A Novel Adapter Protein Employs a Phosphotyrosine Binding Domain and Exceptionally Basic N-terminal Domains to Capture and Localize an Atypical Protein Kinase C

CHARACTERIZATION OF CAENORHABDITIS ELEGANS C KINASE ADAPTER 1, A PROTEIN THAT AVIDLY BINDS PROTEIN KINASE C3*

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Atypical protein kinase C isoforms (aPKCs) transmit regulatory signals to effector proteins located in the cytoplasm, nucleus, cytoskeleton, and membranes. Mechanisms by which aPKCs encounter and control effector proteins in various microenvironments are poorly understood. By using a protein interaction screen, we discovered two novel proteins that adapt a Caenorhabditis elegans aPKC (PKC3) for specialized (localized) functions; protein kinase C adapter 1 (CKA1, 593 amino acids) and CKA1S (549 amino acids) are derived from a unique mRNA by alternative utilization of two translation initiation codons. CKA1S and CKA1 are routed to the cell periphery by exceptionally basic N-terminal regions that include classical phosphorylation site domains (PSDs). Tethering of PKC3 is mediated by a segment of CKA1 that constitutes a phosphotyrosine binding (PTB) domain. Two aromatic amino acids (Phe175 and Phe221) are indispensable for creation of a PKC3-binding surface and/or stabilization of CKA1-aPKC complexes. Patterns of CKA1 gene promoter activity and CKA1/CKA1S protein localization in vivo overlap with patterns established for PKC3 expression and distribution. Transfection experiments demonstrated that CKA1/CKA1S sequesters PKC3 in intact cells. Structural information in CKA1/CKA1S enables delivery of adapters to the lateral plasma membrane surface (near tight junctions) in polarized epithelial cells. Thus, a PTB domain and PSDs collaborate in a novel fashion in CKA1/CKA1S to enable tethering and targeting of PKC3. Avid ligation of a PKC isoform is a previously unappreciated function for a PTB module.

Many hormones and growth factors elicit activation of phospholipases that produce diacylglycerol (DAG)† (1, 2). Protein kinase C (PKC) isoenzymes disseminate signals carried by DAG. Amplification and routing of signals are achieved because classical (α, βI, βII, γ) and novel (δ, ε, η, θ, μ) PKC isoforms (cPKCs and nPKCs, respectively) are DAG-dependent Ser/Thr phosphotransferases that translocate from cytoplasm to membranes where DAG accumulates in response to hormones (1–4). Activated PKCs phosphorylate proteins that control secretion, mitogenesis, cytoskeleton organization, gene transcription, and many other physiological processes (1–3, 5–9).

Atypical PKCs (aPKCs), which include vertebrate PKCζ and PKCι isoforms and Caenorhabditis elegans PKC3 (1, 4, 8), also regulate critical cell functions. PKCζ and/or PKCι activate the Ras-mitogen-activated protein kinase cascade, stimulate gene transcription, inhibit apoptosis, modulate ion channel activities, phosphorylate nucleolin in the nucleus, and mediate translocation of a glucose transporter between internal and plasma membranes (9–16). C. elegans PKC3 is required for polarized accumulation of several regulatory proteins along portions of the periphery of 1-cell embryos (17, 18). Thus, aPKCs apparently regulate effector proteins at multiple intracellular locations.

Mechanisms by which aPKCs are activated and targeted to specific microenvironments are poorly understood. All PKCs have C-terminal catalytic domains and N-terminal pseudosubstrate sites and Cys-rich regions (C1 domains) (1, 2, 4); phosphatidyserine stimulates catalytic activity of all PKCs. However, aPKCs do not bind Ca2+, DAG, or phorbol esters (which mimic DAG) and are not activated or translocated by these molecules (19–21). aPKCs also lack transmembrane domains, cytoskeleton attachment sites, and organelle targeting motifs. Unlike other PKC isoforms, aPKCs escape endosome-mediated degradation when cells are incubated chronically with hormones, DAG analogs, or phorbol esters (19–21). Thus, the paradigm of DAG-mediated membrane translocation/activation and subsequent degradation cannot explain targeting and regulation of aPKCs.

Recent evidence suggests a “recruitment model” for incorporation of aPKCs into signaling pathways. aPKCs exhibit sub-

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1 The abbreviations used are: DAG, diacylglycerol; PKC, protein kinase C; aPKC, atypical protein kinase C; CKA1, C kinase adapter (apparent M_r = 64,000); GST, glutathione S-transferase; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pairs; kb, kilobase; kbp, kilobase pairs; nts, nucleotides; PTB domain, phosphotyrosine binding domain; PSD, phosphorylation site domain; GFP, green fluorescent protein; dNumb, Drosophila Numb protein; mNumb, mouse Numb protein; CMV, cytomegalovirus; FITC, fluorescein isothiocyanate; MARCKS, myristoylated alanine-rich C kinase substrate; MDCK, Madin-Darby canine kidney cells.

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stansional levels of basal activity and are only modestly activated (1–5–2-fold) by hormones and growth factors (16, 22). Coimmonplementation experiments reveal that aPKCs associate (directly or indirectly) with critical signaling enzymes, including p70S6 kinase, Akt/PKB, and PDK-1 (22–24). In addition, several nonenzymic proteins (ZIP, PAR-4, LIP) bind aPKCs and modulate phosphotransferase activity (25–27). Further clues emerged from our investigations on C. elegans PKC3 (10). C. elegans physiology is controlled by signaling molecules and mechanisms that are operative in mammals (28, 29). PKC3 is (a) the sole aPKC encoded in the C. elegans genome (8), (b) is activated by phosphatidylinositol, and (c) possesses catalytic, pseudosubstrate, and C1 domains that share >55% sequence identity with corresponding regions of PKCs ς and ϖ (8). Ablation of PKC3 function in vivo revealed that this kinase is essential for normal progression of embryogenesis and viability of the organism (8, 17). In 1-cell embryos PKC3 apparently binds with the Par-3 protein at the cell periphery, and this complex (in concert with proteins named Par-1, Par-2, and Par-6) establishes cell polarity, controls asymmetric cell divisions, and determines cell fates (17, 18). Mechanisms by which PKC3 influences these processes are currently unknown.

PKC3 is tightly associated with particulate fractions derived from post-embryonic (larvae and adult) C. elegans. The kinase accumulates at inner apical surfaces of intestinal and pharyngeal cells2 and along the cortical cytoskeleton/plasma membrane border of other cells (8). Cells of larval and adult C. elegans that exhibit polarized enrichment of PKC3 are highly differentiated, nonmitotic, and not destined for apoptosis. Thus, anchored PKC3 in larvae and adult animals evidently subserves functions that differ from those mediated by PKC3 in rapidly dividing embryonic cells. The distinctive DAG independence and constitutive activity of aPKCs, the propensity of aPKCs to engage in protein-protein interactions, and the asymmetric localization of aPKCs in cells in vivo suggest that these kinases are adapted for multiple functions by binding with docking proteins. To verify this concept, it is essential to (a) characterize adapter proteins that avidly bind aPKCs; (b) demonstrate that candidate adapters ligate aPKCs in intact cells and target the bound kinase to a specific location; (c) map domains and individual amino acids in the adapter and cognate aPKCs that govern complex formation; and (d) establish the structural basis for attachment of adapter-bound aPKCs to membranes or cytoskeleton. We now report the discovery and characterization of novel PKC3-docking proteins named C kinase adapter-1 (CKA1) and CKA1S that satisfy criteria elaborated above. We also demonstrate a new role for a conserved, PTB domain in CKA1, ligation of a PKC isoform.

**EXPERIMENTAL PROCEDURES**

*Isolation of cDNA Encoding C. elegans CKA1, a PKC3-binding Protein*—A cDNA encoding C. elegans PKC3 was cloned as described previously (8). Complementary DNA encoding amino acids 1–233 in PKC3 (8) was synthesized via the polymerase chain reaction (PCR) and cloned into the yeast expression plasmid pACT (provided by R. Barstead, Oklahoma Medical Research Foundation) which contains an open reading frame for 535 amino acids and a translation initiation codon, was selected for detailed characterization. The cDNA insert was excised from recombinant pACT by digestion with XhoI and was cloned into the plasmid pBluescript SKII. This recombinant plasmid was named pCKA. Alignment of the DNA sequence of the 1.9-kbp insert from pCKA with sequences in the C. elegans EST Data Base (DNA Data base of Japan and NCBI/NIH) identified an overlapping partial cDNA (GenBank™ accession number C99238) that contained a 159-bp extension at the 5′ end. Sequences of the 159-bp extension and the 1.9-kbp insert are joined in the DNA sequence represented for cosmid T03D8 (GenBank™ accession number 292838). A cDNA (clone yk158f8) encoding the 159-bp 5′ extension was provided by Dr. Y. Kohara (Gene Network Laboratory, National Institute of Genetics, Mishima, Japan). Further analysis of the sequence of genomic DNA in cosmids T03D8 revealed that yk158f8 cDNA lacked an initiator AUG and 4 additional codons that are predicted to constitute the 5′ end of the C. elegans PKC3 structural gene. A cDNA that encodes amino acids 1–193 in CKA1 was generated by PCR, using yk158f8 cDNA as a template. The 5′ primer contained a NotI restriction site which preceded codons for predicted amino acids 1–5 (MSASQ) and 22 nts that matched nts 1–22 in yk158f8 cDNA. The 3′ primer sequence corresponded to the inverse complement of nts 357–406 in pCKA (nts 546 to 565 in yk158f8 cDNA) and includes the unique PstI site. The PCR product was cleaved with NotI and PstI and cloned into pCKA, which was cut with the same restriction enzymes. This resulted in assembly of a full-length cDNA (pCKA1) that encodes a protein composed of 593 amino acids.

**DNA Sequencing and Analysis—**cDNA inserts, including full-length CKA1 cDNA, were sequenced as described previously (31). Analysis of sequence data, sequencing reactions, and database searches were performed using programs provided by the NCBI Server at the National Institutes of Health and the SMART website (32–34).

*Isolation of mRNA and Northern Blot Analysis—*Poly(A)′ RNA was isolated, size-fractionated in a denaturing gel, and transferred to a Nitran membrane as described previously (35). A 32P-labeled cDNA probe was generated by random priming, using the 1.9-kbp cDNA cDNA (excised from pCKA) as a template. CKA1 mRNA 5′-EPD-cDNA hybrids were detected by autoradiography on XAR-5 x-ray film (Eastman Kodak Co.) at −75 °C.

*Characterization of the 5′ End and Overall Size of CKA1 cDNA—*Complementary DNA was synthesized by reverse transcriptase (RT), using 2 μg of C. elegans poly(A)′ RNA as a template (35). PCR amplification was performed with a 5′ primer (5′-GAATCTGAGGTTTAAT-TCCCAAATGGTTAG-3′) that contained the added leader 1 (SL1) sequence (see “Results”) downstream from an XbaI restriction site. A series of nested 3′ primers corresponded to a variety of proximal and distal portions of the cDNA sequence for the insert in pCKA1. Nucleotides 5–10 of the 3′ primers constituted an XhoI recognition sequence. PCR products were digested with XbaI and XhoI, cloned into the pBluescript SKII, and sequenced. The same RT-PCR methodology and strategy were employed to synthesize full-length CKA1 cDNA.

*In Vitro Transcription and Translation of CKA1—*35S-Labeled CKA1 and CKA1S were generated by the TNT™-coupled reticulocyte lysate system (Promega Corp.) as described previously (8).

*Production and Affinity Purification of Anti-CKA1 IgGs—*A segment of cDNA encoding amino acids 232–368 of CKA1 was synthesized via the polymerase chain reaction and cloned into the pET-29a vector (Novagen). The expressed kinase was purified with an affinity column using a photoaffinity ligand, and the mAb was expressed in hIgG using a Baculovirus system. The affinity purified antibody was used as an affinity ligand.

**Cell Culture and Transfection—**A cell line (AV-12) derived from a hamster subcutaneous tumor and LLC-PK1 kidney epithelial cells was used as a standard.

**Growth and Extraction of C. elegans—**The Bristol N2 strain of C. elegans was grown randomly or synchronously at 20 °C as described previously (38). Particulate and cytosolic fractions were isolated from L1–L4 larvae and young adult C. elegans as described by Hu and Rubin (38).

**Protein Determination—**Protein concentrations were measured using the Coomassie Plus Protein Assay Reagent (Pierce). Albumin was used as a standard.

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obtained from the American Type Culture Collection. MDCKII cells were provided by Dr. M. Lisanti, Department of Molecular Pharmacology, Albert Einstein College of Medicine. Cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum in an atmosphere of 95% CO₂ and 5% air. Full-length CKA1 cDNA was cloned into the mammalian expression vector pcDNA3.1 at the BamHI-DraI sites. This plasmid, the CKA1 cDNA downstream from a strong, constitutively active CMV promoter and upstream from a polyadenylation signal. Cells were transfected with recombinant plasmid via calcium phosphate precipitation, as described previously (39, 40).

**Immunoprecipitation Assays—**Cells were harvested 24 h after transfection and organized in buffer containing 0.5% (w/v) Triton X-100 as previously reported (40). Lysates were centrifuged at 12,000 x g for 30 min, and samples of the supernatant solution (0.3 mg of protein) were incubated with either 10 μl of affinity purified IgG directed against CKA1 or PKC3 (1:5 relative to serum) or 2 μl of preimmune serum for 16 h at 4 °C. Subsequently, 25 μl of protein A-Sepharose 4B beads (Zymed Laboratories Inc.) was added, and the incubation was continued at 4 °C for 60 min. Beads were collected and washed five times (1 ml per wash) with phosphate-buffered saline (10 mM sodium phosphate, pH 7.4, 0.15 m NaCl) containing 0.1% (w/v) Tween 20.

**Electrophoresis of Proteins and Western Immunoblots—**Proteins (40 μg) from C. elegans, fractions from transfected cells, or immunoprecipitates were denatured in gel loading buffer and subjected to electrophoresis in 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (SDS) as described previously (37). BenchMarker™ prestained proteins (9–182 kDa) (Life Technologies, Inc.) were used as standards for estimation of molecular weight values. Western blots, prepared on Immobilon P membranes (Millipore Corp.), were blocked, incubated with IgGs directed against CKA1 (1:4000) or PKC3 (1:2000) (8), and washed as described previously (40). Antibody-IgG complexes were visualized by an indirect chemiluminescence procedure (40). Signals were recorded on x-ray film.

**Preparation of Transgenic C. elegans—**Transgenic nematodes were created to investigate in vivo activity of the CKA1 gene promoter. A segment of genomic DNA, which includes 1.4 kbp of contiguous 5′-flanking DNA, exon I, intron I, and 51 codons of exon II from the CKA1 structural gene, was synthesized via PCR, using cosmid TO3D8 as a template. This 2-kbp genomic DNA insert was cloned into the C. elegans expression vector pPD65.67 (provided by Dr. A. Z. Fire, Carnegie Institute, Baltimore, MD). CKA1 exon II was fused in-frame with DNA encoding a nuclear localization signal and the green fluorescent protein (GFP) reporter gene. C. elegans were transformed by microinjection, selected, fixed, and stained as reported previously (41). Accumulation of GFP fluorescence was monitored by capturing fluorescence signals using a Nikon Eclipse E400 microscope equipped with Nikon 40× NA 1.4 plan-apo optics and an FITC filter.

**Deletion and Site-directed Mutagenesis—**Deletion mutagenesis was performed on CKA1 cDNA via PCR, as described previously (31, 42). For C-terminal deletions, each 5′ primer contained an EcoRI restriction site followed by nts 175–194 of CKA1 cDNA. Each 5′ primer contained a 3′ probe sequence and created a genomic clone after it is ligated into the C. elegans expression vector pPD65.67 (provided by Dr. A. Z. Fire, Carnegie Institute, Baltimore, MD). CKA1 exon II was fused in-frame with DNA encoding a nuclear localization signal and the green fluorescent protein (GFP) reporter gene. C. elegans were transformed by microinjection, selected, fixed, and stained as reported previously (41). Accumulation of GFP fluorescence was monitored by capturing fluorescence signals using a Nikon Eclipse E400 microscope equipped with Nikon 40× NA 1.4 plan-apo optics and an FITC filter.

**Characterization of cDNA That Encodes a Novel PKC3 Adapter Protein, CKA1—**A C. elegans cDNA library in the yeast plasmid, pACT, was screened for expression of PKC3-binding proteins via the two-hybrid protein interaction assay. A recombinant pACT plasmid, which produced the strongest signal in β-galactosidase reporter assays and exhibited robust growth in selective medium, was characterized. The cDNA insert was excised from recombinant pACT and subcloned into the plasmid pBluescript SKII. The resulting recombinant plasmid was named pCKA. Sequence analysis disclosed that the novel 1.9-kbp cDNA insert contained an open reading frame for 535 amino acids, a translation termination codon, 3′-untranslated sequence (317 nts), and a polyadenylate tail. However, the 5′ terminus of the PKC3 cDNA lacked untranslated nucleotides and an initiating ATG codon. Moreover, the 1.9-kbp cDNA hybridized with a 2.1-kbp transcript on a Northern blot of C. elegans mRNAs.

To characterize the 5′ end of adapter protein cDNA, we used RT-PCR methodology and also extracted critical information from C. elegans EST and genomic data bases (43). Alignment of pCKA cDNA with EST sequences identified an overlapping partial cDNA (GenBank™ accession number C09238) that contained a 159-bp extension at the 5′ end. Adjacent downstream sequence was identical with nts 1–509 in pCKA cDNA. Further insights were obtained by comparing the PKC3 adapter cDNA sequence with predicted exons in the C. elegans genome database (43). The sequence of an exon in cosmid TO3D8 (GenBank™ accession number 292838) confirmed that the 159-bp 5′ cDNA extension was linked with the pCKA cDNA-derived sequence to yield an extended open reading frame. In an upstream exon, a consensus initiator ATG and four additional codons preceded genomic sequence that is identical with the 5′-terminal region established by analysis of cDNA clones. Furthermore, a consensus acceptor site (TTT-GTTTCAAG) for the trans-splicing machinery of C. elegans precedes the putative initiator ATG codon. The 5′ termini of most (~70%) C. elegans mRNAs are modified by incorporation of a 22-nt, nontranslated “leader” sequence that is donated by a distinct 100-nucleotide spliced leader 1 RNA transcript (44). The leader RNA fragment (SL1) is inserted downstream from the acceptor site in a reaction that results in excision of the original 5′ end of the transcript. trans-Splicing generates a short 5′-untranslated region and introduces an essential trimethylguanosine cap at the 5′ terminus of the mRNA (44, 45). Thus, organization of genomic DNA suggested that nucleotides −22 to +15 in Fig. 1A constitute the 5′ end of PKC3 adapter protein cDNA. This idea was tested experimentally. Single-stranded cDNA was synthesized by RT using C. elegans mRNA as a template. cDNA transcripts were amplified via PCR, using the SL1 cDNA sequence for the 5′ primer and series of nested 3′ primer corresponded to either proximal or distal portions of the 1.9-kbp pCKA cDNA sequence. Sequencing of PCR products revealed that all adapter protein cDNAs hybridized with the sequence shown in Fig. 1A, include the entire predicted 159-bp extension, and terminate with cDNA sequence determined for the original 1.9-kbp insert in the recombinant pACT plasmid.

The highly sensitive RT-PCR methodology was used to verify the size of adapter protein mRNA and assess possible heterogeneity among transcripts. A single band corresponding to a
The cDNA sequence is deposited in the GenBank™ data bank and is identified by accession numbers AF286205 and AF286206. The cDNA sequence encodes a predicted PKC3 tethering protein (593 amino acids) that was named C kinase adapter 1 (CKA1). The amino acid sequence for CKA1 is presented in Fig. 1C.

Structural Properties of CKA1—CKA1 is a 66-kDa basic protein (pl ~ 9.9) that carries a (predicted) net charge of +17 at pH 7.4. Computer-mediated comparison with amino acid sequences compiled in protein data bases revealed a tripartite structural organization for CKA1. Two conserved structural modules are embedded within a segment of CKA1 that spans residues 1–231 (Fig. 1C). In contrast, central and C-terminal regions of CKA1 (residues 232–593), which contribute 3% of the amino acid sequence, are not homologous (<15% identity) with previously studied polypeptides. A striking feature of CKA1 is that the sequence bounded by Lys90 and Arg231 aligns with 141-residue portions of the Drosophila Numb (dNumb) protein (46) and a mouse Numb (mNumb) homolog (47) to yield 70–73% identity (Fig. 2A). When conserved amino acid substitutions are included in the calculation, the degree of similarity among the three protein fragments rises to ~85%. Segments of dNumb and mNumb65 shown in Fig. 2A fold into a conserved three-dimensional structural module known as a phosphorytethering domain or PTB domain (48–51). PTB domains are configured by arranging 7 β-strands in a “sandwich” structure that is capped by a C-terminal α-helix (48–51). Some PTB domains also contain one or two α-helices that precede β-strand 1 at the N terminus. PTB domains are incorporated into a structurally diverse group of proteins that perform distinct functions. Initial characterizations suggested that the β-sandwich structure specifically docked with short, contiguous segments (~8–15 amino acids) of target proteins that contained phospho-Tyr within an NPXY motif (where X = any amino acid and P and Y is phospho-Tyr) (48, 49). However, further analysis disclosed that this conclusion only applies to a subset of PTB domains. More than 50% of currently characterized PTB modules bind target sequences that either (a) have a critical but nonphosphorylated NPXY motif or (b) lack Tyr altogether (48–51).

Although all PTB domains fold in a similar manner, their amino acid sequences can vary substantially (48, 49). Moreover, side chains of nonconserved residues may affect binding specificity. The high degree of sequence similarity between a segment of CKA1 (residues 90–231) and Numb PTB domains is a unique relationship. No other PTB domains collected in mammalian, Drosophila, or C. elegans data bases are closely related (typical sequence identity ~26%) to the CKA1 sequence presented in Fig. 2A. Thus, residues 90–231 in CKA1 evidently constitute a specialized PTB domain that subserves functions characteristic of the Numb protein family (one Drosophila and four mammalian Numb isoforms). Numb proteins mediate asymmetric localization of several regulatory proteins in vivo (52, 53). Determination of a solution structure for a peptide-PTB domain complex and peptide binding studies indicate that the prototypic Drosophila Numb PTB module interacts with multiple target proteins by providing a unitary but flexible binding surface (50, 51). This surface accommodates several classes of structurally diverse peptides that are presented in either type I β-turn or α-helical turn conformations (50, 51). Analogies between Numb and CKA1 PTB domains strongly suggest that the cited binding properties will be evident in the PKC3-docking protein. The sequence of the C. elegans CKA1 PTB region can be resolved into probable β-strands and α-helices (as depicted in Fig. 2B) by comparison
with the established folding patterns of the corresponding portions of the dNumb domain.

The N-terminal region of CKA1 (amino acids 1–77, Fig. 1C) is unusually basic. Arg and Lys account for 25% of the N-terminal amino acids, and the predicted pI for this domain is 12.4. The basic residues are not randomly distributed in this region. Instead, they are highly enriched in short clusters that correspond to residues 11–25 and residues 61–77 (Fig. 2C) in the CKA1 sequence (Fig. 1C). In the clusters, Arg and Lys are intermingled with Ser and large hydrophobic amino acids (Leu and Ile). This arrangement and composition of amino acids generates sequences that resemble the “phosphorylation site domains” (PSDs) of the ubiquitous MARCKS protein and MARCKS-related proteins (54–55). The MARCKS PSD constitutes a major, highly specific target site for PKC-catalyzed phosphorylation in many types of mammalian cells. Cycles of PSD phosphorylation/dephosphorylation are associated with dynamic changes in cytoskeleton and reversible targeting of MARCKS to membranes. In nonphosphorylated MARCKS, the high local level of net positive charge in the PSD domain promotes association with membranes by electrostatic interaction with head groups of anionic phospholipids (54–56). By analogy, CKA1 segments that include amino acids 11–25 and/or 61–77 (Fig. 2C) are candidate substrates for PKC3 (or other PKCs). They may also be involved in targeting CKA1-PKC3 complexes to specific intracellular locations.

Examination of the N-terminal portion of dNumb revealed that the fly protein, like CKA1, contains two PSD-like regions (residues 32–41 and residues 44–58, Fig. 2C). In contrast, the short sequence (only 24 amino acids) that precedes the mNumb65 PTB domain accommodates only one PSD-like site (residues 3–12, Fig. 2C). The basic clusters in CKA1, dNumb, and mNumb cannot be aligned to yield high levels of sequence identity. However, these protein segments share the composition and charge characteristics that are hallmarks of previously characterized, functionally homologous PSD domains (see Ref. 57 for detailed discussion of this point).

Central and C-terminal portions of CKA1 (residues 232–593) share only one property with corresponding regions in the Numb homologs, the inclusion of five or more PXXP sequences. The presence of multiple copies of this consensus SH3-binding site raises the possibility of scaffolding functions. However, the physiological significance of these minimal binding sequences must be tested experimentally. Overall, the sequence of the CKA1 central and C-terminal domains diverges markedly (<15% identity) from the sequences of analogous regions of dNumb and mNumb.
Tethering of PKC3 by C Kinase Adapter Proteins 1 and 1S

TABLE I
Organization of the CKA1 gene

| Exon | Nucleotides | Amino acid residues | Downstream intron |
|------|-------------|---------------------|-------------------|
| 1    | 155         | 1–52               | bp                |
| 2    | 97          | 52–86              | bp                |
| 3    | 375         | 86–209             | 208               |
| 4    | 375         | 210–333            | 487               |
| 5    | 111         | 334–370            | 1325              |
| 6    | 274         | 371–462           | 181               |
| 7    | 266         | 462–550           | 53                |
| 8    | 460         | 550–653            | 3–UT*             |

* UT, untranslated sequences.

dNumb and mNumb65. When sequences for the full-length proteins are aligned CKA1 exhibits levels of 31 and 28% identity with dNumb and mNumb, respectively. Various structural relationships among CKA1 and the Numb homologs are summarized diagrammatically in Fig. 2D.

Organization of the CKA1 Gene—A search of the C. elegans genome data base with the cdna sequence for CKA1 disclosed that cosmid T03D8 (GenBank™ accession number Z92838) contains the gene for the docking protein. Alignment of the cdna and cosmid sequences disclosed that the CKA1 gene contains 8 exons and 7 introns that span 5,174 bp of DNA (Table I). Coding sequences for the basic, PSD-like segments of CKA1 (Fig. 2C) are provided by exons I and II. The conserved PTB domain sequence is derived from two exons. Codons in exon III specify the two N-terminal α-helices and the 7 β-strands of the peptide-binding module (Fig. 2B); exon IV encodes the critical α3-helix that caps the “PTB fold” (Fig. 2B) and includes crucial amino acid residues that are directly involved in ligand binding (see below). The gene for CKA1 is located at the far right end of chromosome V (at +25.2), near the grd-1 and jcy-22 genes.

Detection and Expression of Two Isoforms of CKA1—Properties of the PKC3 adapter protein were initially assessed in AV-12 cells that were transiently transfected with the CKA1 transgene. Immunoglobulins in anti-CKA1 serum (see “Experimental Procedures”) avidly bound epitopes in two polypeptides with apparent Mr values of 75,000 and 64,000 (Fig. 3A, lane 1). These proteins were not detected when duplicate blots were probed with preimmune serum or anti-CKA1 IgGs that were incubated with excess GST-CKA1 fusion protein antigen (Fig. 3A, lanes 4 and 5). Moreover, the 64- and 75-kDa proteins were not evident among proteins extracted from nontransfected AV-12 cells (Fig. 3A, lane 6). Since only one species of CKA1 mRNA (2.1 kb) is expressed in vivo (see Fig. 1B and text above), a likely explanation for the unanticipated expression of two PKC3 adapter proteins involves utilization of the Met1 and Met45 codons (Fig. 1C) as alternative sites for translation initiation. To test this idea, codons 1–44 (Fig. 1C) were deleted from CKA1 cDNA. Expression of this truncated (Δ44) transgene in AV-12 cells resulted in the loss of the 75-kDa antigen and exclusive accumulation of the 64-kDa isoform of the PKC3 adapter (Fig. 3A, compare lanes 1 and 2). Exclusion of codons 1–58 (Fig. 2) from the CKA1 transgene forced utilization of Met$^59$ as the translation start site. The Δ58 transgene directed synthesis of a protein with an apparent Mr of 62,000 (Fig. 3A, lane 3). Since this protein is not expressed in cells transfected with either wild type or Δ44 transgenes, it appears that (a) only Met codons 1 and 45 are in an appropriate sequence context to engage the translation initiation complex of ribosomes, and (b) both Met1 and Met45 are used for translation initiation in intact cells. To exclude alternative processing of adapter protein transcripts in the AV-12 cell system, full-length (2.1 kb) CKA1 cDNA was used as a template for in vitro synthesis of CKA1. Translation of the homogeneous 2.1-kb mRNA in a reticulocyte lysate yielded substantial amounts of both the 64- and 75-kDa isoforms of CKA1 (Fig. 3B, lane 2). Thus, a single CKA1 transcript encodes two related proteins; CKA1 is composed of 593 amino acids (Fig. 1C) (pI = 9.9) and has a calculated Mr of 65,500; CKA1S (named as C kinase adapter 1 short isoform) contains 549 amino acids (residues 45–593 in Fig. 1C) (pI = 9.4) and has a calculated Mr of 60,700. Only one PSD-like domain precedes the PTB domain in CKA1S. Both CKA1 (apparent Mr = 75,000) and CKA1S (apparent Mr = 64,000) migrate atypically slowly in denaturing gel electrophoresis, yielding overestimates of their sizes. This property is observed in many basic proteins.

Expression and Localization of CKA1 and CKA1S in Vivo—IgGs directed against CKA1/CKA1S complexed both adapter protein isoforms in samples of total C. elegans proteins (Fig. 4A). CKA1 and CKA1S are expressed in vivo at each stage of C. elegans development (Fig. 4B). Both proteins are recovered in the particulate fraction of C. elegans homogenates, indicating a tight association with organelles and/or cytoskeletal structures. The relative abundance of the 64- and 75-kDa CKA1 isoforms varies during the life span of the nematode. For example, the ratio CKA1S:CKA1 is ~2–3 in embryos and L1 larvae but declines to ~0.5 in adult animals. The predominance of CKA1S in early larvae and CKA1 in adult C. elegans suggests that functions of these closely related PKC3-binding proteins may differ, qualitatively or quantitatively, at distinct developmental stages. Developmentally regulated changes in (a) the frequency of utilization of alternative translation initi-
CKA1 and CKA1S Isoforms Are Differentially Routed to Lateral Junctions Between Polarized Cells—MDCK and LLC cells form a polarized epithelial sheet in culture (59, 60). Apical and basolateral portions of the plasma membrane of polarized epithelial cells have different protein and lipid compositions and subserve distinct physiological functions (61–63). Tight junction complexes, which assemble at points of physical contact between lateral surfaces of adjacent cells, (a) maintain cell polarity by limiting the mixing of apical and basolateral membrane components within the phospholipid bilayer and (b) constitute a stringent permeability barrier between the apical and basal environments. Functions performed by apical, basal, and lateral (tight junctions) segments of MDCK and LLC cell membranes are mediated and regulated by integral membrane proteins in concert with co-clustered peripheral proteins. Signaling and adapter proteins are recruited to specialized segments of the inner surface of the cell membrane by binding with either cytoplasmic domains of transmembrane proteins, certain phospholipids, or docking proteins in adjacent cortical cytoskeleton. Thus, MDCK and LLC cells provide pertinent model systems for assessing the inherent ability of PKC3 adapter proteins to anchor differentially at a discrete target site.

Immunofluorescence microscopy was used to investigate the location of PKC3 adapter proteins in MDCK cells that express the CKA1 transgene. Examination of standard (X-Y) optical

**Fig. 4. Expression of CKA1 and CKA1S in vivo.** A, Western blot analysis of cell proteins was performed as described under “Experimental Procedures” and Fig. 4. Each lane received 40 μg of total proteins derived from a population of *C. elegans* that included animals at all developmental stages. Lanes 1 and 4 were incubated with affinity-purified IgGs (1:4000 relative to serum) directed against CKA1. Excess purified antigen (3 μg) was present when lane 4 was probed with the purified antibodies. Lanes 2 and 3 were incubated with preimmune and nonimmune sera (1:2000), respectively. After application of peroxidase-coupled secondary antibodies, chemiluminescence signals were captured on x-ray film. Only the relevant portion of the immunoblot is shown. No other immunoreactive proteins were observed. B, particulate (P) and soluble cytosolic (C) proteins were isolated from homogenous populations of *C. elegans* embryos (E), L1–L4 larvae and adult animals as indicated under “Experimental Procedures.” Samples of these proteins (30 μg) were assayed for CKA1 (75 kDa) and CKA1S (64 kDa) expression by Western immunoblot analysis as described above.
sections disclosed that PKC3 adapter protein accumulates at sites of cell-cell contact (Fig. 8A). Localization of CKA1/CKA1S was more precisely defined by analyzing optical sections in X-Z planes (Fig. 8, B and C). PKC3 adapter protein is largely excluded from the apical and basal portions of the cell periphery. Instead, CKA1/CKA1S is targeted and concentrated in a limited region of the lateral surface of MDCK cells (Fig. 8, B and C). This region corresponds to the position of tight junctions (61–63). Expression of the CKA1 transgene in polarized LLC cells also resulted in enrichment of the PKC3-binding protein at the cell surface (Fig. 8D). A Z series of optical sections revealed that membrane-associated CKA1/CKA1S accumulates in central segments of LLC cells (e.g. Fig. 8, E2 and E3). In contrast, adapter protein was not abundant at the basal or apical surfaces of the cells (Fig. 8, E1 and E4, respectively).

The disposition of CKA1/CKA1S in LLC cells indicates that the PKC3 adapter is selectively targeted to the lateral surface of polarized plasma membrane. Thus, the amino acid sequence or primary structure of CKA1 isoforms have the ability to bind and target an atypical PKC to a highly specialized segment of the periphery of polarized cells.

The PTB Domain of CKA1 Isoforms Mediates the Binding of PKC3—N- and C-terminal boundaries for the PKC3-tethering module in CKA1/CKA1S were mapped by using the highly sensitive yeast two-hybrid complementation assay in combination with deletion mutagenesis. Segments of CKA1 cDNA indicated in Fig. 9A were tagged with EcoRI and XhoI restriction sites and amplified via PCR methodology. Upon cloning into the yeast target vector pACT, the CKA1 cDNAs aligned inframe with the 3′ end of vector DNA encoding the GAL4 tran-

Fig. 5. C kinase adapter protein accumulation and CKA1 gene promoter activity in situ. A, the location of CKA1/CKA1S in embryos was determined by immunofluorescence analysis. Fluorescence signals that correspond to CKA1/CKA1S IgG complexes are enriched along segments of the cell periphery that are involved in cell-cell junctions (arrows). A 4-cell embryo is shown. B, locations of nuclei in the embryo shown in A are revealed by staining with diamidophenylindole, which intercalates into DNA. Chromosomes in two nuclei are associated with the mitotic spindle apparatus. Positions of plasma membranes in the 4-cell embryo (A) were determined by interference microscopy and traced to generate the diagram in C. Comparison of A and C reveals that CKA1/CKA1S accumulate at junctions of cell 3 with cells 4, 1, and 2, and at points of contact between cells 1 and 2. A representative 4-cell embryo is shown in A–C. Other examples of this pattern of adapter protein accumulation were observed in independent immunostaining experiments. D, transgenic C. elegans that carry the GFP reporter gene under the regulation of CKA1 promoter/enhancer were fixed with paraformaldehyde. Fluorescence signals emanating from GFP in the nucleus were observed and recorded with a Nikon Eclipse 400 microscope. Robust CKA1 promoter activity was evident in both the large nuclei of (>10) intestinal (gut) cells and smaller nuclei located in anal depressor and sphincter muscle cells and cells of the intestinal-rectal valve. A modest level of CKA1 gene transcription was detected in pharyngeal muscle nuclei. (The intensity of fluorescence signals near the pharynx (bounded by the white rectangle) was enhanced 2.5-fold so that high level accumulation of GFP in gut nuclei and the lower abundance of GFP in nuclei of pharyngeal cells could be visualized in a single micrograph.) A representative L3 nematode is shown. Similar patterns of CKA1 gene transcription were obtained for both adults and larvae in several experiments.
scription activation domain. Cotransformation of yeast with a recombinant target vector and a yeast bait plasmid that directs synthesis of a GAL4 DNA binding domain-PKC3 (residues 1–233) chimeric protein enabled detection of PKC3 binding activity via outputs from two complementation assays, growth on His− medium and expression of β-galactosidase. Results from β-galactosidase assays are summarized in Fig. 9A; the ability of transformed yeast to grow on His-deficient plates paralleled β-galactosidase production in all cases. Deletion of residues 236–593 had no effect on the binding of a CKA1 fragment (∆3, Fig. 9A) with partial PKC3 protein. Thus, the novel central and C-terminal regions of CKA1 are not involved in tethering the aPKC. Elimination of one (∆3, Fig. 9A) or both (∆11, Fig. 9A) PSD-like regions at the N terminus did not alter PKC3 ligation. In contrast, truncations that removed either N- or C-terminal portions of the PTB domain (∆6, ∆7, Fig. 9A) abolished PKC3 binding activity. The minimal CKA1 segment that retained avid PKC3 ligation activity included residues 88–235 (∆11, Fig. 9A). This corresponds to an intact PTB domain (amino acids 90–231) that is flanked by only six additional N- or C-terminal residues. Thus, it appears that precise folding of seven β-strands and three α-helices of the PTB module (Fig. 2B) into a specific three-dimensional configuration is required to generate a PKC3-binding surface.

Rational site-directed mutagenesis of the CKA1 PTB domain was undertaken to (a) characterize individual amino acids that are essential for tethering PKC3 and (b) determine whether the same β-strands and α-helices (Fig. 2B) that generate tethering surfaces for ligands of mammalian and Drosophila PTB domains also constitute a binding pocket for the kinase. Conserved residues in the large CKA1 PTB domain were targeted for mutagenesis because corresponding amino acids in Drosophila and mammalian PTB domains are directly involved in binding the epidermal growth factor receptor, LNX, Numbl-associated kinase, JIP-1, and other partner proteins (48, 64). PKC3 binding activity of wild type and mutant CKA1 PTB domains was determined by yeast two-hybrid protein interaction assays (Fig. 9B). Coimmunoprecipitation assays were also used to detect formation of complexes between full-length PKC3 and either wild type or mutant CKA1S adapter proteins in intact cells (Figs. 9, C and D). Several mutations in the α2-helix (Met154 to Ala) and β5-strand (Ser172 to Ala) (Fig. 9, C and D); Cys176 to Ala (Fig. 9B); Cys176 Ala177 to Ala Gly) that compromise partner protein binding in other PTB domains (50, 65) failed to alter coupling of the CKA1-binding module with PKC3. However, substitution of either Phe176 or Phe221 with Leu abrogated ligation of PKC3 (Fig. 9, B and C). Thus, large hydrophobic side chains with aromatic character provide local apolar surfaces that govern (in part) the affinity of CKA1 for PKC3 and/or stability of adapter protein–PKC3 complexes. Mutation of corresponding Phe residues in the dNumb and SHC PTB domains markedly reduces (Phe176 site) or extinguishes (Phe221 site) tethering of target ligands (51, 55). Phe176 is included in the β5-strand, whereas Phe221 is embedded within the α3-helix (Fig. 2B). Therefore, at least two elements of secondary structure cooperate to create a critical component of the PKC3-tethering surface of the CKA1 PTB domain. The deletion and site-directed mutagenesis experiments reveal that classical, conserved features of PTB domains can accomplish a previously unappreciated task, recruitment of an atypical protein kinase C.

Crystal and solution structures for Drosophila and mammalian PTB domains reveal that the orientations of the β5-strand and α2- and α3-helices direct incorporation of side chains from...
Met124, Ser174, Cys176, Ala177, and Phe221 (along with other residues) into a large pocket that accommodates portions of polypeptide ligands. Side chains from the listed amino acids can interact directly with amino acids in the target ligand (50, 51). The PKC3-binding region of the CKA1/CKA1S PTB domain contains key residues (Phe175 and Phe221) contributed by the β5-strand and α3-helix. However, the inability of substitutions for Met124, Ser174, Cys176, or Ala177 to alter tethering activity suggests that PKC3 occupies a binding pocket that is partly similar to and partly distinct from docking sites that accommodate other protein ligands within PTB domains.

Conclusions, Implications, and Perspectives—CKA1 and CKA1S are novel, modular adapter proteins that ligate PKC3, an atypical protein kinase C. A PKC3-binding site is generated by a segment of CKA1 that corresponds to a PTB domain. An exceptionally basic N-terminal region of CKA1 includes two PSD-like sequences and mediates routing/anchoring of the adapter protein to the cell periphery. A single PSD is sufficient to direct differential accumulation of CKA1S in proximity with plasma membrane/cortical actin cytoskeleton; deletion of the CKA1S PSD region creates a cytoplasmic protein with an unaltered binding affinity for PKC3.

Patterns of both CKA1 gene promoter activity and CKA1/CKA1S protein localization (in individual cells in vivo) overlap with previously established patterns of PKC3 expression and intracellular distribution (8). Like PKC3, CKA1 and CKA1S are associated with organelles and/or cytoskeleton throughout the C. elegans life cycle. Therefore, PKC3 is evidently exposed to the PTB domain of CKA1/CKA1S in multiple cells of the nematode. Transfection-expression experiments demonstrate that membrane-associated CKA1 (or CKA1S) sequesters PKC3 in the internal environment of intact cells. Thus, two classical structural modules (a PTB domain and a PSD) collaborate in a novel fashion in the CKA1 adapter protein(s) to enable efficient tethering and targeting of PKC3.

A key physiological consequence of structure/function relationships in CKA1/CKA1S is that the adapter proteins have an intrinsic ability to modulate and diversify functions of an aPKC. Delivery of PKC3 to the vicinity of the plasma membrane exposes the kinase to a large pool of phosphatidylinositol. Phosphatidylinositol binds with PKC3 and promotes high level expression of phosphotransferase activity in the absence of other activators. However, tethering and anchoring the kinase will focus and restrict PKC3-mediated signal transmission to substrate-effector proteins that are co-clustered with CKA1/CKA1S at discrete intracellular locations. In polarized epithelial cells CKA1/CKA1S is targeted to a lateral region of the plasma membrane that is involved in generating tight junctions. Thus, the folded structure of the CKA1 polypeptide contains intrinsic information necessary to guide accumulation in a discrete, highly specialized microenvironment. The unique amino acid sequence of the central and C-terminal regions of CKA1 (residues 232–593) accounts for 60% of the mass of the adapter protein. Since this region of the protein contains several potential SH3-binding sites and is devoid of motifs involved in catalysis, it is logical to consider (in future studies) the possibility that CKA1 (or CKA1S) is a polyvalent scaffold that organizes a multiprotein complex.

Several binding proteins that are unrelated to CKA1 can also modify the properties of aPKCs. An 80-kDa protein named LIP...
binds and activates PKC in vitro and in intact cells (27). In contrast, phosphotransferase activity is markedly diminished when PKC and PKC couple with Par-4, a mammalian, stress-induced 35-kDa protein that facilitates apoptosis (26). A protein alternatively named ZIP or p62 simultaneously binds PKC and either RIP (a Ser/Thr protein kinase involved in TNF signaling) or Kv1.2 (an auxiliary subunit of the Shaker K channel) via distinct domains (11, 66). PKC binds with C. elegans Par-3 in one-cell embryos and at several subsequent stages of early embryonic development (17, 18). Par-3 has a novel binding surface for PKC, and the Par-3 polypeptide contains three PDZ domains that are presumably used to assemble a multiprotein complex that is involved in generating cell polarity and mediating asymmetric cell division. ASIP, the mammalian counterpart of Par-3, binds PKC and PKC and also possesses three analogous PDZ domains (67).

Comparison of key features of the CKA1 binding and targeting domains with properties of the proteins cited above yields new insights and concepts regarding the molecular basis for the diversification of aPKC functions and location. LIP and mammalian Par-4 are evidently aPKC modulators, rather than adapters. These proteins bind with the N-terminal Cys-rich regulatory region of the aPKC ligand (26, 27). However, aPKC-binding sites in Par-4 and LIP are unique, unrelated structures that lack sequence motifs characteristic of classical protein-directed interaction domains (e.g., PDZ, SH3, etc.). The putative ZIP/p62 scaffold complexes an N-terminal region of aPKCs that includes the pseudosubstrate site (25). The novel aPKC binding domain of ZIP/p62 is generated by 65 amino acids located near the N terminus (25). ASIP/Par-3 binds the aPKC catalytic domain via a short protein segment that is well separated from the three PDZ motifs (67). This aPKC-binding sequence is not evident in other proteins. Unlike aPKC-binding proteins cited above, CKA1/CKA1S employs a classical protein interaction module, a PTB domain, to engage PKC.

The intracellular location of CKA1 is governed principally by an N-terminal protein segment (residues 1–77) that includes two
PSDs. Interspersed basic and large hydrophobic amino acids in classical PSDs promote accumulation of MARCKS, DAKAP200, and several other proteins at the cell periphery (54–57). The high concentration of Lys and Arg enables electrostatic binding with anionic head groups of plasma membrane phospholipids, whereas large hydrophobic side chains simultaneously intercalate in the apolar core of the bilayer (56). PSDs can also bind with F-actin at sites where cortical actin cytoskeleton abuts the plasma membrane (54). Thus, juxtaposition of CKA1/CKA1S with the inner surface of plasma membrane is highly consistent with the location of established docking sites for PSDs. (Experiments presented in our companion paper (68) directly demonstrate that PSDs govern targeting/anchoring of CKA1). The more precise routing of CKA1/CKA1S to lateral junctions in MDCK and LLC epithelial cells suggests that the adapter may engage additional docking molecules. This raises the possibility that PSDs cooperate with distal domains, elsewhere in the adapter polypeptide, to achieve increased specificity and establish polarity in the targeting/anchoring of bound PKC3 in some cells.

Precise physiological roles for CKA1-PKC3 complexes remain to be determined. However, general functions are suggested by insights gained from the molecular genetic analysis of asymmetric cell division in C. elegans and Drosophila. Complexes containing PKC3 and Par-3 are asymmetrically targeted to a limited segment of plasma membrane in one-cell C. elegans embryos (17, 18). Cooperative interactions among anchored PKC3 and the Par-1, Par-2, and Par-6 proteins are essential for creating cell polarity, enabling asymmetric cell division (by segregating regulatory proteins), and determining cell fates during the earliest phases of embryogenesis. The membrane-associated asymmetrically localized Drosophila Numb polypeptide is incorporated into a multiprotein complex (52, 53). Regulatory proteins co-clustered with dNumb are differentially transferred to one of two daughter cells, thereby creating two distinct lineages. Proper assembly, localization, and operation of complexes that contain dNumb are essential for balanced generation and positioning of neurons, glia, hair cells, socket cells, sheath cells, and bristles within the body plan of the fly (52, 53).

Together, properties of CKA1/CKA1S proteins, the dNumb PTB domain, and PKC3-Par-3 complexes strongly suggest a straightforward proposition; CKA1-PKC3 complexes are likely to be involved in assembly, maintenance, and/or regulation of protein complexes that execute asymmetric and/or polarized cell functions. For example, anchored PKC3 might be associated with ion or metabolite transporters, tight junctions, apically or basally disposed ion channels, trans-membrane receptor complexes, synapse-based signaling systems, axon guidance proteins, etc. CKA1-bound PKC3 may participate in some asymmetrical cell divisions; however, expression of both proteins in late larval and adult gut and pharynx further suggests that a significant proportion of CKA1-PKC3 may influence highly differentiated functions (see list above) in nondividing cells. A potentially important implication of our current results is that the PTB domain in the four mammalian Numb isoforms may tether PKC3 and/or PKC3′ and adapt these kinases for specialized functions by anchoring at several intracellular locations. Essential prerequisites for rigorously investigating this issue are to characterize the properties of the PTB-binding site in PKC3 and determine the mechanism by which the N-terminal region of CKA1 mediates association with plasma membrane/cytoskeleton. These topics are addressed in the accompanying paper (68).

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REFERENCES

1. Nishizuka, Y. (1995) FASEB J. 9, 484–496
2. Hug, H., and Sarre, T. F. (1993) Biochem. J. 291, 329–343
3. Newton, A. C. (1997) Curr. Opin. Cell Biol. 9, 161–167
4. Mellor, H., and Parker, P. (1998) J. Biol. Chem. 273, 281–292
5. Kikkawa, U., Kishimoto, A., and Nishizuka, Y. (1989) Ann. Rev. Biochem. 58, 31–44
6. Toker, A. (1998) Front. Biosci. 3, 1134–1147
7. Black, J. (2000) Front. Biosci. 5, 406–423
8. Wu, S. L., Staudingher, J., Olsen, E. N., and Rubin, C. S. (1998) J. Biol. Chem. 273, 1130–1141
9. Diaz-Meco, M. T., Dominguez, I., Sanz, L., Dent, P., Lozano, J., Municio, M. M., Berra, E., Hay, R. T., Sturgill, T. W., and Moscat, J. (1994) EMBO J. 13, 2842–2848
10. Berra, E., Diaz-Meco, M. T., Lozano, J., Frutos, S., Municio, M. M., Sanchez, P., Sanz, L., and Moscat, J. (1995) EMBO J. 14, 6157–6163
11. Sanz, L., Sanchez, P., Lallena, M. J., Diaz-Meco, M. T., and Moscat, J. (1999) EMBO J. 18, 3044–3053
12. Sanz, E., Municio, M. M., Sanz, L., Frutos, S., Diaz-Meco, M. T., and Moscat, J. (1997) Mol. Cell. Biol. 17, 4346–4354
13. Diaz-Meco, M. T., Lallena, M. J., Monjas, A., Frutos, S., and Moscat, J. (1997) J. Biol. Chem. 272, 19606–19612
14. Wang, Y. X., Dhubalpa, P. D., Li, L., Benovic, J. L., and Kotlikoff, M. I. (1999) J. Biol. Chem. 274, 13859–13864
15. Zhou, G., Seihuehner, M. L., and Wooten, M. W. (1997) J. Biol. Chem. 272, 31130–31137
16. Standaert, M. L., Bandyopadhyay, G., Perez, L., Price, D., Galloway, L., Pukлевский, A., Sajan, M. P., Cenni, V., Sirri, A., Moscat, J., Toker, A., and Farese, R. V. (1999) J. Biol. Chem. 274, 33508–33516
17. Tabuse, Y., Izumi, Y., Pian, F., Kemphues, K. J., Miwa, J., and Ohno, S. (1998) Development 125, 3607–3614
18. Hung, T. J., and Kemphues, K. J. (1999) Development 126, 127–135
19. Ways, D. K., Cook, P. P., Webster, C., and Parker, P. J. (1992) J. Biol. Chem. 267, 4799–4805
20. Aikawa, K., Minuo, K., Osada, S., Hirai, S., Tanuma, S., Suzaki, K., and Ohno, S. (1994) J. Biol. Chem. 269, 12677–12683
21. Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K., and Nishizuka, Y. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3098–3103
22. Doornbos, R. P., Theelen, M., van der Hoeven, P. C., van Blitterswijk, W. J., Verkleij, A. J., and van Bergen en Henegouwen, P. M. (1999) J. Biol. Chem. 274, 8589–8596
23. Chen, M. M., Hou, W., Johnson, G. L., Lee, M. H., Chen, C. S., Newton, A. C., Schaffhausen, B. S., and Toker, A. (1998) Curr. Biol. 8, 1069–1077
24. Romanelli, A., Martin, K. A., Toker, A., and Blenis, J. (1999) Mol. Cell. Biol. 19, 2921–2928
25. Puls, A., Schmidt, S., Grawe, F., and Stabel, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6191–6196
26. Diaz-Meco, M. T., Municio, M. M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L., and Moscat, J. (1996) Cell 86, 777–786
27. Diaz-Meco, M. T., Municio, M. M., Sanchez, P., Lozano, J., and Moscat, J. (1996) J. Biol. Chem. 271, 14820–14827
28. Bregman, D. B., Bhattacharyya, N., and Rubin, C. S. (1994) J. Biol. Chem. 269, 14820–14827
29. Guan, K. L., and Dixon, J. E. (1994) Anal. Biochem. 226, 262–267
30. Zhang, J., and Rubin, C. S. (1996) J. Biol. Chem. 271, 16862–16869
31. Aderem, A. (1992) Cell 71, 775–778
32. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
33. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
34. Schultz, J., Copley, R. R., Doerks, T., Ponting, C., and Bork, P. (2000) Nucleic Acids Res. 28, 231–234
35. Lu, M., Isaacs-Trejo, A., and Rubin, C. S. (1994) J. Biol. Chem. 269, 14820–14827
36. forman-Kay, J. D., and Pawson, T. (1999) Trends Endocrinol. Metab. 10, 262–267
37. Forman-Kay, J. D., and Pawson, T. (1999) Curr. Opin. Cell Biol. 11, 202–210
38. McLaughlin, S., and Aderem, A. (2000) Trends Biochem. Sci. 25, 272–276
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57. Rossi, E. A., Li, Z., Feng, H., and Rubin, C. S. (1999) J. Biol. Chem. 274, 27201–27210
58. White, J. (1988) in The Nematode Caenorhabditis elegans (Wood, W. B., ed) pp. 81–122, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
59. Viniegra, S., and Rabito, C. A. (1988) J. Biol. Chem. 263, 7089–7104
60. Rodriguez-Boulan, E., and Powell, S. K. (1992) Annu. Rev. Cell Biol. 8, 385–427
61. Balda, M. S., and Matter, K. (1998) J. Cell Sci. 111, 541–547
62. Cereijido, M., Valdes, J., Shoshani, L., and Contreras, R. G. (1998) Annu. Rev. Physiol. 60, 161–177
63. Stevenson, B. R., and Keon, B. H. (1998) Annu. Rev. Dev. Biol. 14, 89–109
64. Meyer, D., Liu, A., and Margolis, B. (1999) J. Biol. Chem. 274, 35113–35118
65. Yaich, L., Ooi, J., Park, M., Borg, J. P., Landry, C., Bodmer, R., and Margolis, B. (1998) J. Biol. Chem. 273, 10381–10388
66. Gong, J., Xu, J., Bezanilla, M., van Huizen, R., Derin, R., and Li, M. (1999) Science 285, 1565–1569
67. Izumi, Y., Hirase, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K. J., and Ohno, S. (1998) J. Cell Biol. 143, 95–106
68. Zhang, L., Wu, S.-L., and Rubin, C. S. (2001) J. Biol. Chem. 276, 10476–10484