitors that did not directly inhibit PKD1 catalytic activity (116), suggesting that rapid PKD1 activation within intact cells is mediated through PKCs. Indeed, cotransfection of PKD1 with active mutant forms of “novel” PKCs (PKCs δ, ε, η, θ), resulted in robust PKD1 activation in the absence of cell stimulation. Studies with multiple receptor agonists (see **Fig.1**), PKD1 mutants and purified PKD1 led to a model that envisages phosphorylation of PKD1 on the activation loop residues Ser^{744} and Ser^{748} (residues #corresponding to the mouse PKD1, **Fig. 1**) as a direct “on/off” switch for catalytic activity [reviewed in (78)]. More recent studies demonstrated that the rapid PKC-dependent PKD1 activation is followed by a sustained, PKC-independent, phase of catalytic activation and phosphorylation induced by stimulation with Gq-coupled receptor agonists, including bombesin and vasopressin (38, 82). These studies also revealed that PKD1

provides an example of a protein kinase in which each residue of the activation loop is regulated by different upstream mechanisms, namely transphosphorylation for Ser^{744} and autophosphorylation for Ser^{748} (38, 82). Thus, PKD1 is a point of convergence and integration of multiple stimuli that is rapidly activated through PKCs and persistently, through PKC-dependent or PKC-independent pathways.

Additional studies showed that PKD family members undergo rapid redistributions in response to cell stimulation. PKD1 and PKD2 translocate from the cytosol to DAG-containing microenvironments in the plasma membrane (75) followed by PKC-dependent reverse translocation of PKD from the plasma membrane to the cytosol and subsequent accumulation in the nucleus (74). In contrast, PKD3 is continuously shuttling between the cytoplasm and the nucleus (73). Pools of PKD family members also localize at the Golgi complex (53, 108) and mitochondria (12). In addition, PKD1 and PKD2 contain short PDZ-binding motifs in their C-termini; VSIL in PKD1 and ISVL in PKD2. Recently, the Na^+/H^+ exchanger regulatory factor 1 (NHERF-1), a PDZ domain-containing protein, has been shown to interact with these sequences of PKD1 and PKD2, implying that PKD can form complexes with NHERF-1 (46). Thus, PKD can regulate targets in a variety of sub-cellular locations and consequently control multiple cellular activities. Accordingly, PKD has been implicated in the regulation of a remarkable array of fundamental biological processes including cell proliferation, polarity, migration and differentiation, membrane trafficking, pain transmission, inflammation, angiogenesis, cardiac contractility and hypertrophy and cancer. The regulation of PKD multi-site phosphorylation, translocation and catalytic activity has been reviewed previously (78). This article focuses on the function of PKD signaling in the regulation of multiple biological processes (**Fig. 2**) and highlights novel concepts emerging from these recent studies (**Fig. 3**).

PKD signaling in cell proliferation and differentiation

Fibroblast cell proliferation and the PKD/RIN1 axis

In fibroblasts, PKD1 can be activated by multiple growth-promoting GPCR agonists (**Fig. 1**) that act through G_q, G_{12} and G_i (112-114, 117), suggesting that PKD functions in mediating mitogenic signaling (78). Indeed, overexpression of either PKD1 or PKD2 potentiated DNA synthesis and cell proliferation induced by Gq-coupled receptor agonists in Swiss 3T3 fibroblasts (82-84, 115). Reciprocally, depletion of PKD1 by transfection of these cells with siRNAs targeting PKD1 markedly attenuated GPCR-induced mitogenesis (82). These results indicate that PKD1 catalytic activation plays a critical and selective role in GPCR mitogenic signaling.

A key pathway involved in mitogenic signaling induced by GPCRs is the extracellular-regulated protein kinase (ERK) cascade (77). The duration and intensity of MEK/ERK/RSK activation are of critical importance for determining specific biological outcomes, including proliferation and differentiation. The potentiating effect of PKD on GPCR-induced cell proliferation has been linked to its ability to increase the duration of the MEK/ERK/RSK pathway leading to accumulation of immediate gene products, (e.g. c-Fos) that stimulate cell cycle progression (82, 83).

Although the targets of PKD responsible for the transmission of its mitogenic signal have not been fully identified, putative substrates are beginning to emerge. A number of scaffolding proteins and endogenous inhibitors have been implicated in the regulation of the intensity and duration of the ERK pathway. Modeling the activation of the ERK pathway indicates that scaffolds regulate its intensity whereas inhibitors modulate its duration (17). The activity and localization of scaffolding and inhibitory proteins are regulated by phosphorylation thereby offering new mechanisms for controlling the MEK/ERK/RSK pathway. PKD1 has been shown to phosphorylate Ras and Rab Interactor 1 (RIN1, see Table 1 for the canonical sequence surrounding the PKD1 phosphorylation site), a multidomain protein that in its unphosphorylated form inhibits Ras/Raf interaction and thereby prevents ERK activation (103). The phosphorylation of RIN1 at Ser^{351} by PKD induces binding of 14-3-3 proteins that restricts RIN1 to the cytosol thereby abrogating its ability of blocking Ras/Raf-1 interaction at the plasma membrane [(103) and **Fig. 3**]. Interestingly, the sustained phase of ERK activation induced by G_q-coupled receptor agonists in PKD-overexpressing 3T3 cells requires epidermal growth factor receptor (EGFR) tyrosine kinase activity (82). It is conceivable that GPCR-induced EGFR transactivation is necessary for the generation of Ras-GTP (77), the partner of Raf. PKD activation facilitates Ras/Raf interaction by phophorylating RIN1, which is then sequestered in the cytosol in a complex with 14-3-3 proteins (**Fig. 3**). The Sprouty (Spry) proteins also act as inhibitors of the Ras/ERK pathway downstream of receptor tyrosine kinases. Interestingly, Spry2 has been shown to interact with PKCδ and prevent PKD activation (10), providing another link in the pathways connecting PKD with ERK activation.

The PKD/ERK/DNA synthesis signaling module is not confined to fibroblasts but operates in a variety of cell types, including cancer cells harboring Ras mutations. For example, the GPCR agonist neurotensin induces PKC-dependent PKD activation (25) and acts as potent growth factor for pancreatic cancer PANC-1 cells (44). PKD1 overexpression in these cells increases DNA synthesis, cell proliferation and anchorage-independent proliferation and potentiates neurotensin-stimulated DNA synthesis, at least in part, via prolongation of ERK signaling (45). Pancreatic cancer cells express RIN1 and neurotensin promotes complex formation between RIN1 and 14-3-3 proteins via PKD in these cells (Sinnett-Smith, Guha and Rozengurt, unpublished results). Additional examples, discussed below, show that the PKD/ERK/DNA synthesis module operates in other cell types, including epithelial and endothelial cells, and will emphasize other mechanisms by which PKD can participate in cell regulation.

Epidermal keratinocyte cell proliferation and reversible differentiation

Regulation of epidermal cell growth and differentiation has been extensively studied in primary cultures of mouse keratinocytes through modulation of extracellular Ca^{2+} levels. PKD is prominently expressed in proliferating primary keratinocytes and down-regulated during differentiation of these cells, suggesting that PKD plays a pro-mitogenic and/or anti-differentiation role in these cells (20). Differentiated keratinocytes, generated by culturing in medium containing millimolar Ca^{2+}, maintain regenerative potential. Lowering Ca^{2+} (to micromolar) stimulates reinitiation of DNA synthesis and cell proliferation in primary cultures of mouse keratinocytes (39). PKD1 has been identified as a major regulator of this

proliferative response through sustained activation of the ERK pathway (39). Interestingly, PKD1 activation was mediated by a PKC-independent pathway involving autophosphorylation at Ser^{748}, similar to the mechanism of PKD1 activation recently elucidated in other cell types (38, 82). These results substantiate a role of PKD in epidermal regeneration and wound healing.

The PKD/HDAC axis and endothelial cell proliferation

Vascular endothelial growth factors (VEGFs) and their endothelial tyrosine kinase receptors play a central role in the regulation of blood and lymphatic vessel development and are essential for normal and abnormal angiogenesis. PKD signaling is necessary for VEGF expression by tumor cells (2, 66) and ERK activation, gene expression and DNA synthesis in VEGF-stimulated endothelial cells (105).

Acetylation/deacetylation of histones is a fundamental mechanism for the control of gene expression. Class II histone deacetylases (i.e. HDACs 4, 5, 7 and 9) regulate chromatin structure by interacting with various transcription factors to repress their activity. PKD phosphorylates specific residues in class II HDACS (see Table 1) leading to their association with 14-3-3 proteins in endothelial cells and other cell types (as illustrated schematically in **Fig. 3**). Sequestration of HDACs in the cytoplasm via 14-3-3 complex formation relieves target genes in the nucleus from HDAC repressive actions, thereby facilitating gene expression.

HDAC7 has been implicated in the regulation of endothelial cells morphology, migration, and capacity to form capillary tube-like structures *in vitro* (62). VEGF induced exit of HDAC7 from the nucleus through PKD-mediated phosphorylation of serines (Table 1) that complex with 14-3-3, promoting nuclear export of HDAC7 and activation of VEGF-responsive genes in endothelial cells (27, 62). Expression of a signal-resistant HDAC7 mutant protein in these cells inhibited proliferation and migration in response to VEGF (102).

HDAC5 has also been identified as a negative regulator of angiogenesis (95). In addition to HDAC7, VEGF stimulates PKD-dependent phosphorylation of HDAC5 on $Ser^{259/498}$ (Table 1), which leads to HDAC5 nuclear exclusion and transcriptional activation (28, 102). Collectively, these studies imply that the complex program of gene expression and migration triggered by VEGF in endothelial cells leading to angiogenesis is orchestrated, at least partly, by PKD-mediated phosphorylation of both HADC5 and HDAC7, leading to their nuclear extrusion in these cells. Accordingly, PKD has emerged as an attractive target for anti-angiogenic therapy.

Osteoblast differentiation and the PKD/HDAC/Runx axis

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor beta (TGFβ) superfamily. They contribute to the formation of bone and connective tissues by inducing the differentiation of mesenchymal cells into bone-forming cells. Recent studies demonstrated that BMP-2 induces PKD activation through a PKC-independent pathway during osteoblast lineage progression (51) and that PKD is

required for the effects of BMP-2 on osteoblast differentiation (8). BMP-2 induces export of HDAC7 from the nucleus in mesenchymal cells that is associated with increased HDAC7 phosphorylation and 14-3-3 binding (40). HDAC7 represses the activity of Runx2, a master transcriptional regulator of skeletal biology. PKD induces HDAC7 nuclear export and thereby alleviates repression of Runx2-mediated transcription (40). Although other pathways may be involved, these results establish a mechanism by which BMP-2 signaling regulates Runx2 activity via PKD-dependent inhibition of HDAC7 transcriptional repression.

PKD function in the regulation of cell vesicle trafficking, secretion and polarity.

In addition to being located at the plasma membrane, cytoplasm and nucleus, a pool of PKD1 and PKD2 is situated at the Golgi complex where it regulates the budding of secretory vesicles from the trans-Golgi network (53, 108). Inactivation of PKD (e.g. by expression of kinase-deficient mutants of PKD) blocks fission of trans-Golgi network (TGN) transport carriers, inducing the appearance of long tubules filled with cargo. At the TGN, active PKD1 and PKD2 phosphorylate Golgi-localized substrates (23, 30, 65), including phosphatidylinositol 4-kinase IIIb (PI4KIIIb), a key player required for fission of TGN-to-plasma membrane carriers (30). PI4KIIIb is recruited to the TGN membrane by the small GTPase ARF, and activated by PKD-mediated phosphorylation to generate PI(4)P, which then recruits the machinery that is required for carrier fission (24). The precise signal that stimulates PKD activation at the Golgi remains unclear. Recent reports proposed intracellular Ca^{2+} released from internal stores in response to Gq-coupled receptor agonists (47) or direct translocation of βγ subunits from the plasma membrane to the Golgi (35, 79).

In agreement with a role in regulating Golgi function, PKD has been implicated in secretion. For example, PKD stimulates secretion of neurotensin from the human endocrine BON cells via the PKD protein substrate Kidins220, [kinase D-interacting substrate of 220 kDa (6, 32) and Table 1] (52). PKD also plays a critical role in regulating angiotensin II-induced cortisol and aldosterone secretion from H295R cells, a human adrenocortical cell line (76). Recent studies revealed a novel p38δ-PKD pathway that regulates insulin secretion and survival of pancreatic β cells, suggesting a critical role for PKD in the development of diabetes mellitus (94).

In addition to secretion, the regulation of Golgi cargo to the plasma membrane has been implicated in fibroblast locomotion, localized Rac1-dependent leading edge activity (72) and integrin recruitment to newly formed focal adhesions (106). *DLC1 (d*eleted in *l*iver *c*ancer*)*, a negative regulator of Rho, is a tumor suppressor gene deleted almost as frequently as p53 in common cancers, including breast, colon, and lung. Activation of PKC/PKD induced association of DLC1 with 14-3-3 proteins (80) via phosphorylation of Ser^{327} and Ser^{431} (Table 1). In turn, Rho activation leads to PKD activation (113). While these studies imply a role of PKD in cell motility, other studies showed that PKD regulates the phosphorylation of cofilin (18, 71) and cortactin (19), leading to reduced cell motility. Although PKD contributes to cell motility and actin dynamics, its role appears complex, depending on cell context and exposure to specific stimuli.

Neuronal and Epithelial Cell Polarity.

Establishing and maintaining cellular polarity is of fundamental importance for the functions of a variety of cell types, including neuronal and epithelial cells. Early neurons develop initial polarity by mechanisms analogous to those used by migrating cells. In line with this notion, PKDs has been shown to play a role in neuronal protein trafficking. In these cells PKD1 and PKD2 regulate TGN-derived sorting of dendritic proteins and axon formation and hence have a role in establishing neuronal polarity (4, 109). PKD has been also implicated in the maintenance of dendritic arborization (14).

In polarized epithelial cells, PKD1 and PKD2, but not PKD3, specifically regulate the production of TGN carriers destined to the basolateral membrane rather than to the apical membrane and consequently, PKD family members may play an important role in the generation of epithelial polarity (108). Another major mechanism involved in establishing cell polarity is mediated by the evolutionary conserved PAR (partitioning-defective) genes, including Par-1. Treatment of cells with phorbol-12-myristate-13-acetate (PMA) induced PKD-mediated phosphorylation of Par-1 on a residue (Ser^{400}; Table1) that promotes Par-1 binding to 14-3-3, thereby promoting its dissociation from lateral plasma membrane and inhibiting its activity [(104); **Fig. 3**). These results suggest that PKD may play a role in regulating cell polarity via phosphorylation of Par-1. Additional experiments using physiological stimuli rather than PMA are necessary to substantiate this important hypothesis. Interestingly, PKD isoforms regulate learning and behavior in Caenorhabditis elegans integrating external information in neuronal and intestinal epithelial cells (22).

PKD signaling and regulation of immune function

A prominent PKC/PKD axis has been demonstrated in B and T lymphocytes (78). PKD is cytosolic in unstimulated T cells (56), but it rapidly polarizes to the immunological synapse in response to antigen/antigen presenting cells. PKD repositioning is driven by the accumulation of DAG at the immunological synapse (87). As in other cell types cells, PKD in lymphocytes phosphorylates and regulates class II HDACs (15, 57, 70). PKD family has also been implicated in regulating the activity of β1 integrins in T cells via Rap1 (59) and IL-2 promoter in response to TCR stimulation (36).

The activation of PKD by antigen receptors is a sustained response associated with changes in PKD intracellular location. The function of PKD at these different locations has been probed in an *in vivo* model using active PKD mutants targeted to either the plasma membrane or the cytosol of pre-T cells of transgenic mice (55). Studies of these mice have shown that PKD can substitute for the pre-T cell receptor and induce both proliferation and differentiation of T cell progenitors in the thymus. Moreover, cellular localization of PKD within a thymocyte is critical; membrane-targeted and cytosolic PKD control different facets of pre-T cell differentiation (55). Subsequent studies probed the Rho requirements for the actions of constitutively active PKD mutants localized at the plasma membrane or the cytosol in pre-T cells of transgenic mice. Membrane-localized PKD regulation of pre- T cell differentiation was shown to be Rho-dependent, but the actions of cytosol-localized PKD were not (63). These studies demonstrated that the link between PKD and Rho is determined by the cellular location of PKD in T lymphocytes.

Toll-like receptors (TLRs) have been identified as primary innate immune receptors. TLRs distinguish between different patterns of pathogens and activate a rapid innate immune response. Recent results implicated PKD in TLR 2, 5 and 9 function in different cell types (37, 69). Specifically, PKD is a downstream target in TLR9 signaling in macrophages (69) and TLR2 in mouse bone marrow-derived mast cells (64). The TLR-interacting protein myeloid differentiation factor 88 (MyD88) has been suggested to play a role in TLR-induced PKD activation (68). In turn, PKD-mediated phosphorylation of TLR5 on Ser^{805} (Table 1) appears necessary for TLR5 response to its ligand, flagellin. TLR5 phosphorylation contributes to p38MAPK activation and production of inflammatory cytokines in epithelial cells (37). Although the precise role of PKD in TLR function remains incompletely understood, these studies provide evidence indicating that PKD plays a role in the regulation of the innate immune response mediated by this class of pattern recognition receptors.

PKD signaling in disease: inflammatory responses, cardiac hypertrophy and cancer PKD, inflammation and oxidative stress

NF-κB is a key transcription factor that is activated by multiple receptors and regulates the expression of a wide variety of proteins that control innate and adaptive immunity. A number of studies indicate that PKD is a mediator of NF-κB induction in a variety of cells exposed to GPCR agonists or oxidative stress (9, 60, 86, 89, 90, 92). In view of the increasing recognition of the interplay between inflammation and cancer development, a possible role of PKD in linking these processes is of importance. However, the precise molecular mechanisms remain incompletely understood.

Stimulation of human colonic epithelial NCM460 cells with the GPCR agonist and bioactive lipid lysophosphatidic acid (LPA) led to a rapid and striking activation of PKD2, the major isoform of the PKD family expressed by these cells (9). LPA stimulated the production of interleukin 8 (IL-8), a potent pro-inflammatory chemokine, and stimulated NF-κB activation. PKD2 gene silencing dramatically reduced LPA-stimulated NF-κB promoter activity and IL-8 production. These results imply that PKD2 mediates LPA-stimulated IL-8 secretion in NCM460 cells through a NF-κB-dependent pathway. PKD2 has also been implicated in mediating NF-κB activation by Bcr-Abl in myeloid leukemia cells (60). Prostaglandins (e.g. PGE2) produced through COX-2 play a critical role in colon cancer development and colonic myofibroblasts are major contributors to their generation. Recent results demonstrated that knockdown of PKD1 in these cells prevented the synergistic increase in COX-2 expression induced by the pro-inflammatory mediators bradykinin and tumor necrosis factor (TNF)-α (110). Thus, these novel results raise the attractive possibility that PKD plays a critical role in mediating COX-2 expression in response to potent pro-inflammatory mediators in human colonic myofibroblasts.

NF-κB also plays a critical role in inflammatory and cell death responses during acute pancreatitis. The PKC isoforms PKCδ and ε are key regulators of NF-κB activation induced by cholecystokinin-8 (CCK-8), an agonist that induces pancreatitis when administered to rodents at supra-maximal doses. PKD was shown to function downstream of PKCδ and PKCε in pancreatic acinar cells stimulated by CCK-8. Specifically, PKD was necessary for NF-κB activation induced by these GPCR agonists in pancreatic cells (111). These results

identify PKD as a novel element in the signaling pathways mediating NF-κB activation in acute pancreatitis. PKD has been also identified as one of the critical factors n the development of hypersensitivity pneumonitis caused by microbial agents (43). Inhibition of PKD1 activation could be an effective way to control acute inflammatory conditions in diverse organs.

Since the original finding that oxidative stress induces PKD activation, partly via PKC-mediated activation loop phosphorylation, and partly through Src-mediated PKD tyrosine phosphorylation (100), a number of reports confirmed that PKD is a sensor of oxidative stress (16, 86, 89-92, 94, 99). Oxidative stress induces PKD1 activation loop phosphorylation on Ser^{744} and Ser^{748} leading to catalytic activation (16). A number of studies have shown that PKD1 opposes the apoptotic effects of oxidative stress in a variety of cells (81, 85, 86, 88, 91, 94).

A recent study using pancreatic β cells, demonstrated that stress signals markedly induced TNFAIP3/A20, a zinc finger–containing, immediate early-response gene with potent antiapoptotic and anti-inflammatory functions (50). In fact, A20 is an early NF-κB-responsive gene that encodes a ubiquitin-editing protein that is involved in the negative feedback regulation of NF-κB signaling (11). Interestingly, other studies demonstrated that PKD induces A20 promoter activity (54). It is plausible that PKD initiates not only an inflammatory response via NF-κB but also stimulates expression of the antiapoptotic and anti-inflammatory A20, as a feedback mechanism that protect cells subjected to stress signals, including oxidative stress.

PKD has also been implicated in pain transmission, a typical response during inflammation. The vanilloid receptor type 1 (VR1 or TRPV1) is a vanilloid-gated, nonselective cation channel that belongs to the transient receptor potential (TRP) channel superfamily which is regulated by phosphorylation by several protein kinases, including PKCε and PKD1 (Table 1). Thus, PKD1 is a modulator of VR1 activity which contributes to allodynia and hyperalgesia development.

Cardiac hypertrophy and contraction

Several years after its identification, PKD family members were shown to be expressed and regulated in ventricular myocytes (31). Treatment of these cells with either PMA or an alpha1-adrenergic receptor (AR) agonist induced rapid PKD activation through PKC (31). Subsequent studies demonstrated that PKD is implicated as a mediator of cardiac hypertrophy, a condition associated with elevated risk for the development of heart failure, and clarified the mechanism by which PKD exerts such profound influence in the heart [see (1) for review].

As mentioned above, PKD directly phosphorylates class II HDAC5, an enzyme that suppresses cardiac hypertrophy (98). PKD-mediated phosphorylation of HDCA5 neutralizes its ability to suppress cardiac hypertrophy by triggering nuclear export (93, 98). The A-Kinase Anchoring Protein (AKAP)-Lbc, which is upregulated in hypertrophic cardiomyocytes, has been proposed to couple PKD activation with the phosphorylation-dependent nuclear export of HDAC5 (7). In turn, other studies demonstrated that augmented

myocardial PKD activity induces cardiac troponin I (TNNI) phosphorylation at Ser^{24} (Table 1) and cardiac myosin binding protein C (MYBPC3) phosphorylation at Ser^{304} (Table 1) which reduces myofilament Ca^{2+} sensitivity and increases cross-bridge cycle rate, implying that altered PKD activity impacts on contractile function (3, 13). Mice with cardiac-specific deletion of PKD1 were viable and showed diminished hypertrophy, fibrosis, and fetal gene activation as well as improved cardiac function in response to pressure overload or chronic adrenergic or angiotensin II signaling (21).

The cAMP-response element-binding protein (CREB) is phosphorylated on Ser^{133} by several upstream kinases. PKD has been identified as a CREB-Ser^{133} kinase (Table 1) that contributes to cardiac remodeling (67). Collectively, these studies indicate that PKD transduces stress stimuli involved in pathological cardiac remodeling and suggest that it could be a novel target in heart disease.

Cancer

Given the widespread role of PKDs in signal transduction, migration, secretion, gene expression, differentiation and proliferation, it is not surprising that PKD signaling has been implicated in a variety of cancer cells, including those originated from the pancreas, liver, gastrointestinal tract, breast, prostate and lung. Recently, two review articles have discussed apparently contrasting roles of the PKD family in cancerous cells from different tissues (26, 49). Consequently, the role of PKD in cancer is not revisited here in detail. However, it is worth noting that in addition of directly promoting cancer cell proliferation, PKD has been implicated in regulating several aspects of the tumor microenvironment, including angiogenesis, inflammation and COX-2 induction. Furthermore, a recent study identified a recurrent mutation of *PRKD1* in human breast and colon cancers (42). Consequently, PKD is a potential target for therapy, at least in certain cancers from some tissues, including pancreas and prostate [(26, 45, 49, 107) but see (5) for a different view].

Concluding Remarks

A great deal of progress has been made in understanding the regulatory mechanisms of activation and sub-cellular localization of PKDs and the role of novel PKCs in mediating rapid PKD family phosphorylation at the activation loop. As in other phosphorylation cascades, inducible activation loop phosphorylation provides a mechanism of signal integration and amplification. Interestingly, new results uncovered that the regulation of the activation loop phosphorylation of PKD is more complex than previously thought, with the participation of different mechanism at different times, especially in cells stimulated by Gq-coupled receptor agonists (82).

Recent advances demonstrate an important role of the PKDs in an array of fundamental biological processes, including cell proliferation, motility, polarity, MAP kinase pathways, cardiac hypertrophy, inflammation and cancer (**Fig. 2**). The involvement of PKDs in mediating such a diverse array of normal and abnormal biological activities in different subcellular compartments is likely to depend on the dynamic changes in their spatial and temporal localization, combined with its distinct substrate specificity (see Table 1). It seems that a variety of biological responses attributed originally to PKCs are in fact executed by

PKDs. Selective PKD inhibitors (29, 48, 61) and animal models using PKD transgenics or tissue specific knockout are emerging and will serve to further clarify the function(s) of PKD isoforms *in vivo*. In this context, it is important to point out that global knockout of PKD1 induces embryonic lethality in mice, with incomplete penetrance (21). Mice deficient in PKD1 enzymatic activity, via homozygous expression of PKD1(S744A/S748A) "knockin" alleles, also induced embryonic lethality (58). These results demonstrated the importance of the phosphorylation of the activation loop residues Ser^{744} and Ser^{748} in the regulation of PKD1 *in vivo*.

In view of the multifunctional roles of PKD, the search for physiological substrates is gathering pace and already a considerable number of interesting molecules have been identified as PKD targets (examples in Table 1). As developed in this article, an emerging theme is that PKD modulates multiple aspects of cell function by altering the sub-cellular localization of its substrates, either interfering with their membrane or nuclear localization, as shown schematically in **Fig. 3**.

In conclusion, studies on PKD thus far indicate a remarkable diversity of both its signal generation and distribution and its potential for complex regulatory interactions with multiple downstream pathways. It is increasingly apparent that the members of the PKD subfamily are key players in the regulation of cell signaling, organization, migration, inflammation and normal and abnormal cell proliferation. PKD emerges as a valuable target for development of novel therapeutic approaches in common diseases, including cardiac hypertrophy and cancer.

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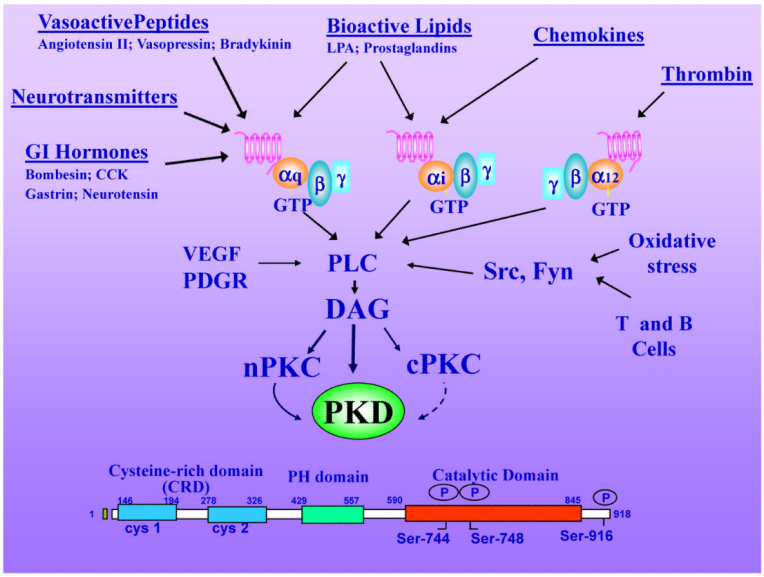

Figure 1. PKDs activation by multiple stimuli

Hormones, growth factors, neurotransmitters, chemokines, bioactive lipids, proteases and oxidative stress induce PLC-mediated hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) to produce DAG at the plasma membrane, which in turn mediates the translocation of inactive PKDs from the cytosol to that cellular compartment. DAG also recruits, and simultaneously activates, novel PKCs to the plasma membrane which mediate transphosphorylation of PKD1 on Ser^{744} (in mouse PKD1). DAG and PKC-mediated transphosphorylation of PKD act synergistically to promote PKD catalytic activation and autophosphorylation on Ser^{748}. The modular structure of PKD (mouse PKD1) is illustrated as an example of the PKD family. PKD1 is the most studied member of the family and its knockout induces embryonic lethality. Further details are provided in the text.

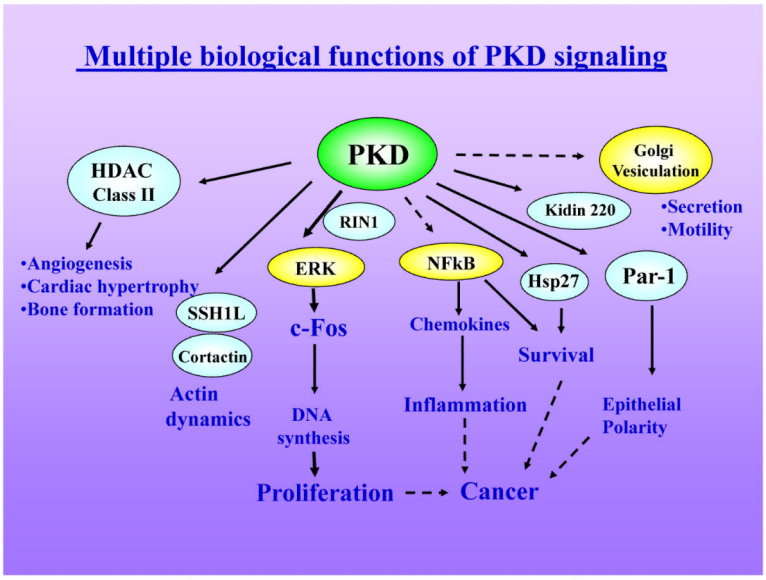

Figure 2. PKD signaling regulates multiple normal and abnormal biological processes

Active PKD phosphorylates a variety of cellular targets at specific sites thereby regulating its sub-cellular localization (as in Fig. 3) or activity (see Table 1 for examples of substrates and of the consensus sequence phosphorylated by PKD). Solid lines indicate direct phosphorylation of substrates (in light blue). Broken lines represent processes in which PKD is implicated but the sequence of molecular events has not been elucidated. Further details are in the text.

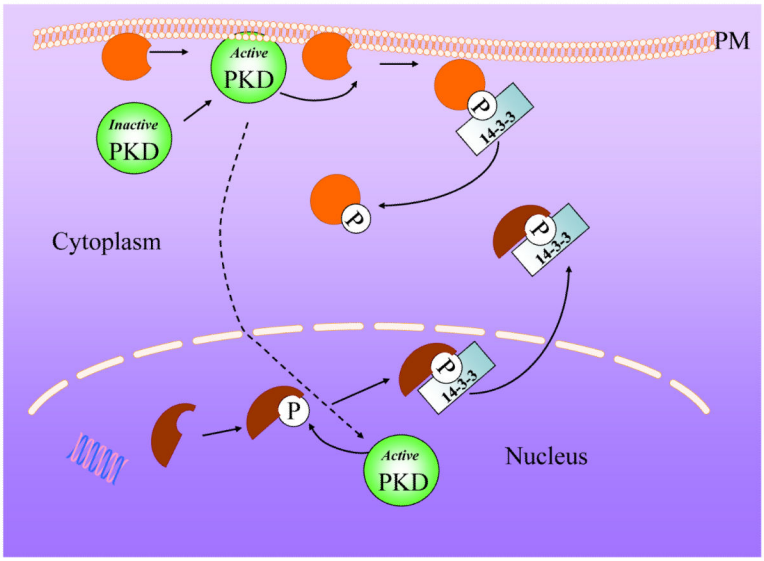

Figure 3. Schematic representation of the mechanism by which PKD modulates intracellular localization of its substrates

In many cases, the phosphorylation of PKD substrates induces binding of 14-3-3 proteins that sequester them to the cytosol, thereby preventing them from acting at the plasma membrane (e.g. RIN1, Par-1, DLC1) or at the nucleus (e.g. HDACS 5 and HDACS 7). An emerging theme is that PKD signaling regulates cell function by altering the sub-cellular localization of its substrates.

TABLE 1

Identified substrates of the PKD family

PKD SUBSTRATE		RESIDUE	TARGET SEQUENCE								
			−5			−3			0		
CERT	(human)	**S132**	S	**L**	R	**R**	H	G	**S**	M	V	S
Cortactin	(human)	**S298**	K	**L**	A	**K**	H	E	**S**	Q	Q	D
CREB	(human)	**S133**	I	**L**	S	**R**	R	P	**S**	Y	R	K
DLC-1	(human)	**S327**	P	**V**	T	**R**	T	R	**S**	L	S	A
HDAC5	(human)	**S259**	P	**L**	R	**K**	T	A	**S**	E	P	N
HDAC5	(human)	**S498**	P	**L**	S	**R**	T	Q	**S**	S	P	L
HDAC7	(human)	**S155**	P	**L**	R	**K**	T	V	**S**	E	P	N
HDAC7	(human)	**S358**	P	**L**	S	**R**	T	R	**S**	E	P	L
HDAC7	(human)	**S486**	P	**L**	S	**R**	A	Q	**S**	S	P	A
HPK1	(human)	**S171**	T	**L**	A	**R**	R	L	**S**	F	I	G
HSP27	(human)	**S82**	A	**L**	S	**R**	Q	L	**S**	S	G	V
Kidins22	(rat)	**S918**	T	**I**	T	**R**	Q	M	**S**	F	D	L
MYBPC3	(human)	**S304**	S	**L**	L	**K**	K	S	S	F	R	T
Par-1	(human)	**S400**	K	**V**	Q	**R**	S	V	**S**	A	N	P
Rin1	(human)	**S351**	P	**L**	L	**R**	S	M	**S**	A	A	F
SSH-1	(human)	**S978**	P	**L**	K	**R**	S	H	**S**	L	A	K
TNNI	(rat)	**S24**	P	**V**	R	**R**	R	S	**S**	A	N	Y
TLR5	(human)	**S805**	Q	**L**	M	**K**	H	Q	**S**	I	R	G
TRPV1	(rat)	**S116**	R	**L**	Y	**D**	R	R	**S**	I	F	D

Note that in most cases, PKD phosphorylates a serine surrounded by a sequence characterized by L/V/I at position −5 and R/K at position −3. Less strict requirements are seen at other positions.