Protein Kinase C Isotypes in *C. elegans*

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The protein kinase C (PKC) family, consisting of multiple isotypes, plays a major role in cellular signaling. In the nematode *Caenorhabditis elegans*, four *pkc* genes, *tpa-1*, *pkc-1*, *pkc-2* and *pkc-3*, have been identified and investigated. Molecular analysis of *tpa-1*, *pkc-1*, and *pkc-2* has shown that each gene encodes multiple PKC isoforms with different expression patterns. One of the *tpa-1* isoforms, which is expressed in vulval cells, is found to play a role in nicotine-induced adaptation. The expression of *pkc-1* seems to be specific to neurons, while that of *pkc-2* is detected in several types of cells including neurons and muscle cells. An aPKC member encoded by *pkc-3* has been shown to play an essential role in establishing the polarity of the zygote. Recent studies have revealed that the mechanism of polarity establishment mediated by aPKC is evolutionarily conserved in diverse organisms from nematodes to mammals. *C. elegans* provides an excellent model system for molecular dissection of the cellular signaling pathways involving PKC.

Key words: *Caenorhabditis elegans*, cell polarity, gene expression, neuron, PKC.

It is now well known that protein kinase C (PKC), first described by Takai *et al.* (*1*), comprises a widespread multigene family of serine/threonine kinases found in diverse eukaryotic organisms from yeast to humans. In mammals, more than ten isotypes with related structures and similar enzymatic properties have been identified. PKC isotypes are categorized into three major subfamilies on the basis of structural similarities and cofactor requirements: conventional PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC). Many *in-vitro* and *in-vivo* studies of PKC family members have revealed their enzymatic properties and have suggested that each isotype plays a specific role in separate signaling cascades (*2*). However, the mode of gene expression and the physiological role of each isotype in organisms are not fully understood.

Caenorhabditis elegans is a small free-living soil nematode and has many advantages to study the function of genes operating in animal development and behavior at the molecular level (*3*). While this animal has various tissues and organs, as in the case of higher organisms, it is composed of a rather small number of somatic cells, and its development can be traced in exceptional detail at the cellular level. It is also amenable to powerful genetic and molecular approaches. The genome sequence has been determined (*4*) and about 40% of the predicted genes are estimated to have human counterparts (*5*). The availability of the genome sequence together with the recent development of an RNA interference technique (RNAi) enables us to identify the function of genes (*6*), even if the mutant for a gene in question is not isolated. These characteristics may make *C. elegans* an excellent system for both genetic and molecular dissection of cellular signaling pathways involving PKC.

Four *pkc* genes, *tpa-1*, *pkc-1*, *pkc-2*, and *pkc-3*, have been identified in *C. elegans*. Recent studies have revealed their structures, expression patterns and functions, and are described in this review. In addition to the four *pkc* genes, *C. elegans* contains two genes encoding PKCμ-like proteins. Table I shows a summary of *pkc* genes including the two PKCμ genes.

The *tpa-1* gene encodes nPKC members involved in phorbol ester–induced developmental and behavioral disorders

Phorbol esters, such as TPA (12-*O*-tetradecanoylphorbol 13-acetate) and PDD (phorbol 12,13-didecanoate), have a wide variety of biological and biochemical influences on many *in-vivo* and *in-vitro* systems. These compounds also cause complete growth arrest and severely uncoordinated behavior in *C. elegans*. The *tpa-1* gene was identified as the target of phorbol esters by a genetic analysis of TPA-resistant mutants, which behave normally as well as develop in the presence of TPA (*7*).

Molecular cloning of the *tpa-1* gene revealed that it encodes a homolog of nPKCδ and θ, thus demonstrating that a PKC mediates the phorbol ester-induced disorders in *C. elegans* (*8*). The *tpa-1* gene, consisting of eleven exons, transcribes two mRNA species: *tpa-1*A, which contains all eleven exons and encodes TPA-1A[2] of 704 amino acid residues, and *tpa-1*B, which consists of exons V through XI and encodes TPA-1B of 567 residues (*9*). The expressions of the two mRNAs are differentially controlled by distinct promoters. The *tpa-1A* mRNA is mainly expressed in a variety of neurons around the nerve ring and a pair of canal-associated neurons, as well as in vulval epidermal cells and vulval muscle cells (*10*). The expression of *tpa-1B* mRNA has

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[2]According to the genetic nomenclature for *C. elegans*, the protein of a gene is represented by the corresponding gene name and is written in non-italic capitals. For example, TPA-1 stands for the protein encoded by *tpa-1*.

TABLE I. *C. elegans* **PKC isotypes.**

Gene name	Linkage group	Corresponding sequence[a]	Predicted isoforms	Residues (amino acids)	Similarity	Tissue expression pattern[b]	Mutant or RNAi phenotype	References
tpa-1	VI	B0545.1	2	704 (A) 567 (B)	nPKCδ/θ	Neurons, Vulval cells (A); Hypoderm (B)	Defective nicotine adaptation, Resistance to TPA	(*7–10*)
pkc-1	V	F10C2.1	2	763 (A) 707 (B)	nPKCε	Neurons (B)	N.D.	(*12, 13*)
pkc-2	X	E01H11.1	6	680–717	cPKCα/β	Neurons, Pharynx, Intestine, Embryo	No apparent phenotype	(*14, 15*)
pkc-3	II	F09E5.1	1	597	aPKCζ, λ/ι	Embryo, Intestine	Defective embryonic polarity	(*16, 17*)
–	I	W09C5.5	1	722	PKCμ	N.D.[c]	N.D.	–
–	V	T25E12.4	1	1101	PKCμ	Spermatheca–uterine junction	N.D.	–

[a]Each sequence name represents a predicted gene on the corresponding cosmid, *e.g.* B0545, by the *C. elegans* genome sequencing consortium. [b]Expression was determined by reporter assay using a promoter–reporter construct and/or staining with a specific antibody [c]N.D.: not determined.

been detected in hypodermal cells arranged in a row on either side of the body and in the head region of newly hatched L1 larvae (Tabuse, Y. and Miwa, J., unpublished study). The distinct tissue expression patterns of *tpa-1A* and *tpa-1B* indicate that each isoform might play separate physiological roles.

In *C. elegans*, acute exposure to nicotinic receptor agonists, such as nicotine and levamisole, is known to cause the stimulation of egg laying. This response is mediated by the *unc-29* (uncoordinated movement) gene, which encodes a subunit of the nicotinic acetylcholine receptor (nAchR) expressed in vulval muscle cells (*11*). Recently, prolonged nicotine treatment has been shown to cause down-regulation of UNC-29 nAchR, resulting in nicotine-adapted animals that no longer respond to further treatment with the agonists. The adaptation to nicotine requires the wild-type *tpa-1* function, since *tpa-1* mutants preserve sensitivity to nicotine and high-level expression of UNC-29 after long-term treatment with nicotine (*10*). These observations, together with the expression pattern of TPA-1A, indicate that TPA-1A plays a role in the signaling pathway for regulating the nAchR level, thus causing nicotine-induced adaptation. How does TPA-1A transmit a signal initiated by chronic nicotine treatment and regulate the nAchR level? Although several reports have suggested the role of PKC in the down-regulation of mammalian nAchR, the results are inconsistent and the molecular mechanism remains obscure. To understand nicotine adaptation, it is important to identify the target of PKC and other components involved in nicotine adaptation. *C. elegans*, equipped with powerful tools for genetic and molecular analysis, may make it possible to dissect the biochemical pathway mediated by TPA-1A, leading to an understanding of nicotine adaptation.

The *pkc-1* gene encodes two neuron-specific nPKC isoforms

The *pkc-1* gene encodes two PKC isoforms, PKC-1A (*12*) and PKC-1B (*13*). The predicted PKC-1B protein consists of 707 amino acid residues and contains structural features characteristic of nPKC isotypes. Consistent with the structural features of an nPKC member, PKC-1B shows Ca^{2+}-independent kinase activity. Predicted PKC-1A contains the entire PKC-1B sequence with an additional N-terminal extension of 56 novel residues. Both PKC-1A and PKC-1B are highly homologous to mammalian nPKCε.

Two *pkc-1* mRNAs are derived from alternate promoters as in the case of the *tpa-1* gene. However, the regulation of *pkc-1* expression is different from that of *tpa-1* and other *pkc* genes, and it constitutes an operon with an upstream gene, *kup-1* (*12*). Although recent surveillance of the whole genome predicted at least 1,000 operons in *C. elegans*, the physiological roles of these operons have not been elucidated. The *kup-1* gene encodes a novel protein and the functional relationship between *pkc-1* and *kup-1*, if any, remains to be examined. The *pkc-1A* mRNA is co-transcribed from the promoter of the upstream gene, whereas the *pkc-1B* mRNA is transcribed from another promoter located internally in the second intron of the *pkc-1* gene. The *pkc-1A* mRNA level is low during larval development but sharply increases in adult animals, while high-level expression of the *pkc-1B* mRNA is observed at larval stages. Thus, the developmental expression of the two *pkc-1* transcripts is differentially controlled. Antibody staining revealed that PKC-1B localizes in the processes and cell bodies of about 75 neurons in the sensory circuitry, thus tending to support some specific roles in neuronal function (*13*). The tissue localization pattern and the function of PKC-1A have not yet been investigated.

The *pkc-2* gene encodes multiple cPKC isoforms

The *pkc-2* gene, comprising 17 exons, is predicted to encode six PKC isoforms of 680–717 amino acid residues (*14*). All isoforms show structural features common to cPKC family members and are similar to cPKCα and β. Their N-terminal and C-terminal sequences diverge due to alternative utilization of the corresponding exons. The *pkc-2* mRNAs are transcribed from three distinct promoters, incorporating a unique 5′-terminal exon that follows each respective promoter. Also, the *pkc-2* mRNAs incorporate one of two 3′-terminal exons *via* alternative splicing. Spatio-temporal expression from each promoter seems to be differentially controlled, suggesting that PKC-2 executes an isoform-specific physiological function. Each promoter is active in a distinct subset of neurons, and certain promoters are active in intestinal cells as well as muscle cells. PKC-2 appears to perform a dispensable or redundant function, since depletion of the normal *pkc-2* function by RNAi does not cause apparent embryonic or postembryonic phenotypes (*15*).

The three *pkc* genes, *tpa-1*, *pkc-1*, and *pkc-2*, produce multiple isoforms through alternative splicing and/or alternate use of distinct promoters. Isoforms produced from each *pkc* gene share the respective common core sequence

with diverse N-terminal and/or C-terminal sequences. Although the physiological roles of diverse terminal sequences have not been fully studied, they might alter the properties of isoforms, such as cofactor requirements, substrate specificity, lifetime, and subcellular localization. As described above, the spatio-temporal expression of each isoform is controlled by the specific promoter. Thus, these elaborate gene expression mechanisms might make it possible to produce diverged PKC members at the proper place to execute the isoform-specific physiological role in *C. elegans*.

PKC-3 encodes an aPKC member with an essential function in early embryogenesis

The protein encoded by the *pkc-3* gene is a member of the aPKC subfamily, as it is highly homologous to mammalian aPKCζ and λ/ι, and expresses Ca^{2+}- and DAG/phorbol ester–independent kinase activity. Recent molecular analysis of PKC-3 has revealed its essential role in early embryogenesis (*16, 17*).

In the *C. elegans* embryo, anterior–posterior (A-P) polarity is established during the first cell cycle along the A-P axis defined by a cue provided by the sperm. The dynamic reorganization of cytoplasm that occurs shortly after fertilization results in the polarized localization of various cellular components including the determinants of cell fate. The first mitotic spindle is placed posteriorly and the first division becomes asymmetric (*18*). The establishment of the A-P polarity of zygotes depends on six maternally expressed *par* (partitioning defective) genes. Mutations in these six *par* genes cause gene-specific polarity defects and some of them lead to a symmetric first division. Consistent with the roles in early A-P polarity, several *par* proteins themselves become localized asymmetrically in zygotes (*18*).

PKC-3 has been shown to interact with PAR-3, a PAR protein with three PDZ domains, to play an indispensable role in establishing A-P polarity (*17*). PKC-3 binds to and co-localizes with PAR-3 at the anterior periphery of zygotes. Later, another PAR protein, PAR-6, which contains a single PDZ domain and a CRIB domain, was also found to co-localize with PKC-3 and PAR-3 (*19*). Thus, these three proteins are thought to form a complex that localizes at the anterior cortex (Fig. 1). The asymmetric localizations of PKC-3, PAR-3 and PAR-6 are interdependent of each other; *i.e.*, disruption of the normal function of any one of them *via* mutation or RNAi results in the mislocalization of all three proteins and causes similar defects in polarity (*17–19*). Based on the role of the PKC/PAR complex described above together with a molecular analysis of other *par* genes, the genetic pathway for A-P polarity is outlined as shown in Fig. 1. Another par protein, PAR-2, localizes to the posterior periphery in zygotes, and the localization of PAR-2 depends on the activity of PAR-3 (*18*). Reciprocally, proper localization of the PKC-3/PAR-3/PAR-6 complex requires *par-2* gene function. Recently, a small G protein, CDC-42, was also reported to function in establishing polarity, binding to PAR-6 and regulating the localization of the complex (*20, 21*).

In mammalian epithelial cells, orthologs of PKC-3, PAR-3, and PAR-6 have been found to localize at the apical domain and play a role in apical-basal polarity (*22–24*). A similar mechanism was reported to operate in the apical–basal polarity of neuroblasts and epithelial cells in *Drosophila* (*25*). Orthologs of PKC-3 and PAR-3 have also been shown to distribute asymmetrically at the hemisphere of *Xenopus* oocytes (*26*). These observations support the notion that PKC/PAR-mediated signaling cascades are conserved evolutionarily and that they might contribute a critical function to the establishment of cellular polarity (*24*). It is not clear how the PKC-3/PAR-3/PAR-6 complex becomes localized asymmetrically, nor how the complex leads to the polarized localization of other cellular components. To understand A-P polarity in *C. elegans*, it is important to identify and analyze other molecules that participate in the PKC/PAR pathway, including the target(s) of PKC-3. The knowledge obtained through such an approach may also provide insight into cellular polarity in higher organisms.

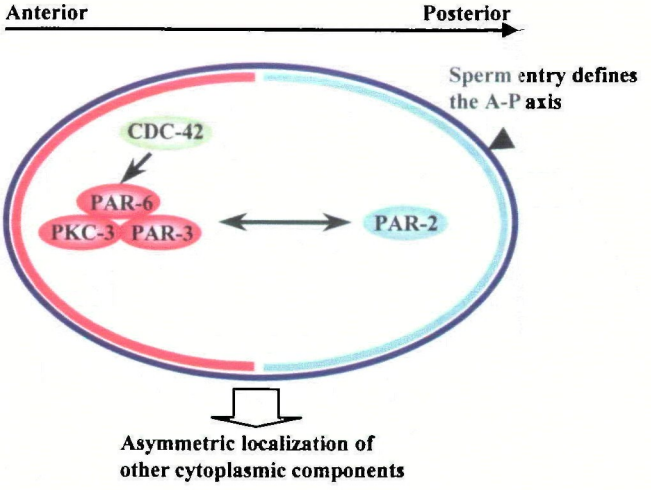

Fig. 1. **Anterior–posterior polarity in the *C. elegans* zygote.** Sperm entry defines the posterior end and initiates the asymmetric localization of PKC-3 and PAR proteins. PKC-3, PAR-3, and PAR-6 form a complex and co-localize to the anterior cortex of the zygote (indicated in red). PAR-2 is a novel protein containing a Zn-binding domain of the ring finger class and a myosin-type ATP-binding site, and localizes to the posterior cortex (blue). CDC-42 binds to PAR-6 and functions in A-P polarity, although its localization in embryos is not confirmed. Polarized PKC-3 and PAR proteins lead to the asymmetric distribution of other cytoplasmic components.

It has been reported that PKC-3 is expressed in intestinal epithelial cells of larval and adult *C. elegans* (*16, 27*). PKC-3 localizes at the apical surface membrane of intestinal cells, suggesting that it also plays some role in apical–basal polarity as in the case of mammalian aPKCs. Does PKC-3 become polarized in intestinal cells through a mechanism similar to that operating in early embryos? In addition to PAR-3 and PAR-6, a new PKC-3 adapter protein, CKA-1 (C-kinase adapter), was recently reported (*28*). The CKA-1 protein is similar to *Drosophila* Numb protein and is expressed in the intestine as well as in embryos. CKA-1 expressed in mammalian cells has been shown to distribute at the cell periphery, and cortical CKA-1 recruits PKC-3, which is co-expressed within the same cells (*29*). Although these observations indicate a role of CKA-1 in the postembryonic localization of PKC-3, it is necessary to examine closely the physiological function of CKA-1 as well as the interaction between CKA-1 and PKC-3 in *C. elegans* before any definitive conclusions can be drawn.

Conclusion

The accumulating data indicate that many biochemical pathways, such as apoptosis and the *ras* signaling pathway, are conserved between *C. elegans* and mammals. A molecular analysis of PKC-3 has successfully shown its physiological role in polarity and provides a clue to clarify the mechanism of cellular polarity in higher organisms. For a greater understanding of the PKC-3 pathway, it is necessary to identify the components of the pathway and the target(s) of PKC-3. The availability of intensive genetic and reverse genetic methods in *C. elegans* will facilitate such research. Similar approaches to the other PKC isotypes may contribute to establishing their functions and to elucidating the whole picture of the pathway mediated by PKC.

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