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Isolation and Characterization of Two New Drosophila Protein Kinase C Genes, Including One Specifically Expressed in Photoreceptor Cells

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Summary

We have isolated and characterized two new protein kinase C (PKC) genes from D. melanogaster. One, dPKC98F, maps to chromosome region 98F and displays over 60% amino acid sequence identity with members of a recently described "PKC-related" subfamily in mammals. The other, dPKC53E(ey), maps to region 53E4–7 on the second chromosome and lies within 50 kb of a PKC gene previously characterized (dPKC). While dPKC98F transcripts are expressed throughout development, expression of the two genes mapping at cytogenetic location 53E is primarily in adults. dPKC98F and the previously reported 53E gene are transcribed predominantly in brain tissue. In contrast, dPKC53E(ey) is transcribed only in photoreceptor cells. We will discuss the significance of this tissue-specific localization with regard to phototransduction.

Introduction

Second-messenger mechanisms by which cells respond to signals from the external environment are currently being intensively studied. One of the most ubiquitous such systems is the phospholipase C–protein kinase C cascade, in which receptor-coupled phospholipase C (PLC) cleaves membrane inositol phospholipids to yield inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mediates mobilization of calcium from intracellular storage sites (Berridge and Irvine, 1984), and DAG activates a family of serine/threonine protein kinases collectively known as protein kinase C (PKC) (Takai et al., 1979; Kishimoto et al., 1980; Nishizuka, 1986).

PKCs appear to be intimately involved in cell growth and proliferation (Rozengurt et al., 1984; Kaibuchi et al., 1985; Persons et al., 1988). Phorbol esters, which are chemical analogs of DAG, can directly stimulate PKC (Niedel et al., 1983; Kikkawa et al., 1983), and this probably accounts for the tumor-promoting properties of these compounds. Examples of biological processes mediated or modulated by PKC include mitogenic responses to growth factors (Sawyer and Cohen, 1981; Habernicht et al., 1981), production of interleukin-2 by T lymphocytes (Truneh et al., 1985), and release of numerous hormones (Negro-Vilar and Lapetina, 1985; Ohmura and Friesen, 1985) and neurotransmitters (Tanaka et al., 1984; Pozzan et al., 1984). PKC in neurons can activate or inactivate specific conductance channels (Farley and Auerbach, 1986; Madison et al., 1986), and its action has also been implicated in long-term potentiation, a learning-like phenomenon observed in the hippocampus (Akers et al., 1986; Lovinger et al., 1987, Malinow et al., 1988). In some neurons PKC may be activated directly by elevated calcium (Malenka et al., 1988) in place of, or in addition to, its activation by DAG. PKC can phosphorylate and desensitize a number of membrane receptors, including the EGF and β-adrenergic receptors (Shoyab et al., 1979; Fearn and King, 1985; Kelleher et al., 1984). The ability of PKC to modulate transduction cascades through phosphorylation of key members provides a sensitive mechanism for fine tuning biological responses to changing stimuli.

The eukaryotic PKC gene family has at least seven members. Four of them (α, β, β′, and γ) have been identified in several species (Parker et al., 1986; Coussens et al., 1986; Knopf et al., 1986; Ono et al., 1986, 1987) and are thought to encode three biochemically distinct isozymes (Huang et al., 1987; Ono et al., 1987). Three recently identified subtypes (δ, ε, and ζ) are more distantly related (Ono et al., 1988; Ohno et al., 1988). The seven isozymes have different responsiveness to calcium and phospholipids (Sekiguchi et al., 1987; Ohno et al., 1988) and different tissue distributions (Ohno et al., 1987; Kosaka et al., 1988); for example, PKCγ and PKCε are particularly enriched in mammalian brain tissue (Nishizuka, 1988; Ono et al., 1988).

PKCs have been extensively characterized biochemically. However, the specific pathways in which they function are poorly understood, as is the reason for the existence of so many different isozymes. To begin to dissect their functional roles, we sought to isolate PKC genes from Drosophila, a system in which they can be studied genetically, and manipulated using molecular techniques. One such PKC gene had previously been reported by Rosenthal et al. (1987). We describe here the isolation and characterization of two additional Drosophila protein kinase C genes. One of these maps to the third chromosome at position 98F, is homologous to mammalian PKCδ, and is expressed throughout the Drosophila central nervous system (CNS). The other maps to position 53E on the second chromosome, within 50 kb of the PKC gene reported by Rosenthal et al. Remarkably, this second 53E gene is expressed only in photoreceptor cells. We will consider this specific expression in terms of invertebrate photoreceptor cell function and phototransduction.

Results

Isolation of Drosophila Genomic and cDNA Clones

To isolate genes encoding Drosophila PKC homologs, we screened a genomic library at reduced stringency with two bovine PKC cDNA clones corresponding to bPKCα and bPKCβ (Parker et al., 1986; Coussens et al., 1986). We isolated 11 positive clones and used them as probes

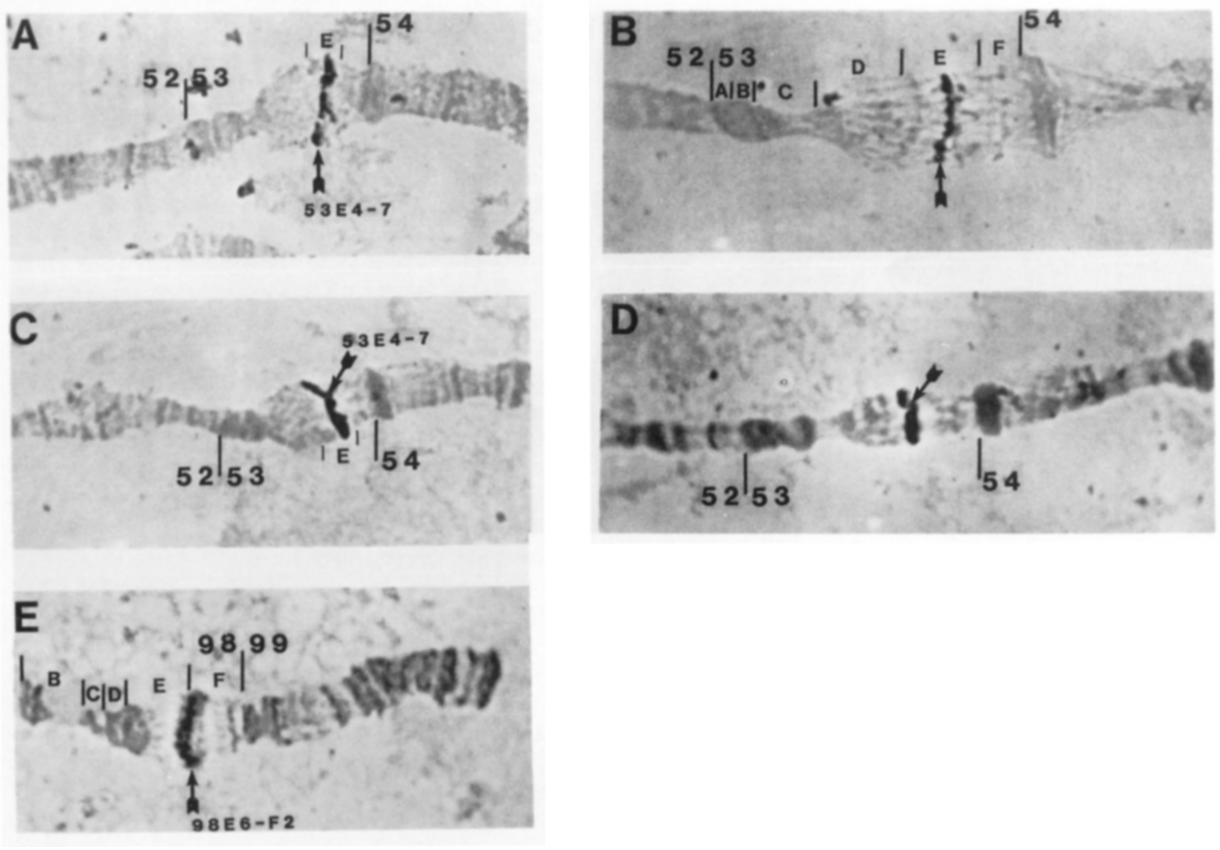

Figure 1. In Situ Hybridization of Drosophila PKC Probes to Larval Salivary Gland Chromosome Squashes

(A, B) λc53–1, (C) λ53–3, and (E) λc98–2 were biotinylated by nick translation and used as hybridization probes to determine their chromosomal location. The arrows at 53E4–7, 53E4–7, and 98E6–98F1 indicate the sites of hybridization of λc53–1 (dPKC53E(ey)), λ53–3 (dPKC53E(br)), and λc98–2 (dPKC98F), respectively; no other sites of hybridization were observed. (B) shows an expanded view of the 53E region. (D) shows hybridization with a mixed probe consisting of λc53–1 and λ53–3. Note a single signal at position 53E4–7.

for in situ hybridization to larval salivary gland chromosomes. Eight clones (λ53–1 to λ53–8) mapped to the second chromosome at position 53E, and three (λ98–1 to λ98–3) mapped to the third chromosome at 98F (Figure 1). Independently, as part of a study on visual transduction in Drosophila, we isolated a collection of genes that were expressed predominantly in the adult visual system (Shieh et al., 1989). One such gene, λEY17, was mapped to position 53E and was shown to cross-hybridize to a subset of the 53E PKC homologs (see below).

We used restriction fragments from the 53E and 98F genomic clones to screen Drosophila adult head cDNA libraries at high stringency (see Experimental Procedures for details). The 98F probe identified two overlapping cDNAs of 2.4 and 2.9 kb (λc98–1, and λc98–2), and the 53E probe identified a series of clones of which the longest was 2.5 kb (λc53–1). The cDNA clones hybridized to the same cytogenetic loci as the genomic clones used to isolate them (Figure 1).

Two PKC Genes at 53E

Rosenthal et al. (1987) had previously isolated a Drosophila PKC gene (dPKC) that mapped to position 53E on the second chromosome. To determine whether our λc53–1 cDNA was identical to dPKC, we sequenced our clone (Figure 2A). Surprisingly, the sequence contained an open reading frame encoding a 700 amino acid protein homologous to other PKCs, but different throughout its length from dPKC. This result raised the possibility that two different PKC genes map to cytogenetic position 53E. To assess whether the dPKC gene was represented among our eight 53E genomic clones, we carried out Southern blot hybridization with a 16-mer oligonucleotide probe specific for the dPKC gene. The results demonstrated that λ53–1, λ53–3, and λ53–8 correspond to dPKC; this was later confirmed by DNA sequencing (data not shown). To verify that both λc53–1 and dPKC map to the same cytogenetic region, we carried out in situ hybridization to polytene chromosomes with each of these clones as well as a mixed probe (Figure 1D). Both probes hybridize to cytogenetic region 53E4–7.

The proximity of these two genes was further demonstrated by using them as probes on a Southern blot of Drosophila genomic DNA fractionated by pulse-field electrophoresis. Both probes hybridized to a 200 kb NotI restriction fragment (data not shown). Although this demonstrates that the two genes are no more than 200 kb apart, the chromosomal in situ hybridization result suggests that they are much closer. Indeed, a mixture of the two probes gave a single hybridization signal at position

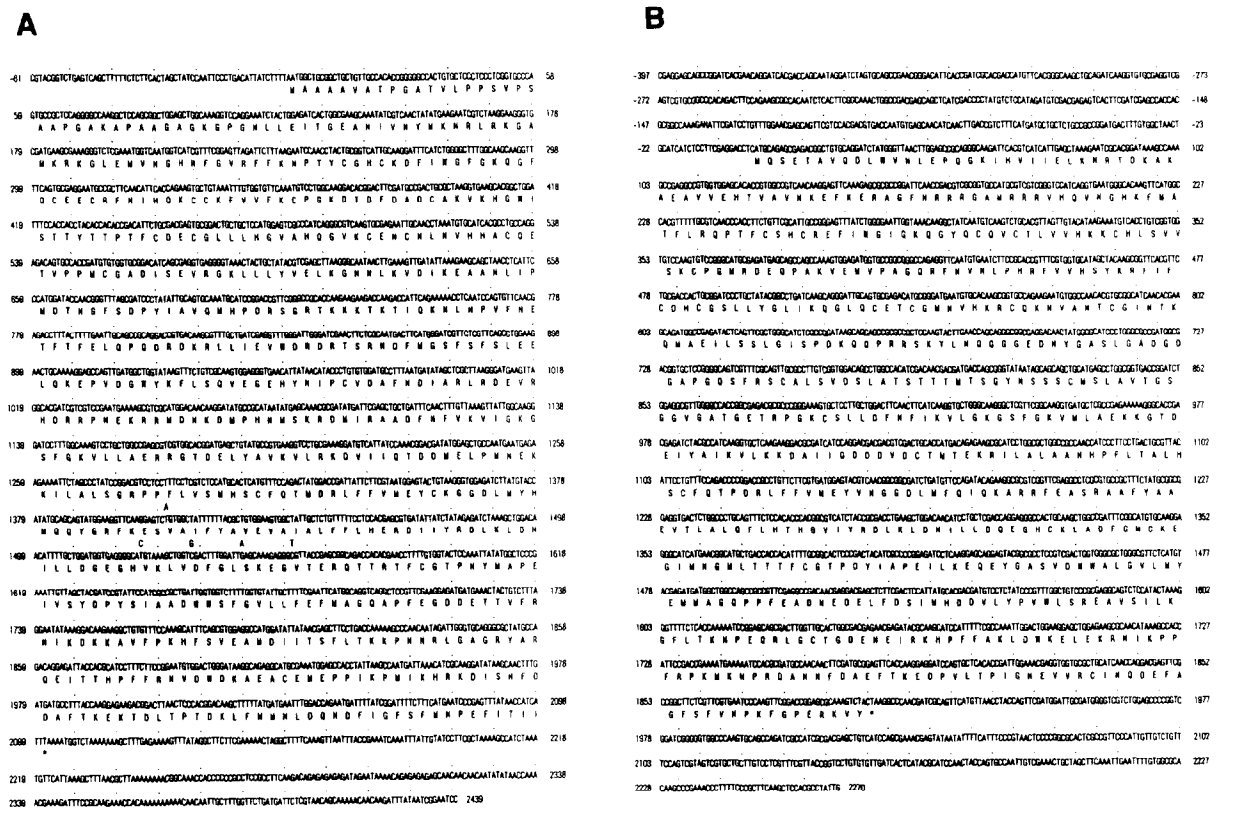

Figure 2. Nucleotide and Deduced Amino Acid Sequences of Two Drosophila PKC Genes

(A) The dPKC53E(ey) sequence was obtained from cDNAs λc53-1 and λc53–2, which were isolated from two different cDNA libraries (see Experimental Procedures). The first nucleotide of the putative ATG initiation codon is numbered one. Nucleotides from λc53–2 that differ from λc53–1 are shown above the sequence. A poly(A) tract was not identified in the sequence, indicating the cDNA may be truncated at the 3′ end. The GenBank accession number for this sequence is J04845.

(B) The dPKC98F sequence was derived from cDNA λc98–2. The first nucleotide of the putative ATG initiation codon is numbered one. No poly(A) tract was found, which suggests that λc98–2 is truncated at the 3′ end. The GenBank accession number for this sequence is J04848.

53E4-7, a region of light chromosomal banding (see Figure 1B). In view of this fact, we estimate that the two genes are less than 50 kb apart. We propose the nomenclature dPKC53E(br) for the gene reported by Rosenthal et al. (1987), and dPKC53E(ey) for the new gene described here (this nomenclature refers to the cytogenetic location and tissue of expression of each gene as discussed below).

A PKC-Related Gene at 98F

As mentioned above, we also isolated two cDNAs that mapped to chromosomal region 98F. The larger of these (λc98–2) was subcloned, and 2668 nucleotides were sequenced (Figure 2B). The sequence, hereafter referred to as dPKC98F, contained a 634 amino acid open reading frame with homology to mammalian PKCs. However, the 98F sequence diverged from "classical" mammalian PKC genes in a region near the amino terminus (region C2 of Figure 3). The more recently identified mammalian PKC-related genes (PKCδ, ε, and ζ) also diverge in this domain (Ono et al., 1988; Ohno et al., 1988). The 98F gene has greater amino acid identity with, and a domain arrangement characteristic of, PKCδ (also termed nPKC) (Table 1).

Sequence Comparisons among PKC Genes

Figure 3 shows an alignment of the three Drosophila genes with three representative mammalian PKC sequences. The Drosophila proteins share structural features with their mammalian counterparts. These include: a consensus ATP binding site located near the middle of the molecule, containing the sequence Gly-X-Gly-X-X-Gly-X_{19}–Lys, where X is any amino acid; a pair of cysteine-containing "zinc finger" motifs near the amino terminus of PKC; and an eight amino acid pseudosubstrate domain located toward the N-terminus, consisting of the sequence Z-Z-Gly-Ala-Leu/Met-Z-Z-Z, where Z is a basic amino acid. This sequence, which resembles the PKC phosphorylation site on target proteins, has been implicated in autoregulation of the enzyme (House and Kemp, 1987).

In general, regions that are the most variable among mammalian PKC genes also show greater variability among the three Drosophila sequences (see Figure 3). The two 53E sequences are more homologous to each other than to 98F. Among the Drosophila genes, dPKC53E(br) is most similar to the classicial mammalian genes (PKCα, β, and γ). Table 1 shows comparisons of the Drosophila sequences to each other and to mammalian sequences.

dPKC53E(ey) Is a Photoreceptor Cell-Specific PKC

To examine the spatial patterns of transcription of the different Drosophila PKC genes, we carried out in situ hybridization to tissue sections of the adult head. In addition, we performed Northern blot analysis with RNA isolated

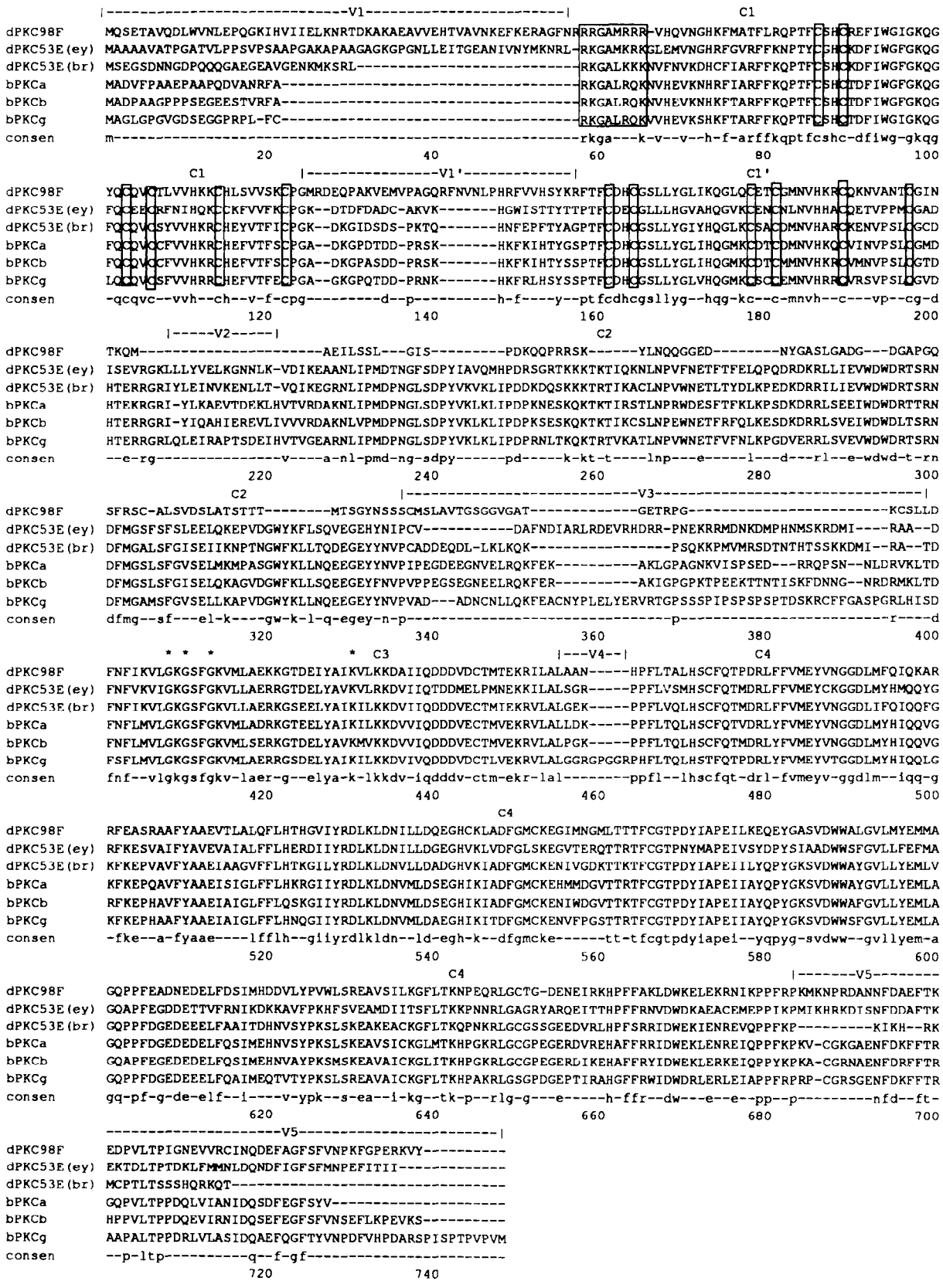
V1
C1
V1'
C1'
V2
C2
V3
V4
C3
C4
V5
dPKC98F
dPKC53E(ey)
dPKC53E(br)
bPKCa
bPKCb
bPKCg
consen

Table 1. Amino Acid Identity Comparisons among Drosophila and Mammalian Protein Kinase C Sequences

	dPKC53E(br)	dPKC53E(ey)	dPKC98F
dPKC53E(br)	—	—	—
dPKC53E(ey)	55%	—	—
dPKC98F	51%	41%	—
bPKCα	70%	52%	51%
bPKCβ	67%	53%	50%
bPKCγ	65%	51%	52%
rPKCδ	49%	38%	61%

Bovine PKCα,β, and γ sequences (bPKCα, β, and γ) are from Parker et al. (1986) and Coussens et al. (1986). Rat PKCδ (rPKCδ) sequence is from Ono et al. (1988).

from the heads and bodies of wild-type and mutant adult flies. Each of the Drosophila PKC genes is expressed in the adult head, but transcripts are not detected in lanes containing body RNA (Figure 4 for 98F and 53E(ey); data not shown for 53E(br)). In view of the ubiquity and importance of PKC activity, it seems implausible that PKC is not expressed in adult bodies. We assume that either our Northern blots are not sensitive enough to detect very low levels of PKC RNA in adult bodies, or that there are other PKC genes yet to be identified.

The dPKC98F gene encodes a major 5.5 kb transcript that is expressed throughout development (Figure 4A). The level of this transcript is greatly reduced in embryos and correlates with the increased expression of two additional transcripts of 4.3 and 4.5 kb. The dPKC98F gene is the only Drosophila PKC that is transcribed during embryonic stages. Figure 5D shows in situ hybridization of dPKC98F to adult heads demonstrating expression in cell bodies of the brain.

Figure 5F shows that dPKC53E(br) is transcribed in all head neural tissue. In contrast, a 2.5 kb transcript that hybridizes to the dPKC53E(ey) gene is specifically expressed in photoreceptor cells (Figures 4B and 5B); note the hybridization signal both in the compound eyes and ocelli. Indeed, the level of this transcript is dramatically reduced upon removal of the visual system by the *eyes absent* (*eya*) mutation (Sved, 1986) (Figure 4B). Developmental Northern blot analysis shows that this 2.5 kb transcript accumulates in late pupal stages (data not shown). Interestingly, this is the time when most of the sequences involved in phototransduction are first transcribed. Thus, dPKC53E(ey) is transcribed specifically in photoreceptor cells, beginning during their terminal differentiation.

Discussion

Paired Genes

There are several examples in Drosophila of homologous genes located close to each other, such as *engrailed* and *invected* (Coleman et al., 1987) and two genes at the *gooseberry* locus (Bopp et al., 1986). Two rhodopsin genes are similarly clustered (Zuker et al., 1987). In all the cases analyzed thus far, the transcripts have similar but nonidentical patterns of expression. Whether sibling genes lie close together because they share regulatory sequences, or merely because they fail to move apart after tandem duplication, is an open question. The sequences of the two 53E PKC gene transcripts, although strongly homologous, show differences throughout their lengths. The longest stretch of identity is 12 nucleotides, a finding that argues against the likelihood of alternative splicing. The two cDNAs also have some sequence homology in their 3′ noncoding portions (data not shown), which suggests that they arose recently by duplication of a single gene.

Structural Features of Drosophila PKCs

The deduced amino acid sequences of the Drosophila PKC genes are generally quite similar to each other and to the mammalian PKC family (Figure 3 and Table 1), particularly in regions to which functions have been ascribed. The homology is greatest in the carboxy-terminal half, which contains the ATP binding site and the catalytic domain. The amino-terminal half of PKC has been implicated in regulation (Lee and Bell, 1986; Muramatsu et al., 1989), and contains several domains that are markedly less well conserved (Nishizuka, 1988). This variability may account for differences in substrate specificity and/or activation conditions among isozymes. The 98F gene diverges extensively from the other Drosophila PKC genes and from classical mammalian PKCs in the amino-terminal half. Specifically, it lacks the C2 domain. This gene shows much greater homology and a similar domain arrangement to members of a recently identified mammalian "PKC-related" subfamily (PKCδ, ε, and ζ, Ono et al., 1988; Ohno et al., 1988). This suggests that the "classicial" and "PKC-related" subfamilies had diverged by the time that the ancestors of arthropods and mammals had evolved apart. The homology also suggests that the 98F PKC enzyme might be relatively independent of calcium concentration, as are its mammalian homologs (Ohno et al., 1988).

While the amino-terminal half of PKC contains several variable regions, there are two highly conserved structural features: a pseudosubstrate domain and a zinc finger do-

Figure 3. Computer Alignment of Drosophila and Mammalian PKC Sequences

Mammalian sequences used in the alignment are bovine PKCα (bPKCa), bovine PKCβ (bPKCb), and bovine PKCγ (bPKCg) (Parker et al., 1986; Coussens et al., 1986). Gaps were introduced into the sequences to generate an optimal alignment. The bottom line represents a consensus sequence (CONSEN), which is defined as an amino acid present in at least five of the six sequences. Conserved and variable regions, as defined in mammalian sequences (Parker et al., 1986; Coussens et al., 1986), are labeled C1–C4 and V1–V5, respectively. An additional variable region has been added in the middle of C1 (V1′), because the Drosophila sequences are not conserved in this region. A box has been drawn around amino acids constituting the pseudosubstate domain (positions 58–65). Boxes are also drawn around cysteine residues in the zinc finger domain (positions 86–197). Asterisks are drawn over residues associated with the ATP binding site (Gly^{408}, Gly^{410}, Gly^{413}, and Lys^{430}). The catalytic domain begins at Asp^{400}, and continues to the end of C4 (position 683).

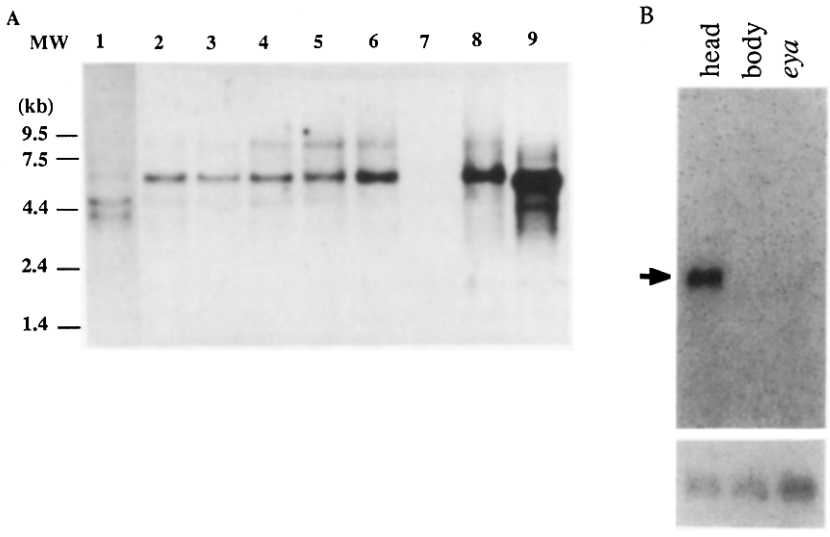

Figure 4. Northern Blot Analysis of PKC Gene Expression

(A) Developmental Northern analysis of dPKC98F gene. Lanes 1–8 contain 20 μg of total RNA from Canton-S embryos, 1st instar larvae, 2nd instar larvae, 3rd instar larvae, early pupae, late pupae, adult bodies, and adult heads, repectively. Lane 9 contains 1.5 μg of poly(A)$^+$ selected RNA from adult heads. The Northern blot was probed with cDNA λc98–2. The migration of RNA molecular weight markers is indicated at the left. Based on intensity of ethidium bromide staining, lanes 1–8 contain equivalent amounts of RNA (data not shown). The slower migration of total RNA (lanes 1–8), compared with poly(A)$^+$ RNA (lane 9), is due to the abundance of ribosomal RNA in the former.

(B) dPKC53E(ey) encodes an eye-specific transcript. Poly(A)$^+$ RNA was extracted from adult heads and bodies of wild-type (Oregon-R) and heads of *eya (eyes absent)* flies. The RNA (3 mg per lane) was gel-fractionated, blotted, and hybridized to a 1.2 kb radiolabeled EcoRI cDNA fragment from λc53–1 following previously described methods (Feiler et al., 1988). The arrow indicates the 2.5 kb eye-specific transcript. An RNA ladder was used as size markers. The blots were also hybridized to an alcohol dehydrogenase gene probe to control for the amount of RNA loaded (lower panel).

main. Drosophila PKCs, like their mammalian counterparts, contain a short segment rich in basic amino acids near their amino termini. This region is present in all PKCs and resembles target sequences found in substrate proteins. However, the sequence in the kinases contains alanine in place of the substrate serine or threonine, and appears to be an inhibitory pseudosubstrate domain involved in regulation of the enzyme. Synthetic peptides based on pseudosubstrate sequences can competitively inhibit bovine and Drosophila PKC activity in vitro (House and Kemp, 1987; E. Schaeffer, unpublished data). The pseudosubstrate sequence RKGALRQK is very highly conserved across mammalian PKC sequences (Figure 3). Among the Drosophila genes, the most closely related pseudosubstrate domain is found in dPKC53E(br). The dPKC53E(ey) and dPKC98F pseudosubstrate domains diverge from 53E(br) and from each other, although they have basic amino acids at the same positions. This may reflect differences in substrate specificity, and raises the possibility that differences between Drosophila pseudosubstrates can be exploited for selective inhibition of individual isozymes.

Adjacent to the pseudosubstrate domain, all PKC sequences contain two cysteine-rich zinc finger motifs that have been found in many nucleic acid–binding proteins. The PKC zinc fingers most closely resemble those found in steroid receptors, which require a divalent metal ion for receptor function (Sabbah et al., 1987). The function of zinc fingers in PKCs remains obscure.

Protein Kinase C and Phototransduction

The finding of a Drosophila PKC gene expressed abundantly and specifically in photoreceptors is tantalizing. Light activation of rhodopsin is the first step in the visual response. In vertebrate rods, photoactivated rhodopsin molecules activate a G protein, transducin, which in turn stimulates a cGMP-phosphodiesterase. This cascade of reactions leads to the transient closure of a cGMP-activated cation-selective channel, and the generation of a hyperpolarizing receptor potential (Stryer, 1986; Stieve, 1986; Kuhn, 1984). In contrast, the identity of the intracellular messenger(s) that mediates excitation in invertebrate photoreceptors has eluded firm identification (Payne, 1986; Fein, 1986). Phosphoinositide metabolism and calcium have been strongly implicated in excitation of both Limulus and dipteran photoreceptors (Payne, 1986; Fein, 1986; Devary et al., 1987; Yoshioka et al., 1985). It is believed that in invertebrates, photoactivated rhodopsin interacts with a GTP binding protein which in turn activates a phospholipase C (PLC). PLC would then catalyze the generation of the second messenger IP_3 and the subsequent mobilization of intracellular calcium. Support for this view is provided by the recent observation that the Drosophila *no-receptor potential A* gene encodes a phospholipase C that is abundantly expressed in the adult retina (Bloomquist et al., 1988); severe mutations of this gene abolish the photoresponse. Interestingly, the other product of PIP_2 breakdown, DAG, normally activates PKC. In Limulus, injection of phorbol esters does not mimic the ef-

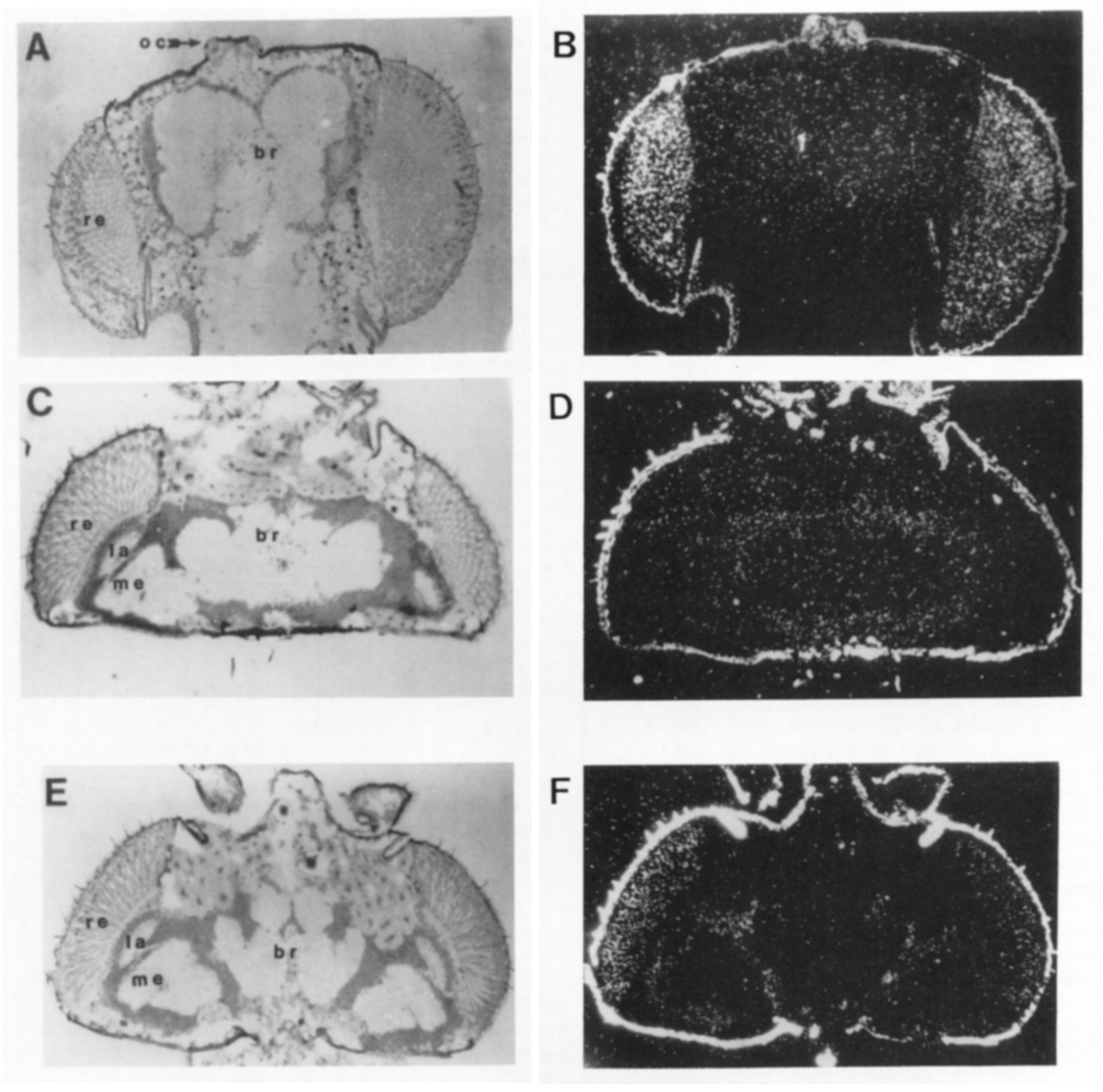

Figure 5. Spatial Localization of PKC RNAs by In Situ Hybridization to Tissue Sections of Adult Fly Heads

Frozen sections of adult fly heads were hybridized to (A, B) dPKC53E(ey), (C, D) dPKC98F, and (E, F) dPKC53E(br) DNA probes nick translated with ^{3}H-labeled dNTPs. A, C, and E, bright-fields; B, D, and F, dark-fields. re, retina; la, lamina ganglionaris; me, medulla; oc, ocelli; br, brain. In the dark-field images (B, D, and F), some light scattering by the cuticle is seen. dPKC53E(br) appears to be transcribed in most neurons in the head, including photoreceptor cells. It is not known whether some of this expression may be due to cross-hybridization to the dPKC53E(ey) transcripts.

fect of light or inositol trisphosphate in depolarizing photoreceptor cells (R. Payne, personal communication). Thus, activation of the kinase is probably not involved in visual excitation. This raises the possibility that PKC may be involved in a subsequent process such as adaptation. One logical way for the kinase to function in visual adaptation would be to phosphorylate rhodopsin or other members of the phototransduction cascade and regulate their activity. In this regard, it is worth noting that PKC is known to modulate ion channels and desensitize a number of receptors (Farley and Auerbach, 1986; Madison et al., 1986; Shoyab et al., 1979; Kelleher et al., 1984). Recently, Kelleher and Johnson (1986) found that PKC purified from bovine rod outer segments can phosphorylate rhodopsin and reduce its ability to activate transducin. It remains to be determined whether Drosophila rhodopsin is a substrate for dPKC53E(ey). Nevertheless, PKC as a mediator of adaptation makes functional sense, because of the coordinate regulation that could be achieved by having different elements of the same signaling cascade involved in both excitation and adaptation.

Currently, we have three PKC genes but no mutants. In the long term it may be possible to generate mutants in PKC structural genes and thus study the effect of each isozyme in development and behavior. Such mutants can also be used as hosts for the introduction of site specifically mutagenized PKC genes by P element–mediated transformation. However, the organization of PKC en-

zymes into separable regulatory and catalytic domains (Kemp and House, 1987; Lee and Bell, 1986; Muramatsu et al., 1989) may provide a quicker means to at least some understanding of the roles played by these kinases. It is experimentally straightforward to transform Drosophila with DNA sequences coding for the catalytic and inhibitory regulatory domains of individual enzymes, under the control of inducible promoters. One could then induce transient increases and decreases in specific PKC isozyme activity, examining transformants for alterations in development, physiology, morphology, and behavior.

Experimental Procedures

Molecular Cloning and Sequencing

Bovine PKCα and β cDNA probes were used to screen 300,000 pfu from a λ charon 4 library generated from DNA of the Canton-S strain (Maniatis et al., 1978). This screen resulted in the isolation of ten strongly hybridizing genomic clones (see Results). In addition to the ten genomic clones mapping to 53E and 98F, another more weakly hybridizing clone was isolated which mapped to the second chromosome at position 21D/22A (hybridization was to two separate closely spaced bands). We have shown by Southern blotting that this clone is identical to the Drosophila cGMP-dependent protein kinase recently reported by Foster et al. (1988), (clone was a gift from Drs. J. Foster and R. Jackson). Restriction fragments from 53E and 98F genomic clones were used to screen 360,000 pfu from two cDNA libraries, resulting in the isolation of seven cDNA clones mapping to 53E and three mapping to 98F. cDNA clones for dPKC98F were isolated from a λgt11 adult head cDNA library (Itoh et al., 1985), and clones for dPKC53E(ey) were isolated from the same λgt11 library as well as a λgt10 adult head cDNA library (Zuker et al., 1985). Filters were prehybridized and hybridized in a buffer containing 50% formamide, 5× SSC, 5× Denhardt's, 50 mM sodium phosphate (pH 7.0), and 250 mg/ml denatured calf thymus DNA. Probes were radioactively labeled by hexanucleotide-primed synthesis to a specific activity of $3–5 \times 10^8$ cpm/mg. Filters were hybridized with Drosophila probes at 5×10^5 cpm/ml hybridization buffer for 18–24 hr at 42°C and washed three times at the same temperature for 15 min each with 0.1× SSC, and 0.1% SDS (high stringency). When bovine probes were used, hybridization was in 35% formamide at 42°C with three washes for 15 min each with 0.5× SSC and 0.1% SDS at 37°C (low stringency). All bacterial manipulations and cloning procedures were carried out by standard methods (Maniatis et al., 1982).

The dPKC98F cDNA (λc98–2) was subcloned into M13 vector mp18 and sequenced by the dideoxy chain termination method (Sanger et al., 1977) using a modified form of T7 DNA polymerase, Sequenase (United States Biochemical Corp.). The dPKC53E(ey) cDNA (λc53–1), was subcloned into pBluescript KS(+) (Stratagene Cloning Systems), and double-stranded sequencing was carried out following the Sequenase protocol. In all cases sequencing was carried out on both strands.

In Situ Hybridization to Polytene Chromosomes

Polytene chromosomes squashes (Canton-S strain) were prepared as previously described (Zuker et al., 1985). Hybridization with biotinylated DNA probes was carried out according to Langer-Sofer et al. (1982) with the following modifications: DNA was nick translated using Bio-16-dUTP (ENZO Biochem), and hybrids were detected using a Detek-1-HRP detection kit (ENZO Biochem).

Northern Blots

For developmental Northern blots of dPKC98F, total RNA was isolated from Canton-S adult flies and larvae following the protocol of Cathala et al. (1983). For Northern blots demonstrating the transcription pattern of dPKC53E(ey), RNA was extracted from the heads and bodies of Oregon-R adult flies as described by O'Hare et al. (1983). Heads were separated from bodies as described by Oliver and Phillips (1970). Poly(A)$^+$ RNA was isolated by affinity chromatography on oligo(dT) cellulose columns (Blumberg and Lodish, 1980). RNA was fractionated on MOPS–formaldehyde 1% agarose gels and transferred to Gene-Screen Plus membranes following the manufacturer's instructions (New England Nuclear/Dupont). Radioactive probes were prepared as described above. Hybridization and washing conditions were also as recommended by the membrane manufacturer.

In Situ Hybridization to Tissue Sections

Preparation of 8 μm frozen sections of adult heads and hybridization of ^{3}H-labeled probes were as described by Hafen et al. (1983) except that the acid and pronase treatments were omitted in the pretreatment of the slides.

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