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*MINIREVIEW*

# Regulation of Protein Kinase D1 Activity

Susan F. Steinberg

*Department of Pharmacology, Columbia University, New York, New York*

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**ABSTRACT**

Protein kinase D1 (PKD1) is a stress-activated serine/threonine kinase that plays a vital role in various physiologically important biological processes, including cell growth, apoptosis, adhesion, motility, and angiogenesis. Dysregulated PKD1 expression also contributes to the pathogenesis of certain cancers and cardiovascular disorders. Studies to date have focused primarily on the canonical membrane-delimited pathway for PKD1 activation by G protein-coupled receptors or peptide growth factors. Here, agonist-dependent increases in diacylglycerol accumulation lead to the activation of protein kinase C (PKC) and PKC-dependent phosphorylation of PKD1 at two highly conserved serine residues in the activation loop; this modification increases PKD1 catalytic activity, as assessed by PKD1 autophosphorylation at a consensus phosphorylation motif at the extreme C terminus. However, recent studies expose additional controls and consequences for PKD1 activation loop and C-terminal phosphorylation as well as additional autophosphorylation reactions and *trans*-phosphorylations (by PKC and other cellular enzymes) that contribute to the spatiotemporal control of PKD1 signaling in cells. This review focuses on the multisite phosphorylations that are known or predicted to influence PKD1 catalytic activity and may also influence docking interactions with cellular scaffolds and trafficking to signaling microdomains in various subcellular compartments. These modifications represent novel targets for the development of PKD1-directed pharmaceuticals for the treatment of cancers and cardiovascular disorders.

## Introduction

Protein kinase D1 (PKD1) is the founding member of a family of stress-activated enzymes that play multifunctional roles in fundamental biological processes that regulate cell proliferation, differentiation, apoptosis, immune regulation, cardiac contraction, cardiac hypertrophy, angiogenesis, and cancer (Rozengurt et al., 2005; Avkiran et al., 2008; Guha et al., 2010; LaValle et al., 2010; Steiner et al., 2010). PKD1 is structurally characterized by a C-terminal kinase domain and a N-terminal regulatory domain that contains tandem C1A/C1B motifs that anchor full-length PKD1 to diacylglycerol-/phorbol ester-containing membranes and a pleckstrin homology (PH) domain that participates in intramolecular autoinhibitory interactions that limit catalytic activity (Fig. 1) (Iglesias and Rozengurt, 1998; Chen et al., 2008). PKD1 activation is generally attributed to growth factor-dependent mechanisms that promote diacylglycerol accumulation, colocalize PKD1 at lipid membranes with allosterically activated novel PKC isoforms (nPKCs), and promote nPKC-dependent *trans*-phosphorylation of PKD1 at two highly conserved serine residues in the activation loop ($Ser^{738}$/$Ser^{742}$; nomenclature based upon human PKD1; Fig. 2A) (Waldron et al., 1999). The activated form of PKD1 then autophosphorylates at $Ser^{910}$, a serine at the extreme C terminus that resides in a consensus PKD1 phosphorylation motif (Nishikawa et al., 1997).

### Other PKD1 Activation Mechanisms

Recent studies indicate that the common stereotypic PKD1 activation mechanism involving activation loop phosphorylation by nPKCs does not account for PKD1 activation in all cell

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**ABBREVIATIONS:** PKD, protein kinase D; PH, pleckstrin homology; PKC, protein kinase C; nPKC, novel PKC isoform ($\delta$, $\varepsilon$, $\eta$, $\theta$); AR, adrenergic receptor; CREB, cAMP response element-binding protein; cTnI, cardiac troponin I; NF$\kappa$B, nuclear factor $\kappa$B; WT, wild type; PMA, phorbol 12-myristate 13-acetate; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; DYRK, dual-specificity tyrosine-phosphorylation regulated kinase; GSK-3, glycogen synthase kinase-3; Gö6976, 12-(2-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5*H*-indolo(2,3-*a*)pyrrolo(3,4-*c*)-carbazole; HADC, histone deacetylase; BPKDi, bipyridyl PKD inhibitor; PSSA, phosphorylation site-specific antibody; PDZ, postsynaptic density 95/discs-large/zona occludens; JNK, c-jun-N-terminal kinase.

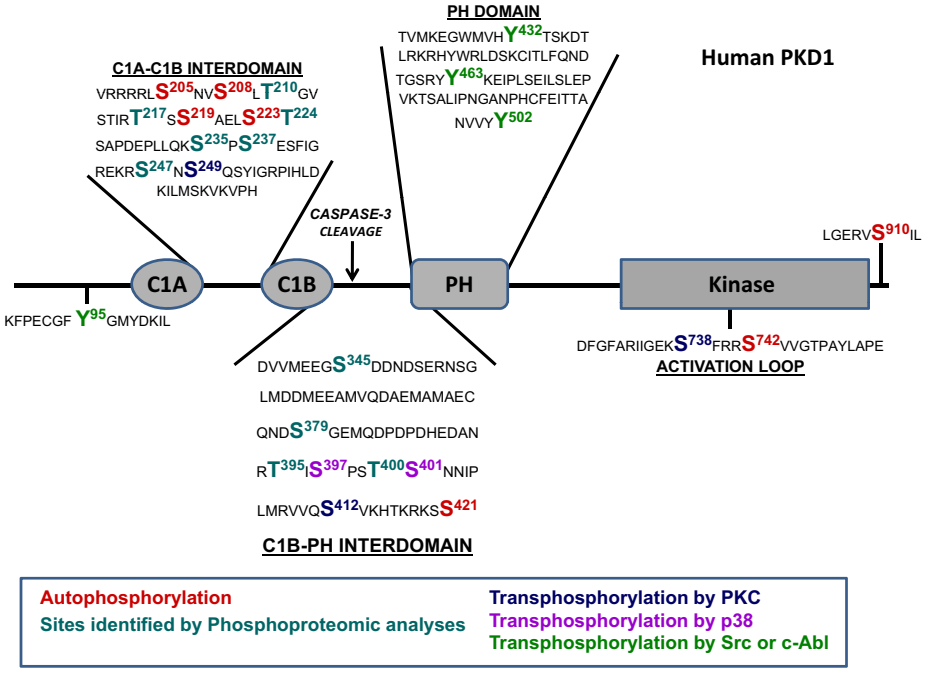


**Fig. 1.** Domain structure and regulatory phosphorylation sites in PKD1. C1A/C1B, cysteine-rich Zn finger domains; Kinase, kinase domain. Numbering based upon the human PKD1 enzyme.

types (or even by all G protein-coupled receptors). Rather, studies in cardiomyocytes identify stimulus-specific differences in PKD1 activation by $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs) and endothelin-1 receptors, two seemingly similar $G_q$-coupled receptors. Here, $\alpha_1$-ARs induce a rapid increase in PKD1 activity that is sustained for at least 1 h; the rapid and sustained phases of $\alpha_1$-AR-dependent PKD1 activation both require PKC activity (Guo et al., 2011). In contrast, endothelin-1 receptors induce a transient PKC-dependent increase in PKD1 activity that is followed by a more sustained increase in PKD1 that does not require PKC activity (Guo et al., 2011). This PKC-independent mechanism for PKD1 activation may have evolved to support signaling responses at late time points, when PKC isoforms are down-regulated. Spatiotemporal differences in PKD1 activation also have been detected in adult cardiomyocytes. Here, phenylephrine ($\alpha_1$-AR agonist) and endothelin-1 act in a similar manner to induce rapid PKD1 translocation to the sarcolemma (Bossuyt et al., 2011). However, the activated form of PKD1 remains stably associated with the sarcolemma only in endothelin-1-treated cardiomyocytes. In phenylephrine-treated cardiomyocytes, activated PKD1 shuttles to the nucleus, where it phosphorylates the class IIa histone deacetylase HDAC5 (Haworth et al., 2000; Harrison et al., 2006; Bossuyt et al., 2008, 2011); because HDAC5 phosphorylation creates docking sites for 14-3-3 proteins that escort HDAC5 from the nucleus, this pathway provides a mechanism to derepress pathologic gene programs that promote cardiomyocyte hypertrophy (Fig. 2A). In theory, these subtle differences in PKD1 activation by $\alpha_1$-AR agonists and endothelin-1 also might influence the phosphorylation of cAMP response element-binding protein (CREB), sarcomeric proteins such as cardiac troponin I (cTnI) or cardiac myosin-binding protein C, or other cardiac PKD1 substrates that regulate contraction, influence tissue remodeling, and contribute to the pathogenesis of certain cardiomyopathies (Ozgen et al., 2008; Bardswell et al., 2010).

PKD1 is activated during oxidative stress through a mechanism that requires nonreceptor tyrosine kinases (c-Abl and Src) and PKCδ (and probably not other PKCs; Fig. 2B). Here, c-Abl-dependent PKD1 phosphorylation at $Tyr^{463}$ (in the PH domain) releases intramolecular autoinhibition, and Src-dependent PKD1 phosphorylation at $Tyr^{95}$ creates a docking site for the C2 domain of PKCδ; PKCδ then phosphorylates the PKD1 activation loop at $Ser^{738}/Ser^{742}$ (Storz and Toker, 2003; Storz et al., 2003; Döppler and Storz, 2007). A redox-dependent pathway involving Src and c-Abl also promotes PKD1-PH domain phosphorylation at $Tyr^{432}$ and $Tyr^{502}$ (Fig. 1), but the significance of these modifications is uncertain because they do not lead to gross changes in PKD1 activity (Storz et al., 2003). There is evidence that the reactive oxygen species-activated PKD1 enzyme is localized (although not necessarily restricted) to mitochondria and that it recruits a nuclear factor κB (NFκB) pathway that induces expression of antioxidant/antiapoptotic genes (such as manganese superoxide dismutase) and promotes cell survival (Storz et al., 2004; Storz et al., 2005). It is noteworthy that the canonical growth factor-dependent PKD1 signaling pathway does not activate NFκB or induce manganese superoxide dismutase, emphasizing that the signaling repertoire and cellular actions of PKD1 can be highly contextual.

PKD1 also is cleaved by caspase-3; it is a component of the signaling machinery mobilized by proapoptotic stimuli (Fig. 2C). Although there is general consensus that caspase-3 cleaves PKD1 at a site in the C1-PH interdomain, the precise cleavage site remains uncertain (Häussermann et al., 1999; Endo et al., 2000; Vántus et al., 2004). The consequences of this proteolytic event (which removes the C1 domain, but not the "autoinhibitory" PH domain) also have been disputed. Vántus et al. (2004) concluded that PKD1 is a proteolytically activated enzyme on the basis of evidence that the PKD1 cleavage product generated during apoptosis displays a modest increase in basal activity compared with WT-PKD1. However, Häussermann et al. (1999) showed that the C-terminal cleavage product (which lacks a C1 domain) does not respond to lipid cofactors (phosphatidylserine/PMA); as a result, the maximal activity of this catalytic fragment is inconsequential

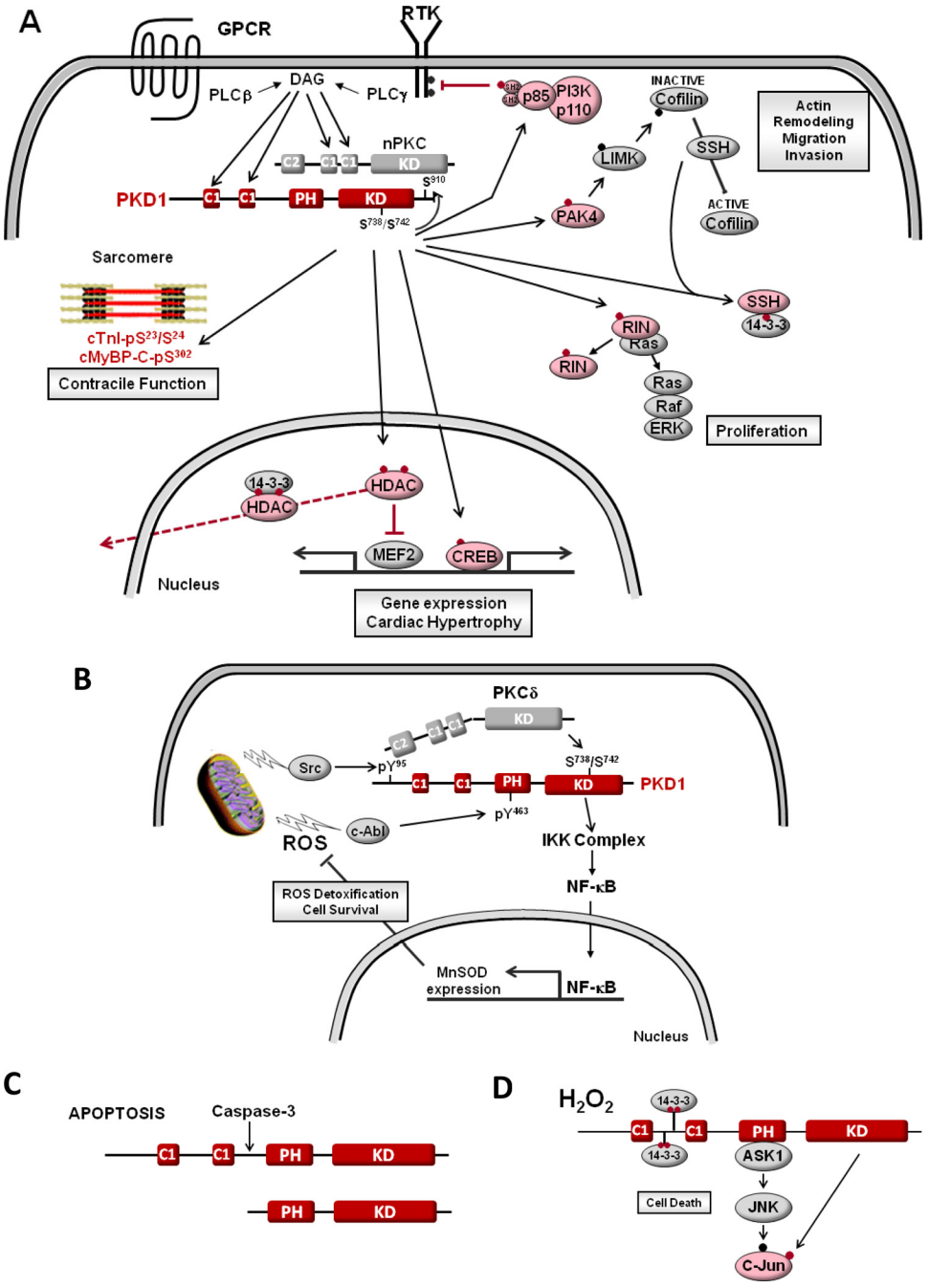


**Fig. 2.** PKD1 activation mechanisms. A, G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) activate PKD1 via an allosteric mechanism involving lipid cofactors and phosphorylation by nPKC isoforms. PKD1 then phosphorylates a range of cellular substrates, including HDAC5, the sarcomeric proteins cTnI and cardiac myosin binding protein-C (cMyBP-C), CREB, the 27-kDa heat shock protein (HSP27), p21 protein (Cdc42/Rac)-activated kinase 4 (PAK4), c-Jun, Bit1 (Bcl-2 inhibitor of transcription, a mitochondrial protein that induces caspase-independent apoptosis), the F-actin-binding protein cortactin, the cofillin phosphatase slingshot 1, RIN1 (a Ras effector protein that influences ERK and c-Abl pathways), and the p85 regulatory subunit of PI3K (which is inhibited—no longer binds to RTKs—when phosphorylated in the SH2 domain by PKD1); direct substrates of PKD1 are in pink (Hurd et al., 2002; Döppler et al., 2005; Biliran et al., 2008; Eiseler et al., 2009, 2010; Peterburs et al., 2009; Barišić et al., 2011; Lee et al., 2011; Spratley et al., 2011; Ziegler et al., 2011). B and C depict alternative mechanism for PKD1 regulation by reactive oxygen species (ROS) or caspase-3 in the setting of oxidative stress or apoptosis (see *Other PKD1 Activation Mechanisms*).

compared with the activity of the phosphatidylserine/PMA-activated full-length PKD1 enzyme. The notion that cleavage limits maximal PKD1 activity also is more consistent with recent results in cardiomyocytes, where the action of PKD1 to regulate lipoprotein lipase-mediated triglyceride accumulation is lost during apoptosis (under conditions associated with the activation of caspase-3 and caspase-3-dependent cleavage of PKD1) (Kim et al., 2009).

## Phosphorylation and the Control of PKD1 Activity

The prevailing dogma regarding the structural basis for PKD1 activation is based upon early studies that relied primarily on Ser$^{910}$ autophosphorylation or PKD1 phosphorylation of syntide-2 (a peptide substrate) as measures of PKD1 activity. Recent studies indicate that these measures do not necessarily provide valid surrogates for PKD1 activity toward more physiologically relevant protein substrates (Rybin et al., 2009). This review summarizes recent studies that use more comprehensive experimental approaches and expose novel controls and consequences of PKD1 phosphorylation at the activation loop, C terminus, and elsewhere in the enzyme.

**Mechanisms and Consequences of PKD1-Ser$^{910}$ Phosphorylation.** The observations that PKD1-Ser$^{910}$ phosphorylation increases in the context of PKD1 activation by growth factor receptors or phorbol esters and that constitutively active forms of PKD1 (such as the PH domain-deleted or S738E/S742E-substituted mutants) display high levels of basal Ser$^{910}$ phosphorylation led to the widespread use of PKD1-Ser$^{910}$ phosphorylation as a surrogate marker of

PKD1 activity, in place of more cumbersome direct enzyme activity measurements (Matthews et al., 1999). The assumption inherent in this experimental approach is that PKD1 activation loop phosphorylation is followed by PKD1 autophosphorylation at $Ser^{910}$ and that $Ser^{910}$ phosphorylation provides a valid measure of the activity of that particular PKD1 molecule. However, there is ample evidence that these assumptions do not apply to all experimental conditions. First, several laboratories have described agonist-dependent increases in PKD1 activation loop phosphorylation and catalytic activity that are not accompanied by increased PKD1-$Ser^{910}$ phosphorylation (Brändlin et al., 2002; Storz et al., 2004; Celil and Campbell, 2005). Second, we and others reported that PKD1-K612W (a catalytically inactive form of PKD1 that by definition cannot undergo a intramolecular *cis*-autophosphorylation) is phosphorylated at $Ser^{910}$ in *trans* by endogenous PKD1 or other enzymes with $Ser^{910}$ kinase activity in several cell types (Sánchez-Ruiloba et al., 2006; Rybin et al., 2009). We also identified major discrepancies between the controls of PKD1-$Ser^{910}$ autophosphorylation versus PKD1 phosphorylation of target substrates. In particular, we showed that PKD1-$Ser^{910}$ autophosphorylation is a privileged catalytic reaction that proceeds at exceedingly low ATP concentrations, does not require prior PKD1 phosphorylation at $Ser^{738}$/$Ser^{742}$, and is not necessarily accompanied by increased PKD1 activity toward heterologous protein substrates (Rybin et al., 2009). Collectively, these results seriously undermine the assumption that immunoblotting studies that track PKD1-$Ser^{910}$ phosphorylation provide a reliable measure of PKD1 activity under all experimental conditions.

The distinct mechanisms for PKD1-$Ser^{910}$ autophosphorylation and PKD1 *trans*-phosphorylation of target substrates are consistent with findings recently reported for several other protein kinases. For example, the epidermal growth factor receptor family member ErbB3 binds ligands, forms heterodimeric complexes with other epidermal growth factor receptor family members, and possesses some *trans*-autocatalytic activity, but ErbB3 does not phosphorylate exogenous target substrates (Shi et al., 2010). DYRK and GSK-3 are serine/threonine kinases that autoactivate through an intramolecular autophosphorylation at tyrosine residues in the activation loop (Lochhead et al., 2006; Lochhead, 2009). The mitogen-activated protein kinase p38$\alpha$ phosphorylates target substrates as a proline-directed serine/threonine kinase, but under some stimulatory conditions p38$\alpha$ autophosphorylates at $Thr^{180}$ and $Tyr^{182}$ in the activation loop as a dual-specificity kinase (Ge et al., 2002). It is noteworthy that although the p38$\alpha$ inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole (SB203580) blocks all p38$\alpha$ activities, the inhibitor sensitivities of the *cis*-autophosphorylation versus target substrate phosphorylation reactions catalyzed by DYRKs and GSK-3 are quite different. These results emphasize that conventional drug screens—designed to identify compounds that prevent phosphorylation of peptide substrates—may miss compounds that specifically block intramolecular *cis*-autophosphorylations. Recent studies suggest that this caveat may be pertinent to the development of therapeutics targeted to PKD1, because PKD1-$Ser^{910}$ autophosphorylation (a reaction that requires only very low concentrations of ATP) is relatively resistant to inhibition by 12-(2-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5*H*-indolo(2,3-*a*)pyrrolo(3,4-*c*)-carbazole (Gö6976; an inhibitor that is competitive with ATP) (Rybin et al., 2009). As a result, Gö6976 treatment protocols that effectively block PKD1 phosphorylation of target substrates (such as HDAC5, CREB, or cTnI) do not induce coordinate decreases in PKD1-$Ser^{910}$ autophosphorylation (Rybin et al., 2009). Similar discrepancies have been identified in studies of BPKDi, another ATP competitive inhibitor recently identified in a high-throughput medicinal chemistry screen (Meredith et al., 2010). Here, typical BPKDi treatment protocols that inhibit HDAC5 phosphorylation do not block PKD1-$Ser^{910}$ autophosphorylation or confer protection from thoracic aortic constriction-induced cardiac hypertrophy. It is interesting to note that BPKDi exerts a modest growth inhibitory effect only at very high doses that induce a modest decrease in PKD1-$Ser^{910}$ autophosphorylation. These results raise the intriguing hypothesis that $Ser^{910}$ phosphorylation may be critical for PKD1 regulation of cardiac growth responses (and that PKD1 exerts some cardiac actions as a scaffold).

PKD1 can act as a signal-regulated scaffold, because sequence flanking the PKD1 autophosphorylation site at $Ser^{910}$ conforms to a type I PDZ domain-binding motif (S/T-X-$\phi$, where X is any amino acid and $\phi$ is a hydrophobic amino acid); $Ser^{910}$ phosphorylation disrupts this PKD1 docking interaction with PDZ domain-containing scaffolding proteins. Recent studies identify another docking interaction between PKD1 and $Na^+/H^+$ exchanger regulatory factor, a PDZ domain-containing protein that colocalizes PKD1 with phosphatases that "fine tune" the local amplitude and tempo of PKD1 responses (relative to PKD1 responses in the bulk cytosol) (Kunkel et al., 2009). Other studies identified an additional role for $Ser^{910}$ to structure the kinase core for some aspect of catalysis, showing that a S910A substitution abrogates PKD1 autophosphorylation at $Ser^{742}$ and prolongs in vivo PKD1 signaling responses (Rybin et al., 2009). The S910A substitution does not lead to other gross changes in PKD1 activity; PKD1-S910A autophosphorylates at $Ser^{738}$ and *trans*-phosphorylates various protein substrates (Rybin et al., 2009). Collectively, these results suggest that a S910A substitution alters the dynamics of PKD1 cellular responses by disrupting docking interactions with PDZ domain containing scaffolding proteins that regulate PKD1 trafficking or terminate PKD1's cellular actions. Alternatively, a secondary $Ser^{742}$ autophosphorylation defect induced by the S910A substitution regulates these processes; phosphorylation at the homologous activation loop site in D kinase family-2 (a PKD-related enzyme in *Caenorhabditis elegans*) enhances enzyme stability (Feng et al., 2007).

**PKD1-$Ser^{738}$/$Ser^{742}$ Loop Phosphorylation.** Concepts regarding the controls and consequences of PKD1-$Ser^{738}$/$Ser^{742}$ (activation loop) phosphorylation are based largely on early studies that used an anti-PKD1-Ser(P)$^{738}$/Ser(P)$^{742}$ PSSA (from Cell Signaling Technology, Danvers, MA) and showed that PMA increases PKD1 activation loop phosphorylation in many cell types via a mechanism that requires nPKC isoform activity (PKC$\delta$, PKC$\varepsilon$, PKC$\eta$, and/or PKC$\theta$). In vitro kinase assays showing direct phosphorylation of the PKD1 activation loop by certain nPKC isoforms also have been published (Brändlin et al., 2002). However, there is recent evidence that the Cell Signaling Technology anti-PKD1-Ser(P)$^{738}$/Ser(P)$^{742}$ PSSA primarily recognizes PKD1 phosphorylation at $Ser^{738}$ and that PKD1 phosphorylation at

Ser$^{742}$ can be tracked with a different PSSA (commercially available from Abcam Inc., Cambridge, MA). Experiments that use a combined approach with these two PSSAs expose differences in the controls and consequences of PKD1 phosphorylation at Ser$^{738}$ and Ser$^{742}$ (Jacamo et al., 2008; Rybin et al., 2009). First, there is evidence that the kinase-inactive PKD1-K612W mutant displays a high level of *trans*-phosphorylation at Ser$^{738}$ but only a low level of phosphorylation at Ser$^{742}$. In the context of in vitro studies showing that Ser$^{742}$ is a target for autocatalytic phosphorylation, these results suggest that PKD1-Ser$^{742}$ phosphorylation in vivo is mediated primarily by a *cis*-autophosphorylation reaction that is defective in the catalytically inactive enzyme (Rybin et al., 2009). Second, there is evidence that certain G protein-coupled receptor agonists induce a rapid/coordinate PKC-dependent increase in PKD1 phosphorylation at Ser$^{738}$/Ser$^{742}$ that is followed by a more sustained increase in PKD1 phosphorylation at Ser$^{742}$; Ser$^{742}$ phosphorylation during the late phase of G protein-coupled receptor activation occurs via an autocatalytic mechanism that does not require PKC activity (Jacamo et al., 2008; Guo et al., 2011). This sustained GPCR-dependent mechanism for PKD1 activation loop phosphorylation (via an autocatalytic mechanism that does not require PKC activity) promotes extracellular signal-regulated kinase activation and mitogenic signaling in some cell types (Sinnett-Smith et al., 2009).

Mutagenesis studies expose a mechanism for PKD1 autophosphorylation at Ser$^{742}$, showing that PKD1-Ser$^{742}$ autophosphorylation is abrogated by a S910A substitution (Rybin et al., 2009). The observation that PKD1-Ser$^{742}$ autophosphorylation is a hierarchical process that requires a prior priming phosphorylation at Ser$^{910}$ is important for two reasons. First, these results identify a heretofore-unrecognized role for the Ser$^{910}$-phosphorylated C terminus to structure the kinase core for some aspects of catalysis. The observation that Ser$^{910}$ is specifically required for PKD1 autophosphorylation at Ser$^{742}$ (a site that does not conform to a PKD1 consensus phosphorylation motif) but not PKD1 phosphorylation of target substrates is intriguing—given evidence that other autoactivating kinases (such as GSK-3, p38a, or DYRK) autophosphorylate at their activation loops (sites that do not conform to conventional substrate sequences) only when stabilized in unique conformations as a result of intramolecular interactions or docking interactions with protein chaperones (Lochhead, 2009). Second, these results indicate that Ser$^{742}$ phosphorylation plays little to no role in the control of PKD1 activity, because WT-PKD1 and the PKD1-S910A mutant (which is not phosphorylated at Ser$^{742}$) display similar high levels of activity toward target substrates (Rybin et al., 2009). The notion that PKD1 activity is regulated by activation loop phosphorylation at Ser$^{738}$—and not at Ser$^{742}$—is at odds with previous conclusions derived from mutagenesis studies, where a single S738A or S742A substitution decreases—and a double S738A/S742A substitution abrogates—PKD1 catalytic activity; the previous studies were interpreted as evidence that phosphorylation reactions at Ser$^{738}$ and Ser$^{742}$ play similar roles to regulate PKD1 activity (Iglesias et al., 1998). However, activation loop Ser→Ala substitutions can have dual effects to prevent phosphorylation and to remove hydroxyl groups that may engage in structurally important electrostatic interactions (Steichen et al., 2010). Our results with the PKD1-S910A mutant—a catalytically active enzyme with a nonphosphorylated position 742 serine—argue that the position 742 serine plays a structural role to stabilize the active site of the enzyme—and that phosphorylation at this site has little to no effect on PKD1 activity. Finally, it is interesting to note that the observation that PKD1 activity is regulated primarily via a phosphorylation at Ser$^{738}$ (and not Ser$^{742}$) resonates with results obtained for the *Caenorhabditis elegans* PKD enzyme D kinase family-2, where sites in the homologous $^{925}$SFRRS$^{929}$ activation loop sequence (corresponding to $^{738}$SFRRS$^{742}$ in PKD1) play distinct roles to regulate activity (through phosphorylation at Ser$^{925}$) or the duration of the signaling response (through phosphorylation at Ser$^{929}$) (Feng et al., 2007).

Although activation loop phosphorylation is critical for PKD1 activation by agonists that signal via PKC, PKD1 also is activated via a PKC-independent mechanism that is not associated with (and does not require) activation loop phosphorylation in bone morphogenetic protein 2-treated MC3T3-E1 osteoblast-like cells (Lemonnier et al., 2004), reactive oxygen species-activated endothelial cells (Zhang et al., 2005), and UVB-treated keratinocytes (Arun et al., 2011). Although the molecular underpinnings for PKC-independent modes of PKD1 activation remain uncertain, mutagenesis studies provide some hints regarding mechanism. In particular, the observation that S738A/S742A substitutions abrogate WT-PKD1 catalytic activity, but PKD1 truncation mutants lacking either the isolated PH domain or the entire regulatory domain tolerate S738A/S742A substitutions without a significant loss of catalytic activity, have been interpreted as evidence that activation loop phosphorylation activates the enzyme by relieving intramolecular autoinhibitory constraints involving the regulatory domain that limit catalytic activity (Waldron and Rozengurt, 2003). According to this formulation, other events (such as other post-translation modifications or protein-protein interactions) that disrupt autoinhibitory constraints might also increase PKD1 activity via a mechanism that does not involve (or require) activation loop phosphorylation. In this regard, dextran sulfate activates PKD1 without increasing activation loop phosphorylation—and dextran sulfate is a potent agonist for both WT and S738A/S742A-substituted PKD1 enzymes (Gschwendt et al., 1997; Rybin et al., 2009). Gschwendt et al. (1997) speculated that dextran sulfate activates PKD1 by disrupting an intramolecular interaction between a highly acidic region in the C1-PH interdomain and basic regions elsewhere in the enzyme. This formulation provides a framework to consider whether some agonist-dependent increases in PKD1 activity that develop over protracted intervals and are not associated with increased activation loop phosphorylation might be attributable to the de novo synthesis of a PKD1 binding partner that disrupts intramolecular autoinhibitory constraints in the enzyme (Lemonnier et al., 2004).

### Other Phosphorylation Sites That Regulate PKD1 Activity

The PKD1 regulatory domain contains other phosphorylation sites that are known or predicted to regulate signaling by PKD1 (Fig. 1). For example, the C1A-C1B interdomain contains a cluster of autophosphorylation sites (at Ser$^{205}$, Ser$^{208}$, Ser$^{219}$, and Ser$^{223}$) that reside in 14-3-3 consensus binding motifs. Autophosphorylation at these sites leads to the formation of PKD1-14-3-3$\tau$ complexes, recruitment of apoptosis signal-regulated kinase 1 to the PKD1-PH domain,

activation of the apoptosis signal-regulated kinase 1-JNK pathway, c-Jun phosphorylation, and induction of apoptosis in $H_2O_2$-treated endothelial cells (Fig. 2D) (Hausser et al., 1999; Zhang et al., 2005). This seems to be a kinase-independent mechanism for PKD1 activation of the JNK signaling pathway, because C1A-C1B interdomain autophosphorylation is not linked to gross changes in PKD1 activity; the docking interaction between 14-3-3$\tau$ and PKD1 actually decreases PKD1 catalytic activity. Moreover, catalytically active PKD1 phosphorylates c-Jun at N-terminal regulatory sites (that are distinct from the sites phosphorylated by JNK) and actually inhibits JNK-dependent c-Jun phosphorylation (Hurd et al., 2002; Waldron et al., 2007). The PKD1 C1A-C1B interdomain also contains another phosphorylation site at position 249; studies to date suggest that $Ser^{249}$ is a target for *trans*-phosphorylation by PKC and that $Ser^{249}$ phosphorylation may contribute to optimal PKD1 activation by PKC (but it is not required for PKD1 activation by lipid cofactors) (Vertommen et al., 2000).

Two additional phosphorylation sites have recently been identified adjacent to the autoinhibitory PH domain at positions 421 and 412 (Fig. 1). $Ser^{421}$ is a target for an autophosphorylation reaction or *trans*-phosphorylation by protein kinase A (Smith et al., 2011). $Ser^{412}$ is phosphorylated via a PKC-dependent mechanism (and not an autophosphorylation reaction) in neonatal cardiomyocytes treated with PMA or hypertrophic agonists such phenylephrine or endothelin-1 (Phan et al., 2011). Phosphorylation at these sites could in theory influence intramolecular interactions involving the PH domain that limit catalytic activity (or influence the PH domain-mediated mechanism that controls nuclear export of PKD1) (Rey et al., 2001). However, mutagenesis studies to date do not link $Ser^{421}$ phosphorylation to changes in PKD1 localization or catalytic activity; the consequences of a S412A substitution have not been examined (Phan et al., 2011; Smith et al., 2011). Finally, p38MAPK-dependent phosphorylation of PKD1 at $Ser^{397}$ and $Ser^{401}$ in the C1-PH interdomain is identified in pancreatic $\beta$-cells, where it is implicated as a mechanism that controls insulin secretion by inhibiting PKD1 activity (Sumara et al., 2009).

Recent improvements in methods for large scale phosphoproteomics analyses have led to the identification of a large number of additional phosphorylation sites in PKD1 (Villén et al., 2007; Cantin et al., 2008; Zanivan et al., 2008; Brill et al., 2009; Old et al., 2009; Oppermann et al., 2009; Chen et al., 2010; Huttlin et al., 2010). It is interesting to note that these phosphorylation sites map primarily to the C1A-C1B and C1B-PH interdomains. These phosphorylation "hot spots"—in unstructured regions of the enzyme that share little homology with corresponding regions of PKD2 and PKD3—might contribute to PKD1 isoform- and organelle-specific functions.

## Conclusions and Future Directions

PKD1 has recently emerged as a signaling enzyme with multifunctional roles in both physiologic and pathologic cellular responses. Recent studies identify an elaborate network of phosphorylation reactions at the activation loop, C terminus, and other regions of the enzyme that contribute to the control of PKD1's cellular actions. These multisite phosphorylations also provide a mechanism to integrate input from diverse inciting stimuli; stimulus-specific differences in the ensemble phosphorylation pattern (or pools of PKD1 with different phosphorylation profiles) could underlie stimulus-specific PKD1 signaling repertoires and cellular responses. Although this review focused primarily on the phosphorylation reactions that influence PKD1 signaling efficiency or specificity, docking interactions with small molecules, protein scaffolds, or protein substrates also may contribute to the allosteric control of PKD1 activity (Sharlow et al., 2008). With this in mind, it is worth noting that high-throughput screens currently in use to identify PKD1 inhibitors typically screen for compounds that inhibit peptide substrate phosphorylation by the resting (unphosphorylated) form of PKD1. This approach may miss clinically useful PKD1 inhibitors for two reasons. First, it assumes that PKD1 inhibitor sensitivity is an inherent property of the enzyme that is not altered (or fine-tuned) by events that accompany enzyme activation. This approach does not allow for possible phosphorylation-dependent changes in PKD1 inhibitor sensitivity (i.e., the notion that differentially phosphorylated forms of PKD1 might effectively constitute distinct drug targets). Second, although peptide substrates typically bind to a single site within the catalytic pocket, physiologically relevant protein substrates typically bind to protein kinases at both the active site and at distal docking motifs outside the catalytic cleft. These docking interactions may serve two functions. First, by tethering or orienting protein substrates on the enzyme, they may facilitate phosphorylation of sites that do not conform to optimal consensus phosphorylation motifs (perhaps explaining the known effects of PKD1 to phosphorylate sites in c-Jun, $\beta$-catenin, c-TnI, and type II$\alpha$ phosphatidylinositol 4-phosphate kinase that do not conform to LxRxxpS/T motifs) (Hinchliffe and Irvine, 2006; Qin et al., 2006; Waldron et al., 2007; Du et al., 2009). Second, a docking interaction with a protein substrate or scaffold may influence inhibitor sensitivity; there is recent evidence that PKC is rendered insensitive to inhibitors that compete with ATP when anchored to AKAP79 (Hoshi et al., 2010). Although similar docking interactions that alter the pharmacologic profile of PKD1 have not yet been identified, PKD1 is a conformationally flexible enzyme that could be regulated in this manner. The phosphorylation reactions and docking mechanisms that influence the pharmacologic profile or signaling specificity of PKD1 present both challenges and opportunities for the development of novel PKD1-targeted pharmaceuticals for the treatment of cardiac hypertrophy/failure and certain intractable cancers.

### Authorship Contributions

*Wrote or contributed to the writing of the manuscript:* Steinberg.

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**Address correspondence to:** Dr. Susan F. Steinberg, Department of Pharmacology, College of Physicians and Surgeons, Columbia University, 630 West 168 Street, New York, NY 10032. E-mail: sfs1@columbia.edu