

REGULATION OF PROTEIN KINASE D1 ACTIVITY

Susan F. Steinberg

Department of Pharmacology, Columbia University,
New York, NY 10032

A) RUNNING TITLE: PKD1 PHOSPHORYLATION

B) CORRESPONDING AUTHOR

Susan F. Steinberg, M.D.
Department of Pharmacology
College of Physicians and Surgeons
Columbia University
630 West 168 Street
New York, NY 10032
Tel: 212-305-4297
E-mail: sfs1@columbia.edu

C) Text pages: 23
Tables: 0
Figures: 2
References: 76
Abstract: 208 words
manuscript: 6966 words

D) Nonstandard abbreviations:

α_1 -AR, α_1 -adrenergic receptor; ASK-1, apoptosis signal-regulating kinase 1; CREB, cAMP-response element binding protein; cTnl, cardiac troponin I; DYRK, dual-specificity tyrosine-phosphorylation regulated kinase; EGFR, epidermal growth factor receptor; ET-1-R, endothelin-1 receptor; GSK-3, glycogen synthase kinase-3; HDAC, histone deacetylase; JNK, c-jun-N-terminal kinase; NF κ B, nuclear factor κ B; NHERF-1, Na⁺/H⁺ exchanger regulatory factor 1; nuclear factor κ B; PSSA, phosphorylation site-specific antibody; PKC, protein kinase C; nPKC, novel PKC isoforms (δ , ϵ , η , θ); PKD1, protein kinase D1; PH domain, pleckstrin homology domain; PMA, phorbol 12-myristate 13-acetate; PS, phosphatidylserine.

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's 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 transphosphorylations (by PKC and other cellular enzymes) that contribute to the spatiotemporal control of PKD1 signaling in cells. This review focuses on the multi-site phosphorylations that are known or predicted to influence PKD1's catalytic activity and also may 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 (Steiner et al., 2010; Rozengurt et al., 2005; Avkiran et al., 2008; Lavalley et al., 2010; Guha 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, co-localize 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 (Ser⁷³⁸/Ser⁷⁴²; nomenclature based upon human PKD1; Figure 2A (Waldron et al., 1999)). The activated form of PKD1 then autophosphorylates at Ser⁹¹⁰, 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 types (or even by all G protein coupled receptors). Rather, studies in cardiomyocytes identify stimulus-specific differences in PKD1 activation by α_1 -adrenergic receptors (α_1 -ARs) and endothelin-1 (ET-1-Rs), two seemingly similar Gq-coupled receptors. Here, α_1 -ARs induce a rapid increase in PKD1 activity that is sustained for at least 1 hour; the rapid and sustained

phases of α_1 -AR-dependent PKD1 activation both require PKC activity (Guo et al., 2011). In contrast, ET-1-Rs 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 (α_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; Bossuyt et al., 2011); since 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 (Figure 2A). In theory, these subtle differences in PKD1 activation by α_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 (Bardswell et al., 2010; Ozgen et al., 2008).

PKD1 is activated during oxidative stress through a mechanism that requires non-receptor tyrosine kinases (c-Abl and Src) and PKC δ (and probably not other PKCs; Figure 2B). Here, c-Abl-dependent PKD1 phosphorylation at Tyr⁴⁶³ (in the PH domain) releases intramolecular autoinhibition and Src-dependent PKD1 phosphorylation at Tyr⁹⁵ creates a docking site for the C2 domain of PKC δ ; PKC δ then phosphorylates the PKD1 activation loop at

Ser⁷³⁸/Ser⁷⁴² (Storz and Toker, 2003;Storz et al., 2003;Doppler and Storz, 2007). A redox-dependent pathway involving Src and c-Abl also promotes PKD1-PH domain phosphorylation at Tyr⁴³² and Tyr⁵⁰² (Figure 1) but the significance of these modifications is uncertain since they do not lead to gross changes in PKD1 activity (Storz et al., 2003). There is evidence that the ROS-activated PKD1 enzyme is localized (although not necessarily restricted) to mitochondria and that it recruits a nuclear factor κ B (NF κ B) pathway that induces expression of antioxidant/anti-apoptotic genes (such as MnSOD) and promotes cell survival (Storz et al., 2005;Storz et al., 2004). Of note, the canonical growth factor-dependent PKD1 signaling pathway does not activate NF κ B or induce MnSOD, 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 pro-apoptotic stimuli (Figure 2C). While there is general consensus that caspase-3 cleaves PKD1 at a site in the C1-PH interdomain, the precise cleavage site remains uncertain (Endo et al., 2000;Vantus et al., 2004;Haussermann et al., 1999). The consequences of this proteolytic event (which removes the C1 domain, but not the 'autoinhibitory' PH domain) also have been disputed. Vantus et al. concluded that PKD1 is a proteolytically-activated enzyme, based upon evidence that the PKD1 cleavage product generated during apoptosis displays a modest increase in basal activity, compared to WT-PKD1 (Vantus et al., 2004). However, Häussermann et al. showed that the C-terminal cleavage product (which lacks a C1 domain) does not respond to lipid cofactors (PS/PMA); as a result, the maximal activity of this catalytic fragment is inconsequential when compared to the activity of the PS/PMA-activated full-length PKD1 enzyme (Haussermann et al., 1999). The notion that cleavage limits maximal PKD1 activity also is more consistent with recent results in cardiomyocytes, where PKD1's action 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 primarily relied on Ser⁹¹⁰ autophosphorylation or PKD1 phosphorylation of syntide-2 (a peptide substrate) as measures of PKD1 activity. Recent studies indicate that these readouts 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-Ser⁹¹⁰ PHOSPHORYLATION: The observations that PKD1-Ser⁹¹⁰ 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 Ser⁹¹⁰ phosphorylation lead to the widespread use of PKD1-Ser⁹¹⁰ 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 Ser⁹¹⁰ and that Ser⁹¹⁰ phosphorylation provides a valid readout 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-S⁹¹⁰ phosphorylation (Brandlin et al., 2002;Storz et al., 2004;Celil and Campbell, 2005). Second, we and others reported that PKD1-K612W (a catalytically-inactive forms of PKD1 that by definition cannot undergo a intramolecular *cis* autophosphorylation) is phosphorylated at S⁹¹⁰ in *trans* by endogenous PKD1 or other enzymes with S⁹¹⁰ kinase activity in several cell types (Sanchez-Ruiloba et al., 2006;Rybin et al., 2009). We also identified major discrepancies between the controls of PKD1-Ser⁹¹⁰ autophosphorylation versus PKD1 phosphorylation of target substrates. In particular, we showed that PKD1-Ser⁹¹⁰ autophosphorylation is a privileged catalytic reaction that proceeds at exceedingly low ATP concentrations, does not require prior PKD1 phosphorylation at Ser⁷³⁸/Ser⁷⁴², 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-Ser⁹¹⁰ phosphorylation provide a reliable readout of PKD1 activity under all experimental conditions.

The distinct mechanisms for PKD1-Ser⁹¹⁰ 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 (EGFR) family member ErbB3 binds ligands, forms heterodimeric complexes with other EGFR 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, 2009;Lochhead et al., 2006). The mitogen-activated protein kinase p38 α phosphorylates target substrates as a proline-directed serine/threonine kinase, but under some stimulatory conditions p38 α autophosphorylates at Thr¹⁸⁰ and Tyr¹⁸² in the activation loop as a dual-specificity kinase (Ge et al., 2002). Of note, while the p38 α inhibitor SB203580 blocks all p38 α 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, since PKD1-Ser⁹¹⁰ autophosphorylation (a reaction that requires only very low concentrations of ATP) is relatively resistant to inhibition by 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-Ser⁹¹⁰ 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-Ser⁹¹⁰ autophosphorylation or confer protection from thoracic aortic constriction (TAC)-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-S⁹¹⁰ autophosphorylation. These results raise the intriguing hypothesis that Ser⁹¹⁰ 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, since sequence flanking the PKD1 autophosphorylation site at Ser⁹¹⁰ conforms to a type I PDZ domain-binding motif (S/T-X- ϕ , where X is any amino acid and ϕ is a hydrophobic amino acid); Ser⁹¹⁰ phosphorylation disrupts PKD1 docking to PDZ domain-containing scaffolding proteins. Recent studies identify a docking interaction between PKD1 and Na⁺/H⁺ exchanger regulatory factor (NHERF-1), a PDZ domain-containing protein that co-localizes 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 Ser⁹¹⁰ to structure the kinase core for some

aspect of catalysis, showing that a S910A substitution abrogates PKD1 autophosphorylation at Ser⁷⁴² and it 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 Ser⁷³⁸ and it *trans* phosphorylates various protein substrates (Rybin et al., 2009)). These results could 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. However, the secondary Ser⁷⁴² autophosphorylation defect induced by the S910A substitution also could be pertinent, since phosphorylation at the homologous activation loop site in DKF-2 (a PKD-related enzyme in *C. elegans*) enhances enzyme stability (Feng et al., 2007).

PKD1-Ser⁷³⁸/Ser⁷⁴² loop phosphorylation. Concepts regarding the controls and consequences of PKD1-Ser⁷³⁸/Ser⁷⁴² (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) and showed that PMA increases PKD1 activation loop phosphorylation in many cell types via a mechanism that requires nPKC isoform activity (PKC δ , PKC ϵ , PKC η and/or PKC θ). *In vitro* kinase assays showing direct phosphorylation of the PKD1 activation loop by certain nPKC isoforms also have been published (Brandlin 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 Ser⁷³⁸ and that PKD1 phosphorylation at Ser⁷⁴² can be tracked with a different PSSA (commercially available from Abcam). Experiments that use a combined approach with these two PSSAs expose differences in the controls and consequences of PKD1 phosphorylation at Ser⁷³⁸ and Ser⁷⁴² (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 Ser⁷³⁸, but only a low level of phosphorylation at Ser⁷⁴². In the context of *in*

vitro studies showing that Ser⁷⁴² is a target for autocatalytic phosphorylation, these results suggest that PKD1-Ser⁷⁴² 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 Ser⁷³⁸/Ser⁷⁴² that is followed by a more sustained increase in PKD1 phosphorylation at Ser⁷⁴²; Ser⁷⁴² phosphorylation during the late phase of G protein-coupled receptor activation is 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 ERK activation and mitogenic signaling in some cell types (Sinnott-Smith et al., 2009)

Mutagenesis studies expose a mechanism for PKD1 autophosphorylation at Ser⁷⁴² showing that PKD1-Ser⁷⁴² autophosphorylation is abrogated by a S910A substitution (Rybin et al., 2009). The observation that PKD1-Ser⁷⁴² autophosphorylation is a hierarchical process that requires a prior priming phosphorylation at Ser⁹¹⁰ is important for two reasons. First, these results identify a heretofore unrecognized role for the Ser⁹¹⁰-phosphorylated C-terminus to structure the kinase core for some aspects of catalysis. The observation that Ser⁹¹⁰ is specifically required for PKD1 autophosphorylation at Ser⁷⁴² (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 Ser⁷⁴² phosphorylation plays little-to-no role in the control of PKD1 activity, since WT-PKD1 and the PKD1-S910A mutant (which is not phosphorylated at Ser⁷⁴²) 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 Ser⁷³⁸ - and not at Ser⁷⁴² - 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 Ser⁷³⁸ and Ser⁷⁴² play similar roles to regulate PKD1 activity (Iglesias et al., 1998). However, activation loop S→A 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 non-phosphorylated 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 Ser⁷³⁸ (and not Ser⁷⁴²) resonates with results obtained for the *Caenorhabditis elegans* PKD enzyme DKF-2, where sites in the homologous ⁹²⁵SFRRS⁹²⁹ activation loop sequence (corresponding to ⁷³⁸SFRRS⁷⁴² in PKD1) play distinct roles to regulate activity (through phosphorylation at Ser⁹²⁵) or the duration of the signaling response (through phosphorylation at Ser⁹²⁹ (Feng et al., 2007)).

While 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), ROS-activated endothelial cells (Zhang et al., 2005), and UVB-treated keratinocytes (Arun et al., 2011). While 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. 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 (Gschwendt et al., 1997). 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 predict to regulate signaling by PKD1 (Figure 1). For example, the C1A-C1B interdomain contains a cluster of autophosphorylation sites (at Ser²⁰⁵, Ser²⁰⁸, Ser²¹⁹ and Ser²²³) that reside in 14-3-3 consensus binding motifs. Autophosphorylation at these sites leads to the formation of PKD1-14-3-3 τ complexes, recruitment of ASK1 to the PKD1-PH domain, activation of the ASK1-JNK pathway, c-Jun phosphorylation, and induction of apoptosis in H₂O₂-treated endothelial

cells (Figure 2D; (Hausser et al., 1999;Zhang et al., 2005)). This appears to be a kinase-independent mechanism for PKD1 activation of the JNK signaling pathway, since C1A-C1B interdomain autophosphorylation is not linked to gross changes in PKD1 activity; the docking interaction between 14-3-3 τ 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 (Waldron et al., 2007;Hurd et al., 2002). The PKD1 C1A-C1B interdomain also contains another phosphorylation site at position 249; studies to date suggest that Ser²⁴⁹ is a target for *trans*-phosphorylation by PKC and that Ser²⁴⁹ 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 (Figure 1). Ser⁴²¹ is a target for an autophosphorylation reaction or *trans*-phosphorylation by protein kinase A (Smith et al., 2011). Ser⁴¹² 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 Ser⁴²¹ 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 Ser³⁹⁷ and Ser⁴⁰¹ in the C1-PH interdomain is identified in pancreatic β -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 lead to the identification of a large number of additional phosphorylation sites in PKD1 (Oppermann et al., 2009;Old et al., 2009;Cantin et al., 2008;Huttlin et al., 2010;Zanivan et al., 2008;Villen et al., 2007;Brill et al., 2009;Chen 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. While this review focuses primarily on the phosphorylation reactions that influence PKD1's 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's inhibitor sensitivity is an inherent property of the enzyme that it is not altered (or 'fine-tuned') by events

that accompany enzyme activation. This approach does not allow for possible phosphorylation-dependent changes in PKD1's inhibitor sensitivity (i.e., the notion that differentially-phosphorylated forms of PKD1 might effectively constitute distinct drug targets). Second, while 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, β -catenin, c-TnI, and type II α PI4P kinase that do not conform to LxRxxpS/T motifs (Waldron et al., 2007; Du et al., 2009; Hinchliffe and Irvine, 2006; Qin et al., 2006)). 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). While 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 PKD1's pharmacologic profile or signaling specificity 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

Participated in research design: *not applicable*

Conducted experiments: *not applicable*

Contributed new reagents or analytic tools: *not applicable*

Performed data analysis: *not applicable*

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

References

- Arun SN, Kaddour-Djebbar I, Shapiro B A and Bollag W B (2011) Ultraviolet B Irradiation and Activation of Protein Kinase D in Primary Mouse Epidermal Keratinocytes. *Oncogene* **30**:1586-1596.
- Avkiran M, Rowland A J, Cuello F and Haworth R S (2008) Protein Kinase D in the cardiovascular system: Emerging roles in health and disease. *Circ Res* **102**:157-163.
- Bardswell SC, Cuello F, Rowland A J, Sadayappan S, Robbins J, Gautel M, Walker J W, Kentish J C and Avkiran M (2010) Distinct sarcomeric substrates are responsible for protein kinase D-mediated regulation of cardiac myofilament Ca^{2+} sensitivity and cross-bridge cycling. *J Biol Chem* **285**:5674-5682.
- Barisic S, Nagel A C, Franz-Wachtel M, Macek B, Preiss A, Link G, Maier D and Hausser A (2011) Phosphorylation of Ser 402 impedes phosphatase activity of Slingshot 1. *EMBO Rep* **12**:527-533.
- Biliran H, Jan Y, Chen R, Pasquale E B and Ruoslahti E (2008) Protein Kinase D is a positive regulator of Bit1 apoptotic function. *J Biol Chem* **283**:28029-28037.
- Bossuyt J, Chang C W, Helmstadter K, Kunkel M T, Newton A C, Campbell K S, Martin J L, Bossuyt S, Robia S L and Bers D M (2011) Spatiotemporally distinct PKD activation in adult cardiomyocytes in response to phenylephrine and endothelin. *J Biol Chem* **286**:33390-400.
- Bossuyt J, Helmstadter K, Wu X, Clements-Jewery H, Haworth R S, Avkiran M, Martin J L, Pogwizd S M and Bers D M (2008) Ca^{2+} /calmodulin-dependent protein kinase II δ and protein kinase D overexpression reinforce the histone deacetylase 5 redistribution in heart failure. *Circ Res* **102**:695-702.
- Brandlin I, Hubner S, Eiseler T, Martinez-Moya M, Horschinek A, Hausser A, Link G, Rupp S, Storz P, Pfizenmaier K and Johannes F J (2002) PKC η -Mediated PKC μ activation modulates ERK and JNK signal pathways. *J Biol Chem* **277**:6490-6496.
- Brill LM, Xiong W, Lee K B, Ficarro S B, Crain A, Xu Y, Terskikh A, Snyder E Y and Ding S (2009) Phosphoproteomic analysis of human embryonic stem cells. *Cell Stem Cell* **5**:204-213.
- Cantin GT, Yi W, Lu B, Park S K, Xu T, Lee J D and Yates J R, III (2008) Combining protein-based IMAC, peptide-based IMAC, and MudPIT for efficient phosphoproteomic analysis. *J Proteome Res* **7**:1346-1351.
- Celil AB and Campbell P G (2005) BMP-2 and Insulin-Like Growth Factor-I Mediate Osterix (Osx) Expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways. *J Biol Chem* **280**:31353-31359.
- Chen J, Deng F, Li J and Wang Q J (2008) Selective binding of phorbol esters and diacylglycerol by individual C1 domains of the PKD family. *Biochem J* **411**:333-342.
- Chen L, Giorgianni F and Beranova-Giorgianni S (2010) Characterization of the phosphoproteome in LNCaP prostate cancer cells by in-gel isoelectric focusing and tandem mass spectrometry. *J Proteome Res* **9**:174-178.

Doppler H and Storz P (2007) A novel tyrosine phosphorylation site in protein kinase D contributes to oxidative stress-mediated activation. *J Biol Chem* **282**:31873-31881.

Doppler H, Storz P, Li J, Comb M J and Toker A (2005) A phosphorylation state-specific antibody recognizes Hsp27, a novel substrate of protein kinase D. *J Biol Chem* **280**:15013-15019.

Du C, Jaggi M, Zhang C and Balaji K C (2009) Protein kinase D1-mediated phosphorylation and subcellular localization of β -catenin. *Cancer Res* **69**:1117-1124.

Eiseler T, Doppler H, Yan I K, Kitatani K, Mizuno K and Storz P (2009) Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. *Nat Cell Biol* **11**:545-556.

Eiseler T, Hausser A, De K L, van L J and Pfizenmaier K (2010) Protein kinase D controls actin polymerization and cell motility through phosphorylation of cortactin. *J Biol Chem* **285**:18672-18683.

Endo K, Oki E, Biedermann V, Kojima H, Yoshida K, Johannes F J, Kufe D and Datta R (2000) Proteolytic Cleavage and activation of protein kinase C μ by caspase-3 in the apoptotic response of cells to 1- β -D-arabinofuranosylcytosine and other genotoxic Agents. *J Biol Chem* **275**:18476-18481.

Feng H, Ren M, Chen L and Rubin C S (2007) Properties, regulation, and in vivo functions of a novel protein kinase D: caenorhabditis elegans DKF-2 links diacylglycerol second messenger to the regulation of stress responses and life span. *J Biol Chem* **282**:31273-31288.

Ge B, Gram H, Di P F, Huang B, New L, Ulevitch R J, Luo Y and Han J (2002) MAPKK-independent activation of p38 α mediated by TAB1-dependent autophosphorylation of p38 α . *Science* **295**:1291-1294.

Gschwendt M, Johannes F J, Kittstein W and Marks F (1997) Regulation of protein kinase C μ by basic peptides and heparin. putative role of an acidic domain in the activation of the kinase. *J Biol Chem* **272**:20742-20746.

Guha S, Tanasanvimon S, Sinnott-Smith J and Rozengurt E (2010) Role of protein kinase D signaling in pancreatic cancer. *Biochem Pharmacol* **80**:1946-1954.

Guo J, Gertsberg Z, Ozgen N, Sabri A and Steinberg S F (2011) Protein kinase D isoforms are activated in an agonist-specific manner in cardiomyocytes. *J Biol Chem* **286**:6500-6509.

Harrison BC, Kim M S, van Rooij E, Plato C F, Papst P J, Vega R B, McAnally J A, Richardson J A, Bassel-Duby R, Olson E N and McKinsey T A (2006) Regulation of cardiac stress signaling by protein kinase D1. *Mol Cell Biol* **26**:3875-3888.

Hausser A, Storz P, Link G, Stoll H, Liu Y C, Altman A, Pfizenmaier K and Johannes F J (1999) Protein kinase C μ is negatively regulated by 14-3-3 signal transduction proteins. *J Biol Chem* **274**:9258-9264.

- Haussermann S, Kittstein W, Rincke G, Johannes F J, Marks F and Gschwendt M (1999) Proteolytic cleavage of protein kinase C μ upon induction of apoptosis in U937 cells. *FEBS Lett* **462**:442-446.
- Haworth RS, Goss M W, Rozengurt E and Avkiran M (2000) Expression and activity of protein kinase D/protein kinase C μ in myocardium: evidence for α_1 -adrenergic receptor- and protein kinase C-mediated regulation. *J Mol Cell Cardiol* **32**:1013-1023.
- Hinchliffe KA and Irvine R F (2006) Regulation of type II PIP kinase by PKD phosphorylation. *Cell Signal* **18**:1906-1913.
- Hoshi N, Langeberg L K, Gould C M, Newton A C and Scott J D (2010) Interaction with AKAP79 modifies the cellular pharmacology of PKC. *Mol Cell* **37**:541-550.
- Hurd C, Waldron R T and Rozengurt E (2002) Protein kinase D complexes with c-Jun N-terminal kinase via activation loop phosphorylation and phosphorylates the c-Jun N-terminus. *Oncogene* **21**:2154-2160.
- Huttlin EL, Jedrychowski M P, Elias J E, Goswami T, Rad R, Beausoleil S A, Villen J, Haas W, Sowa M E and Gygi S P (2010) A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell* **143**:1174-1189.
- Iglesias T and Rozengurt E (1998) Protein kinase D activation by mutations within its pleckstrin homology domain. *J Biol Chem* **273**:410-416.
- Iglesias T, Waldron R T and Rozengurt E (1998) Identification of *in vivo* phosphorylation sites required for protein kinase D activation. *J Biol Chem* **273**:27662-27667.
- Jacamo R, Sinnott-Smith J, Rey O, Waldron R T and Rozengurt E (2008) Sequential PKC-dependent and PKC-independent protein kinase D catalytic activation via Gq-coupled receptors: differential regulation of activation loop Ser744 and Ser748 phosphorylation. *J Biol Chem* **283**:12877-12887.
- Kim MS, Wang F, Puthanveetil P, Kewalramani G, Innis S, Marzban L, Steinberg S F, Webber T D, Kieffer T J, Abrahani A and Rodrigues B (2009) Cleavage of protein kinase D after acute hypoinsulinemia prevents excessive lipoprotein lipase-mediated cardiac triglyceride accumulation. *Diabetes* **58**:2464-2475.
- Kunkel MT, Garcia E L, Kajimoto T, Hall R A and Newton A C (2009) The protein scaffold NHERF-1 controls the amplitude and duration of localized protein kinase D activity. *J Biol Chem* **284**:24653-24661.
- Lavalle CR, George K M, Sharlow E R, Lazo J S, Wipf P and Wang Q J (2010) Protein kinase D as a potential new target for cancer therapy. *Biochim Biophys Acta* **1806**:183-192.
- Lee JY, Chiu Y H, Asara J and Cantley L C (2011) Inhibition of PI3K binding to activators by serine phosphorylation of PI3K regulatory subunit p85 α Src homology-2 domains. *Proc Natl Acad Sci U S A* **108**:14157-14162.
- Lemonnier J, Ghayor C, Guicheux J and Caverzasio J (2004) Protein Kinase C-independent activation of protein kinase D is involved in BMP-2-induced activation of stress mitogen-

activated protein kinases JNK and p38 and osteoblastic cell differentiation. *J Biol Chem* **279**:259-264.

Lochhead PA (2009) Protein kinase activation loop autophosphorylation in cis: overcoming a catch-22 situation. *Sci Signal* **2**:e4.

Lochhead PA, Kinstrie R, Sibbet G, Rawjee T, Morrice N and Cleghon V (2006) A chaperone-dependent GSK3 β transitional intermediate mediates activation-loop autophosphorylation. *Mol Cell* **24**:627-633.

Matthews SA, Rozengurt E and Cantrell D (1999) Characterization of S⁹¹⁶ as an *in vivo* autophosphorylation site for protein kinase D/protein kinase C μ . *J Biol Chem* **274**:26543-26549.

Meredith EL, Beattie K, Burgis R, Capparelli M, Chapo J, Dipietro L, Gamber G, Enyedy I, Hood D B, Hosagrahara V, Jewell C, Koch K A, Lee W, Lemon D D, McKinsey T A, Miranda K, Pagratis N, Phan D, Plato C, Rao C, Rozhitskaya O, Soldermann N, Springer C, van E M, Vega R B, Yan W, Zhu Q and Monovich L G (2010) Identification of potent and selective amidobipyridyl inhibitors of protein kinase D. *J Med Chem* **53**:5422-5438.

Nishikawa K, Toker A, Johannes F J, Songyang Z and Cantley L C (1997) Determination of the specific substrate sequence motifs of protein kinase C isozymes. *J Biol Chem* **272**:952-960.

Old WM, Shabb J B, Houel S, Wang H, Coutts K L, Yen C Y, Litman E S, Croy C H, Meyer-Arendt K, Miranda J G, Brown R A, Witze E S, Schweppe R E, Resing K A and Ahn N G (2009) Functional proteomics identifies targets of phosphorylation by B-Raf signaling in melanoma. *Mol Cell* **34**:115-131.

Oppermann FS, Gnad F, Olsen J V, Hornberger R, Greff Z, Keri G, Mann M and Daub H (2009) Large-scale proteomics analysis of the human kinome. *Mol Cell Proteomics* **8**:1751-1764.

Ozgen N, Obrezhtchikova M, Guo J, Elouardighi H, Dorn G W, Wilson B A and Steinberg S F (2008) Protein kinase D links Gq-coupled receptors to cAMP response element-binding protein (CREB)-Ser¹³³ phosphorylation in the heart. *J Biol Chem* **283**:17009-17019.

Peterburs P, Heering J, Link G, Pfizenmaier K, Olayioye M A and Hausser A (2009) Protein kinase D regulates cell migration by direct phosphorylation of the cofilin phosphatase slingshot 1 like. *Cancer Res* **69**:5634-5638.

Phan D, Stratton M S, Khai H Q and McKinsey T A (2011) A novel protein kinase C target site in protein kinase D is phosphorylated in response to signals for cardiac hypertrophy. *Biochem Biophys Res Commun* **411**:335-341.

Qin L, Zeng H and Zhao D (2006) Requirement of protein kinase D tyrosine phosphorylation for VEGF-A¹⁶⁵-induced angiogenesis through its interaction and regulation of phospholipase C γ phosphorylation. *J Biol Chem* **281**:32550-32558.

Rey O, Sinnott-Smith J, Zhukova E and Rozengurt E (2001) Regulated nucleocytoplasmic transport of protein kinase D in response to G protein-coupled receptor activation. *J Biol Chem* **276**:49228-49235.

Rozengurt E, Rey O and Waldron R T (2005) Protein kinase D signaling. *J Biol Chem* **280**:13205-13208.

Rybin VO, Guo J and Steinberg S F (2009) Protein kinase D1 Autophosphorylation via distinct mechanisms at Ser⁷⁴⁴/Ser⁷⁴⁸ and Ser⁹¹⁶. *J Biol Chem* **284**:2332-2343.

Sanchez-Ruiloba L, Cabrera-Poch N, Rodriguez-Martinez M, Lopez-Menendez C, Martin Jean-Mairet R, Higuero A M and Iglesias T (2006) Protein kinase D intracellular localization and activity control Kidins220 traffic through a PDZ-binding motif. *J Biol Chem* **281**:18888-18900.

Sharlow ER, Giridhar K V, Lavallo C R, Chen J, Leimgruber S, Barrett R, Bravo-Altamirano K, Wipf P, Lazo J S and Wang Q J (2008) Potent and selective disruption of protein kinase D functionality by a benzoxoloazepinolone. *J Biol Chem* **283**:33516-33526.

Shi F, Telesco S E, Liu Y, Radhakrishnan R and Lemmon M A (2010) ErbB3/HER3 intracellular domain is competent to bind ATP and catalyze autophosphorylation. *Proc Natl Acad Sci U S A* **107**:7692-7697.

Sinnett-Smith J, Jacamo R, Kui R, Wang Y M, Young S H, Rey O, Waldron R T and Rozengurt E (2009) Protein kinase D mediates mitogenic signaling by Gq-coupled receptors through PKC-independent regulation of activation loop Ser744 and Ser748 phosphorylation. *J Biol Chem*.

Smith FD, Samelson B K and Scott J D (2011) Discovery of cellular substrates for protein kinase A using a peptide array screening protocol. *Biochem J* **438**:103-110.

Spratley SJ, Bastea L I, Doppler H, Mizuno K and Storz P (2011) Protein kinase D regulates cofilin activity through p21-activated kinase 4. *J Biol Chem* **286**:34254-34261.

Steichen JM, Iyer G H, Li S, Saldanha S A, Deal M S, Woods V L, Jr. and Taylor S S (2010) Global consequences of activation loop phosphorylation on protein kinase A. *J Biol Chem* **285**:3825-3832.

Steiner TS, Ivison S M, Yao Y and Kifayet A (2010) Protein kinase D1 and D2 are involved in chemokine release induced by toll-like receptors 2, 4, and 5. *Cell Immunol* **264**:135-142.

Storz P, Doppler H, Johannes F J and Toker A (2003) Tyrosine phosphorylation of protein kinase D in the pleckstrin homology domain leads to activation. *J Biol Chem* **278**:17969-17976.

Storz P, Doppler H and Toker A (2004) Activation loop phosphorylation controls protein kinase D-dependent activation of nuclear factor κ B. *Mol Pharmacol* **66**:870-879.

Storz P, Doppler H and Toker A (2005) Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol* **25**:8520-8530.

Storz P and Toker A (2003) Protein kinase D mediates a stress-induced NF- κ B activation and survival pathway. *EMBO J* **22**:109-120.

Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, Ramracheya R, Caille D, Jiang H, Platt K A, Meda P, Aebersold R, Rorsman P and Ricci R (2009) Regulation of PKD by the MAPK p38 δ in insulin secretion and glucose homeostasis. *Cell* **136**:235-248.

Vantus T, Vertommen D, Saelens X, Rykx A, De Kimpe L, Vancauwenbergh S, Mikhlap S, Waelkens E, Keri G, Seufferlein T, Vandenabeele P, Rider M H, Vandenheede J R and Van Lint J (2004) Doxorubicin-induced activation of protein kinase D1 through caspase-mediated proteolytic cleavage: identification of two cleavage sites by microsequencing. *Cell Signal* **16**:703-709.

Vertommen D, Rider M, Ni Y, Waelkens E, Merlevede W, Vandenheede J R and Van Lint J (2000) Regulation of protein kinase D by multisite phosphorylation. identification of phosphorylation sites by mass spectrometry and characterization by site-directed mutagenesis. *J Biol Chem* **275**:19567-19576.

Villen J, Beausoleil S A, Gerber S A and Gygi S P (2007) Large-scale phosphorylation analysis of mouse liver. *Proc Natl Acad Sci U S A* **104**:1488-1493.

Waldron RT, Iglesias T and Rozengurt E (1999) The pleckstrin homology domain of protein kinase D interacts preferentially with the eta isoform of protein kinase C. *J Biol Chem* **274**:9224-9230.

Waldron RT and Rozengurt E (2003) Protein kinase C phosphorylates protein kinase D activation loop Ser⁷⁴⁴ and Ser⁷⁴⁸ and releases autoinhibition by the pleckstrin homology domain. *J Biol Chem* **278**:154-163.

Waldron RT, Whitelegge J P, Faull K F and Rozengurt E (2007) Identification of a novel phosphorylation site in c-Jun directly targeted in vitro by protein kinase D. *Biochem Biophys Res Commun* **356**:361-367.

Zanivan S, Gnad F, Wickstrom S A, Geiger T, Macek B, Cox J, Fassler R and Mann M (2008) Solid tumor proteome and phosphoproteome analysis by high resolution mass spectrometry. *J Proteome Res* **7**:5314-5326.

Zhang W, Zheng S, Storz P and Min W (2005) Protein kinase D specifically mediates apoptosis signal-regulating kinase 1-JNK signaling induced by H₂O₂ but not tumor necrosis factor. *J Biol Chem* **280**:19036-19044.

Ziegler S, Eiseler T, Scholz R P, Beck A, Link G and Hausser A (2011) A novel protein kinase D phosphorylation site in the tumor suppressor rab interactor 1 is critical for coordination of cell migration. *Mol Biol Cell* **22**:570-580.

FOOTNOTES. This work was supported by the National Institutes of Health, National Heart, Lung, and Blood Institute [Grant HL77860].

LEGENDS FOR FIGURES

Figure 1: Domain structure and regulatory phosphorylation sites in PKD1. C1A/C1B, cysteine-rich Zn finger domains; PH, pleckstrin homology domain; Kinase, kinase domain. Numbering based upon the human PKD1 enzyme. See text.

Figure 2: PKD1 Activation Mechanisms. *Panel 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 cMyBP-C, CREB, HSP27, PAK4, c-Jun, Bit1 (Bcl-2 inhibitor of transcription, a mitochondrial protein that induces caspase-independent apoptosis), the F-actin-binding protein cortactin and 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 (Doppler et al., 2005; Spratley et al., 2011; Eiseler et al., 2009; Eiseler et al., 2010; Biliran et al., 2008; Peterburs et al., 2009; Ziegler et al., 2011; Barisic et al., 2011; Hurd et al., 2002; Lee et al., 2011). Panels B-C depict alternative mechanism for PKD1 regulation by reactive oxygen species (ROS) or caspase-3 in the setting of oxidative stress or apoptosis (see text).

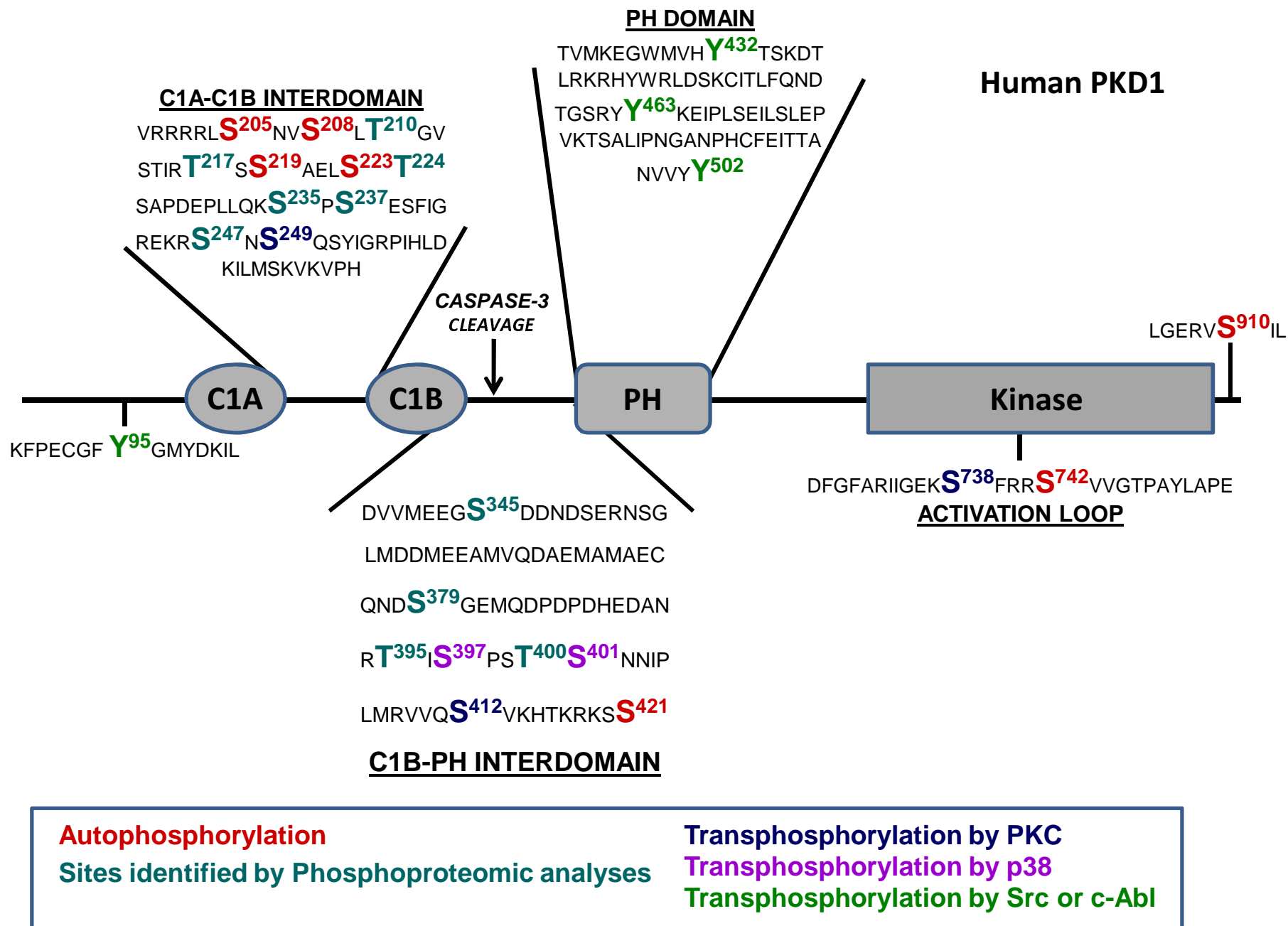


Figure 1

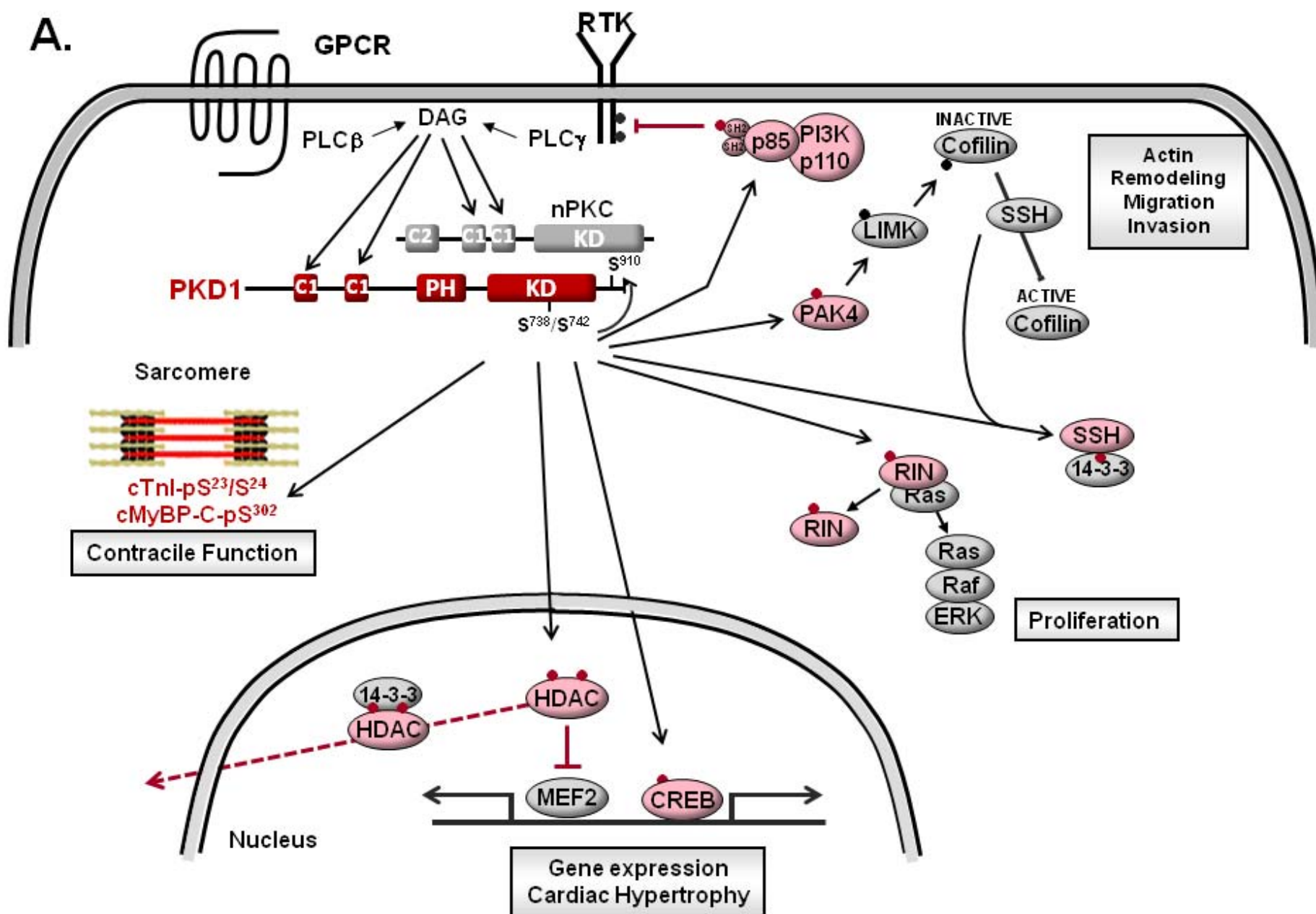
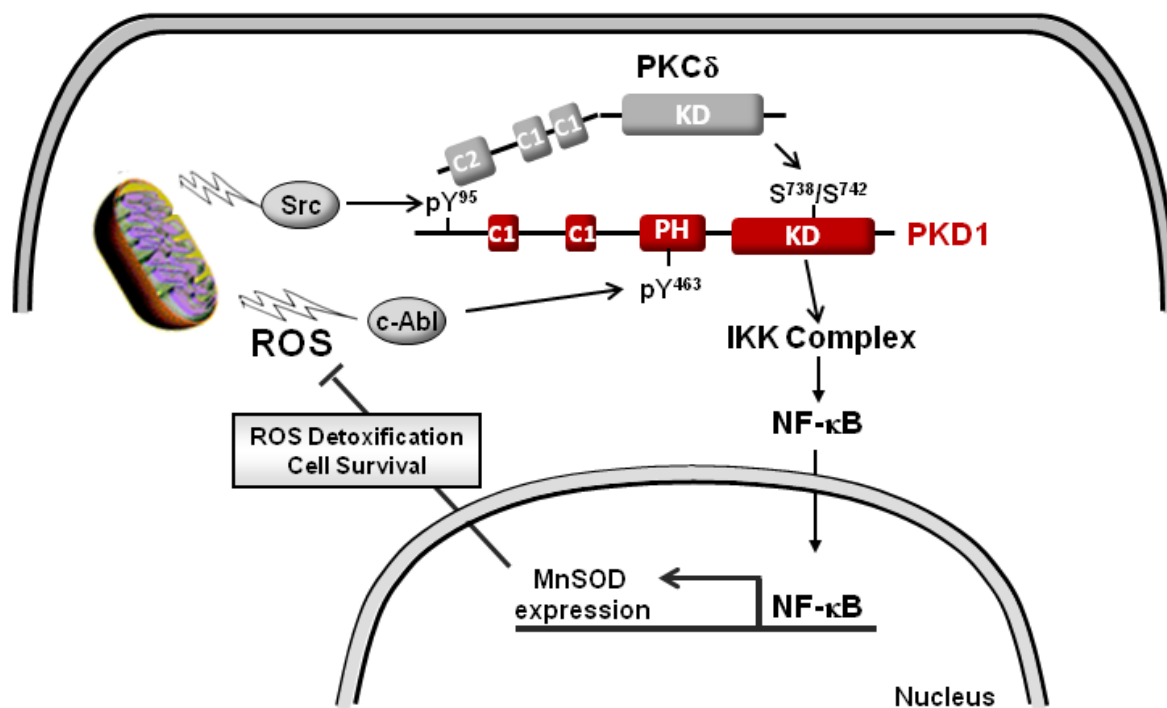
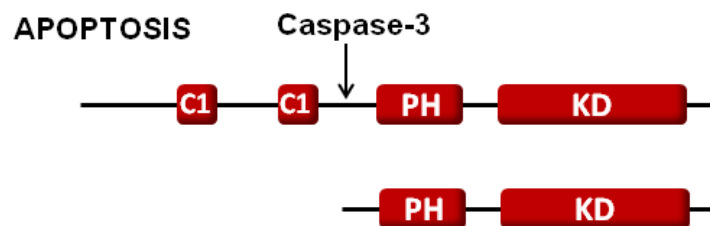


Figure 2

B.



C.



D.

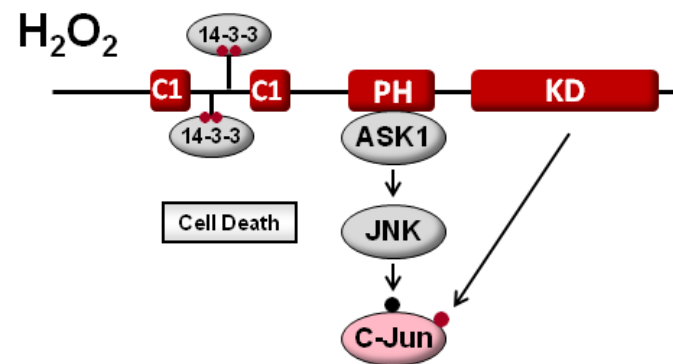
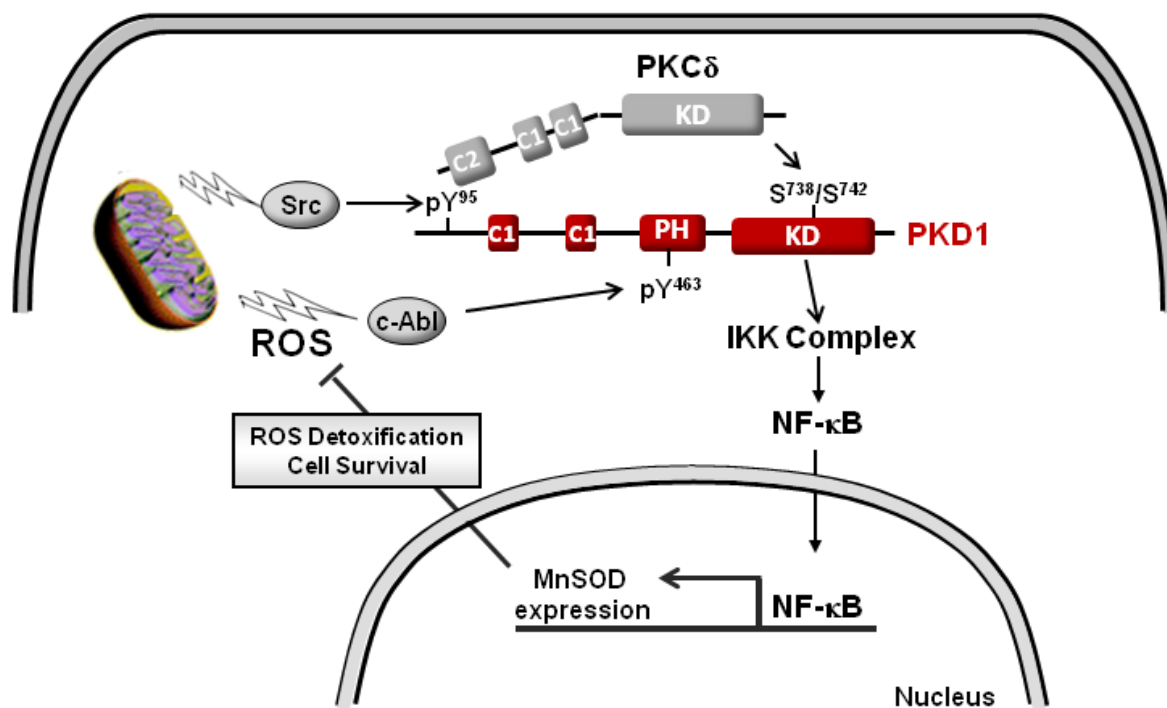
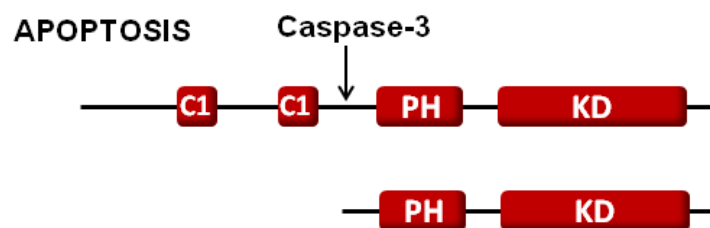


Figure 2

B.



C.



D.

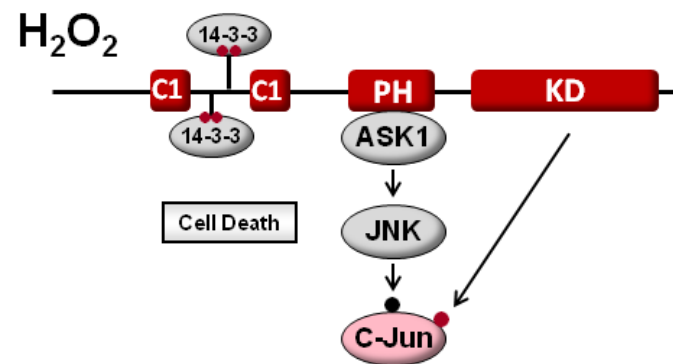


Figure 2