Structure and Expression of the *Caenorhabditis elegans* Protein Kinase C2 Gene

ORIGINS AND REGULATED EXPRESSION OF A FAMILY OF Ca\(^{2+}\)-ACTIVATED PROTEIN KINASE C ISOFORMS*

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Alma Islas-Trejo, Marianne Land, Irina Tcherepanova, Jonathan H. Freedman‡, and Charles S. Rubin§

From the Department of Molecular Pharmacology, Atran Laboratories, Albert Einstein College of Medicine, Bronx, New York 10461

The molecular and cellular basis for concerted Ca\(^{2+}\)/lipid signaling in *Caenorhabditis elegans* was investigated. A unique gene (*pkc-2*) and cognate cDNAs that encode six Ca\(^{2+}\)/diacylglycerol-stimulated PKC2 isoenzymes were characterized. PKC2 polypeptides (680–717 amino acid residues) share identical catalytic, Ca\(^{2+}\)-binding, diacylglycerol-activation and pseudosubstrate domains. However, sequences of the N- and C-terminal regions of the kinases diverge. PKC2 diversity is partly due to differential activation of transcription by distinct promoters. Each promoter precedes an adjacent exon that encodes 5′-untranslated RNA, an initiator AUG codon and a unique open reading frame. PKC2 mRNAs also incorporate one of two 3′-terminal exons via alternative splicing. Cells that are capable of receiving and propagating signals carried by Ca\(^{2+}\)/diacylglycerol were identified by assessing activities of *pkc-2* gene promoters in transgenic *C. elegans* and visualizing the distribution of PKC2 polypeptides via immunofluorescence. Highly-selective expression of certain PKC2 isoforms was observed in distinct subsets of neurons, intestinal and muscle cells. A low level of PKC2 isoforms is observed in embryos. When L1 larvae hatch and interact with the external environment PKC2 content increases 10-fold. Although 77- and 78-kDa PKC2 isoforms are evident throughout post-embryonic development, an 81-kDa isoform appears to be adapted for function in L1 and L2 larvae.

Numerous hormones, growth factors, and neurotransmitters elicit receptor-mediated activation of phospholipases (C\(_a\), C\(_b\), D, and A\(_2\)) and the concomitant production of phospholipid-derived second messenger molecules (1–3). Signals carried by diacylglycerol and other phospholipid metabolites are received, amplified, and distributed to multiple intracellular compartments by various protein kinase C (PKC) isoforms (1–8). Activated PKCs catalyze the phosphorylation of substrate effector proteins at specific Ser and/or Thr residues, thereby modulating their functions. PKC-mediated protein phosphorylation is involved in the regulation of many important processes, including secretion, ion channel activity, mitogenesis, gene transcription, and cell differentiation (1–5).

Functions controlled by lipid second messengers vary with cell type (1–5). This reflects differences in expression of hormone/growth factor receptors, phospholipases, and PKC substrates. Distinctive properties of PKC isoenzymes also contribute to the diversity of responses elicited by phospholipid metabolites. In mammals nine genes encode 10 PKC isoforms (1–7). These kinases differ in their substrate specificities, susceptibility to activation by calcium and lipid second messengers, intracellular destinations after activation, and ability to undergo down-regulation. Reconstitution of PKC-mediated signaling in permeabilized cells and manipulation of PKC signaling pathways via transfection and microinjection indicate that individual PKCs control discrete physiological functions. Examples include the regulation of secretion, phospholipase C\(_g\) activity, and mitogenesis by PKCs β, ε, and ζ, respectively (9–11). An important caveat is that these studies were performed with immortalized or tumor-derived cultured cells. The relevance of the observations to physiological roles for individual PKCs in specific cells in intact organisms remains to be established.

The spectrum of cellular responses to lipid second messengers also reflects differential expression of genes encoding PKC isoforms. Qualitative and quantitative differences in PKC isoform content are evident in many mammalian tissues (12–15). Moreover, types and levels of PKC isoenzymes expressed in a given cell can be altered by differentiation, hormones, and phorbol esters (1–5, 16–20). In model cell systems, an increase in PKC isoform content has been correlated with elevations in the rate of transcription of the cognate gene and the level of mRNA encoding the isoform (17, 18). Although direct transcriptional activation probably plays a central role in generating PKC diversity (20–22), underlying control mechanisms have not been elucidated. Little is known about cis-regulatory elements and trans-acting proteins that govern activation or inhibition of PKC gene transcription.

The non-parasitic nematode *Caenorhabditis elegans* pro-

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‡ Present address: School of the Environment, Box 90328, Duke University, Durham, NC 27708.

§ To whom correspondence should be addressed. Tel.: 718-430-2505; Fax: 718-430-8922; E-mail: rubin@secom.yu.edu.

1 The abbreviations used are: PKC, protein kinase C; cPKCs, classical PKC isoforms (α, βI, βII, γ); nPKCs, novel PKC isoforms (δ, ε, η, θ); MES, 4-morpholineethanesulfonic acid; kbp, kilobase pair(s); bp, base pair(s); RACE, rapid amplification of cDNA ends; RACK, receptors for activated protein kinase Cs.
vides a powerful system for investigations on functions and regulated expression of PKC isoforms. Adult *C. elegans* are composed of 959 somatic cells, which are organized into tissues that constitute digestive, reproductive, muscular, hypodermal, and nervous systems (23–25). The cellular and developmental biology of *C. elegans* have been characterized in exceptional detail and the lineage of each cell in the animal has been determined (23–25). Numerous aspects of *C. elegans* development and homeostasis are controlled by signal transduction systems that are analogous to, or identical with, those operative in mammals (26, 27). Methods for induction of mutant, transgenic and “knock-out” strains of *C. elegans* enable the analysis of gene promoter activities and gene functions in individual cells in situ (28–32). Confocal immunofluorescence microscopy (33) permits detection of specific proteins in individual cells of intact *C. elegans* at all developmental stages.

We demonstrated the utility of this system by characterizing the *C. elegans* PKC1 gene (34). This gene encodes a novel calcium-independent, diacylglycerol-activated PKC (nPCK) that is expressed exclusively in ~75 sensory neurons and related interneurons. Another *C. elegans* nPKC (the product of the tpa-1 gene) has been characterized by Miwa and colleagues (35, 36). It is thought that nPKCs mediate sustained, long-term, lipid-controlled signaling. In contrast, rapid (but transient) binding sites for diacylglycerol and calcium. Concerted actions of the two activators promote translocation of cytoplasmic cPKCs to membranes and cytoskeleton and generate maximal levels of kinase activity (1–8). Previous studies documented the occurrence of a cPKC-like enzyme in *C. elegans* (37), suggesting that concerted lipid/calcium signaling is operative in *C. elegans*. However, *C. elegans* cPKCs have not been characterized at the molecular level. Thus, to initiate investigations on functions and regulated expression of *C. elegans* cPKCs it is essential to clone and characterize relevant cDNAs and genes; discover mechanisms that govern the generation of cPKC diversity; identify cells in which the cPKC promoter(s) is/are active and cPKC polypeptides accumulate (to provide clues regarding isofrom function in vivo); and characterize alterations in cPKC expression and intracellular localization during development.

**Experimental Procedures**

**Growth of C. elegans—**The Bristol N2 strain of *C. elegans* was grown at 20 °C as described previously (38). To synchronize *C. elegans* for developmental studies nematodes were hatched in the absence of nutrients and then transferred to plates containing *Escherichia coli* as a food source. Under these conditions, the worms develop synchronously into reproductive adults (39). L1 larvae were harvested 6 h after feeding, L2 larvae at 20 h, L3 larvae at 29 h, L4 larvae at 40 h, young adult worms at 53 h, and egg-laying adult nematodes at 75 h. A purified population of embryos was obtained by alkaline hypochlorite treatment of gravid *C. elegans*, as described by Sulston and Hodgkin (40).

**Isolation of cDNAs Encoding PKC2 Isoforms—**A cDNA that encodes a segment (residues 287–665, Fig. 1, A and B) of a novel *C. elegans* protein kinase C (named PKC2) was isolated from a complementary DNA library in bacteriophage ggt10, as described previously (34). A fragment (394 bp) was excised from the 5’-end of the cDNA (by digestion with EcoRI and NcoI) and used as a template to generate a random-primed, 32P-labeled probe. This probe was used to screen the *C. elegans* cDNA library in bacteriophage zAP II (Clontech) as indicated in previous papers (34, 38). Seven positive recombinant phage clones were plaque purified and the cDNAs (0.4–2.4 kbp) were subcloned in the plasmids pGEM7Z (Promega) and pBluescript (Stratagene) and sequenced.

**Computer Analysis—**Analyses of sequence data, sequence comparisons, and data base searches were performed using PCGENE-IntelliGenetics software (IntelliGenetics, Mountainview, CA) and the BLAST and FASTA programs (41, 42) provided by the NCBI server and the National Library of Medicine/National Institutes of Health.

**Southern Gel Analysis—**Fragment of *C. elegans* genomic DNA were generated by digestion with restriction endonucleases, fractionated in a 0.6% agarose gel, and transferred to a Nytrocel membrane described previously (38). The Southern blot was probed with the 32P-labeled PKC2 cDNA (2 × 106 cpm/ml) described below. Conditions for hybridization, as well as high and low stringency washing of the membrane, are given in Hu and Rubin (38).

**Preparation of RNA and Northern Gel Analysis—**Total *C. elegans* RNA was prepared in a previous paper (38). Poly(A)− mRNA was purified according to Sambrook et al. (43). Northern blot analysis was performed as described previously (34). A 32P-labeled, EcoRI fragment of PKC2 cDNA (nucleotides 885–2019, Fig. 1, A and B), which encodes the catalytic domain of the kinase, was used as a probe.

**DNA Sequence Analysis—**PKC2 cDNAs and genomic DNA fragments containing the phc-2 gene were subcloned into the plasmid pGEM7Z. DNA inserts were sequenced by the dideoxynucleotide chain termination procedure of Sanger et al. (44) using T7, SP6, and custom oligonucleotide primers as described previously (38).

**Characterization of the Extreme 5′-Ends of PKC2 cDNAs—**Complementary DNAs corresponding to the 5′-terminal regions of PKC2 mRNA were synthesized, amplified, cloned, and sequenced as described in Lane et al. (34, 43). These rounds of amplification, via the polymerase chain reaction, were used to obtain PKC2 cDNAs. The 5′-termini were described previously (34). The initial 3′ primer (5′-GCCTCTAGATATTCTGCCTGGCAGGATCT-3′) contains the inverse complement of nucleotides 321–340 in the cDNA encoding PKC2 isoforms (Fig. 1A); the second 3′ primer (5′-GCCTCTAGATATTCTGCCTGGCAGGATCT-3′) contains the inverse complement of nucleotides 123–142 in PKC2 cDNA. The first 8 nucleotides of each primer correspond to two irrelevant nucleotides and an XbaI recognition sequence (TCTAGA). Before the final round of cDNA amplification, primers and template were incubated at 100 °C for 2 min and then annealed for 15 s at −70 °C in 50 mM Tris-HCl, pH 8.3, containing 70 mM KCl. Amplified cDNAs were cloned into the Xbal site of plasmid pGEM7Z and sequenced.

**RNase Protection Analysis—**Complementary DNAs encoded by three alternative 5′-terminal exons in the *C. elegans* phc-2 gene were synthesized and cloned in pGEM7Z as described above (see Fig. 2 for sequences and nomenclature). Recombinant plasmids were linearized by digestion with PvuII and used as a template for antisense RNA synthesis. Antisense RNA was synthesized by bacteriophage T7 RNA polymerase as described previously (45). Antisense RNAs for exons 1A, 1B, and 1C contain unique sequences of 122, 70, and 94 nucleotides, respectively, that are complementary to corresponding 5′-termini in subsets of PKC2 mRNA. RNase protection analysis was performed with 20 μg of total RNA isolated from *C. elegans* at seven stages of development as described previously (34, 45).

**Expression and Purification of Recombinant PKC2 Fusion Protein—**A 729-bp HpaI-NcoI restriction fragment of PKC2 cDNA was subcloned into the pRSET-A expression plasmid (Invitrogen). This plasmid cDNA encoding the calcium-binding domain and part of the catalytic domain of PKC2 isoforms (residues 176–417, Fig. 1C) was expressed from pRSET-A under control of the lac promoter. Bacteria were harvested, disrupted, and separated into soluble and particulate fractions as described for previous studies (34). The PKC2 fusion protein was recovered in the pellet fraction. Recombinant PKC2 fusion protein was dissolved in 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl supplemented with 8 μM urea and purified to near-homogeneity by nickel-chelate chromatography (in the presence of 6 μM urea) as described previously (46). When urea was eliminated by extensive dialysis against 50 mM sodium phosphate, pH 5.0, the purified protein remained soluble. Approximately 3 mg of highly-purified PKC2 fusion protein was obtained from a 500-ml culture of *E. coli*.
Affinity Purification of Anti-PKC2 Immunoglobulins—Pured PKC2 fusion protein (0.7 mg) was coupled to 1 ml of Affi-Gel 10 resin (Bio-Rad), in 2 ml of 0.1 M sodium acetate, pH 5.0, at 4 °C for 4 h. Antiserum (2 ml) was adjusted to a final concentration of 20 mM MES buffer, pH 6.0, and mixed with the affinity resin for 2 h at room temperature. Next, the resin was packed into a column with 20 ml of MES, pH 6.0, containing 0.5 M NaCl until the flow-through reached an absorbance of zero at 280 nm. Bound IgGs were eluted with 3 ml of 0.5% acetic acid containing 0.15 M NaCl. Fractions (0.5 ml) were collected into tubes containing sufficient 1 N NaOH, to neutralize the acid and adjust the pH to 7.5. The IgG concentration was estimated from the absorbance at 280 nm. Fractions containing IgGs were pooled and supplemented with 5 mg of albumin/mg of IgG. Subsequently, affinity purified IgGs were dialyzed against 10 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl and 50% (v/v) glycerol and stored at −20 °C.

Electrophoresis of Proteins and Western Immunoblot Assays—Samples of proteins were denaturated in gel loading buffer and subjected to electrophoresis in a 9% polyacrylamide gel containing 0.1% SDS as described previously (47). Phosphorylase (Mₙ = 97,000), transferrin (77,000), albumin (67,000), ovalbumin (43,000), and carbonic anhydrase (29,000) were used as standards for the estimation of Mₙ values. Cytoolic and particulate fractions of C. elegans and Sf9 cell homogenates were prepared as described previously (34). Western blots of C. elegans proteins and anti-PKC2 antibodies from Sf9 cells were blocked with 5% nonfat dry milk and incubated with affinity purified IgGs, washed, and visualized using an indirect chemiluminescence procedure as previously reported (48).

Expression of PKC2 in Insect Cells—Complementary DNAs containing the complete coding sequences for PKC2A and PKC2B (see Fig. 1) were excised from recombinant pBluescript plasmids by digestion with BamHI and SpeI. These inserts were subcloned into the baculovirus transfer vector pVL1392, which was cleaved with BamHI and XbaI. Recombinant baculoviruses were produced and used to infect Sf9 cells as indicated in previous papers (34, 45). Aliquots of infected cells were harvested every 24 h over a period of 5 days. Cytosolic and particulate fractions of infected Sf9 cells were prepared as described previously (49). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). PKC activity was determined in the presence and absence of calcium, using the synthetic peptide RFARKGSLRQKNV as a substrate (48). 

Preparation of Transgenic C. elegans—The cosmid E01H11, which contains the gene encoding PKC2 isoforms and 5′-flanking DNA (see "Results"), was obtained from Dr. Alan Coulson, Medical Research Countc Laboratories of Molecular Biology, Cambridge, United Kingdom. Fragments of genomic DNA that flank the 5′-ends of exons 1A, 1B, and 1C were excised from cosmid E01H11 by digestion with restriction enzymes and identified by hybridization (on Southern blots) with 32P-labeled cDNA probes corresponding to the unique 5′-terminal cDNAs described above and in Fig. 1. Lines of transgenic nematodes were created in order to investigate the cell-specific in vivo promoter activity of the flanking DNA segments. The basic strategy involves the insertion of 5′ flanking DNA into the multiple cloning site of a C. elegans expression vector (plasmid pPD16.51) devised by Fire et al. (29). Inserted promoter sequences will drive the expression of a β-galactosidase reporter gene (lacZ) that is immediately preceded by 27 nucleotides encoding an initiator ATG and the nuclear targeting region of SV40 large T antigen (29). The lacZ coding region is followed by translation termination and poly(A) addition signals.

A 1.5-kbp segment of cosmid DNA that terminates 13 bp upstream from the initiator ATG in exon 1A was obtained by digestion with BsaXI, creation of blunt ends with T4 DNA polymerase, and subsequent cleavage with SpH1. The DNA fragment was ligated into plasmid pPD16.51 that was cut with Smal and SpH1. A 3-kbp DNA fragment that termina 9 bp upstream from the initiator ATG in exon 1B was obtained by digestion with BssHII and XhoI. This DNA insert was ligated into plasmid pPD16.51 that was cleaved with BssHII and SalI. Finally, a 1.4-kbp fragment that terminates at codon 20 (in exon 1B) was obtained by digesting cosmid DNA with ApaLI, filling in with Klenow DNA polymerase, and cleaving with SpH1. The fragment was cloned into the vector pPD16.51, which was cleaved with Smal and SpH1. In the resulting construct, the DNA segment that encodes β-galactosidase precedes the nuclear targeting sequence is positioned, in-frame, downstream from the exon 1C initiator ATG. In recombinant pPD16.51 plasmids containing DNA that flank exons 1A and 1B, translation is initiated at the ATG adjacent to the SV40 nuclear localization signal. 

C. elegans were transformed by microinjecting both recombinant reporter plasmid DNA containing the putative 1A, 1B, or 1C promoter and a plasmid containing the dominant selectable marker gene rol-6, as described previously (30, 49). Transgenic C. elegans were selected and maintained as indicated in a previous paper (49). Transgenic C. elegans were fixed and stained for β-galactosidase activity as reported in Freedman et al. (49).

Immunofluorescence Analysis—C. elegans were fixed, washed, and incubated essentially with affinity purified anti-PKC2 IgGs and fluorescent isothiocyanate-tagged goat IgGs directed against rabbit immunoglobulins, as described by Land et al. (34). Fluorescence signals corresponding to PKC2-IgG complexes were obtained with a Bio-Rad MRC 600 laser scanning confocal microscope (Image Analysis Facility, Albert Einstein College of Medicine).

RESULTS

Cloning and Sequence Analysis of cDNAs Encoding C. elegans PKC2 Isoforms—Complementary DNAs that encode PKC2 were retrieved from bacteriophage libraries. Two near-full length (~2.4 kbp) and six partial (0.4 to 2.0 kbp) cDNAs were sequenced from both DNA strands. All partial sequences were identical with segments of the larger cDNAs. The 2.4-kbp cDNAs encoded two related, but distinct PKC2 isoforms (designated PKC2A and PKC2B) (Fig. 1). PKC2A and 2B cDNAs are identical between nucleotides 1 and 1918 (Fig. 1A). The shared cDNA segment contains 5′-untranslated nucleotides, a Met codon (nucleotides 27–29) in a C. elegans consensus context for translation initiation (AAG/NNAATGT) and a contiguous 3′-open reading frame that encodes 630 amino acids (Fig. 1A). The sequences of PKC2A and 2B cDNAs diverge after codon 631. The novel 3′ portion of PKC2A cDNA contains 150 bp of coding sequence followed by a translation termination codon (nucleotides 2067–2069) and 309 untranslated nucleotides (Fig. 1B). A consensus poly(A) addition signal (AATAAA, nucleotides 2363–2368) precedes the poly(A) tail by 11 nucleotides. The unique 3′ region in PKC2B cDNA includes 52 codons, a translation termination signal (nucleotide 2073–2075) and 287 untranslated nucleotides (Fig. 1C). An atypical poly(A) addition signal (ACTAAA, nucleotides 2343–2348) is evident 14 nucleotides upstream from the poly(A) tail. The data suggest that the divergent sequences in PKC2A and 2B mRNAs correspond to alternatively-spliced, 3′-terminal exons comprising 462 and 446 nucleotides, respectively. Subsequent characterization of genomic DNA demonstrated directly that sequences presented in Fig. 1, B and C, constitute the final and penultimate exons, respectively, of the kin-112 (pkc-2) gene (see below).

The predicted amino acid sequences of the C-terminal regions of PKC2A and PKC2B are divergent (only 48% identical). Dissimilarities in C-terminal domain sequences and higher order structure may differentially affect kinetic properties, stability, and/or intracellular targeting of PKC2 isoforms (e.g. see Ref. 50). Overall, the PKC2A isoform is composed of 680 amino acids and has a Mₙ of 77,800; PKC2B contains 682 residues and has a calculated Mₙ of 78,100.

PKC2 Isoform Diversity Is Amplified by the Utilization of Three Types of 5′-Terminal Exons—An anchored polymerase chain reaction procedure, known as RACE (for rapid amplification of cDNA ends), was used to establish the exact length and complete sequence for the 5′-ends of PKC2 mRNAs. Three distinct 5′-terminal cDNA sequences were cloned and were assigned the names 1A, 1B, and 1C (Fig. 2). 1A-1C were incorporated at the 5′-ends of PKC2 cDNAs in a mutually exclusive

² In accordance with standard C. elegans nomenclature, genes are named with three lower case letters and a number (pkc-2); the same upper case letters (PKC2) are used to designate mRNAs and proteins encoded by the corresponding gene. The gene encoding PKC2 isoforms is designated “kin-112” on the C. elegans genetic map. In this paper the alternative gene name pkc-2, is used to (a) clearly delineate relationships among the gene and its cognate mRNA and protein products and (b) emphasize the ultimate functional significance of this genetic locus.
FIG. 1. Sequences of PKC2A and PKC2B cDNAs. Panel A presents the nucleotide sequence that is identical in the PKC2A and -2B isoforms. The derived amino acid sequence is given below the corresponding codons. The cDNA sequences for the divergent 3′-regions of the 2A and 2B isoforms are shown in Panel B.
manner. Each of the novel 5’ cDNA sequences contains untranslated nucleotides upstream from a potential initiator Met codon and a contiguous coding region, that in turn, fuses in-frame with the second nucleotide in codon 14 (Fig. 1A). The 1B sequence was previously identified by sequencing the PKC2A and 2B cDNAs, as shown in Fig. 1. 1C comprises 86 nucleotides and encodes an N-terminal extension of 15 residues. 1AA, which is derived from two novel exons (see below), encompasses 159 nucleotides and contains an open reading frame for an N-terminal segment of 50 amino acid residues. Polypeptide sequences encoded in the three 5’-terminal cDNAs are unrelated to each other and to protein sequences in the standard data bases. The results suggest that the C. elegans pkc-2 gene can direct the synthesis of six calcium/diacylglycerol-dependent PKC isoenzymes. Diversity is generated by differential incorporation of alternative 5’- and 3’-exons into PKC2 mRNA that contains an invariant core of 1848 nucleotides (codons 15–630, Fig. 1A).

Determination of the Size of PKC2 mRNAs and the Chromosomal Location of the pkc-2 Gene—A Northern blot that contains size-fractionated C. elegans poly(A)+ RNA was probed with 32P-labeled PKC2 cDNA. A hybridization signal was obtained for mRNA that is composed of ~2500 nucleotides (Fig. 3A). Detection of 2.5-kilobase mRNA is consistent with sizes of cDNA sequences that encode various PKC2 isoforms. Although six distinct mRNAs can be derived from the pkc-2 gene, their predicted sizes cluster in a narrow range: 2360–2480 nucleotides plus poly(A) tails. Given the limited resolving power of a 0.8% agarose gel (Fig. 3A), it is probable that the apparent 2.5-kilobase transcript corresponds to a mixture of several or all six PKC2 mRNAs.

C. elegans genomic DNA was cleaved with restriction enzymes and analyzed by Southern gel analysis (Fig. 3B). The hybridization pattern observed when the blot was probed with 32P-labeled PKC2 cDNA probe indicated that multiple PKC2 isoenzymes are encoded by a single copy gene. The chromosomal location of the pkc-2 gene was elucidated by hybridizing a panel of yeast artificial chromosomes, which contain ~90% of the C. elegans genome in overlapping segments (51), with a radiolabeled PKC2 cDNA probe. Four overlapping genomic DNA fragments hybridized. Comparison of the positions of the genomic DNA segments on the C. elegans physical map of chromosomes (Harvard Medical School electronic map) places pkc-2 on the left arm of the X chromosome in the vicinity of genes named sup-28 and unc-115.

Structure/Function Relationships in C. elegans PKC2 Isoforms—Functional roles for amino acids that are conserved in S/T protein kinases have been established by a single copy gene. The chromosomal location of the pkc-2 gene was elucidated by hybridizing a panel of yeast artificial chromosomes, which contain ~90% of the C. elegans genome in overlapping segments (51), with a radiolabeled PKC2 cDNA probe. Four overlapping genomic DNA fragments hybridized. Comparison of the positions of the genomic DNA segments on the C. elegans physical map of chromosomes (Harvard Medical School electronic map) places pkc-2 on the left arm of the X chromosome in the vicinity of genes named sup-28 and unc-115.

isoforms are shown in panels B and C, respectively. Numbering of the nucleotides and amino acids in panels B and C is in register with the numbering presented in panel A.
Fig. 2. Characterization of the 5'-ends of PKC2 cDNAs. Nucleotide and predicted amino acid sequences are presented for alternative exons that are incorporated at the 5' termini of PKC2 cDNAs. The sequence designated IAA' includes two exons (1A plus 1A'); the IB and IC sequences correspond to single exons.

Fig. 3. A single-copy gene encodes PKC2 mRNA. Panel A, a Northern blot containing 5 µg of C. elegans poly(A)+ RNA was probed with 32P-labeled cDNA corresponding to nucleotides 885 to 2019 in Fig. 1, A and B (see "Experimental Procedures"). An autoradiogram is shown. The gel was calibrated by running an RNA ladder in a parallel lane and staining with ethidium bromide. Panel B, samples of high molecular weight DNA (10 µg) were digested with SpeI (lane 1), NcoI (lane 2), BglII plus KpnI (lane 3), HpaI (lane 4), and HindIII (lane 5) and processed as described under "Experimental Procedures." The Southern blot was probed with the 32P-labeled cDNA as described above. An autoradiogram is presented. The gel was calibrated with DNA markers that were electrophoresed in a parallel lane.

biochemical analysis, comparisons of sequences of hundreds of phosphotransferases and determination of three-dimensional structures for several S/T protein kinases (52–55). Application of this knowledge to the derived amino acid sequences in Fig. 1 enables tentative identification of functional domains in C. elegans PKC2 isoforms. The catalytic domain of all PKC2 isoenzymes consists of residues 347–620 (Fig. 1A). A GXXXGX4K motif (residues 355–377) probably contributes hydrogen bonds and charged side chains that anchor the α and β phosphates of the substrate ATP. Lys377 is essential for expression of catalytic activity. Asp249, which appears in the conserved DFG motif (residues 355–377) probably contributes hydrogen bonds to the "signature" sequence for a S/T protein kinase (52) and is highly homologous with a critical portion of the catalytic loop in protein kinase A (53, 54).

C. elegans PKC2 isoforms contain two copies of a Cys-rich, zinc binding motif (residues 52–88 and 117–157). These domains mediate the binding of phosphatidyserine, diacylglycerol, and phorbol esters in mammalian cPKCs and nPKCs (1–7). The binding of zinc (2 atoms per Cys-rich repeat) is required for proper higher-order folding of these regulatory domains (56). The N-terminal Cys-rich region is preceded by a pseudosubstrate sequence (residues 22–35, Fig. 1A). Ala37 and flanking basic residues generate a PKC2 substrate site that lacks Ser and Thr (57). When intracellular levels of diacylglycerol and free Ca2+ are low (unstimulated cells) the pseudosubstrate site occupies the catalytic cleft and inhibits PKC activity.

Residues 184–259 (Fig. 1A) are homologous (Table I) with the calcium-binding region (C2 domain) in mammalian cPKCs and nPKCs (58). Six Asp residues (Asp186, Asp187, Asp205, Asp206, Asp250, and Asp251) that ligate Ca2+ ions and hydrophobic amino acids (Trp250, Trp251, and Phe256) that orient PKC interactions with membranes are conserved in C. elegans PKC2. Binding of diacylglycerol and calcium to cPKCs results in expulsion of the pseudosubstrate domain from the catalytic site and expression of phosphotransferase activity (57).

Activation and intracellular translocation of cPKCs are governed (in part) by the sequential phosphorylation of three residues in the C-terminal portion of the enzymes (7, 59). By analogy with mammalian PKCs, these residues are identified as Thr510, Thr651, and Ser670 in nematode PKC2A (Fig. 1, A and B). Thr510 is trypsophosphorylated by an unidentified protein kinase (59); subsequent incorporation of phosphate at the latter two residues is due to autophosphorylation.

The utilization of alternative 3'-exons to encode the C termini of PKC2 isoforms (Fig. 1) documents the conservation (from nematode to man) of a splicing mechanism that generates PKC diversity (20, 60). Mammalian PKCβ and ι isoforms have divergent C termini encoded by alternative exons. In nematodes and mammals the two C-terminal sequences include 50 or 52 amino acids, but share only ~48% sequence identity. Human PKCβII binds F-actin via a unique site located near the C terminus of the kinase; PKCβI does not interact with F-actin (50). The sequence that sequesters actin in man (50) is not conserved in C. elegans PKC2 isoforms. However, a substantial proportion of C. elegans PKC2 is associated with the particulate fraction of homogenates (see below).

Mammalian cPKCs bind 35-kDa proteins known as RACKs (receptors for activated protein kinase Cs) (64). RACKs facilitate translocation and anchoring of activated cPKCs to membranes. Mammalian PKCβ isoforms contain an autoregulatory region (Ser-Val-Glu-Ile-Trp-Asp) in the C2 (calcium-binding) domain that occludes the RACK-binding domain in the absence of lipid second messengers, thereby blocking PKC activation/translocation in unstimulated cells (65). The pseudo-RACK, autoregulatory region is conserved (Ser244-Ile-Glu-Val-Trp-Asp249) in C. elegans PKC2 isoforms (Fig. 1A), suggesting that RACK-mediated PKC routing operates in C. elegans.
homology (67% identity) with both the α and β isoforms of
mammalian cPKCs (Fig. 4). Maximal levels of sequence iden-
tity are evident in the pseudosubstrate, catalytic, and Cys-rich
regulatory regions (Table I). However, the differential utiliza-
tion of alternative exons to produce distinct C termini is ob-
served only for the mammalian PKCβ and the C. elegans pgc-2
genes. This suggests that these two genes are most closely
related and derived from a common ancestor.

Organization of the C. elegans pgc-2 Gene—A 12-kbp frag-
ment of DNA that contains a portion of the pgc-2 gene was
obtained from a C. elegans genomic DNA library in bacterio-
phage EMBL4. Sequence analysis of a 2-kbp segment of the
genomic DNA elucidated sequences for 5 introns and 6 exons
that encode residues 14–312 in the common core of all PKC2
isoforms (Fig. 1 A and B). During the course of our studies the
C. elegans genome project (67) deposited the DNA sequence for
large portions of the X chromosome in the GenBank database.
Searches of the database with sequences of the 5' and 3'
ends of PKC2 cDNAs (Figs. 1, B and C, and 2) revealed that cosmid
E01H11 (accession number U29376) contained the entire pgc-2
gene.
gene. Comparison of cDNA sequences determined for all PKC2 isoforms (Figs. 1 and 2) with the cosmid DNA sequence enabled the elucidation of the intron/exon organization of the pck-2 structural gene (Table II). The GENEFINDER program (67) used in the sequencing project predicted an incorrect amino acid sequence for this region of the DNA. No portion of the gene or its transcripts was previously characterized experimentally.

The pck-2 gene contains 17 exons that are dispersed over 25 kbp of DNA (Table II). Alternative 5'- and 3'-exons are separated by large introns that account for 80% of the total length of the gene. In contrast, invariant exons 2–12 are embedded in a relatively compact DNA segment (3 kbp). All introns in this region are small, ranging in size from 46 to 281 bp. The 1A-1C sequences that appear at the 5' termini of discrete PKC2 mRNAs are encoded by 4 exons. Exons 1A and 1A' (Table II) are spliced together to generate a novel 5'-terminal cDNA sequence (Fig. 2). Alternative exons 1B and 1C encode the 1B and 1C cDNA sequences, respectively. Alternative exons (13A and 13B, Table II) at the 3'-end of the gene are separated by a 1.6-kbp intron and contain open reading frames for 50 or 52 residues, translation termination codons, 3'-untranslated sequences, and poly(A) addition signals linked in a contiguous fashion. Since only one 3'-exon and one or two 5'-exon(s) are incorporated in mature mRNAs, PKC2 isoforms are encoded by 13 or 14 exons.

Systematic characterization of PKC2 cDNAs and RACE cDNA products revealed the sequence GTTATACCGATTTA-ACCAAG at the extreme 5'-end of 1B cDNA sequences. This sequence is donated from the 5'-end of a spliced leader RNA (encoded by ~100 tandemly-repeated SL1 RNA genes) in a trans-splicing reaction (68). C. elegans mRNAs undergo trans-splicing only when transcripts contain an unpaired splice acceptor signal (TTTCAG) in their 5'-untranslated regions (69). Since the pck-2 gene contains upstream splice donor sequences at the 3'-boundaries of exons 1A and 1A', the results indicate that a promoter (designated P1B) is positioned upstream from exon 1B and downstream from exon 1A'. A distinct promoter (P1A) must drive transcription of exons 1A and 1A'. A third promoter (P1C) may be associated with exon 1C because the 1A' or 1B exons cannot be excised from transcripts initiated by promoters P1A and P1B to yield the 1C 5'-cDNA sequence. In contrast, when transcription begins at promoter P1A or P1B, exons 1B and/or 1C readily become introns.

C. elegans PKC2 Isoforms Are Ca2+-stimulated Phosphotransferases—Sf9 insect cells were infected with recombinant baculovirus that contained full-length PKC2A cDNA (Fig. 1) downstream from a powerful polyhedron promoter. Proteins in Sf9 cell cytosol were size-fractionated in a 0.1% SDS-9% polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and probed with affinity-purified IgGs directed against PKC2; antigen-IgG complexes were visualized by an enhanced chemiluminescence procedure. Lane 1 received protein from uninfected Sf9 cells; lanes 3 and 4 contained proteins from baculovirus-infected Sf9 cells (3 and 4 days post-infection, respectively), lane 2 was identical with lane 3, except the antibodies were preincubated with excess (5 μg) purified antigen.

TABLE II

| Exon number | Exon size | Amino acid residues | Downstream intron size |
|-------------|-----------|---------------------|------------------------|
| 1A          | 67        | 1–17                | 643                    |
| 1A'         | 99        | 18–50               | 8857                   |
| 1B          | 66        | 1–13                | 8516                   |
| 1C          | 85        | 1–15                | 269                    |
| 2           | 71        | 14–37               | 219                    |
| 3           | 98        | 38–70               | 142                    |
| 4           | 84        | 71–98               | 73                     |
| 5           | 270       | 99–178              | 148                    |
| 6           | 160       | 179–231             | 47                     |
| 7           | 241       | 232–312             | 46                     |
| 8           | 320       | 313–419             | 281                    |
| 9           | 203       | 420–487             | 48                     |
| 10          | 211       | 488–557             | 47                     |
| 11          | 141       | 558–604             | 140                    |
| 12          | 77        | 605–630             | 375                    |
| 13A         | 447       | 631–682             | 1661                   |
| 13B         | 463       | 631–680             |                        |

* Amino acid residues encoded by exons 2–13B are numbered in register with the derived amino acid sequence of exon 1B.

Fig. 5. Expression of recombinant PKC2A. Complementary DNA encoding PKC2A was inserted into a baculovirus expression vector (“Experimental Procedures”). Infected Sf9 cells were extracted with buffer containing Triton X-100 and soluble proteins (25 μg) were size-fractionated by SDS-polyacrylamide gel electrophoresis. Resolved proteins were transferred to an Immobilon P membrane and incubated with affinity-purified IgGs directed against PKC2; antigen-IgG complexes were visualized by an enhanced chemiluminescence procedure. Lane 1 received protein from uninfected Sf9 cells; lanes 3 and 4 contained proteins from baculovirus-infected Sf9 cells (3 and 4 days post-infection, respectively), lane 2 was identical with lane 3, except the antibodies were preincubated with excess (5 μg) purified antigen.
RNA fragment is 122 nucleotides in length. PKC2 mRNAs were relatively constant throughout development. PKC2 mRNAs were isolated from C. elegans embryos (E), L1-L4 larvae, young adults (A), egg-laying adults (A+E), and newly hatched (H) animals as indicated under "Experimental Procedures." Samples of protein (40 μg) were assayed for expression of PKC2 isoforms by Western immunoblot analysis as described under "Experimental Procedures."

The principal cytosolic PKC2 has an apparent Mr of 78,000 at all developmental stages. However, a closely spaced 77/78-kDa PKC2 doublet usually appears in particular fractions of C. elegans extracts (Fig. 7, lanes 5, 7, 9, 11, 13, and 15). Thus, distinct PKC2 isoforms may differentially associate with membranes and/or cytoskeleton. Both the 77- and 78-kDa kinases are potentially heterogeneous because four PKC2 isoforms have predicted Mr values of 77,000–78,000: PKC2A1B, PKC2A1C, PKC2B1B, and PKC2B1C (where A and B correspond to C-terminal regions encoded by alternative 3′-exons; 1B and 1C denote N-terminal domains encoded by the alternate 1B and 1C 5′-exons, respectively (Table II)). In addition, each isoform may exhibit an altered electrophoretic mobility because of differential autophosphorylation (59).

Larger PKC2 isoforms (Mr, ~80,000–82,000) accumulate immediately after C. elegans embryogenesis terminates (Fig. 7, lane 3). An 8-fold increase in an 81-kDa PKC2 polypeptide in membranes (Fig. 7, lanes 1 and 3) is coordinated with a 6-fold elevation in content of mRNA that contains exons 1A and 1A′ (Fig. 6). Expression of 80–82 kDa PKC2 proteins persists in L1 and L2 larvae (Fig. 7, lanes 5–8). The level of 80–82-kDa PKCs declines sharply in L3 animals and these proteins are not detected in L4 and adult C. elegans (Fig. 7, lanes 9–16). A decrease in mRNA containing exons 1A and 1A′ parallels the loss of the larger PKC2 enzymes (Fig. 6). However, a low level of 81-kDa PKC2 accumulates in embryos (Fig. 7, lane 1), which contain the same or a lesser amount of the cognate mRNA than L4 and adult nematodes (Fig. 6). Incorporation of 50 amino acids encoded by the 1A and 1A′ exons (Table II) at the N terminus of PKC2 generates two 81-kDa isoforms, PKC2A1A and PKC2B1A. Different stoichiometries of autophosphorylation could contribute another level of heterogeneity (59).

Three Promoters Drive pkc-2 Gene Transcription in Distinct Subsets of Neuronal and Non-neuronal Cells—Together, RNase protection analysis, cDNA sequencing, and elucidation of the organization of the pkc-2 gene suggest that three promoters...
The orientation of the L1 larva is the same as the animals shown in "indicated. Cells with lightly stained nuclei that lie between the positionsofmusclecells (M observed. the spermatheca). A single, heavily-stained extra-gonadal cell is also end of the intestine (anterior and posterior to the nerve ring, cells comprising the anterior portion of the micrograph) in an L1 larval nematode. The positions of the various cell types are explicitly labeled in wildtype (unstained) adult C.elegans the various cell types are explicitly labeled in Fig. 8. The P1C promoter was active in only region PKC2 isoforms are also detected in cell bodies of pharyngeal neurons and in neuronal processes that contribute to the nerve ring and ventral nerve cord. P1A promoter activity is also observed in sensory neurons in the tail ganglion. The cellular patterns of P1A and P1C promoter activities do not overlap. The P1B promoter activates reporter gene transcription in ~35 cells in L1-L4 larvae. The pattern of β-galactosidase accumulation in transgenic C. elegans containing the pck2P1B: lacZ chimera (Fig. 8C) is partially congruent with that observed for the pck2P1A: lacZ construct. Nuclei in body wall muscle, several sensory neurons posterior to the nerve ring, and in tail ganglia and a few intestinal cells evidently employ the P1A and P1B promoters to produce mRNAs encoding multiple PKC2 isoforms (Fig. 8, B and C). However, the P1B promoter also stimulates lacZ transcription in neuronal, intestinal, and muscle nuclei that lack β-galactosidase in C. elegans carrying pck2P1A: lacZ and pck2P1C: lacZ (Fig. 8C). Intense 1B promoter activity is evident in four cells that comprise the top of the intestine, whereas weaker activity is observed in other intestinal nuclei and in body wall muscle nuclei that lie near the tip of the head. Promoter P1B also directs β-galactosidase expression in nuclei of somatic cells in distal regions of the symmetrical adult gonad (Fig. 8D).

No positively staining cells were observed when transgenic nematodes carrying a promoterless reporter gene or lacZ downstream from a metal-inducible promoter (49) were assayed under similar conditions. The specificity of utilization of the P1A-P1C promoters was documented further by the lack of β-galactosidase staining in ~900 somatic nuclei in the transgenic lines of C. elegans.

Expression and Distribution of PKC2 Polypeptides in Vivo—Accumulation and localization of PKC2 proteins were analyzed by confocal immunofluorescence microscopy. PKC2 polypeptides accumulate in the cell bodies of multiple neurons that are components of sensory ganglia. These neurons are positioned anterior and posterior to the nerve ring in the head of C. elegans (Figs. 9, A-C). Representative micrographs reveal that the Ca2⁺-activated kinases are maximally enriched in neuronal processes that are incorporated into the nerve ring (the principal site of integration of neuronal signaling) and cells that constitute the anterior end of the intestine. In the head region PKC2 isoforms are also detected in cell bodies of pharyngeal neurons and in neuronal processes that contribute to the amphid and labial nerve fibers and the ventral nerve cord (Fig. 9, A and B). PKC2 expression is also evident in cell bodies located in neurons. In the head region, the neurons are constituents of sensory ganglia (Fig. 8B). Their cell bodies are positioned immediately anterior and posterior to the nerve ring and their processes contribute to the nerve ring and ventral nerve cord. P1A promoter activity is also observed in sensory neurons in the tail ganglion. The cellular patterns of P1A and P1C promoter activities do not overlap. The P1B promoter activates reporter gene transcription in ~35 cells in L1-L4 larvae. The pattern of β-galactosidase accumulation in transgenic C. elegans containing the pck2P1B: lacZ chimera (Fig. 8C) is partially congruent with that observed for the pck2P1A: lacZ construct. Nuclei in body wall muscle, several sensory neurons posterior to the nerve ring, and in tail ganglia and a few intestinal cells evidently employ the P1A and P1B promoters to produce mRNAs encoding multiple PKC2 isoforms (Fig. 8, B and C). However, the P1B promoter also stimulates lacZ transcription in neuronal, intestinal, and muscle nuclei that lack β-galactosidase in C. elegans carrying pck2P1A: lacZ and pck2P1C: lacZ (Fig. 8C). Intense 1B promoter activity is evident in four cells that comprise the top of the intestine, whereas weaker activity is observed in other intestinal nuclei and in body wall muscle nuclei that lie near the tip of the head. Promoter P1B also directs β-galactosidase expression in nuclei of somatic cells in distal regions of the symmetrical adult gonad (Fig. 8D).

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of neurons that are included in the rectal and tail ganglia (Fig. 9C). Finally, a high level of PKC2 is observed in somatic cells of the distal portions of the gonad, including the spermatheca (Fig. 9D). Patterns of PKC2 protein expression and P1A-P1C promoter activities are similar.

DISCUSSION

A unique C. elegans gene (the pkc-2 gene) encodes a family of calcium-stimulated PKC isoenzymes. PKC2 polypeptides contain 680–717 amino acid residues and have molecular weights of 77,000–82,000. The catalytic domain, pseudosubstrate site, calcium-binding segment, and Cys-rich regulatory regions are identical in each PKC2 isoform. However, the proteins diverge at their N and C termini. Complementary DNAs encoding PKC2 isoforms directed the expression of calcium-activated, lipid-dependent protein kinases in S9 cells infected with recombinant baculovirus. Anti-C. elegans PKC2 IgGs bound transgene products on Western blots and precipitated calcium-stimulated phosphotransferase activity, thereby confirming that pkc-2 gene products are members of the cPKC superfamily.

In mammals, three genes encode four cPKCs: the α, β, βII, and γ isoforms (1–7). Although these kinases have similar sizes, sequences, and kinetic properties, they subserve distinct physiological roles in certain cultured cells and the immune system (1–7, 70). They also differ (somewhat) in intracellular distribution, sensitivity to lipid activators and inhibitors, and stability. Levels of α, β, βII, and γ isoforms vary markedly and independently with cell/tissue type, development, and the ambient concentrations of hormones and growth factors. Thus, distinctive properties of cPKC genes and isoenzymes enable an organism to generate a broad spectrum of integrated physiological responses to hormones/growth factors that initiate concerted Ca²⁺/diacylglycerol-mediated signaling. In contrast, extensive screening of cDNA libraries, reverse transcriptase-PCR analysis, and the impressive progress of the C. elegans Genome Project (~50% complete in Sept. 1996) have revealed only one nematode gene that codes for a Ca²⁺-activated PKC, pkc-2. This raises the question of whether multiple transcriptional and/or post-transcriptional control mechanisms can diversify the pkc-2 gene products sufficiently to mediate, target, and fully integrate a variety of input signals in C. elegans.

Systematic cDNA sequencing and application of an anchored PCR procedure (RACE) to characterize extreme 5’-ends of cDNAs revealed that six distinct mRNAs can be derived from the pkc-2 gene. PKC2 mRNAs incorporate one of two 3’-terminal exons via alternative splicing. Further diversity in PKC2 mRNAs is contributed by the complex organization of multiple promoters that govern pkc-2 gene transcription. The 25-kbp gene has 17 exons and is located on the X chromosome. Exons 2–12 encode shared domains that are present in all PKC2 isoforms. However, transcription can be initiated from three distinct promoters. Each promoter precedes an adjacent exon that encodes 5’-untranslated RNA, an initiator ATG codon, and a unique open reading frame. Differential promoter utilization and splicing results in the incorporation of the promoter-proximal exon as exon 1 in the processed PKC2 mRNA. Exons adjacent to the alternative, non-utilized promoters are processed as “introns” and are excluded from the mRNA. Thus, three distinct N termini (1A-1C) contribute to PKC diversity. Derived amino acid sequences encoded by alternative 5’-exons are not homologous with each other or protein sequences in standard data bases. The divergent nature of the N- and C-terminal segments of the isoforms suggests that these structural cassettes may mediate specialized functions. Possible roles for the different N and C termini include: the modulation of substrate specificity, selective intracellular targeting and anchoring of PKC2 isoforms to various organelles and cytoskeleton; modulation of the susceptibility of PKC2 to regulation by a variety of lipid-derived activators and inhibitors; and control of the stability of the kinases.

Differential utilization of pkc-2 promoters and 3’-terminal exons provides mechanisms for generating novel, cell- and development-stage specific patterns of PKC2 isoform accumulation and intracellular distribution. In situ promoter analysis and RNase protection studies demonstrated that some neurons and non-neuronal cells restrict PKC2 diversity by expressing mRNAs that contain only one type of 5’-terminal exon. This implies that only one or two PKC2 isoforms are sufficient to mediate Ca²⁺/diacylglycerol signaling in those cells. Regulation of 5’-exon selection suggests the speculation that properties conferred by distinct 1A-1C termini adapt PKC2 isoforms for distinct functions in different cells. For example, the 15-residue sequence encoded by exon 1C will appear exclusively at the N terminus of PKC2 isoforms expressed in nine neurons. Various clusters of neurons, muscle, and intestinal cells, as well as somatic cells of the gonad, accumulate mixtures of PKC2 mRNAs that begin with either exon 1A or exon 1B. Thus, some cells of intact C. elegans will contain four PKC2 isoforms if alternative 3’ splicing is also operative. An increase in PKC2 diversity could alter the magnitude and duration of cellular responses to external stimuli and facilitate recruitment of an enlarged group of effector proteins from multiple cell compartments. Phosphorylation of different types and increased numbers of downstream substrate/effectector molecules by several PKC2 isoforms would enable activation and integration of multiple responding pathways to a concerted Ca²⁺/diacylglycerol signal.

PKC2 isoforms are minimally expressed in C. elegans embryos. A 10-fold increase in total PKC2 content was observed in newly-hatched animals. Substantial levels of 77/78-kDa PKC2 were evident at all stages of post-embryonic development (larval stages L1-L4 and reproductive adults). The abundance of mRNAs encoding these isoforms is nearly invariant throughout development. Thus, accumulation of the 77/78-kDa PKC2 isoforms is negatively regulated at the level of translation during embryogenesis. The concentrations of 80–82-kDa PKC2 isoforms and cognate mRNAs are coordinately increased 8- and 6-fold, respectively, in newly-hatched larvae. These larger isoenzymes persist only in a relatively brief developmental period (~18 h) that terminates with the molt that demarcates the transition from L2 to L3 larvae. Thus, 80–82-kDa PKC2 isoforms apparently subserve physiological/regulatory functions associated with early stages of post-embryonic development. Expression of 80–82-kDa PKC2 isoforms seems to be regulated transcriptionally since 1A promoter activity was observed principally in L1 and L2 animals.

Compartmentalization of PKC2 varied markedly during development. For example, approximately 80% of PKC2 was in the particulate fraction of homogenates of L1 larvae, whereas 70% of PKC2 in adult nematodes partitioned with cytosol. This could be due to developmental regulation of alternative 3’-terminal exon splicing and/or promoter selection. One or more of the unique amino acid sequences encoded by the variable 5’- and 3’-exons may include compartment-specific targeting/anchoring domains. The observation that the 77-kDa isoform is restricted to the particulate fraction of C. elegans homogenates is consistent with this idea. In addition, immunofluorescence microscopy revealed differential intracellular targeting of PKC2 isoforms in neurons. The kinases are moderately abundant in cell bodies, highly enriched in processes that comprise the nerve ring (the key locus of interneuronal communication and integration of signaling), and excluded from nuclei.
The low level of PKC2 expression in embryos indicates that this family of protein kinases does not play an essential role in early development. The abrupt increase in expression of PKC2 polypeptides in newly-hatched animals and the persistence of PKC2 and PKC2 gene promoter activity in sensory neurons throughout post-embryonic development suggests that, in part, these kinases mediate the reception and integration of environmental signals and the animal's responses to such signals. The occurrence of a high level of PKC2 in the somatic tissue of the hermaphrodite gonad raises the possibility that this family of lipid activated kinases may play a prominent role in supporting the development of oocytes and spermatoocytes. Finally, the shift from a predominantly particulate localization in early larval to a cytoplasmic distribution in adult C. elegans indicates that PKC2 isoenzymes may perform different functions in different cell compartments at various stages of development.

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