REVIEW ARTICLE
The extended protein kinase C superfamily

Harry MELLOR¹ and Peter J. PARKER²

*Protein Phosphorylation Laboratory, Imperial Cancer Research Fund, 44 Lincoln’s Inn Fields, London WC2A 3PX, U.K.

Members of the mammalian protein kinase C (PKC) superfamily play key regulatory roles in a multitude of cellular processes, ranging from control of fundamental cell autonomous activities, such as proliferation, to more organismal functions, such as memory. However, understanding of mammalian PKC signalling systems is complicated by the large number of family members. Significant progress has been made through studies based on comparative analysis, which have defined a number of regulatory elements in PKCs which confer specific location and activation signals to each isotype. Further studies on simple organisms have shown that PKC signalling paradigms are conserved through evolution from yeast to humans, underscoring the importance of this family in cellular signalling and giving novel insights into PKC function in complex mammalian systems.

INTRODUCTION

Although some 20 years after the discovery of phosphorylase kinase, protein kinase C (PKC) was one of the very first protein kinases to be identified. It was first defined as a histone kinase activity from rat brain which could be activated by limited proteolysis [1]. This was followed by the discovery that this new kinase could also be activated by phosphatidylserine (PS) and diacylglycerol (DAG) in a Ca²⁺-dependent manner and also by tumour-promoting phorbol esters such as PMA (synonym TPA) (reviewed in [2]). It soon became apparent from chromatographic purifications of PKC that this activity was composed of at least three distinct species, which were designated the α, β and γ isotypes [3]. After 22 years, exhaustive genetic screening has defined a superfamily of mammalian PKC isotypes that currently comprises some twelve distinct genes.

The number of PKC isotypes in existence in some ways represents the greatest challenge to the understanding of PKC function. PKCs have a multitude of cellular substrates and are involved in a bewildering array of biological processes. Against such a broad canvas it is hard to pick out and define physiologically relevant functions and to assign them to individual PKC isotypes. The difficulties of satisfactorily determining the individual contributions of each of the PKC isotypes to any particular process are such that few such studies have been accomplished. The broadly overlapping substrate specificities of the PKC isotypes in vitro has led to suggestions of redundancy of function in the PKC superfamily. Although this may be true, it is an intellectually unsatisfying explanation for the diversity of mammalian PKCs. Several recent studies have suggested that specificity within the PKC superfamily is more likely to be effected through regulatory inputs, with PKC isotypes responding to different activation and localization signals.

The wealth of DNA sequence information now available has made possible the comparison of PKCs from a wide range of organisms. This has allowed us to refine our understanding of the domain structure of PKCs and has given valuable insight into the many regulatory elements present in these proteins. Additionally, it has allowed comparison of the mammalian PKC signalling systems with those of simpler organisms where more complete studies have been achieved.

The present review focuses on the information that has been obtained from the comparative analysis of the PKC superfamily and discusses how the potential parallels between PKC signalling in simple organisms may help to elucidate the more complex mammalian signalling pathways.

PKC ISOTYPES

The first PKCs to be identified and cloned were the α, β and γ isotypes, which were initially isolated from brain cDNA libraries [4,5]. This tissue has proven to be a rich source of PKC isotypes, and low-stringency screening of brain cDNA libraries with probes derived from the α, β and γ isotypes yielded three additional PKCs, the δ, ε and ζ isotypes [6]. Further low-stringency screens of other tissue cDNA libraries has delivered PKCθ [7], PKCθ [8], PKCε (of which PKCε is the mouse homologue) [9] and, most recently, the PKC-related kinases (PRKs) [10,11]. The mammalian PKC isotypes have been grouped into smaller subfamilies on the basis of their enzymic properties. The best understood and most studied of these groups is the conventional PKCs (cPKCs), which comprise the α, βI, βII and γ isotypes (the PKCβ gene is alternatively spliced to produce two gene products which differ only in their extreme C-terminal ends [12]). These PKC isotypes are activated by PS in a Ca²⁺-dependent manner; they also bind DAG, which both increases the specificity of the enzyme for PS and also shifts the affinity for Ca²⁺ into the physiological range [13]. The cPKCs are targets of the tumour-promoting phorbol ester PMA, which
These kinases are Ca²⁺-insensitive, nor do they respond to PMA. DAG and phorbol esters activate these enzymes by eliminating the requirement for Ca²⁺ ions for CaMKK activation [14].

The nPKCs and cPKCs are Ca²⁺-insensitive, but are still activated by DAG or phorbol esters in the presence of PS (see, for example, [15]). The atypical PKCs (aPKCs), ι and ζ, comprise a third category. Like the nPKCs, these protein kinases are Ca²⁺-insensitive, nor do they respond to PMA/DAG [16]. Finally, the recently discovered PRKs define a fourth grouping consisting of at least three members, PRKs 1–3. PRK1 (PKN) was isolated in PCR-based and low-stringency screening, simultaneously by this laboratory [10] and by Ono and co-workers [11]. Like the aPKCs, PRKs are insensitive to Ca²⁺, DAG and phorbol esters [17,18]. However, PRK1 has been shown to bind to the activated RhoA GTase, which leads to a 4-fold activation of the kinase in vitro [19,20]. Recently it has been shown that the other fully cloned member of the PRK subfamily, PRK2, is also capable of binding RhoA [21,22], suggesting that this is a general property of this group.

The categorization of PKC isotypes based on their enzymic properties is strengthened by comparing the relatedness of these enzymes at the protein-sequence level. Sequence comparisons place PKC isotypes into five subgroups (Figure 1). These groupings are identical with those produced by the enzymic criteria described above, with the only exception being the splitting of the nPKCs into two pairs of very closely related kinases, namely δ and θ, and ε and γ.

**PKC BUILDING BLOCKS**

Closer examination of protein-sequence alignments between PKC isotypes reveals the presence of blocks of homology between family members (Figure 2). In all cases these conserved regions have been shown to define protein domains (or motifs) which confer a specific localization and/or activation input to the isotype. In this way, the functional significance of these groupings becomes more obvious. The distribution of these regulatory protein modules between isotypes also allows a more precise categorization of PKC isotypes and demonstrates that these groupings represent PKC subfamilies.

**The C1 domain**

The cPKCs and nPKCs contain a C1 domain that is defined by the presence of two repeated zinc-finger motifs, C1a and C1b [23]. Each motif has a conserved pattern of cysteine and histidine residues (H-X₇-C-X₇-C-X₁₁₋₁₂₃₋₄₋₅₋₆₋₆₋₇₋₈₋₉₋₁ₒ₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋んですよ

![Figure 2](image-url)
Table 1 Mammalian C1 domain-containing proteins

The table summarizes all of the mammalian proteins (in addition to the PKCs) containing consensus C1 domains which have been isolated to date. In many cases these proteins have been shown to bind PMA and/or DAG with a concomitant activation of protein function. Abbreviation: n.d., not done.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Zn fingers</th>
<th>PMA binding</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAG kinase</td>
<td>2</td>
<td>No</td>
<td>C1 not required for DAG binding or catalysis</td>
<td>[32]</td>
</tr>
<tr>
<td>Chimaerin</td>
<td>1</td>
<td>Yes</td>
<td>PMA stimulates GAP activity against Rac</td>
<td>[33]</td>
</tr>
<tr>
<td>PKD</td>
<td>2</td>
<td>Yes</td>
<td>Activated by PMA and DAG</td>
<td>[34]</td>
</tr>
<tr>
<td>Munc-13</td>
<td>1</td>
<td>n.d.</td>
<td>Mammalian homologue of unc-13; synaptosomal</td>
<td>[35]</td>
</tr>
<tr>
<td>Raf</td>
<td>1</td>
<td>No</td>
<td>C1 binds to Ras prenyl group</td>
<td>[36]</td>
</tr>
<tr>
<td>Ksr</td>
<td>1</td>
<td>n.d.</td>
<td>Raf-related kinase</td>
<td>[37]</td>
</tr>
<tr>
<td>Vav</td>
<td>1</td>
<td>Yes</td>
<td>GEF for Ras</td>
<td>[38]</td>
</tr>
<tr>
<td>Stac</td>
<td>1</td>
<td>n.d.</td>
<td>Novel SH3-containing protein from brain</td>
<td>[39]</td>
</tr>
</tbody>
</table>

effect on responsiveness to phorbol ester, whereas mutation of the equivalent amino acid residue in C1b caused a 125-fold decrease in phorbol-ester-binding affinity [31]. Interestingly, the single zinc-finger motif of the αPKCs shows much greater homology to the C1a motif than to the C1b. The function of the single zinc finger in the PKCs is unknown. C1 domains occur in several mammalian proteins other than PKC (Table 1). In some proteins, such as DAG kinase, the domain binds to neither PMA nor DAG [32]. Other proteins have been shown to bind to, and be activated by, phorbol esters. Protein kinase D (PKD) is a human phorbol-ester- and DAG-stimulated protein kinase [34]. The mouse isotype has been termed PKCβ, and it has been suggested that it is a member of the αPKC subfamily [40]. However, examination of the catalytic domain of PKD shows that it is related to the CaMKII-like protein kinases and, indeed, both its substrate specificity [34] and its sensitivity to inhibitors [41] are unlike those of the PKC family. The chimaerin family are GTPase-activating proteins (GAPs) for the small GTPase Rac [42]. Their cellular function seems to be involved with the regulation of the actin cytoskeleton by Rac (and perhaps Cdc42) as they stimulate the formation of lamellipodia and filopodia in a Rac-dependent fashion [43]. The GAP activity against Rac has been shown to be stimulated by PMA treatment in vitro [33]. Unc-13 is a Caenorhabditis elegans neural-specific protein which binds to both PMA and DAG [44]. Defects in this protein lead to severe disruptions in neurotransmitter release. Three related mammalian homologues have been identified, of which the best studied is Munc13-1. This mammalian Unc-13 has been shown to interact with two synaptic-vesicle proteins, namely Doc2 [35] and syntaxin [45], suggesting a role for this protein in synaptic-vesicle exocytosis. Doc itself contains two C2 domains (see below) and is implicated in Ca<sup>2+</sup>-dependent exocytosis [46].

The identification of PMA-responsive, non-PKC proteins in mammalian cells raises the need for caution in the interpretation of studies of PKC function that rely solely on the use of phorbol esters as an investigative tool. This is particularly pertinent for the chimaerin and Munc families which are involved in cellular processes which are also potentially regulated by PKCs.

The C2 domain

The C2 domain is found in the εPKCs immediately C-terminal to the C1 domain. Like the C1 domain, the C2 domain has been found to be present in many other proteins, including the synaptotagmins, rabphilin-3A, phospholipases and GAPs [47]. As many of these proteins bind phospholipid in a Ca<sup>2+</sup>-dependent manner, it has been assumed that the C2 domain confers Ca<sup>2+</sup>/PS binding to the εPKCs. Recently it has been shown that the isolated C2 domain from PKCε does indeed bind to phospholipid vesicles in a Ca<sup>2+</sup>-dependent fashion [48]. Synaptotagmin contains two copies of the C2 domain (C2A and C2B), and it has been shown that the C2A domain confers Ca<sup>2+</sup>-dependent PS binding to the protein [49]. The three-dimensional structure of the C2A domain of synaptotagmin I has recently been solved in the presence and absence of Ca<sup>2+</sup>. The C2 polypeptide assumes a compact β-sandwich fold formed by two four-stranded anti-parallel β-sheets with Ca<sup>2+</sup> bound in a cup-shaped depression [50]. Further information has been obtained by NMR-spectroscopic studies on PKCβ, which have shown that two Ca<sup>2+</sup> ions are co-ordinated by five conserved aspartate residues [48]. Determination of the X-ray crystal structure of the PLCδ1 C2 domain has suggested that Ca<sup>2+</sup> binding triggers a conformational change which opens a cleft to allow binding of a phospholipid headgroup [51].

Although the classical C2 domain is missing from the Ca<sup>2+</sup>-independent PKCs, close inspection of these enzymes reveals that regions of homology originally termed V<sub>α</sub> in aPKCs and nPKCs, and HR2 in PRKs, are related to the C2 domain [47,52]. In these C2-like domains, one or more of the conserved aspartate residues required for Ca<sup>2+</sup> binding are missing. It is unclear whether these domains are capable of binding to phospholipids, although activation of these PKCs by phospholipid has been demonstrated in vitro (for example, for PRK1 see [53]). It is possible that the general function of these C2-like domains is to make protein–protein interactions. The C2A and C2B domains of synaptotagmin have been shown to bind to syntaxin and clathrin AP-2 respectively [54]. Similarly, a class of proteins has been defined which interacts with the C2 domains of PKCs, i.e. the receptors for activated PKCs (RACKs) [55]. RACKs are not PKC substrates, but instead seem to be important in targeting active PKCs to specific membrane compartments. At present only two RACKs have been fully characterized, namely annexin 1 [56] and RACK1, a novel protein showing some homology to the β-subunit of heterotrimeric G-proteins [57]. The RACK1-binding site has been mapped to a short region of the PKCζ/C2 domain [58]. Peptides derived from this region block the translocation of PKCζ to the plasma membrane in response to stimuli [59], suggesting a general role for PKCζ and C2-like domains in mediating protein–protein interactions which target PKCs to cellular compartments. Recent studies have shown that the V<sub>α</sub>/C2 domain of PKCζ is a binding site for the growth associated protein GAP-43 [60], providing evidence for the idea that the function of the C2 domain in Ca<sup>2+</sup>-unresponsive PKCs may be to make protein–protein interactions. GAP-43 does not fit the description of a RACK on two counts: (i) it is a well-characterized substrate of PKC and (ii) the association with PKCζ does not depend on the activation state of the kinase [60]. However, it seems likely that the interaction serves to impose a level of isotype specificity on the phosphorylation reaction by co-localizing the substrate with a specific PKC. Interestingly, although PKCζ does not bind Ca<sup>2+</sup>, its association with GAP-43 is Ca<sup>2+</sup>-dependent, adding a further level of complexity to the regulation of this phosphorylation. Clearly a model in which specific RACKs (or even PKC substrates, such as GAP-43) target individual PKC isoforms to distinct cellular compartments is an attractive one.
that would go some way towards untangling the PKC signalling network. The recent description of the coatamer protein β-COP as a RACK specific for PKCe [61] provides the required selectivity. Ultimately, the validity of this hypothesis rests upon the identification of further isotype-specific protein-binding partners for PKC C2 domains.

The HR1 domain

The HR1 domain, initially identified as a region of homology between PRK1 and PRK2, is composed of three repeats of an approx. 55-amino-acid motif [10]. A single copy of this repeat is found in two other RhoA-binding proteins, rhophilin [20] and rhotekin [62]. Protein alignments of known HR1 repeats from various sources reveals several conserved features (Figure 3). The motif seems to consist of two blocks of homology with a short variable length of linking sequence. The first half of the repeat consists of a block of basic amino acids which have a predicted α-helical structure. This is terminated by a short, partially conserved sequence: Gly-Ala-Glu-Asn. The second half of the motif shows some homology to a leucine zipper in its primary sequence, although the presence of helix-breaking glycine and proline residues mean that it is unlikely to fold as a leucine zipper in vivo. The HR1 motif represents the single point of homology between PRKs, rhophilin and rhotekin. This suggests that this sequence defines a Rho-interacting module, and this has indeed been shown to be the case. The first HR1 repeat in PRK1 (HR1a) binds to the active GDP–RhoA complex, but not to the inactive GDP-bound form [63]. The second HR1 motif (HR1b) also binds RhoA, but this binding is weaker and occurs with either the activated (GTP-bound) or inactive (GDP-bound) forms of RhoA ([64]. Further binding analysis has suggested that the HR1a and HR1b motifs make distinct contacts on the RhoA protein and probably both contribute to binding in vivo [64]. We have recently shown that PRK1 is targeted to the endosomal compartment by another member of the Rho GTPase family, RhoB. Localization studies have shown that the wild-type RhoB (of which only a fraction is in the GTP-bound state) is as efficient at targeting PRK1 to endosomes as a constitutively GTP-bound mutant RhoB [65]. This suggests that a weak interaction between PRK1 HR1b and RhoB is sufficient to confer binding in vivo and that the HR1 domain can act as a localization signal as well as controlling kinase activation.

The HR1c repeat does not bind Rho [64], and its function is unknown. Small protein motifs are often stacked together to form more stable domains [66]. It may be that the three HR1 motifs in PRKs assemble into a higher-order structure that allows further points of contact with Rho. In this respect it is important to note that the published binding studies have been performed with recombinant RhoA which lacks the C-terminal prenylation modification found in the authentic molecule. It is possible that further contact points are made with the modified Rho that may require the complete HR1 domain (or indeed other PKC domains). Alternatively the HR1c motif may confer binding to some other protein, perhaps another member of the Rho GTPase family.

The pseudosubstrate site

Juxtaposed to the N-terminus of the C1 domains of the cPKCs, aPKCs and nPKCs is a sequence which retains the hallmarks of a PKC phosphorylation site, but has an alanine at the predicted serine/threonine phosphorylation site [67]. Mutation of this region confers effector-independent activity on the mutant protein [68]. This pseudosubstrate site interacts with the catalytic
domain and is responsible for the intramolecular suppression of catalytic activity prior to effector binding. For PRKs, which lack the C1 domain, studies using the yeast two-hybrid system have defined an interaction between the catalytic domain of PRK1 and the HR1a motif in the regulatory domain. This sequence contains a consensus phosphorylation site for PKCs centred around Ile\(^{68}\), and synthetic peptides in this region have been shown to be inhibitors of PRK1 activity in vitro. It has therefore been proposed that the HR1a motif acts as a pseudosubstrate site to inhibit kinase function [69]. This is an attractive proposal, as it suggests a model whereby Rho binding to PRK could release the pseudosubstrate site from the catalytic domain and lead to activation of the kinase. Interestingly, it has been shown that the activation of PRK1 is accompanied by autophosphorylation within the HR1a motif at Thr\(^{41}\), although the contribution of this phosphorylation to activity is unknown [70].

The V5 region

Although too short (approx. 50 amino acids) to be considered a protein domain, the V5 region of PKC plays an important regulatory role in kinase function. The initial assignment of the V5 region was based upon the deterioration in sequence conservation between PKC \(\alpha, \beta\) and \(\gamma\) towards the end of the kinase domain [5]. The subsequent demonstration that the PKC\(\beta\) gene produced two alternatively spliced transcripts specifically covering this region, provides an objective boundary for this C-terminal V5 region. In itself, this PKC\(\beta\) alternative splicing and its conservation in other species, imply that this region confers specific properties. In the case of PKC\(\beta I\) and PKC\(\beta II\) there is very good evidence that the V5 domain plays a critical role in localization. Studies in the human U937 monocyte cell line have shown that PKC\(\beta I\) is localized to the microtubules, whereas PKC\(\beta II\) is localized in part to secretory granules [71]. As the only difference between these two proteins is the V5 region, it would seem that this acts as a differential localization signal in these isoforms. Presumably, the V5 region makes a protein–protein interaction, although the protein targets of the PKC\(\beta I\) and \(\beta II\) isoforms in U937 cell have not been identified. Differential localization of PKC\(\beta I\) and \(\beta II\) has also been observed in the MOLT-4 T-lymphoblastoid cell line. Here it has been shown that PKC\(\beta I\), but not \(\beta II\), translocates to actin microfilaments upon PMA treatment of cells [72]. In in vitro binding studies, PKC\(\beta I\) was seen to associate directly with F-actin; however, it did not bind to polymerized actin, suggesting that translocation to actin microfilaments in vitro may be mediated by association with some other cytoskeletal component.

The V5 regions of PKCs also play key roles with respect to their phosphorylation. This is most clearly elucidated for the cPKCs, where two V5 region phosphorylation sites have been identified, at least one of which is the target of an upstream, non-PKC kinase. Phosphorylation at these sites has been shown to control both the net ‘on’ rate for PKC\(\alpha\) phosphorylation at subsequent regulatory sites [73] and also the rate of PKC\(\alpha\) and \(\beta\) dephosphorylation [73–75]. The basis for these effects seems to be in part due to the interaction of the phosphorylated V5 region with the kinase domain itself, inducing a closed, stable conformation. A precedent for this is provided by the equivalent C-terminal region of protein kinase A, which makes extensive contacts, wrapping back around the lower and upper lobes of the kinase domain [76]. Indirect evidence that this region of PKC\(\alpha\) is important for stability/activity derives from a deletion analysis of the mammalian protein expressed in \textit{Saccharomyces cerevisiae}, where removal of just the C-terminal 26 amino acids destroys activity [77]. It is noteworthy that the most C-terminal phosphorylation site in the V5 regions of PKCs (the so-called ‘FSY’ site) is replaced by a glutamic acid residue or an aspartic acid residue in the aPKCs and PRKs, respectively. In PKC\(\alpha\) the equivalent mutation (S657D) partially restores wild-type PKC\(\alpha\) properties [73]. Thus it appears that for aPKCs and PRKs, the requirement for an upstream kinase to act on this hydrophobic site is by-passed.

Pke1: An Archetypal PKC?

PKCs have been isolated from a wide-range of eukaryotic sources. Perhaps the simplest of these is the budding yeast \textit{S. cerevisiae}, demonstrating both the age and fundamental importance of this protein kinase. The yeast PKC (Pke1) was originally identified by low-stringency screens of yeast genomic libraries with mammalian PKCs [78] and has subsequently been re-isolated as a product of a genetic screen for resistance to the PKC inhibitor staurosporine [79]. With the sequencing of the \textit{S. cerevisiae} genome now complete [80], it is clear that Pke1 represents the sole PKC in this organism. At 132 kDa, Pke1 is a much larger protein than any of the mammalian PKCs. Examination of its protein sequence shows that this is due to an extended regulatory domain. In the mammalian PKC superfamily the various regulatory modules are differentially distributed between the different isoforms. However, in \textit{S. cerevisiae}, all of these elements are present together in one enzyme, Pke1 (Figure 4), suggesting that this protein kinase represents an archetypal PKC.

Two copies of the HR1 motif are present at the N-terminus of the kinase [47]. Sequence comparison with the PRKs indicates that these are most closely related to the HR1a and HR1b motifs which are responsible for the binding of RhoA GTPase. Like the PRKs, Pke1 has been shown to be a downstream effector of Rho GTPase, both genetically and biochemically. Dominant active forms of Pke1 suppress the phenotypes of mutations in Rho1, the \textit{S. cerevisiae} Rho GTPase [81, 82], and Rho1 directly activates purified Pke1 in vitro [82]. The site of interaction between Rho1 and Pke1 has been examined by yeast two-hybrid analysis. Use of Pke1 truncation mutants has defined a binding site for Rho1 within the C1 domain of the kinase, but not in the first 337 residues of Pke1 that contain the HR1 and C2 domains.

![Figure 4](image)

**Figure 4** Protein architecture of Pke1

The structure of Pke1 comprises an HR1 domain with two copies of the HR1 motif (residues 6–61 and 121–177), a C2 domain (195–369), a C1 domain (415–544) (preceded by the pseudosubstrate site (narrow white box)), the kinase domain (818–1004) and a V5 region (1105–1151). A key to the colourings of these domains is given in Figure 2. The percentage similarities of the respective domains to their counterparts in the mammalian PKC isoforms are shown in tabular form below the structure.
[81]. This is a somewhat surprising result given that the PRKs lack a C1 domain, whereas this domain is present in PKC isotypes which do not interact with Rho GTPases. We have shown that another yeast PKC, the S. pombe enzyme Pck2, does interact with Rho GTPase through its HR1 domain (L. Sayers and P. J. Parker, unpublished work). It would seem that Rhol may make two interactions with Pck1: an HR1 interaction and an interaction with the C1 domain. Many small GTPases, including Rhol [83], are modified by the addition of a prenyl group at the C-terminus. It has been recently shown that the small GTPase Ras interacts through its prenyl modification with the C1 domain of the Raf kinase [36]. This interaction appears to stabilize the specific protein–protein interaction between Ras and the Ras-binding domain of Raf. It seems possible that a similar situation exists for the Rholl–Pck1 interaction, although clearly further work is required to clarify this matter.

The HR1 domain is followed by a C2 like domain and then a C1 domain with two zinc fingers. Sequence comparison suggests the existence of a second C2-like domain between the C1 domain and the catalytic region [78], although the sequence similarity is too weak to be proven conclusively [47]. Pck1 therefore has the potential to respond to all of the physiological activators of the mammalian PKCs. This question has been addressed to some extent by in vitro studies of the enzymic properties of the purified protein. From these studies it is clear that Pck1 does not respond to Ca$^{2+}$ [84], and this is not surprising, given that only one of the four aspartic acid residues required for Ca$^{2+}$ binding are present in the Pck1 C2 domain [47]. A similar lack of responsiveness to Ca$^{2+}$ has been noted for the S. pombe Pck1 [85] and PKCs from the filamentous fungi Trichoderma reesei and Aspergillus niger [86]. Purified Pck1 is not activated by combinations of PS, DAG or PMA in vitro [84]. However, recent studies have shown that Pck1 is potently stimulated by PS in the presence of activated Rhol [82]. Thus it would seem that dual inputs from the C2 domain (PS) and the HR1 and/or C1 domains (Rhol) are required for activation. The possible contribution of DAG to Pck1 activation is unclear. Addition of DAG to Pck1 incubated with PS and Rhol produces no further activation of kinase activity [82]. Unfortunately, the effect of DAG and Rhol on Pck1 activity over a range of concentrations of PS has not been published. There is some indirect evidence for a role of DAG in Pck1 regulation in vivo. Mutation of conserved cysteine residues in the Pck1 C1 domain has been shown to mimic some of phenotypes of Pck1 deletion, although this could be due to an inhibition of Rhol binding [87]. More compelling are studies with the S. cerevisiae cyclin-dependent kinase cdcl28. Genetic evidence has shown that cdcl28 activation in late G$_{1}$ phase leads to a stimulation of DAG production, probably through the activation of a phosphatidylycholine-dependent phospholipase C and that this DAG production parallels Pck1 activation. Although strictly correlative, these data suggest a possible role for DAG in the regulation of Pck1 in vivo [88]. No doubt further studies will resolve this question and provide the biochemical base upon which to establish links to the genetic analysis.

EXTENDING THE FAMILY: NEMATODE PKCS

More clues to the development of the PKC superfamily come from the study of the nematode worm *C. elegans*. This extremely simple organism contains only approx. 1000 somatic cells and yet has a primitive neural system and exhibits a range of behavioural responses to its environment. Treatment with PMA causes uncoordinated movement and growth arrest [89]. Screening for mutant worms resistant to PMA led to the identification of the *tpa-1* gene (*tpa* referring to TPA, a synonym of PMA) [90]. The *tpa-1* gene product encodes a protein (TPA1A) with substantial homology with the novel PKCs, especially to the PKC8 and 0 grouping [90]. It has been shown recently that a second product of the *tpa-1* gene, TPA1B, is produced by alternative splicing. Interestingly, the effect of this splice is to remove very neatly the N-terminal V$_{δ}$/C2-like domain while leaving the C1 domain intact [91]. Mutations in the *tpa-1* gene lead to a reversal of both the growth-inhibitory and locomotory effects of PMA, suggesting that TPA1 is responsible for the observable phenotype of phorbol ester on the organism [90]. A second *C. elegans* PKC gene, *pck-1*, has been isolated through screening of cDNA libraries with a PKC$_{δ}$/probe [92]. *PKC1B* is most related to the second grouping of novel PKCs, 6 and 9. Its expression in *C. elegans* is restricted to a subset of sensory neurons, suggesting parallels with the distribution of PKCs 6 and 9 in mammals [92]. As with *tpa-1*, the *pck-1* gene is also alternatively spliced to produce a second PKC, PKC1A. This isotype differs only in the presence of an additional 56 amino acids at the N-terminus [93]. The *PKC1B* isotype shows differential expression during development with a peak of protein in the early larval stages [92]. It would seem likely that this isotype is required for post-embryonic development of the neurosensory system.

The *pck-2* (*kin-11*) gene was identified through the *C. elegans* genome sequencing project. The predicted product of this gene shows high homology with the cPKCs, and its structure suggests that it will have similar enzymic properties. It has a C2 domain which contains the invariant residues required to confer Ca$^{2+}$ binding and also a well-conserved C1 domain. Recent isolation and cloning of the *PKC2* cDNAs has revealed a complex pattern of alternative splicing, generating variation at both ends of the protein [94]. The *pck-2* gene gives rise to two 3’-splice variants, PKC2A and 2B, which differ in their C-termini. This has an obvious parallel with the splice variants of ePKC$_{β}$ and, indeed, comparison of the protein sequences reveals that the length and relative position of the alternatively spliced exon is the same for PKC2 and PKC$_{β}$. These findings suggest that PKC$_{β}$ and PKC2 share a common ancestor, perhaps the original ePKC [94], and that this alternative splicing of the C-terminus has been conserved from nematodes to man. Indeed, closer examination of the protein sequences suggests that PKC2A corresponds to PKC$_{β}$/I and PKC2B to βII. Three splice variants have been isolated at the 5’-end of the PKC2 cDNA that encode alternative N-terminal extensions of 50 (exons 1A plus 1A’), 13 (exon 1B) and 15 (exon 1C) residues [94]. This gives a potential six isoforms of PKC2, although it is not known whether all combinations are expressed. The kinase activity of recombinant PKC2 is stimulated by Ca$^{2+}$ [94] and may correspond to the Ca$^{2+}$- and DAG/PS-dependent PKC-like kinase activity isolated from *C. elegans* tissue extracts [95]. The observed Ca$^{2+}$-dependence demonstrates that the enzyme is a cPKC both by structural and enzymic criteria. The level of expression of the protein kinase rises dramatically upon hatching and then remains relatively constant throughout adult life, although, as with PKC1, there appears to be stage-specific regulation of the expression of splice variants. As with the other nematode PKCs, the cellular role of PKC2 is at yet unknown. Clues to its function may come from its localization; immunodetection of the endogenous protein shows that the distribution of PKC2 in adult *C. elegans* is restricted largely to a subset of neurons and to somatic cells in the gonad [94].

The sequencing of the *C. elegans* genome is now 75% complete, with well over 5000 genes identified so far. Analysis of these sequences predicts the presence of five PKC-related proteins, three of which being the products of the *tpa-1*, *pck-1* and *pck-2* genes.
genes. As the mammalian PKC superfamily appears split into five groups of related enzymes, so a single *C. elegans* PKC seems to correspond to each of these groups (Figure 5). The predicted *F09E1.1* gene product is highly homologous with the aPKCs and shares the same domain structure, whereas the PRK-A kinase (H. Mellor and P. J. Parker, unpublished work), encoded by the *F46F6.2* gene, contains a highly conserved HR1 domain (containing all three HR1 motifs) and appears to be the nematode equivalent of the PRK subfamily. While other PKCs may be identified within the remaining 25% of the *C. elegans* genome remaining to be sequenced, it seems clear that expansion of the PKC family occurred early in metazoan evolution to create five main PKC subfamilies. It also seems likely that subsequent gene duplication has lead to the appearance of multiple members of these subfamilies in mammals. Whilst the first expansion would appear to have involved gene rearrangement, the products of the second expansion have retained their modular architecture and are presumably products of gene duplication.

*C. elegans* is an extremely manipulatable and powerful genetic system. The presence of five representative isotypes of the PKC superfamily in this organism greatly simplifies the study of PKC function. As yet only the *tpa-1* gene has been mutated. It is to be hoped that genetic dissection of the other four genes will allow a functional distinction of PKC subfamilies with relevance for the more complex mammalian system.

**PKC SIGNALLING IN YEAST**

Genetic studies in yeast have made rapid progress in delineating the PKC signalling pathways in this simple organism. In the interests of clarity, the present review will focus on Pkc1 signalling in *S. cerevisiae* which represents the most complete story, although significant progress has been made in studies of PKC pathways in the fission yeast *S. pombe* (see, for example [96]).

Deletion of Pkc1 in *S. cerevisiae* leads to an arrest of protein synthesis and cell growth at a point in the cell cycle prior to mitosis and after DNA synthesis [78]. Growth can be restored to ΔPkc1 mutants by the addition of osmotic stabilizing agents, such as sorbitol, to the medium, suggesting that the defect involves a weakening of the cell wall. When ΔPkc1 yeasts are returned to a hypertonic medium, budding cells undergo immediate lysis, further suggesting that the lethality of the cell-wall defect is manifest on bud formation [97]. Electron-microscopic studies have shown this to be true, revealing thinned cell walls in ΔPkc1 yeast and holes at the bud tip [98,99]. The Pkc1 pathway has been shown to be stimulated by at least two signals. The first is cellular stress in the form of hypotonic osmotic shock [100] or heat shock [101]. The second is cell-cycle-dependent establishment of polarized growth. Budding yeast exhibit polarized growth in the vegetative state during bud formation (reviewed in [102]). Conditional mutants of Pkc1 arrest with small buds, indicating that Pkc1 is not required for bud establishment but for the subsequent bud emergence [97]. *S. cerevisiae* also exhibits polarized growth under two other conditions. Haploid cells will respond to mating pheromone by undergoing sexual development involving cell cycle arrest and the production of a mating projection (reviewed in [103]). Haploid or diploid cells will also exhibit polarized growth under conditions of nitrogen starvation, where they switch to a pseudohyphal growth mode in order to explore the environment for nutrient sources (reviewed in [102]). Pkc1 signalling pathways have also been shown to be required for polarized growth in both of these conditions; i.e. in response to mating pheromone or nitrogen starvation [104–106], demonstrating a general role for Pkc1 in this process. Polarized growth and the cellular response to hypotonic shock or heat shock all require remodelling of the cell wall, and this commonality between the various activating signals for Pkc1 suggests that the dynamic regulation of the cell wall is the cellular site of Pkc1 action. Dissection of the Pkc1 signalling pathways indicates how this may be achieved.

Genetic studies have led to an understanding of PKC signalling pathways in yeast that is more extensive and, in many ways, more conclusive than our current understanding of signalling in the mammalian PKC signalling networks (Figure 6). As mentioned above, Pkc1 is immediately downstream of the Rho GTPase Rhoc1 [81]. The activity of Rhoc1 is governed by the opposing actions of GDP/GTP exchange factors (GEFs) and GTPase-activating proteins (GAPs). The GEFs Rom1 and Rom2 both contain a DH domain which catalyses the exchange of GDP for GTP on Rhoc1, causing its activation [107]. The GAPs Bem2 [108] and Sac7 [109] have both been shown to catalyse the hydrolysis of GTP on Rhoc1 *in vitro* and therefore are potential negative regulators of Rhoc1. Whether both proteins fulfil this role *in vivo* is uncertain, as only Sac7 deletions are capable of suppressing the phenotype of mutations in Rom2 [109]. Further upstream is the yeast Tor2 protein, which is related to the PI 3-kinases. Genetic evidence suggests that Tor2 stimulates Rom2 activity (and hence Pkc1 activity) through the Rom2 PH domain [109]. It is not known whether Tor2 acts directly on Rom2, although an attractive proposal is that stimulation of

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**Figure 5 Expansion of the PKC superfamily**

Five PKC genes have been identified in the nematode worm *C. elegans*. Each of these corresponds to a specific subgroup of the mammalian PKC superfamily, both by overall similarity and protein architecture. The diagram shows the percentage similarities between each of the *C. elegans* PKCs and their corresponding mammalian isotypes.
Rom2 activity is mediated by the binding of a PI lipid product of Tor2 to the Rom2 PH domain. However, the identity of the presumed lipid product(s) of the Tor2 kinase is as yet unknown. Genetic evidence has also placed another PI lipid kinase upstream of Pkc1; the phosphatidylinositol (PI) 4-kinase, Stt4 [110,111]. Interestingly, both the mammalian homologue of Tor2 [112] and yeast Tor2 (F. Cooke, unpublished work) have been shown to co-purify with an associated, uncharacterized, PI 4-kinase activity. This suggests the possibility that Stt4 may lie in the pathway leading to Tor2-dependent activation of Pkc1, although further work is clearly needed to clarify the biological relevance of the genetic interaction between Stt4 and Pkc1. Looking further upstream, recent studies have identified a candidate cell-surface receptor for the Pkc1 pathway [113]. Hcs77 (also termed Wsc1 [114]) is an integral plasma-membrane protein that has been shown genetically to be upstream of Pkc1 [113]. Deletion mutants of Hcs77 show the ΔPkc1 cell-lysis phenotype and also fail to activate Mpk1 (see below) in response to heat shock. These findings, combined with the cell-surface localization of the protein, have lead to the proposal that Hcs77 is the mechanosensor for the Pkc1 pathway [113]. It will be interesting to see how mutations of Hcs77 interact with the Tor2/Rom2/Rho1 arm of the Pkc1 pathway.

Downstream of Pkc1 is a mitogen-activated protein kinase (MAPK) module of the paradigm MEKK > MEK > MAPK, where MEK is MAPK kinase and MEKK is MEK kinase. Genetic screens for extragenic suppressors of a conditional Pkc1 deletion have identified Bck1 (bypass of C kinase, also termed Sltk [104]) as a downstream effector of the yeast PKC [115]. Bck1 is upstream of two related MEKs, Mkk1/Mkk2, which are functionally redundant in the Pkc1 signalling pathway [116]. These proteins are in turn upstream of the last member of the MAP kinase module, Mpk1 ([116,117], also termed Slt2 [118]). Deletion of either Bck1, Mkk1/Mkk2 or Mpk1 leads to a cell lysis phenotype similar to ΔPkc1 mutants; however, the magnitude of the phenotype is less severe, only being evident at higher temperatures. This has lead to the proposal that a bifurcation in the pathway occurs at the level of Pkc1, with a second, as-yet-uncharacterized signalling pathway parallel with the Bck1/Mkk/Mpk1 MAP kinase module [116]. Genetic analysis has also suggested that there may be an alternative downstream partner for Pkc1 within the MAP kinase module. The activation of Mpk1 during heat shock is absolutely dependent on Bck1 [101]. However, whereas the activation of Mpk1 that occurs in response to mating pheromone requires Pkc1 and Mkk1/Mkk2, there is only a partial effect of the deletion of Bck1. This has lead to the suggestion of an alternative mechanism for activation of Mkk/Mkk2 by Pkc1 under certain conditions [119]. Hopefully, further studies will resolve this issue.

MAP kinase modules are generally seen to regulate transcription-factor activity, and this would seem to be the case with the Pkc1 pathway. Mutation of Pkc1 causes reduced expression of several genes involved in cell-wall synthesis, most notably FKS1 [subunit of (1–3)-β-glucan synthase], MNN1 (α-1,3-mannosyltransferase) and CS2 (chitin synthase III) in mid-exponential-phase cells [120]. This indicates that at least part of the effects of the Pkc1 pathway on cell-wall integrity are mediated through regulated gene expression. Two transcription factors, namely SBF and Rlm1, have, on the basis of their genetic interactions with the Pkc1 pathway, been proposed as candidates to execute this regulation. Genetic studies of this part of the pathway are particularly difficult. Transcriptional regulation of the genes involved in cell-wall synthesis in yeast appears to be complex and multifactorial. Mutation of any transcription factor involved the expression of cell-wall genes could potentially aggravate the phenotypes of mutations of the Pkc1 pathway and/or display a cell-wall-lysis phenotype. This gives rise to difficulties in addressing the biological relevance of such genetic data to Pkc1 signalling.

SBF is composed of a regulatory subunit (Swi6) and a DNA-binding protein (Swi4, reviewed in [121]). Swi4 controls the expression of several genes involved in cell-wall synthesis at the G1/S-phase boundary [120], and mutants in Swi4 have temperature-sensitive cell-wall defects [120,122]. It has been shown that Mpk1 can phosphorylate Swi6 and Swi4 in vitro, leading to the proposal that Swi4 is downstream of the Pkc1 pathway [122]. However, whereas expression of Swi4 suppresses ΔBck1 and ΔMpk1 mutants [122], overexpression of Pkc1 can suppress mutants in Swi4 [113]. Further, Pkc1 deficient mutants still show Swi4-dependent transcription of cell-wall genes at the G1/S-phase boundary [120]. Taken together, the data suggest that Swi4 acts in a pathway governing cell-wall integrity that may be parallel with the Pkc1 pathway, rather than co-linear.

Stronger evidence exists for a role for the Rlm1 transcription factor as a downstream effector of the Pkc1 MAP kinase module. This protein was isolated as a suppressor mutant of cells expressing an constitutively active Mkk1 [123]. Rlm1 shows homology with the MADS family of transcription factors which...
includes the mammalian serum response factor (SRF, reviewed in [124]). Rlm1 interacts with Mpk1 in a two-hybrid binding assay [123] and is phosphorylated by Mpk1 in vitro [125]. Most importantly, expression of Rlm1 reporter constructs is entirely dependent on Mpk1 activity in vitro [126]. As yet, the identity of the genes under the control of Rlm1 is unknown. However, they most likely represent only a subset of the genes regulated by the Pkc1 pathway, as deletion of Rlm1 does not lead to the cell-lysis phenotype seen with ΔPkc1 mutants [123].

A recent detailed study of the FKS2 gene has shed light on this. Fks2 is an alternative subunit to Fks1 for the (1–3)-β-glucan synthase. Fks1 is constitutively expressed in cells with some cell-cycle-dependent fluctuations. However, Fks2 expression is normally very low, but is induced under three conditions: exposure to mating pheromone, heat shock or growth on a non-fermentable carbon source [127]. Levin and co-workers have shown that two genetically separate pathways control the expression of the FKS2 gene, one dependent on calcineurin and the other on the Pkc1 MAP kinase module [128]. Pkc1 signalling controls only one of the three triggers to FKS2 expression, the induction during heat-shock. The Pck1-dependent regulatory element in the FKS2 gene promoter has been mapped and shows no requirement for Rlm1 for activation through Pkc1 [128]. It would seem that at least one other, as-yet-unknown transcription factor acts downstream of Pkc1 to control expression of the FKS2 gene.

Regulated gene expression is only one of the probable outcomes of Pkc1 signalling pathways in yeast. As mentioned above, the phenotypes of deletions of members of the Pck1 MAP kinase module are less severe than that of Pck1-deleted strains, suggesting the existence of other downstream pathways from Pck1. Additionally, mutations of members of the Pck1 MAP kinase module have some phenotypes that are hard to explain in terms of regulated gene expression. Polarized growth involves the transport of secretory vesicles along actin cables to the site of cell-wall synthesis. Conditional Mpk1 mutants show disruption of this process, with an accumulation of vesicles in the cytoplasm and a delocalization of cortical actin structures [105]. Clearly other targets of the Pck1 MAP kinase pathway remain to be identified.

Although still incomplete, our current knowledge of PKC signalling pathways in yeast presents interesting and potentially informative parallels with the mammalian system. Broadly speaking, this information can be used in two ways. The most concrete of these is the use of interactions defined in the yeast Pck1 pathway to predict possible signalling partners in higher organisms. The majority of the members of the yeast Pck1 pathway have mammalian homologues, and comparative studies would focus on these proteins as candidate PKC effectors. The interaction between Pck1 and Rho1 [81] was reported over a year before the discovery of the analogous interaction between PRK1 and RhoA [19,20], illustrating the potential predictive value of yeast studies, albeit with hindsight in this case. On a larger scale, comparisons between yeast and mammals can also be made for entire pathways. Such arguments based upon analogy are more diffuse and may have less predictive power. However, in three cases the parallels are compelling and suggest potential directions for future research in mammalian studies.

The most obvious parallel between PKC signalling in yeast and mammals is the regulation of gene expression by the Rho GTPase. In mammalian cells, RhoA induces the expression of a number of immediate early genes in response to serum, including c-fos [129]. The response is mediated by a member of the MADS box family of transcription factors, the SRF (reviewed in [130]). In yeast the Rho1 GTPase also activates a member of the MADS box family of transcription factors, Rlm1, through the Pkc1 MAP kinase pathway. Although currently nothing is known of the signalling pathway to the SRF from RhoA, it is possible that an uncharacterized MAP kinase pathway may be involved. By analogy with yeast, PRKs are an obvious candidate effector for this aspect of RhoA signalling. As yet, only a modest stimulation of SRF activity has been reported in cells overexpressing PRK2 [21]. However, it is possible that these studies require another PRK (1 or 3) or some additional regulatory input to achieve PRK activation.

In mammals, a pathway parallel with that involving Rho and SRF involves the classical MAP kinase cascade (i.e. Raf/MEK/ERK) and the transcription factors of the TCF subfamily of ets-related proteins (reviewed in [130,131]). This pathway can be triggered by phorbol esters and the evidence indicates the involvement of PKC. Thus activated PKC mutants will stimulate the pathway and dominant negative PKC mutants will inhibit, showing that PKC is both necessary and sufficient for the response to phorbol esters. The actual mechanism involved is far from clear, with conflicting evidence as to whether PKC acts directly on c-Raf-1 or not (see [132]. Interestingly, there is evidence that direct PKC phosphorylation of c-Raf-1 contributes to desensitization rather than activation [132]. The control of c-Raf-1 through Ras, tyrosine and serine/threonine protein kinases has yet to be crystallized into a coherent story, and this has complicated the elucidation of the PKC input(s). It is clear that certain PKC isotypes can activate MEK independent of c-Raf-1 [132]. This has parallels with the yeast Pck1 pathway where, as mentioned previously, alternative routes to Bck1 can be used by Pck1 to activate Mkk1/Mkk2 (Figure 6). It may be that the resolution of this signalling junction in yeast will help to clarify PKC signalling to the classical MAP kinase cascade in mammals.

Finally, Bussey has remarked on the potential parallels between Rho action at the polarised bud site in yeast and focal adhesions in mammalian cells [133,134]. Focal adhesions are concentrated aggregates of cytoskeletal proteins and signalling molecules at the plasma membrane which mediate signalling between the cytoskeleton and the extracellular matrix through integrins (reviewed in [135]). Rho action at the bud site and at focal adhesions involves some PKC-independent functions. Thus, in yeast, Rho1 stimulates (1–3)-β-glucan synthase at the bud site directly, independent of Pck1 [136,137]. In mammalian cells RhoA stimulates the formation of actin stress fibres at the focal adhesion through the actions of two non-PKC proteins, p140mDia [138] and ROK [139]. This has parallels with the localization of actin cables at the yeast bud site. However, it seems that other parallels exists between the yeast bud site and focal adhesions that do involve PKC function. In yeast it has been proposed that a mechanosensor (perhaps Hcs77) triggers a Rho1-dependent Pck1 response to heat shock or osmotic shock. Mammalian cells also have signalling pathways to respond to mechanical stress which includes the tensile stress exerted by neighbouring cells and the substratum as well as specialized conditions of stress such as the fluid shear stress experienced by vascular endothelial cells. Several lines of evidence point to integrins as the mechanosensors involved in these responses (reviewed in [140]). PKCz has been shown to be localized to focal adhesions in rat embryo fibroblasts [142], and recently it has been shown that RACK1 binds directly to β-type integrins in a PMA-stimulated fashion, suggesting how PKCs may localize to this compartment [143]. Perhaps the most compelling data is the recent finding that the specific depletion of PKCz using antisense oligonucleotides prevents the stimulation of the ERK1/ERK2 MAP kinase pathway caused by shear stress.
of human umbilical-vein endothelial cells [144]. This presents the best evidence to date for a direct parallel between PKC-dependent mechanosensor pathways in yeast and mammals.

**OTHER GENETIC SYSTEMS**

Genetic analysis of PKC pathways in higher organisms can be expected to give additional information to studies in unicellular yeast. Transgenic mouse knockouts have been established for PKCy [145] and β [146] with associated mild neurological and immune disorders respectively. It is possible that the reason for the fairly benign phenotypes of the single PKC knockouts in mice is that other members of the same PKC subfamilies are able to substitute partially for the deleted gene. In this case, the crossing of single knockout mice to remove an entire PKC subfamily may give more definitive results. As mentioned above, genetic studies in *C. elegans* may represent a complementary way of addressing this problem, as each PKC subfamily is represented by a single isotype. While such studies are still in their early stages, Rubin and co-workers have recently made rapid progress in characterizing the nematode PKCs and it would seem likely that this system will contribute significantly to our understanding of PKC signalling.

Further information may come from other genetic study systems. In *Drosophila* it has been shown that an eye-specific PKC isoform (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148]. Recently PKC isotype (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148]. Recently PKC isotype (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148]. Recently PKC isotype (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148]. Recently PKC isotype (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148]. Recently PKC isotype (InaC) interacts with InaD [147], a scaffolding protein for the phototransduction cascade. This interaction has been shown to occur through one of the five PDZ domains in InaD and serves to regulate the subcellular location of InaC to the rhabdomere [148].

**PERPECTIVES**

Dissecting the complex mesh of mammalian PKC signalling pathways presents several problems. First is the presence of so many PKC isoforms, all with seemingly broadly overlapping substrate specificities. Secondly is the number of potential substrates for these enzymes. The substrate specificity of many protein kinases is governed by the catalytic domain, making the identification of cellular substrates relatively straightforward. However, most proteins contain consensus phosphorylation sites for PKC, and many of these are phosphorylated by PKC in *vitro*. Addressing the biological relevance of these phosphorylations is far from easy. Comparative studies can help to address this problem by providing information about specificity in the PKC signalling pathway. Comparisons of the protein sequences of PKCs from various sources shows that most of the diversity between isoforms is in the regulatory region, and it is here that we can look for specificity in PKC signalling. Comparative studies have defined a number of conserved domains in the regulatory region, and in all cases these have been shown to confer an activation and/or localization input to the enzyme. Each PKC subfamily contains a different arrangement of these regulatory domains, suggesting a method by which isoform specificity may be determined by a unique pattern of localization and activation. The identification of these domains allows us to design experiments to find their binding partners. It also has predictive power; for example, a RhoA-dependent signalling pathway containing a PKC substrate is more likely to involve a PRK than a cPKC. Comparative studies in simple organisms can give further information about specificity in the PKC signalling pathway. The reduced number of PKC isoforms makes the results of genetic studies more conclusive. Thus deletion of one of the 12 PKC isoforms in mice has a mild phenotype, whereas deletion of the sole PKC in *S. cerevisiae* causes growth arrest. Presumably there are the same degree of biologically irrelevant in *vitro* PKC substrates in yeast as there are in mammals. However, the ability to analyse a clear phenotype has made it possible to identify biologically relevant PKC substrates directly and to elucidate the signalling pathway in yeast to much greater detail than has been possible in mammals.

Evidence so far suggests that many of the paradigms of PKC signalling are conserved across species from yeast to mammals. It seems likely that this information gained form the study of PKCs in unicellular systems will help to unravel these complex and important mammalian signalling pathways.

**Note added in proof (received 27 April 1998)**

The cDNA corresponding to the *C. elegans* gene sequence F-46F6.2 has recently been characterized by Rubin and co-workers and designated PKC3 [151].

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