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SIGNALING THROUGH PROTEIN KINASE C

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1. ABSTRACT

Protein kinase C (PKC) comprises a large family of serine/threonine kinases which are activated by many extracellular signals. Inside the cell, PKCs are regulated by a variety of lipid second messengers, including the ubiquitous diacylglycerol and phosphatidylserine. Phosphorylation has also emerged as an important mechanism of regulation of all PKCs. Work in the last 20 years has provided evidence that these enzymes are involved in a multitude of physiological processes. Similarly, a number of proteins which are phosphorylated by PKCs have also been discovered and their role in cell biology has been investigated. More recently, there has been considerable interest in a number of specific PKC isoforms and their role in signaling pathways in the cell. This review will focus on recent findings on the mechanism of regulation of PKCepsilon, PKCmu and PKCzeta, and how these enzymes regulate cell growth, the actin cytoskeleton, apoptosis and other biological functions.

2. INTRODUCTION

Protein kinase C comprises a family of serine/threonine protein kinases which play a critical role in many signal-transducing pathways in the cell. Up to 12 distinct family members have been discovered in mammalian cells, and several non-mammalian PKCs have also been described. PKC has been implicated in a multitude of physiological functions in the cell, and considerable evidence exists which suggests that PKC plays a fundamental role in signaling mechanisms leading to mitogenesis and proliferation of cells, apoptosis, platelet activation, remodeling of the actin cytoskeleton, modulation of ion channels and secretion. PKCs can be considered as 'classical' mediators of many extracellular agonists which elicit the production of multiple lipid second messengers. Indeed the initial discovery of the enzyme as a calcium-activated enzyme, some 21 years ago by Nishizuka co-workers was immediately followed by the realization that PKC was the major target of the lipid metabolite diacylglycerol (DAG) (1,2). In addition, the observation that PKC is also the major receptor for tumor-promoting phorbol esters provided a key reagent for studying the mechanism of action of this enzyme (3). These initial findings heralded a new and important area of lipid signaling, which in the present day continues to provide evidence for an essential role of this enzyme in cell biology. In the first decade since its discovery, considerable progress was made in the understanding of the regulation of PKC by activators such as calcium, DAG and the co-factor phosphatidylserine (PS). The discovery that multiple isoforms of PKC exist in any one tissue or cell made for some interesting studies on the distribution and cellular localization of PKCs. It is only in the last decade that progress has been made on the role of PKC in signaling pathways and regulation of cell function. Many excellent reviews recently have addressed the regulation of PKC by multiple lipid mediators (4-6), as well as the complexity of the structure and function of the extended PKC family (7,8). In this review, we will focus on some recent, exciting observations concerning the regulation of the novel PKC family members and their role in signal transduction.

3. THE PKC SUPERFAMILY OF ENZYMES

cDNA cloning efforts over the past decade have revealed that as with many other signaling molecules, PKC comprises a large family of isoforms each with distinct

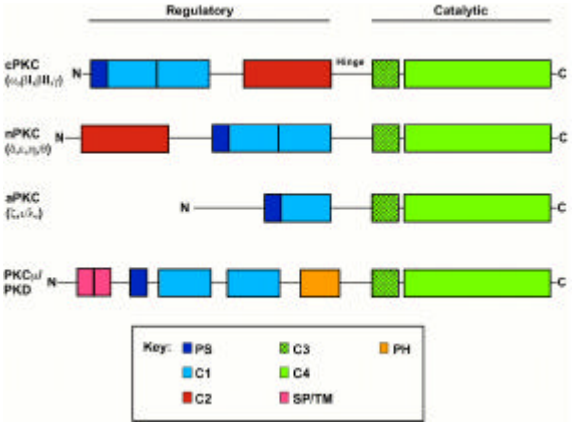

Figure 1. Schematic of the primary structure of the extended PKC family. A comparison of the primary structure of cPKCs (alpha, betaI, betaII and gamma), nPKCs (delta, epsilon, eta and theta), aPKCs (PKCzeta, iota/lambda) and PKCmu/PKD. Shown are the pseudosubstrate domain (PS), cysteine-rich region (C1), calcium-binding domain (C2), catalytic domain (C3 + C4), pleckstrin homology domain (PH) and the putative signal peptide (SP) and transmembrane (TM) domains in PKCmu/PKD.

properties. 12 distinct members have been discovered to date in mammalian cells, and several non-mammalian isoforms have also been found (7). The mammalian isoforms have been subdivided into three distinct sub-families as follows: conventional PKCs (cPKC) including alpha, betaI and the splice variant betaII, and gamma; novel PKCs (nPKC), delta, epsilon, eta and theta; atypical PKCs (aPKC) comprising zeta and lambda (also known as iota) (figure 1). An additional family may be considered by the more recently discovered PKCmu. This PKC was independently discovered by two laboratories, and is also known as protein kinase D (PKD) (9,10). Yet another more distantly related family includes the PKC-related kinases (PRK), PRK 1-3, which are also known as PKN (11). The different PKC sub-families were created partly due to primary sequence similarities between certain PKCs, as well as the activation profiles of each isoform. Figure 1 shows the primary structure of the three sub-families of PKC, as well as PKCmu/PKD. cPKCs alpha, beta I/II and gamma were the first described and are activated in vitro and in vivo by PS in a calcium-dependent manner. In addition, they bind to and are activated by DAG, which increases the specificity of the enzyme for PS and also increases the affinity of the enzyme for calcium (reviewed in (12)). Novel PKCs are also activated by DAG and require PS as a co-factor, but have lost the requirement for calcium. As discussed below, this may partly be due to the absence of a classical C2 domain. Both the atypical PKCs zeta and lambda/iota as well as PKCmu/PKD do not respond to either DAG or calcium, though apparently still require PS as a cofactor. The regulation of various PKCs by multiple lipid second messengers is further discussed below.

4. REGULATION OF PKC

4.1. Multiple lipid mediators

Multiple lipid second messengers have been shown to activate PKC family members both in vitro and in vivo. Perhaps the most studied and best understood mechanism of activation is that of the conventional PKCs. These enzymes have a number of regulatory and membrane-targeting domains, some of which are also found in other PKCs and other unrelated protein kinases. All PKCs have an N-terminal pseudosubstrate domain which confers auto-inhibition in the native enzyme (figure 1). This module closely resembles the optimal substrate recognition motif of the PKC except that the serine/threonine is replaced by an alanine. The pseudosubstrate domain binds to the substrate-binding pocket in the inactive molecule. Agonist-stimulated activation of phospholipases type C (PLC; beta, gamma and delta subtypes) results in the hydrolysis of membrane inositol phospholipids. This leads to the generation of 1,2-sn-diacylglycerol (DAG) and soluble inositol phosphates (e.g., InsP, Ins-1,4-P2 or Ins-1,4,5-P3). The latter are responsible for stimulating release of calcium from intracellular stores. Generation of DAG or addition of exogenous phorbol esters (PMA (phorbol 12-myristate 13-acetate), also known as TPA), which bind to the C1 domain, relieves autoinhibition leading to activation and phosphorylation of relevant substrates. The cPKC C1 domain comprises a cysteine-rich motif, capable of binding two zinc ions. Despite this, the DAG/PMA - PKC interaction has a stoichiometry of 1:1 (13,14). Binding of the co-factor phosphatidyl-L-serine (PS) to the regulatory domain is also required to promote the conformational change which results in removal of the pseudosubstrate from the catalytic site. In the case of cPKCs, binding of calcium greatly increases the affinity of the enzyme for acidic phospholipids such as PS (12). However, an isolated C2 domain from PKCbetaII has been shown not to bind to PS in a calcium-dependent manner (15). C2 domains are also found in other signaling proteins such as synaptotagmin, phospholipases and GTPase activating proteins (GAPs). Thus, calcium/PS synergize with DAG or PMA to promote a remarkably high affinity interaction of PKCs with membrane (1.5x10-5 mol% for PMA and two orders of magnitude lower for DAG). The precise binding site for phosphatidylserine on PKCs remains elusive. Novel PKCs such as delta and epsilon also require DAG and PS for activation, but have lost the requirement for calcium. Although the classical C2 domain is absent in this family, recently, a C2-like domain has been identified in novel PKCs at the very N-terminus of the enzyme (16) (figure 1), though it is not clear whether this domain is capable of binding to phospholipids in a calcium-dependent manner. One possibility is that the C2-like domain of nPKCs exists in a pre-existing conformation similar to that found in the C2-calcium complex in cPKCs. Atypical PKCs only have one C1 domain and are not activated by either DAG or PMA. Similarly, they do not have a C2 domain and thus are unresponsive to calcium. Whether the isolated C1 domain can act as a membrane-targeting module remains to be established, although this idea is supported by the finding that a PKCdelta mutant with only

Figure 2. Alignment of phosphorylation sites of mammalian PKCs. Shown is an alignment of 11 mammalian PKC isoforms to highlight the homology surrounding the activation loop threonine (top, in red), and the two autophosphorylation sites in the catalytic domain (middle and bottom, in green). The number of the first amino acid in the sequence is shown left, and the position of the phosphorylated Thr or Ser is shown right. Also shown is substitution of a glutamate residue (highlighted in pink) at the second autophosphorylation motif in PKCzeta and PKClambda.

the second C1 domain is efficiently translocated in vivo in response to PMA (17).

Several other lipid second messengers and mediators have been shown to either activate PKCs both in vitro and in vivo. In particular, the phosphoinositide 3-kinase (PI 3-K) products PtdIns-3,4-P2 and PtdIns-3,4-5-P3 have been shown to activate both the novel (delta, epsilon and eta) and the atypical (zeta) PKCs in vitro (18-21). One problem interpreting the mechanism of activation of PKCs by these lipids is that some discrepancy exists concerning which PKCs are activated by these polyphosphoinositides. The reason for this is perhaps the use of different PKC and lipid preparations for these in vitro studies. However, as discussed later considerable evidence now exists that certain PKCs are activated downstream of PI 3-K in vivo. The domain or residues responsible for the phosphoinositide-PKC interaction have yet to be identified. It is possible that these highly negatively charged lipids may mimic PS, thus increasing the affinity for PKC with the membrane. Other lipid mediators which have been shown to activate PKCs include several cis-unsaturated fatty-acids, most notably arachidonic acid (for a detailed review, see (5)). These lipids activate PKC by potentiating the effects of DAG at basal calcium concentrations and are inactive towards PKC if either DAG or PMA are absent. Similarly, both lyso-phosphatidic acid (LPA) and lyso-phosphatidylcholine (lysoPC) have been shown to activate PKC, again in the presence of DAG. In addition, ceramide, which is produced upon tumor necrosis factor (TNF-alpha) stimulation of cells, activates and binds to both the atypical PKCzeta family member (22) and to the related Raf-1 kinase (23) in vivo. Although the relevance of these lipid mediators in the activation of PKCs is largely unexplored, it is possible that they serve as membrane anchors allowing for efficient localization of the enzyme in proximity to other upstream regulators. Although this notion requires additional investigation, it is clear that there exists considerable heterogeneity in the lipid-dependent regulation of PKCs. This regulation is further complicated by the role of phosphorylation in the activation of PKC.

4.2 Phosphorylation

After the initial discovery of PKC as a lipid-regulated enzyme, it took some 10 years for workers in the field to appreciate that phosphorylation of the enzyme plays a critical role in its activation (24). Considerable information concerning phosphorylation sites on PKCs and their role in regulation of the enzyme now exists. Three key residues in the catalytic domain (C4) have been mapped, and these are likely to represent the major in vivo phosphorylation sites. Figure 2 shows an alignment of these three sites in 11 mammalian isoforms. A comprehensive analysis of PKC phosphorylation has been carried out for PKCbetaII, and has demonstrated that the initial, rate-limiting step is phosphorylation of the activation loop Thr500 (25). Similar observations have been made for PKCalpha (26). This Thr residue, and the motif surrounding it, is not only highly conserved in all PKCs (figure 2), but is also found in a large number of other protein kinases and phosphorylation of this site is usually required for protein kinase activity. Substitution of PKCbetaII Thr500 to an Ala residue results in an inactivatable enzyme, whereas mutation to an acidic Glu residue mimics the effect of phosphate resulting in a cofactor activatable enzyme (27). A recent study of the activation loop threonine of both novel (epsilon) and atypical (zeta) PKCs has shown that phosphorylation of the activation loop threonine is a conserved mechanism for these enzymes, and that substitution of this residue to an alanine confers dominant negative properties in vivo (28). The PKC activation loop threonine cannot undergo autophosphorylation, and it has been postulated that a PKC upstream kinase exists which carries out this reaction. Recent studies from this laboratory and the Parker laboratory have provided evidence that the phosphoinositide-dependent kinase-1 (PDK-1) is the PKC-upstream kinase (29,30). PDK-1 has been shown to phosphorylate the equivalent activation loop Thr of both the Akt/PKB protein kinase as well as the p70S6-kinase, leading to activation of the enzymes (reviewed in (31)). PKCzeta and PKCdelta are also phosphorylated at the corresponding Thr410 at Thr505 residues, and this is sufficient to activate the PKCs both in vitro and in vivo (29,30). In stimulated cells, PDK-1 binds with high affinity and specificity to the PI 3-K lipids PtdIns-3,4-P2 and PtdIns-3,4-5-P3, resulting in translocation from the cytosol to the membrane (32). Interestingly, both phosphorylation

and activation of PKCzeta in vitro requires PtdIns-3,4-5-P3, providing additional evidence for a role of this PKC in PI 3-K signaling pathways (29). Similarly, optimal phosphorylation and activation of PKCdelta in vitro requires PDK-1, PtdIns-3,4,5-P3 and PMA (30). Consistent with the notion that PKCs are enzymes which are translocated to the plasma membrane as part of their regulation, an artificially membrane-targeted PKCzeta mutant is constitutively active in vivo. As the motif surrounding the PKCzeta activation loop Thr is highly conserved in all PKC family members (figure 2), it is likely that PDK-1 is the universal PKC upstream kinase, though this has not been formally tested. Modeling studies have indicated that the activation loop Thr is masked in the inactive molecule by the binding of the pseudosubstrate domain to the active site (12). Thus it is likely that binding of DAG (and other lipids) which relieves this inhibition also uncovers the Thr residue allowing phosphorylation and activation to occur.

Once PKCs are phosphorylated at the activation loop threonine, two additional phosphorylations occur in the catalytic domain. In the case of PKCbetaII, these have been shown to be due to autophosphorylation, rather than phosphorylation by an upstream kinase (25). In the case of PKCbetaII, autophosphorylation of Thr641 precedes that of Ser660. Both of these residues are also conserved in all mammalian PKCs except for PKCmu (figure 2). Interestingly, in PKCzeta and PKClambda, the second C-terminal residue is substituted to an acidic Glu residue (figure 2). As the negative charge on the Glu is likely to infer the same net effect as phosphorylation, this naturally occurring substitution may account for the high basal activity of these PKCs in vivo. It is also worth noting that although the C-terminal sites in PKCbetaII are autophosphorylation sites, this may not be the case for all PKCs. The motif surrounding the second C-terminal residue (Ser729 in PKCepsilon and Ser664 in PKCdelta) is very similar to the corresponding motif in other protein kinases such as Akt/PKB and p70S6-kinase (33). In the case of Akt/PKB, this has been shown to be phosphorylated by a putative second upstream kinase, tentatively named PDK-2 (34). The identity of this enzyme has not yet been established, though it will clearly be interesting to see if it can also phosphorylate the equivalent residue in PKCs.

One unresolved issue in the field is the role played by agonist-stimulated signaling in the regulation of PKC. Clearly, generation of the lipid second messenger DAG is an agonist-dependent event which stimulates the recruitment of inactive PKC from the cytosol to the membrane where activation occurs. The role of 'signaling' in the regulation of PKC by phosphorylation is less clear. As PDK-1 is an agonist-regulated enzyme which is only translocated to the plasma membrane following stimulation with growth factors (e.g., PDGF) or hormones (e.g., insulin), then one might expect that its substrates would also be phosphorylated in an agonist-dependent manner. However, in the case of conventional PKCs (e.g., betaII), phosphorylation of the activation loop threonine appears to be constitutive, such that at least 50% of the native molecule in unstimulated cells is phosphorylated at this residue (25). In contrast, atypical PKCs such as PKCzeta, as well as Akt/PKB are phosphorylated at this residue by PDK-1 in a mitogen-dependent manner (29). PDK-1 is a constitutively active enzyme and its intrinsic protein kinase activity does not appear to change upon agonist stimulation (35), such that accessibility and phosphorylation of relevant substrates is determined only by localization within the cell. One resolution to these apparent contradictions is that the precise mechanism of regulation of one PKC isoform may not apply to others. Thus, PKCalpha and/or betaII may be constitutively phosphorylated by PDK-1 in unstimulated cells, whereas PKCzeta may only be phosphorylated upon stimulation of cells with agonists which recruit PKC and PDK-1 to the plasma membrane. Clearly more work in this area is required to resolve these issues.

4.3. Structure

Valuable information on PKC regulation has been gained from the crystal structure of the isolated C1 domain of PKCdelta, with and without phorbol ester bound (36). This revealed a globular structure comprising two beta-sheets which form the ligand-binding cavity. Surprisingly, binding of phorbol ester to the C1 domain does not induce significant conformational change, but allows for membrane targeting by altering the hydrophobicity of the surface of the C1 domain, promoting hydrophobic interactions. Closer examination of this structure and comparison with other C1 domains in PKCs which do not bind phorbol ester, or the Raf-1 kinase, has provided an explanation why these enzymes do not respond to PMA, as they lack the residues necessary to form the hydrophobic surface at the ligand-binding site (37).

Although there is as yet no structure of the C2 domain of PKCs, the crystal structures of the homologous C2 domains of synaptotagmin (38) and phospholipase C (39) have been solved. The structures have revealed a domain rich in beta-sheets which form a novel calcium-binding pocket which co-ordinates two metal ions. Divalent ion binding induces a conformational change which exposes lysine residues on the anterior face of the C2 domain. As with C1 domains, PKCs which do not respond to calcium lack the necessary residues which co-ordinate metal ions. Similarly, although the catalytic kinase core of PKCs has not been solved at the structural level, the high similarity of the PKC kinase domain with that of protein kinase A (PKA) has enabled modeling studies for both PKCbetaII (40) and PKCalpha (41). These studies have revealed that the autoinhibitory pseudosubstrate domain interacts with the kinase core via electrostatic interaction with acidic residues. Similarly, a model of the structure of catalytic kinase core of PKCzeta is shown in figure 3. Highlighted is the activation loop which lies between the two lobes of the kinase core, and shown is the Thr410 residue which is phosphorylated by PDK-1. Although these modeling studies have provided useful information, clearly the crystal structure of the full length kinase with and without the necessary phosphorylation sites is necessary for a complete understanding of the regulation of PKC.

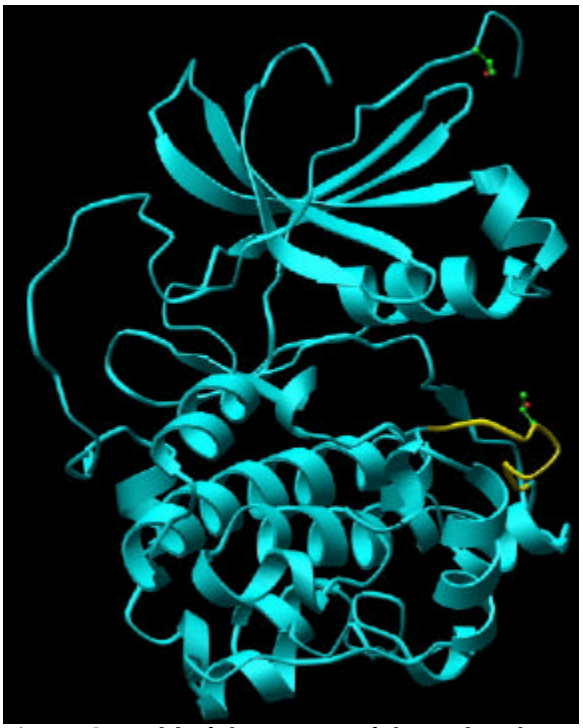

Figure 3. Model of the structure of the catalytic kinase core of PKCzeta. A model of the structure of the kinase domain of PKCzeta is shown. The structure was obtained by threading the amino acid sequence of PKCzeta through the PredictProtein program at European Molecular Biology Laboratory (EMBL: http://www.embl-heidelberg.de/predictprotein/). The pdb co-ordinates obtained were used for modeling the structure on a Silicon Graphics Octane Workstation. Highlighted in yellow is the activation loop which interfaces the two lobes of the kinase core. In green is the activation loop threonine 410, and in red is the hydroxyl group which serves as the phospho acceptor. Also shown at the top in green is the putative autophosphorylation site Thr563. Glu579 is not shown in this structure.

5. SIGNALING THROUGH PKC

5.1. Substrate specificity

A large number of proteins have been shown to be phosphorylated either in vitro or in vivo by PKC. Based on these data as well as studies using synthetic peptides, a basic consensus PKC phosphorylation motif was originally determined, as follows: RXXS/TXRX, where X indicates any amino acid. These studies underscored the importance of basic residues N- and C-terminal to the phosphoacceptor residue. Similarly, a large number of 'artificial' substrates of PKC are also known and used for activity measurements, such as myelin basic protein, various histones, protamine and protamine sulfate. Recently, and oriented peptide library approach was used to determine the optimal substrate selectivity of nine PKC family members (42). The results confirmed the requirement for basic residues surrounding the Thr or Ser, and indicated that although the optimal phosphorylation for each PKC is similar, there are some subtle differences between family members, particularly between conventional, novel and atypical PKCs. All PKCs prefer basic residues at positions -6, -4 and -2 to the Ser/Thr. cPKCs also preferred basic residues at +2, +3 and +4, whereas nPKC and aPKCs preferred hydrophobic residues at these positions. The only deviation to this selectivity was PKCmu, which had a strikingly distinct optimal motif from all other PKCs, with a strong selectivity for Leu at the -5 position (42). This approach has enabled database searches for potential PKC substrates, as well as providing an explanation for the substrate selectivity of certain PKCs over other family members. For example, the MARCKS protein (myristoylated alanine-rich C-kinase substrate), a ubiquitous PKC substrate in all cells, is phosphorylated by all PKCs except for PKCzeta (43). Conversely, pleckstrin (platelet and leukocyte C-kinase substrate protein), a PKC substrate found in cells of hematopoietic origin appears to be phosphorylated by all PKCs (44). The heterogeneous ribonucleo-protein A1 (hnRNP A1) is efficiently phosphorylated by PKCzeta but not other PKCs (45), whereas elongation factor eEF-1alpha is a good PKCdelta substrate (46). Similarly, cPKCs and nPKCs efficiently phosphorylate GAP-43 (growth-associated protein of 43kDa, also known as neuromodulin), whereas PKCzeta does not (47). Despite these subtle differences, it is clear that all PKCs generally prefer to phosphorylate very similar sequences, suggesting that other mechanisms must exist to discriminate which particular PKC isoform participates in signaling mechanisms. These include regulation by different cofactors as discussed above, and importantly, proper cellular localization. The latter is achieved by a number of anchoring proteins which specifically bind to activated PKC, hence the name RACKs (receptors for activated C-kinase) (48,49). Binding of activated PKCs to RACKS may ensure appropriate access to substrates. In addition, AKAPs (A-kinase anchoring protein) have also been shown to bind to PKCs as well as other related protein kinases (50). Finally, a PKCzeta interacting protein, named ZIP (zeta-interacting protein) has been described, which specifically interacts with the regulatory domain of PKCzeta but not other PKCs and may act as a scaffolding protein for this isoform (51). A PKClambda interacting protein (LIP) has also been described which specifically interacts with this PKC (52). Regulation of PKC by RACKS and related proteins has been extensively reviewed in Refs. (53,54).

5.2. PKCepsilon

PKCepsilon is a novel PKC family member which responds to both DAG and PS in vivo. In addition, PKCepsilon is also activated by the PI 3-K lipids PtdIns-3,4-P2 and PtdIns-3,4-5-P3 in vitro (19,55). Consistent with these observations, studies with PDGF-receptor mutants have indicated that in vivo, PKCepsilon can be activated by both PLC-gamma and PI 3-K-dependent pathways (55). Whether both proteins and their lipid products are required for maximal activation of PKCepsilon in vivo is still not clear. Ohno et al. were the first to demonstrate that both PKCepsilon and PKCdelta could be activated by mitogenic stimuli in vivo, as judged by hyperphosphorylation shift and in vivo 32P-labelling of the PKCs, as well as enhancement of transcriptional

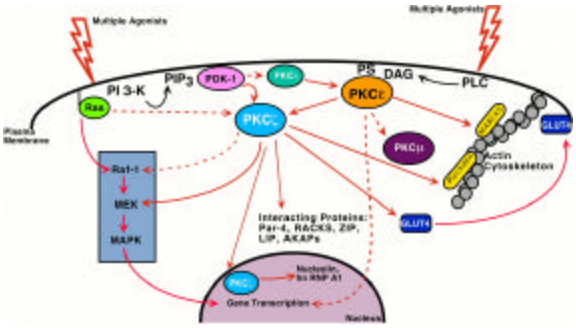

Figure 4. Signaling through PKCs. PKCepsilon, PKClambda, PKCzeta and PKCmu can be activated by multiple lipid-signaling pathways, including PI 3-K and PLC. The PDK-1 protein kinase may be the universal PKC upstream kinase. Once activated, PKCs regulate multiple physiological processes. Both PKCepsilon and PKCzeta are involved in mitogenic signal transduction, and it is likely that they exert their effects on gene transcription, at least in part, by activating the MAPK pathway. Both enzymes are also implicated in insulin-stimulated signaling, and in the case of PKCzeta, may regulate glucose uptake through the glucose transporter GLUT4. PKCzeta is also found in the nucleus, and a number of PKCzeta-specific nuclear substrates have been identified (nucleolin, hn RNP A1). In the cytosol, PKCs interact with several different proteins including RACKS (receptor for activated C kinase), the product of the par-4 gene, ZIP (zeta-interacting protein), LIP (lambda interacting protein) and AKAPs (A kinase-anchoring protein). PKCs are also involved in remodeling of the actin cytoskeleton, partly by phosphorylating specific PKC substrates such as the MARCKS protein and pleckstrin. Thus, PKCs influence many distinct aspects of cell function. One exciting possibility is that there exists a hierarchical PKC cascade in which one PKC isoform activates another (e.g., PKClambda → PKCepsilon → PKCzeta, and PKCepsilon → PKCmu). Whether this is a general phenomenon, or whether this is restricted to these isoforms, remains to be seen.

activation through PMA- and serum-response elements (56). At about the same time, two independent studies demonstrated that PKCepsilon can function as an oncogene when overexpressed in fibroblasts (57,58). Increased PKCepsilon activity in these cells correlated with formation of dense foci in monolayer cultures, decreased doubling times, increased cell saturation densities decreased serum requirements, growth in soft agar and tumor formation in nude mice, all characteristics of neoplastic transformation. More recent studies from the same laboratories has indicated that PKCepsilon may function as an oncogene by enhancing the activity of the Raf-1 kinase, and thus modulate the MAPK pathway (59). Evidence has also been presented that the oncogenic potential of PKCepsilon is due in part to the production of autocrine growth factors, in particular transforming growth factor-beta 2 and 3 (TGF-beta) (60,61). The oncogenic potential of PKCepsilon has also been observed in rat colonic epithelial cells, where overexpression leads to anchorage-independent colony formation in soft agar and formation of tumors in nude mice (62,63). Furthermore, PKCepsilon is required for initiating DNA synthesis in mouse erythroleukemia cells stimulated with erythropoietin, leading to the up-regulation of c-Myc (64). There is evidence that PKCepsilon can directly activate Raf-1 in vitro and in vivo (65,66), and this may be a mechanism for mediating the oncogenicity of this PKC family member. It is worth stating however, that there are reports to the contrary; in particular, Ueda et al. have reported that PKCepsilon cannot stimulate activation of the MAPK pathway (67). However, it is likely that PKCepsilon does regulate the MAPK pathway, either directly or indirectly (figure 4). One interesting possibility is that the atypical PKC isoform PKCzeta may actually be regulated by PKCepsilon and lead to activation of MAPK, as discussed below. Whether there are additional yet-to-be discovered mechanisms by which PKCepsilon regulates cell growth and transformation remains to be seen.

PKCepsilon also participates in nerve growth factor (NGF) signaling. PKCepsilon is activated following NGF-stimulation of the PC12 rat pheochromocytoma cell line (68). Overexpression of PKCepsilon in the same cells induces both neurite extension as well as activation of MAPK in an NGF-dependent manner (69,70). Similar results have been obtained in human neuroblastoma cells (71,72), and PKCepsilon has been found to be enriched at the growth cones of extending neurites in differentiating neural cells (71). It therefore appears that PKCepsilon regulates certain aspects of neural function and this is consistent with the fact that this PKC isoform is very abundant in brain and other neural tissues.

PKCepsilon has also been linked to the expression of certain transcription factors, and the induction of immediate-early genes. This may account, at least in part, for its effects on cell growth. PKCepsilon has been shown to induce the accumulation of both c-fos and c-Jun mRNAs, and this is dependent on the activation of high-affinity IgE receptors (73). Moreover, PKCepsilon has been shown to regulate the transcription factors NF-AT-1 and AP-1 in activated T cells, similar to the stimulatory effects of activated Ras in the same system (74). In the same study, PKCepsilon also induced the activity of NF-kappa B, and this was not observed with conventional PKCalpha. As discussed below, PKCzeta has also been shown to regulate similar transcription factors.

PKCs are known to be important regulators of the cytoskeleton in cells. In particular, certain PKC substrates such as the MARCKS protein as well as pleckstrin are known to associate with the cytoskeleton in a PKC-dependent manner (44,75,76). Although little is known about the PKC isoform specificity of these events, recent data indicates that PKCepsilon plays an important role in cytoskeletal organization (figure 4). Firstly, an actin-binding motif that is unique to PKCepsilon has been identified, and it has been proposed that this serves to localize the active PKC within intact nerve terminals (77). A separate and distinct actin-binding motif has also been identified in the PKCbetaII isoform (78), and PKCzeta has also been shown to interact with actin in the presence of

Zn^{2+} (79). PKCepsilon translocates to the membrane fraction when HeLa cells are plated an a gelatin substratum, but not when cells are plated on plastic (80). In addition, inhibition of PKCepsilon with antisense oligodinucleotides inhibits cell spreading on a fibronectin matrix (81). In the same study, PKCepsilon was seen to translocate to focal adhesions following integrin activation. Thus, PKCepsilon may be involved in the regulation of adhesion of cells to the extracellular matrix. Finally, PKCepsilon has been shown to mediate smooth muscle cell contractility on a Ca2+-independent manner, and to phosphorylate the smooth muscle cells proteins calponin and caldesmon in vitro (82). For an extensive review on the role of PKCs in cytoskeletal function, see ref. (83).

A number of reports have suggested that stimulation of a variety of cell types with insulin leads to activation and membrane translocation of PKCepsilon. Insulin activates PKCepsilon in fetal chick neurons (84), and there is evidence that this activation is concomitant with a translocation of PKCepsilon from the cytosol to the plasma membrane (85,86). These results are somewhat unexpected as insulin stimulation of cells is not thought to activate PLC-gamma, leading to generation of DAG. In addition, a careful analysis of all PKC isoforms in PMA-downregulated and insulin-stimulated cells has indicated that PMA-responsive PKCs (including PKCepsilon) do not lead to expression of c-fos (87). One way to rationalize these contrasting studies is that activation of PKCepsilon in insulin-stimulated cells may occur through PI 3-K, which is a central mediator of many of insulin's physiological functions. Alternatively, a recent study indicated that PLC-gamma is in fact activated following insulin stimulation of 3T3-L1 adipocytes, by coupling directly to the insulin-receptor, and not to IRS-1 (insulin-receptor substrate 1) (88). What specific role(s) PKCepsilon plays in insulin-stimulated signaling remains to be seen.

5.3. PKCzeta and PKClambda

Over the last five years, evidence has been accumulating which places PKCzeta, and its close relative PKClambda, as critical mediators of mitogenic signal transduction. Much of the current interest in PKCzeta signaling stems from the observation by Berra et al. which demonstrated that this PKC isoform is required for maturation of Xenopus oocytes, and for DNA synthesis in fibroblasts as a consequence of Ras activation (89). This was one of the first indications that PKCzeta participates in mitogenic signaling. At about the same time, Nakanishi and Exton reported that PKCzeta could be activated in vitro by the lipid product of PI 3-K, PtdIns-3,4-5-P3 (90). Consistent with these initial observations, it was subsequently reported that the related PKClambda isoform could be activated in PDGF- or EGF-stimulated fibroblasts in a PI 3-K-dependent manner (91). Activation of PKCzeta by PtdIns-3,4-5-P3 in vitro has also been reported by other laboratories (92,93). As discussed above, the PDK-1 enzyme and PtdIns-3,4-5-P3 synergize to activate PKCzeta both in vitro and in vivo. Therefore, there appears to be conclusive evidence placing PKCzeta and PKClambda downstream of receptor-tyrosine kinases, and more specifically, downstream of PI 3-K. What has emerged more recently is the role played by these PKCs in mediating mitogenic signaling.

After the initial proposal that PKCzeta participates in mitogenic signaling via the Ras pathway, two reports presented evidence for a role for PKCzeta in activation of an NF-kappa B-like activity in Xenopus oocytes and mouse fibroblasts (94,95). More recently, it was also shown that expression of the small t antigen of SV40 leads to inhibition of protein phosphatase 2A (PP2A), which in turns leads to constitutive activation of PKCzeta (96). The net outcome was activation of NF-kappa B-dependent gene transcription and cell proliferation. In addition, PKCzeta associates with and appears to directly phosphorylate a novel I kappa B kinase activity capable of modulating I kappa B-alpha function in vivo (97). A role for PKCzeta as a Ras effector has also been proposed based on the direct interaction of GTP-loaded Ras and PKCzeta in vitro, as well as association of Ras with PKCzeta in vivo (98). An attractive model which takes these data into account is that Ras.GTP efficiently localizes PKCzeta at the membrane, where it is regulated by PtdIns-3,4-5-P3 and PDK-1. However, contradictory results have been reported in which a direct interaction between PKCzeta and Ras was not detected (99), and in a separate study no evidence for a link between Ras signaling and PKCzeta activation leading to oocyte maturation was found (100). Similarly, activation of the mitogen-activated protein kinase (MAPK) by exogenously added PC-specific phospholipase C (PC-PLC) is mediated by PKCzeta, but in a Ras-independent manner (101). Thus more work is required to establish whether PKCzeta can be considered as a true Ras effector. In addition, a number of studies have provided conflicting evidence as to the role of PKCzeta in cell transformation and proliferation. Firstly, Montaner et al. were unable to detect any differences in the growth properties of NIH 3T3 fibroblasts transfected with wild-type PKCzeta or any differences in the regulation of NF-kappa B (102), as originally reported (94). Moreover, PKCzeta has been reported to actually suppress the neoplastic transformation of fibroblasts mediated by the v-raf oncogene (103). In this study, PKCzeta antagonized cell transformation and transformation, possibly by modulating the expression of the transcription factors junB and egr-1. Thus, PKCzeta has been reported to possess both growth-promoting and growth-inhibitory properties. It is difficult to rationalize how this is possible, and so one is left with the somewhat unsatisfactory explanation that these contradictory results are due to differences in the cell types and experimental assays used. One potential resolution to this problem comes from a very recent study which suggested that the concerted action of a hierarchical signaling cascade involving PKClambda, PKCepsilon and PKCzeta (in that order) is required to mediate transcriptional activation of the c-fos promoter in cells expressing oncogenic Ha-ras (104). Previous studies have not taken into account this novel idea of a hierarchy of PKC signaling involving multiple isoforms, although an analogous model has been proposed for the regulation of PKCmu / PKD (see below, and figure 4). Thus, the weight of evidence currently is in favor of PKCzeta as an important element in mitogenic signal transduction, though

more work will help to clarify some of the apparent contradictions.

How could PKCzeta regulate transcriptional activation leading to mitogenesis? A large body of literature suggests that activation of the MAPK pathway is downstream of PKCzeta. One of the first reports providing evidence for a link between PKCzeta and the MAPK pathway made use of an activated PKCzeta allele which in co-transfection experiments stimulated the activation of both MAPK-kinase (MEK) and MAPK (105). Conversely, a dominant negative PKCzeta mutant suppressed activation of MEK and MAPK by serum and tumor necrosis factor (TNF alpha). Similarly, a role for PKCzeta in activation of MAPK in angiotensin II-stimulated vascular smooth muscle cells has also been proposed (106). Subsequent studies demonstrated that activation of MAPK is not restricted to PKCzeta, but that other PKC family members, including cPKCs are also capable of activating MAPK in vivo. Two more recent studies have extensively addressed the role of several PKC isoforms in the activation of Raf-1, MEK and MAPK in cells (65,66). Cai et al. have shown that both PKCalpha and PKCepsilon activate Raf-1 in vivo, as discussed above (66). In contrast, whereas PKCalpha and PKCeta are potent activators of Raf-1, PKCzeta was not able to activate Raf-1 (65), in agreement with a previous report which failed to detect activation or phosphorylation in vitro (107). However, in the same study PKCzeta was a potent activator of MEK, and consequently MAPK, indicating that PKC isotypes differ in their ability to activate MAPK. It remains to be established by what mechanism PKCzeta, and indeed other PKCs, activate Raf-1/MEK/MAPK, whether there is a direct phosphorylation event, as proposed by one study (66), or whether an intermediary step(s) is required, as suggested by another (65). Activation of MAPK by PI 3-K has also been reported, though this appears to be dependent on the cell type and stimulus used (108). Therefore, one attractive hypothesis is that this activation is mediated by PKCzeta, which as discussed above has been shown to be downstream of PI 3-K and upstream of MAPK. Understanding the mechanism of MAPK regulation by PKCs is in part confounded by the complexity of the activation of Raf-1.

PKCzeta also appears to participate in nuclear signaling pathway. Stimulation of PC12 cells with nerve growth factor stimulates neurite extension which has been shown to require the activity of PKCzeta (109), and this is correlates with a translocation of the enzyme from the cytosol to the nucleus (110). More recently, the nuclear protein nucelolin was found shown to be phosphorylated by PKCzeta both in vitro and in vivo in an NGF-dependent manner (111). This may provide a link between cell surface receptors, PKCzeta and nuclear signaling (figure 4).

There is also evidence that atypical PKCs participate in apoptosis and cell survival mechanisms. PKCzeta has been shown to specifically interact with the product of par-4, a gene which is induced during apoptosis (112). Par-4 both interacts with and potently inhibits the protein kinase activity of PKCzeta and PKClambda/iota, supporting a role for these PKCs in the control of cell growth and survival mechanisms. PKCiota (also known as PKClambda) has been shown to protect cells against drug-induced apoptosis, implicating this PKC in cell survival (113). Thus, positive and negative regulation of PKCzeta/lambda activity can have profound effects on the fate of a cell.

Finally, PKCzeta is also an important mediator of some of the physiological actions of insulin. Stimulation of 3T3 L1 adipocytes with insulin results in the activation of PKCzeta, as well as other PKCs (114). Activation of PKCzeta by insulin is dependent on PI 3-K (92,115), consistent with results obtained EGF and PDGF-stimulated cells. Over-expression of PKCzeta also leads to an increase in insulin-stimulated glucose transport (116). A subsequent study demonstrated that insulin-stimulated glucose uptake is mediated by GLUT4 acting downstream of PKCzeta (92). GLUT4 is the major glucose transporter in insulin-responsive tissues. Thus, PKCzeta may regulate glucose homeostasis in part by stimulating GLUT4 translocation, though clearly multiple mechanisms exist which regulate the localization of GLUT4 (figure 4). PKCzeta is also required for insulin-stimulated protein synthesis, and this does not appear act to through the transcriptional regulator p70S6-kinase (115). Again, the targets downstream of PKCzeta which are responsible for the regulation of protein synthesis are not known. Activation of PKCzeta downstream of PI 3-K is also required for macrophage differentiation in insulin-like growth factor-I (IGF-I) stimulated cells (117). Thus, PKCzeta is emerging as an important signal-transducing molecule which mediates some of the physiological effects of mitogens, hormones and agonists which induce programmed cell death. Unraveling the mechanisms and targets by which this PKC isoform elicits these responses is clearly a priority in the field.

5.4. PKCmu / PKD

Although PKCmu (also known as PKD) was the most recently discovered and cloned PKC family member, there is already good evidence that it participates in agonist-stimulated signaling. PKCmu was independently discovered by two laboratories (9,10), and it was readily apparent that it is only distantly related to other PKCs in terms of primary sequence similarities. However, PKCmu does have a cysteine-rich domain capable of binding phorbol esters, but interestingly, also has a pleckstrin homology (PH) domain (figure 1). Thus, PKCmu has some features in common with both PKCs as well as Akt/PKB and PDK-1, and these enzymes share the highest degree of homology in the kinase domain. Perhaps the most striking feature of PKCmu signaling is its mode of regulation. PKCmu is potently and rapidly activated in cells stimulated with a variety of agonists, including bombesin, vasopressin, bradykinin (118), bryostatin 1 (119), angiotensin II, PDGF, as well as phorbol esters (120). These are all agonists which activate one or more PKC family member in vivo. The Rozengurt laboratory was the first to demonstrate that activation of PKCmu in cells occurs through a PKC-dependent pathway, such that

PKCepsilon and PKCeta are upstream kinases for PKCmu (118,121). Consistent with this, activation of PKCmu using PDGF-receptor mutants lacking the binding sites for either PI 3-K or PLC-gamma demonstrated that activation of PLC-gamma, leading to activation of PKCs is both necessary and sufficient to activate PKCmu in vivo (122). To date, no experiment has been presented demonstrating a direct phosphorylation of PKCmu by PKCepsilon or PKCeta, and thus it is unclear whether one or more intermediary steps between these enzymes are present in vivo (figure 4). As PKCmu has an N-terminal PH domain, one might have predicted that it would bind to PtdIns-3,4-P2 and/or PtdIns-3,4-5-P3 and thus be downstream of PI 3-K in vivo, but in fact there is evidence against this model (122). However, deletion or mutations in the PH domain of PKCmu results in constitutive activation of the enzyme (123), though it is unclear how this relates to in vivo regulation and whether lipids or proteins, or both, bind to the PKCmu PH domain. There is also evidence that PKCmu participates in B-cell receptor (BCR) signaling in lymphocytes and is activated following cross-linking of the BCR (124). A model has been proposed that PKCmu may function in a negative-feedback loop to regulate BCR signaling pathways. Finally, antisense oligodeoxynucleotide experiments in 3T3-F442A pre-adipocytes have demonstrated a negative role for PKCmu (as well as PKCalpha and PKCdelta) in adipocyte differentiation (125). Clearly, there are exciting prospects for an important role for this unusual PKC in signaling pathways.

6. PERSPECTIVE

The importance of PKCs in physiology and signaling mechanisms is not a novel concept. The fact that these enzymes phosphorylate and regulate many different proteins such as ion channels, cytoskeletal proteins and other enzymes which regulate cell growth underscores the essential role played by these kinases in cell function. What has remained elusive until recently, is the precise mechanism by which PKCs regulate these events. Initial studies on the role of PKC in cell function made use of the phorbol ester PMA, and although some valuable information was gained, it was difficult to ascribe a particular response to any one of the 12 different PKC family members. Similarly, considerable efforts went into understanding the precise mechanism of activation of PKCs by both lipids and phosphorylation. These regulatory mechanisms are now largely understood at the molecular level, and thus the focus has now shifted to understanding the mechanisms by which PKCs themselves regulate signaling pathways. Through the use of dominant negative and constitutively active mutants of individual PKCs, as well as the availability of isoform specific antibodies and specific substrates, a picture is beginning to emerge that novel PKCs such as PKCepsilon and PKCmu, and in particular atypical PKCzeta play a key role in cell biology. Although it is clearly still early days, there is a growing consensus that these PKCs are involved in mitogenic signal transduction, mediate some of the metabolic effects of insulin, are involved in remodeling of the actin cytoskeleton as well as apoptosis (figure 4). It is also clear that one common mechanism by which PKCs elicit these events is by activating the MAPK pathway, although the precise mechanism is still unclear. Given the complexity of MAPK regulation, it will not be surprising if PKCs regulate MAPK by multiple mechanisms. The outcome of some of the recent studies reviewed here begs one important question; what are the immediate downstream targets of these PKCs which regulate these responses? Identification of these proteins is likely to represent the next major area of research in the field.

7. REFERENCES

1. Inoue, M., Kishimoto, A., Takai, Y., & Nishizuka, Y.: Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain. *J Biol Chem* 252, 7610-6 (1977)

2. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U., & Nishizuka, Y.: Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J Biol Chem* 255, 2273-6 (1980)

3. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., & Nishizuka, Y.: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* 257, 7847-51 (1982)

4. Nishizuka, Y.: Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 258, 607-14 (1992)

5. Nishizuka, Y.: Protein kinase C and lipid signaling for sustained cellular responses. *Faseb J* 9, 484-96 (1995)

6. Newton, A. C.: Regulation of protein kinase C. *Curr Opin Cell Biol* 9, 161-7 (1997)

7. Mellor, H., & Parker, P. J.: The extended protein kinase C superfamily. *Biochem J* 332, 281-92 (1998)

8. Dekker, L. V., Palmer, R. H., & Parker, P. J.: The protein kinase C and protein kinase C related gene families. *Curr Opin Struct Biol* 5, 396-402 (1995)

9. Johannes, F. J., Prestle, J., Eis, S., Oberhagemann, P., & Pfizenmaier, K.: PKCu is a novel, atypical member of the protein kinase C family. *J. Biol. Chem.J Biol Chem* 269, 6140-8 (1994)

10. Valverde, A. M., Sinnett-Smith, J., Van Lint, J., & Rozengurt, E.: Molecular cloning and characterization of protein kinase D: a target for diacylglycerol and phorbol esters with a distinctive catalytic domain. *Proc Natl Acad Sci U S A* 91, 8572-6 (1994)

11. Palmer, R. H., Ridden, J., & Parker, P. J.: Cloning and expression patterns of two members of a novel protein-

kinase-C-related kinase family. *Eur J Biochem* 227, 344-51 (1995)

12. Newton, A. C.: Protein kinase C: structure, function, and regulation. *J Biol Chem* 270, 28495-8 (1995)

13. Mosior, M., & Newton, A. C.: Mechanism of interaction of protein kinase C with phorbol esters. Reversibility and nature of membrane association. *J Biol Chem* 270, 25526-33 (1995)

14. Mosior, M., & Newton, A. C.: Calcium-independent binding to interfacial phorbol esters causes protein kinase C to associate with membranes in the absence of acidic lipids. *Biochemistry* 35, 1612-23 (1996)

15. Johnson, J. E., Edwards, A. S., & Newton, A. C.: A putative phosphatidylserine binding motif is not involved in the lipid regulation of protein kinase C. *J Biol Chem* 272, 30787-92 (1997)

16. Sossin, W. S., & Schwartz, J. H.: Calcium-independent protein kinase Cs contain an amino-terminal domain similar to the C2 consensus sequence. *Trends Biochem Sci* 18, 207-8 (1993)

17. Szallasi, Z., Bogi, K., Gohari, S., Biro, T., Acs, P., & Blumberg, P. M.: Non-equivalent roles for the first and second zinc fingers of protein kinase Cdelta. Effect of their mutation on phorbol ester-induced translocation in NIH 3T3 cells. *J Biol Chem* 271, 18299-301 (1996)

18. Nakanishi, H., Brewer, K. A., & Exton, J. H.: Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 268, 13-6 (1993)

19. Toker, A., Meyer, M., Reddy, K. K., Falck, J. R., Aneja, R., Aneja, S., Parra, A., Burns, D. J., Ballas, L. M., & Cantley, L. C.: Activation of protein kinase C family members by the novel polyphosphoinositides PtdIns-3,4-P2 and PtdIns-3,4,5-P3. *J Biol Chem* 269, 32358-67 (1994)

20. Singh, S. S., Chauhan, A., Brockerhoff, H., & Chauhan, V. P.: Activation of protein kinase C by phosphatidylinositol 3,4,5- trisphosphate. *Biochem Biophys Res Commun* 195, 104-12 (1993)

21. Palmer, R. H., Dekker, L. V., Woscholski, R., Le Good, J. A., Gigg, R., & Parker, P. J.: Activation of PRK1 by phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate. A comparison with protein kinase C isotypes. *J Biol Chem* 270, 22412-6 (1995)

22. Muller, G., Ayoub, M., Storz, P., Rennecke, J., Fabbro, D., & Pfizenmaier, K.: PKC zeta is a molecular switch in signal transduction of TNF-alpha, bifunctionally regulated by ceramide and arachidonic acid. *Embo J* 14, 1961-9 (1995)

23. Muller, G., Storz, P., Bourteele, S., Doppler, H., Pfizenmaier, K., Mischak, H., Philipp, A., Kaiser, C., & Kolch, W.: Regulation of Raf-1 kinase by TNF via its second messenger ceramide and cross-talk with mitogenic signalling. *Embo J* 17, 732-42 (1998)

24. Borner, C., Filipuzzi, I., Wartmann, M., Eppenberger, U., & Fabbro, D.: Biosynthesis and posttranslational modifications of protein kinase C in human breast cancer cells. *J Biol Chem* 264, 13902-9 (1989)

25. Keranen, L. M., Dutil, E. M., & Newton, A. C.: Protein kinase C is regulated in vivo by three functionally distinct phosphorylations. *Curr Biol* 5, 1394-403 (1995)

26. Cazaubon, S., Bornancin, F., & Parker, P. J.: Threonine-497 is a critical site for permissive activation of protein kinase C alpha. *Biochem J* 301, 443-8 (1994)

27. Orr, J. W., & Newton, A. C.: Requirement for negative charge on "activation loop" of protein kinase C. *J Biol Chem* 269, 27715-8 (1994)

28. Garcia-Paramio, P., Cabrerizo, Y., Bornancin, F., & Parker, P. J.: The broad specificity of dominant inhibitory protein kinase C mutants infers a common step in phosphorylation. *Biochem J* 333, 631-6 (1998)

29. Chou, M. M., Hou, W., Johnson, J., Graham, L. K., Lee, M. H., Chen, C. S., Newton, A. C., Schaffhausen, B. S., & Toker, A.: Regulation of protein kinase C zeta by PI 3-kinase and PDK-1. *Curr Biol* 8, 1069-77 (1998)

30. Le Good, J. A., Ziegler, W. H., Parekh, D. B., Alessi, D. R., Cohen, P., & Parker, P. J.: Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* 281, 2042-5 (1998)

31. Downward, J.: Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* 10, 262-7 (1998)

32. Anderson, K. E., Coadwell, J., Stephens, L. R., & Hawkins, P. T.: Translocation of PDK-1 to the plasma membrane is important in allowoing PDK-1 to activate protein kinase B. *Curr Biol* 8, 684-91 (1998)

33. Hanks, S. K., & Hunter, T. (1995). The protein kinase facts book: protein-serine kinases, G. Hardie and S. Hanks, eds. (London: Academic Press, London).

34. Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., & Hemmings, B. A.: Mechanism of activation of protein kinase B by insulin and IGF-1. *Embo J.Embo J* 15, 6541-51 (1996)

35. Alessi, D. R., Deak, M., Casamayor, A., Caudwell, F. B., Morrice, N., Norman, D. G., Gaffney, P., Reese, C. B., MacDougall, C. N., Harbison, D., Ashworth, A., & Bownes, M.: 3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. *Curr Biol* 7, 776-89 (1997)

36. Zhang, G., Kazanietz, M. G., Blumberg, P. M., & Hurley, J. H.: Crystal structure of the cys2 activator-

binding domain of protein kinase C delta in complex with phorbol ester. *Cell* 81, 917-24 (1995)

37. Hurley, J. H., Newton, A. C., Parker, P. J., Blumberg, P. M., & Nishizuka, Y.: Taxonomy and function of C1 protein kinase C homology domains. *Protein Sci* 6, 477-80 (1997)

38. Sutton, R. B., Davletov, B. A., Berghuis, A. M., Sudhof, T. C., & Sprang, S. R.: Structure of the first C2 domain of synaptotagmin I: a novel Ca2+/phospholipid-binding fold. *Cell* 80, 929-38 (1995)

39. Grobler, J. A., Essen, L. O., Williams, R. L., & Hurley, J. H.: C2 domain conformational changes in phospholipase C-delta 1. *Nat Struct Biol* 3, 788-95 (1996)

40. Orr, J. W., & Newton, A. C.: Intrapeptide regulation of protein kinase C. *J Biol Chem* 269, 8383-7 (1994)

41. Srinivasan, N., Bax, B., Blundell, T. L., & Parker, P. J.: Structural aspects of the functional modules in human protein kinase-C alpha deduced from comparative analyses. *Proteins* 26, 217-35 (1996)

42. Nishikawa, K., Toker, A., Johannes, F. J., Songyang, Z., & Cantley, L. C.: Determination of the specific substrate sequence motifs of protein kinase C isozymes. *J. Biol. Chem.J Biol Chem* 272, 952-60 (1997)

43. Uberall, F., Giselbrecht, S., Hellbert, K., Fresser, F., Bauer, B., Gschwendt, M., Grunicke, H. H., & Baier, G.: Conventional PKC-alpha, novel PKC-epsilon and PKC-theta, but not atypical PKC-lambda are MARCKS kinases in intact NIH 3T3 fibroblasts. *J Biol Chem* 272, 4072-8 (1997)

44. Brumell, J. H., Craig, K. L., Ferguson, D., Tyers, M., & Grinstein, S.: Phosphorylation and subcellular redistribution of pleckstrin in human neutrophils. *J Immunol* 158, 4862-71 (1997)

45. Municio, M. M., Lozano, J., Sanchez, P., Moscat, J., & Diaz-Meco, M. T.: Identification of heterogeneous ribonucleoprotein A1 as a novel substrate for protein kinase C zeta. *J Biol Chem* 270, 15884-91 (1995)

46. Kielbassa, K., Muller, H. J., Meyer, H. E., Marks, F., & Gschwendt, M.: Protein kinase C delta-specific phosphorylation of the elongation factor eEF-alpha and an eEF-1 alpha peptide at threonine 431. *J Biol Chem* 270, 6156-62 (1995)

47. Oehrlein, S. A., Parker, P. J., & Herget, T.: Phosphorylation of GAP-43 (growth-associated protein of 43 kDa) by conventional, novel and atypical isotypes of the protein kinase C gene family: differences between oligopeptide and polypeptide phosphorylation. *Biochem J* 317, 219-24 (1996)

48. Mochly-Rosen, D., Khaner, H., & Lopez, J.: Identification of intracellular receptor proteins for activated protein kinase C. *Proc Natl Acad Sci U S A* 88, 3997-4000 (1991)

49. Mochly-Rosen, D., Khaner, H., Lopez, J., & Smith, B. L.: Intracellular receptors for activated protein kinase C. Identification of a binding site for the enzyme. *J Biol Chem* 266, 14866-8 (1991)

50. Faux, M. C., & Scott, J. D.: Regulation of the AKAP79-protein kinase C interaction by Ca2+/Calmodulin. *J Biol Chem* 272, 17038-44 (1997)

51. Puls, A., Schmidt, S., Grawe, F., & Stabel, S.: Interaction of protein kinase C zeta with ZIP, a novel protein kinase C- binding protein. *Proc Natl Acad Sci U S A* 94, 6191-6 (1997)

52. Diaz-Meco, M. T., Municio, M. M., Sanchez, P., Lozano, J., & Moscat, J.: Lambda-interacting protein, a novel protein that specifically interacts with the zinc finger domain of the atypical protein kinase C isotype lambda/iota and stimulates its kinase activity in vitro and in vivo. *Mol Cell Biol* 16, 105-14 (1996)

53. Mochly-Rosen, D., & Gordon, A. S.: Anchoring proteins for protein kinase C: a means for isozyme selectivity. *Faseb J* 12, 35-42 (1998)

54. Newton, A. C.: Protein kinase C: ports of anchor in the cell. *Curr Biol* 6, 806-9 (1996)

55. Moriya, S., Kazlauskas, A., Akimoto, K., Hirai, S., Mizuno, K., Takenawa, T., Fukui, Y., Watanabe, Y., Ozaki, S., & Ohno, S.: Platelet-derived growth factor activates protein kinase C epsilon through redundant and independent signaling pathways involving phospholipase C gamma or phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* 93, 151-5 (1996)

56. Ohno, S., Mizuno, K., Adachi, Y., Hata, A., Akita, Y., Akimoto, K., Osada, S., Hirai, S., & Suzuki, K.: Activation of novel protein kinases C delta and C epsilon upon mitogenic stimulation of quiescent rat 3Y1 fibroblasts. *J Biol Chem* 269, 17495-501 (1994)

57. Cacace, A. M., Guadagno, S. N., Krauss, R. S., Fabbro, D., & Weinstein, I. B.: The epsilon isoform of protein kinase C is an oncogene when overexpressed in rat fibroblasts. *OncogeneOncogene* 8, 2095-104 (1993)

58. Mischak, H., Goodnight, J. A., Kolch, W., Martiny, B. G., Schaechtle, C., Kazanietz, M. G., Blumberg, P. M., Pierce, J. H., & Mushinski, J. F.: Overexpression of protein kinase C-delta and -epsilon in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. *J Biol Chem* 268, 6090-6 (1993)

59. Cacace, A. M., Ueffing, M., Philipp, A., Han, E. K., Kolch, W., & Weinstein, I. B.: PKC epsilon functions as an oncogene by enhancing activation of the Raf kinase. *OncogeneOncogene* 13, 2517-26 (1996)

60. Ueffing, M., Lovric, J., Philipp, A., Mischak, H., & Kolch, W.: Protein kinase C-epsilon associates with the Raf-1 kinase and induces the production of growth factors that stimulate Raf-1 activity. *Oncogene* 15, 2921-7 (1997)

61. Cacace, A. M., Ueffing, M., Han, E. K., Marme, D., & Weinstein, I. B.: Overexpression of PKCepsilon in R6 fibroblasts causes increased production of active TGFbeta. *J Cell Physiol* 175, 314-22 (1998)

62. Perletti, G. P., Folini, M., Lin, H. C., Mischak, H., Piccinini, F., & Tashjian, A.: Overexpression of protein kinase C epsilon is oncogenic in rat colonic epithelial cells. *Oncogene* 12, 847-54 (1996)

63. Perletti, G. P., Concari, P., Brusaferri, S., Marras, E., Piccinini, F., & Tashjian, A. H., Jr.: Protein kinase Cepsilon is oncogenic in colon epithelial cells by interaction with the ras signal transduction pathway. *Oncogene* 16, 3345-8 (1998)

64. Li, Y., Davis, K. L., & Sytkowski, A. J.: Protein kinase C-epsilon is necessary for erythropoietin's up- regulation of c-myc and for factor-dependent DNA synthesis. Evidence for discrete signals for growth and differentiation. *J Biol Chem* 271, 27025-30 (1996)

65. Schonwasser, D. C., Marais, R. M., Marshall, C. J., & Parker, P. J.: Activation of the mitogen-activated protein kinase/extracellular signal- regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. *Mol Cell Biol* 18, 790-8 (1998)

66. Cai, H., Smola, U., Wixler, V., Eisenmann-Trappe, I., Diaz-Meco, M. T., Moscat, J., Rapp, U., & Cooper, G. M.: Role of DAG-regulated PKC isotypes in growth factor activation of the raf-1 protein kinase. *Mol Cell Biol* 17, 732-41 (1997)

67. Ueda, Y., Hirai, S., Osada, S., Suzuki, A., Mizuno, K., & Ohno, S.: Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. *J Biol Chem* 271, 23512-9 (1996)

68. Ohmichi, M., Zhu, G., & Saltiel, A. R.: Nerve growth factor activates calcium-insensitive protein kinase C-epsilon in PC-12 rat pheochromocytoma cells. *Biochem J* 295, 767-72 (1993)

69. Hundle, B., McMahon, T., Dadgar, J., & Messing, R. O.: Overexpression of epsilon-protein kinase C enhances nerve growth factor- induced phosphorylation of mitogen-activated protein kinases and neurite outgrowth. *J Biol Chem* 270, 30134-40 (1995)

70. Hundle, B., McMahon, T., Dadgar, J., Chen, C. H., Mochly-Rosen, D., & Messing, R. O.: An inhibitory fragment derived from protein kinase Cepsilon prevents enhancement of nerve growth factor responses by ethanol and phorbol esters. *J Biol Chem* 272, 15028-35 (1997)

71. Parrow, V., Fagerstrom, S., Meyerson, G., Nanberg, E., & Pahlman, S.: Protein kinase C-alpha and -epsilon are enriched in growth cones of differentiating SH-SY5Y human neuroblastoma cells. *J Neurosci Res* 41, 782-91 (1995)

72. Fagerstrom, S., Pahlman, S., Gestblom, C., & Nanberg, E.: Protein kinase C-epsilon is implicated in neurite outgrowth in differentiating human neuroblastoma cells. *Cell Growth Differ* 7, 775-85 (1996)

73. Razin, E., Szallasi, Z., Kazanietz, M. G., Blumberg, P. M., & Rivera, J.: Protein kinases C-beta and C-epsilon link the mast cell high-affinity receptor for IgE to the expression of c-fos and c-jun. *Proc Natl Acad Sci U S A* 91, 7722-6 (1994)

74. Genot, E. M., Parker, P. J., & Cantrell, D. A.: Analysis of the role of protein kinase C-alpha, -epsilon, and -zeta in T cell activation. *J Biol Chem* 270, 9833-9 (1995)

75. Hartwig, J. H., Thelen, M., Rosen, A., Janmey, P. A., Nairn, A. C., & Aderem, A.: MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. *Nature* 356, 618-22 (1992)

76. Myat, M. M., Anderson, S., Allen, L. A., & Aderem, A.: MARCKS regulates membrane ruffling and cell spreading. *Curr Biol* 7, 611-4 (1997)

77. Prekeris, R., Mayhew, M. W., Cooper, J. B., & Terrian, D. M.: Identification and localization of an actin-binding motif that is unique to the epsilon isoform of protein kinase C and participates in the regulation of synaptic function. *J Cell Biol* 132, 77-90 (1996)

78. Blobe, G. C., Stribling, D. S., Fabbro, D., Stabel, S., & Hannun, Y. A.: Protein kinase C beta II specifically binds to and is activated by F- actin. *J Biol Chem* 271, 15823-30 (1996)

79. Gomez, J., Martinez, C., Garcia, A., & Rebollo, A.: Association of phosphatidylinositol 3 kinase to protein kinase C zeta during interleukin-2 stimulation. *Eur J Immunol* 26, 1781-7 (1996)

80. Chun, J. S., Ha, M. J., & Jacobson, B. S.: Differential translocation of protein kinase C epsilon during HeLa cell adhesion to a gelatin substratum. *J Biol Chem* 271, 13008-12 (1996)

81. Haller, H., Lindschau, C., Maasch, C., Olthoff, H., Kurscheid, D., & Luft, F. C.: Integrin-induced protein kinase Calpha and Cepsilon translocation to focal adhesions mediates vascular smooth muscle cell spreading. *Circ Res* 82, 157-65 (1998)

82. Horowitz, A., Clement-Chomienne, O., Walsh, M. P., & Morgan, K. G.: Epsilon-isoenzyme of protein kinase C induces a Ca(2+)-independent contraction in vascular smooth muscle. *Am J Physiol* 271, C589-94 (1996)

83. Keenan, C., & Kelleher, D.: Protein kinase C and the cytoskeleton. *Cell Signal* 10, 225-32 (1998)

84. Heidenreich, K. A., Toledo, S. P., Brunton, L. L., Watson, M. J., Daniel-Issakani, S., & Strulovici, B.: Insulin

stimulates the activity of a novel protein kinase C, PKC-epsilon, in cultured fetal chick neurons. *J Biol Chem* 265, 15076-82 (1990)

85. Zhao, L., Standaert, M. L., Cooper, D. R., Avignon, A., & Farese, R. V.: Effects of insulin on protein kinase-C (PKC) in HIRC-B cells: specific activation of PKC epsilon and its resistance to phorbol ester-induced down-regulation. *Endocrinology* 135, 2504-10 (1994)

86. Standaert, M. L., Bandyopadhyay, G., Zhou, X., Galloway, L., & Farese, R. V.: Insulin stimulates phospholipase D-dependent phosphatidylcholine hydrolysis, Rho translocation, de novo phospholipid synthesis, and diacylglycerol/protein kinase C signaling in L6 myotubes. *Endocrinology* 137, 3014-20 (1996)

87. Stumpo, D. J., Haupt, D. M., & Blackshear, P. J.: Protein kinase c isozyme distribution and down-regulation in relation to insulin stimulated c-fos induction. *J. Biol. Chem.* 269, 21184-90 (1994)

88. Kayali, A. G., Eichhorn, J., Haruta, T., Morris, A. J., Nelson, J. G., Vollenweider, P., Olefsky, J. M., & Webster, N. J.: Association of the insulin receptor with phospholipase C-gamma (PLCgamma) in 3T3-L1 adipocytes suggests a role for PLCgamma in metabolic signaling by insulin. *J Biol Chem* 273, 13808-18 (1998)

89. Berra, E., Diaz, M. M., Dominguez, I., Municio, M. M., Sanz, L., Lozano, J., Chapkin, R. S., & Moscat, J.: Protein kinase C zeta isoform is critical for mitogenic signal transduction. *Cell* 74, 555-63 (1993)

90. Nakanishi, H., Brewer, K. A., & Exton, J. H.: Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 268, 13-6 (1993)

91. Akimoto, K., Takahashi, R., Moriya, S., Nishioka, N., Takayanagi, J., Kimura, K., Fukui, Y., Osada, S., Mizuno, K., Hirai, S., Kazlauskas, A., & Ohno, S.: EGF or PDGF receptors activate atypical PKClambda through phosphatidylinositol 3-kinase. *EMBO J* 15, 788-98 (1996)

92. Standaert, M. L., Galloway, L., Karnam, P., Bandyopadhyay, G., Moscat, J., & Farese, R. V.: Protein kinase C-zeta as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes. Potential role in glucose transport. *J Biol Chem* 272, 30075-82 (1997)

93. Herrera-Velit, P., Knutson, K. L., & Reiner, N. E.: Phosphatidylinositol 3-kinase-dependent activation of protein kinase C- zeta in bacterial lipopolysaccharide-treated human monocytes. *J Biol Chem* 272, 16445-52 (1997)

94. Diaz-Meco, M. T., Berra, E., Municio, M. M., Sanz, L., Lozano, J., Dominguez, I., Diaz-Golpe, V., Lain de Lera, M. T., Alcami, J., Paya, C. V., & et al.: A dominant negative protein kinase C zeta subspecies blocks NF-kappa B activation. *Mol Cell Biol* 13, 4770-5 (1993)

95. Dominguez, I., Sanz, L., Arenzana, S. F., Diaz, M. M., Virelizier, J. L., & Moscat, J.: Inhibition of protein kinase C zeta subspecies blocks the activation of an NF-kappa B-like activity in Xenopus laevis oocytes. *Mol Cell Biol* 13, 1290-5 (1993)

96. Sontag, E., Sontag, J. M., & Garcia, A.: Protein phosphatase 2A is a critical regulator of protein kinase C zeta signaling targeted by SV40 small t to promote cell growth and NF-kappaB activation. *Embo J* 16, 5662-71 (1997)

97. Diaz-Meco, M. T., Dominguez, I., Sanz, L., Dent, P., Lozano, J., Municio, M. M., Berra, E., Hay, R. T., Sturgill, T. W., & Moscat, J.: zeta PKC induces phosphorylation and inactivation of I kappa B-alpha in vitro. *Embo J* 13, 2842-8 (1994)

98. Diaz-Meco, M. T., Lozano, J., Municio, M. M., Berra, E., Frutos, S., Sanz, L., & Moscat, J.: Evidence for the in vitro and in vivo interaction of Ras with protein kinase C zeta. *J Biol Chem* 269, 31706-10 (1994)

99. Warne, P. H., Viciana, P. R., & Downward, J.: Direct interaction of Ras and the amino-terminal region of Raf-1 in vitro. *Nature* 364, 352-5 (1993)

100. Carnero, A., Liyanage, M., Stabel, S., & Lacal, J. C.: Evidence for different signalling pathways of PKC zeta and ras-p21 in Xenopus oocytes. *Oncogene* 11, 1541-7 (1995)

101. van Dijk, M., Muriana, F. J., van Der Hoeven, P. C., de Widt, J., Schaap, D., Moolenaar, W. H., & van Blitterswijk, W. J.: Diacylglycerol generated by exogenous phospholipase C activates the mitogen-activated protein kinase pathway independent of Ras- and phorbol ester-sensitive protein kinase C: dependence on protein kinase C-zeta. *Biochem J* 323, 693-9 (1997)

102. Montaner, S., Ramos, A., Perona, R., Esteve, P., Carnero, A., & Lacal, J. C.: Overexpression of PKC zeta in NIH3T3 cells does not induce cell transformation nor tumorigenicity and does not alter NF kappa B activity. *Oncogene* 10, 2213-20 (1995)

103. Kieser, A., Seitz, T., Adler, H. S., Coffer, P., Kremmer, E., Crespo, P., Gutkind, J. S., Henderson, D. W., Mushinski, J. F., Kolch, W., & Mischak, H.: Protein kinase C-zeta reverts v-raf transformation of NIH-3T3 cells. *Genes Dev* 10, 1455-66 (1996)

104. Kampfer, S., Hellbert, K., Villunger, A., Doppler, W., Baier, G., Grunicke, H. H., & berall, F.: Transcriptional activation of c-fos by oncogenic Ha-Ras in mouse mammary epithelial cells requires the combined activities of PKC- lambda, epsilon and zeta. *Embo J* 17, 4046-55 (1998)

105. Berra, E., Diaz-Meco, M. T., Lozano, J., Frutos, S., Municio, M. M., Sanchez, P., Sanz, L., & Moscat, J.: Evidence for a role of MEK and MAPK during signal transduction by protein kinase C zeta. *Embo J* 14, 6157-63 (1995)

106. Liao, D. F., Duff, J. L., Daum, G., Pelech, S. L., & Berk, B. C.: Angiotensin II stimulates MAP kinase kinase kinase activity in vascular smooth muscle cells, Role of Raf. *Circ Res* 79, 1007-14 (1996)

107. Sozeri, O., Vollmer, K., Liyanage, M., Frith, D., Kour, G., Mark, G. E. d., & Stabel, S.: Activation of the c-Raf protein kinase by protein kinase C phosphorylation. *Oncogene* 7, 2259-62 (1992)

108. Duckworth, B. C., & Cantley, L. C.: Conditional inhibition of the mitogen-activated protein kinase cascade by wortmannin. Dependence on signal strength. *J Biol Chem* 272, 27665-70 (1997)

109. Coleman, E. S., & Wooten, M. W.: Nerve growth factor-induced differentiation of PC12 cells employs the PMA-insensitive protein kinase C-zeta isoform. *J Mol Neurosci* 5, 39-57 (1994)

110. Wooten, M. W., Zhou, G., Wooten, M. C., & Seibenhener, M. L.: Transport of protein kinase C isoforms to the nucleus of PC12 cells by nerve growth factor: association of atypical zeta-PKC with the nuclear matrix. *J Neurosci Res* 49, 393-403 (1997)

111. Zhou, G., Seibenhener, M. L., & Wooten, M. W.: Nucleolin is a protein kinase C-zeta substrate. Connection between cell surface signaling and nucleus in PC12 cells. *J Biol Chem* 272, 31130-7 (1997)

112. Diaz-Meco, M. T., Municio, M. M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L., & Moscat, J.: The product of par-4, a gene induced during apoptosis, interacts selectively with the atypical isoforms of protein kinase C. *Cell* 86, 777-86 (1996)

113. Murray, N. R., & Fields, A. P.: Atypical protein kinase C iota protects human leukemia cells against drug-induced apoptosis. *J Biol Chem* 272, 27521-4 (1997)

114. Bandyopadhyay, G., Standaert, M. L., Zhao, L., Yu, B., Avignon, A., Galloway, L., Karnam, P., Moscat, J., & Farese, R. V.: Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. *J Biol Chem* 272, 2551-8 (1997)

115. Mendez, R., Kollmorgen, G., White, M. F., & Rhoads, R. E.: Requirement of protein kinase C zeta for stimulation of protein synthesis by insulin. *Mol Cell Biol* 17, 5184-92 (1997)

116. Bandyopadhyay, G., Standaert, M. L., Galloway, L., Moscat, J., & Farese, R. V.: Evidence for involvement of protein kinase C (PKC)-zeta and noninvolvement of diacylglycerol-sensitive PKCs in insulin-stimulated glucose transport in L6 myotubes. *Endocrinology* 138, 4721-31 (1997)

117. Liu, Q., Ning, W., Dantzer, R., Freund, G. G., & Kelley, K. W.: Activation of protein kinase C-zeta and phosphatidylinositol 3'-kinase and promotion of macrophage differentiation by insulin-like growth factor-I. *J Immunol* 160, 1393-401 (1998)

118. Zugaza, J. L., Waldron, R. T., Sinnett-Smith, J., & Rozengurt, E.: Bombesin, vasopressin, endothelin, bradykinin, and platelet-derived growth factor rapidly activate protein kinase D through a protein kinase C-dependent signal transduction pathway. *J Biol Chem* 272, 23952-60 (1997)

119. Matthews, S. A., Pettit, G. R., & Rozengurt, E.: Bryostatin 1 induces biphasic activation of protein kinase D in intact cells. *J Biol Chem* 272, 20245-50 (1997)

120. Abedi, H., Rozengurt, E., & Zachary, I.: Rapid activation of the novel serine/threonine protein kinase, protein kinase D by phorbol esters, angiotensin II and PDGF-BB in vascular smooth muscle cells. *FEBS Lett* 427, 209-12 (1998)

121. Zugaza, J. L., Sinnett-Smith, J., Van Lint, J., & Rozengurt, E.: Protein kinase D (PKD) activation in intact cells through a protein kinase C-dependent signal transduction pathway. *Embo J.Embo J* 15, 6220-30 (1996)

122. Van Lint, J., Ni, Y., Valius, M., Merlevede, W., & Vandenheede, J. R.: Platelet-derived growth factor stimulates protein kinase D through the activation of phospholipase Cgamma and protein kinase C. *J Biol Chem* 273, 7038-43 (1998)

123. Iglesias, T., & Rozengurt, E.: Protein kinase D activation by mutations within its pleckstrin homology domain. *J Biol Chem* 273, 410-6 (1998)

124. Sidorenko, S. P., Law, C. L., Klaus, S. J., Chandran, K. A., Takata, M., Kurosaki, T., & Clark, E. A.: Protein kinase C mu (PKC mu) associates with the B cell antigen receptor complex and regulates lymphocyte signaling. *Immunity* 5, 353-63 (1996)

125. Fleming, I., MacKenzie, S. J., Vernon, R. G., Anderson, N. G., Houslay, M. D., & Kilgour, E.: Protein kinase C isoforms play differential roles in the regulation of adipocyte differentiation. *Biochem J* 333, 719-27 (1998)

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