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Molecular cloning of a *Drosophila* diacylglycerol kinase gene that is expressed in the nervous system and muscle

(signal transduction/phosphatidylinositol metabolism)

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ABSTRACT We have isolated a Drosophila melanogaster diacylglycerol kinase (DGK, EC 2.7.1.107) homologue by using a porcine DGK cDNA probe and we have characterized its structure and expression. The DGK cDNA has a single open reading frame that encodes 791 amino acids. The Drosophila and porcine DGKs share a similar carboxyl-terminal region, a putative catalytic domain, which is divided into two separate domains in Drosophila. The DGK gene was mapped to the cytogenetic position 43F1, and its DGK mRNA is abundant both in embryo and in adult fly. By in situ hybridization to sections of adult flies, we demonstrated that the mRNA is present predominantly in the nervous system and muscles, including compound eyes, brain cortex, fibrillar muscle, and tubular muscle. In a 10- to 11-hr embryo, the DGK gene is expressed abundantly in a limited number of cells in the procephalic region and in the ventral nerve cord. The pattern of temporal and spatial expression suggests that the DGK protein has an important function in the adult nervous system and muscle and during the development of the embryonic nervous system.

In a variety of cell types, phosphatidylinositol 4,5-bisphosphate is hydrolyzed by the action of phosphatidylinositol-specific phospholipase C (PI-PLC) in response to external stimuli, generating two second messengers (1). They are inositol 1,4,5-trisphosphate and 1,2-diacylglycerol (DG), which constitute a bifurcating signaling pathway (2).

Upon cell stimulation, diacylglycerol kinase (DGK, EC 2.7.1.107) initiates the resynthesis of phosphatidylinositols by converting the released DG to phosphatidic acid (3). Furthermore, this enzyme is thought to reduce the level of DG, resulting in the regulation of protein kinase C (PKC) activity. DGK activity is known to exist in a wide variety of tissues and organisms, from Escherichia coli to mammals. Recent biochemical studies revealed the existence of various DGK isoforms that differ from each other with respect to cellular localization, substrate specificity, and molecular weight (3-8). However, details of the structural and functional differences among the various types are not yet known. In mammalian cells, the 80-kDa species was initially purified from pig brain and later from thymus cytosol (4). Cloning of the cDNA of 80-kDa DGK recently revealed that the enzyme has E-F hand and cysteine-rich zinc-finger motifs (9, 10).

To elucidate the role of DGK both in the signaling pathway and during development of organisms, we cloned a *Drosophila melanogaster* DGK homologue, which has conserved domains and biochemical features essential to DGK protein function. Here we report the nucleotide and amino acid sequence of the DGK homologue and its spatial and temporal expression.* We previously reported that DGK activity is defective in a retinal degeneration mutant, rdgA (11). How-

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ever, the DGK homologue reported here was mapped to a cytogenetic position different from *rdgA* and is thought to be a second DGK species that is expressed predominantly in the embryonic and adult stages. The DGK gene is expressed predominantly in the nervous system and muscle in an adult fly. In a 10- to 11-hr embryo, the DGK mRNA was detected abundantly in three clusters on each segment in the ventral nerve cord and in sensory organs in the procephalic region. These results suggest that the DGK protein plays important roles both in the adult neuromuscular system and during development of the embryonic nervous system.

MATERIALS AND METHODS

Nucleotide Sequence Analysis. A series of deletion plasmids were prepared by using the exonuclease III/mung bean nuclease and sequenced by the dideoxy chain termination method (12) with modified T7 DNA polymerase (13), using double-stranded templates and specific oligonucleotide primers.

Northern Blot Analysis. $Poly(A)^+$ mRNA was electrophoresed in a formaldehyde/1% agarose gel and blotted to a nylon membrane (14).

In Situ Hybridization to Adult Tissue Sections. Probes for in situ hybridization were prepared from a recombinant plasmid constructed by subcloning the 2-kilobase (kb) Xba I fragment of no. 40 phage insert in the Bluescript II SK(-) vector (Stratagene). This does not carry such repetitive sequences as the opa repeat, and it contains the coding region homologous to the porcine DGK cDNA. Frozen sections (8 μ m) of white (w) adult flies were cut on a cryostat and pretreated according to the methods of Hafen *et al.* (15). After pretreatment and air-drying, mild alkali hydrolysis of the RNA probe, overnight RNA RNA hybridization at 50°C, posthybridization washes, and immunological detection of the hybridized probe were performed as described by Coen *et al.* (16).

Whole-Mount in Situ Hybridization to Embryos. Digoxigenin-labeled single-stranded probes were prepared, using the polymerase chain reaction, from the same recombinant plasmid as used for *in situ* hybridization to adult tissue sections. Whole-mount *in situ* hybridization was carried out as described by Tautz and Pfeifle (17).

Chromosome *in Situ* **Hybridization.** Chromosome squashes for hybridization were prepared as described by Engels *et al.* (18). The procedure for hybridization and post-hybridization was according to de Frutos *et al.* (19).

RESULTS

Cloning of a Drosophila DGK Gene and Its Genomic Organization. We screened a Drosophila genomic library with the

Abbreviations: DGK, 1,2-diacylglycerol kinase; PI-PLC, phosphatidylinositol-specific phospholipase C; DG, 1,2-diacylglycerol; PKC, protein kinase C.

^{*}The sequence reported in this paper has been deposited in the GenBank data base (accession no. D11120).



porcine DGK cDNA (λ DGK1) (9) under low-stringency conditions and isolated several positive clones. Since the pattern of hybridization of *Drosophila* DNA with phage designated no. 40 (Fig. 1) was identical to that of the Southern blot using the porcine DGK cDNA (data not shown), we

-438										AGAT	TTCT	таат	TTAT	TCGA	AATT	АТАА	ACTA	AATA	CAAA	AGG
- 395	TATATTTATTGTGGTAGTTTGAACTAATTTGACACTTCCAATATATACAAATTTAATAGTATATTCTTAGAAATTTCT																			
-316	ATT	ATTTGGTGTGATTAAATATAAATACATAAGTAGTTTATAATTGGAATTCGAGTTACATCAGCTGGCAAATTCGGTTCAT																		
-237	TCAC	CACAATCAATCCGTAACAAGACCAAATCAGATCGGTTCTGGCCGAGATTGCCGGCATTGTTTCGAAACAAAATTCCAA																		
-158	TTAT	TATCCGGAATAACAAGGCAGCCTTTGGCCCAGCACTCCCAATGTAGTTTCGTTGGAGCTGTGAACTACACAAAATAC																		
-79	AACA	AACACCAACATTTCCCACCAGCGAAATCCCCCCCAATTCCCGGCACGGCAGGGACTCGGATGCGTCCCGGACAC																		
1	ATG	AGA	TGG	GAA	CTT	CGT	TAT	GGC	TGG	CAA	ATT	GTC	ACG	TTC	CGA	ACA	AAT	TGC	GCT	ACT
1	Met	Arg	Trp	Glu	Leu	Arg	Tyr	Gly	Trp	Gln	Ile	Val	Thr	Phe	Arg	Thr	Asn	Cys	Ala	Thr
~	0.00			000		-	010		0000	mma	mam	005	ama	mom	mmo	101	» om	003	omo	010
21	GCT Ala	ala.	BLC.	Wal.	GCA 81a	TGC	Lic	CAA	Lou	Loui	TGT	Dro	Un1	For	Low	mbr	AGT	ALA.	Up 1	GAG
21	AId	ALA	ALA	vai	AId	Сув	HIS	GTII	Deu	Deu	Cys	PIO	vai	Ser	Бец	1111	Ser	Ald	vai	Giù
121	ATA	ATG	CCG	AAT	TCC	TTT	CCG	CCA	GAG	GGT	CGT	CGG	CGG	GAG	CGA	AGG	TCG	GGA	AAC	TTG
41	Ile	Met	Pro	Asn	Ser	Phe	Pro	Pro	Glu	Gly	Arg	Arg	Arg	Glu	Arg	Arg	Ser	Gly	Asn	Leu
181	GTC	CTT	CGA	GCC	GGA	TGC	TGC	TGC	ATC	GGC	AAA	TAC	TTT	CTC	CAC	AAC	CGC	TGC	GCC	TCG
61	Val	Leu	Arg	Ala	Gly	Cys	Cys	Cys	Ile	Gly	Lys	Tyr	Phe	Leu	His	Asn	Arg	Cys	Ala	Ser
241	TCG	GTG	AAG	AAG	GAG	TGC	ACC	TTG	GGC	GAA	TAC	TCC	GAG	CTA	ATT	GTC	CCG	ccc	ACG	GCC
81	Ser	Val	Lys	Lys	Glu	Cys	Thr	Leu	Gly	Glu	Tyr	Ser	Glu	Leu	Ile	Val	Pro	Pro	Thr	Ala
201	200	maa	000	000	0777	oma	020	000	010	003	maa	000	2 2 00	010	000	010	no l	1000	200	CAC
101	TIO	TGC	Dro	GCG Ala	Ual	Loui	Ban	CGC Nrc	CIN	X.ra	For	GIG	AAT	CAG	Al-	Lic	THE	ala.	Mbr	CAC .
101	776	CAP	FLU	MTG	var	Leu	Moh	my	GTH	ALY	Der	VAL	MBII	Gin	ALA	HTP	пур	ALG	****	nis
361	TTC	CAA	ATC	ACG	CCG	CCG	GAC	GAG	CTG	AGC	TGC	CCG	CTG	CTG	GTG	TTC	GTC	AAT	CCG	AAG
121	Phe	Gln	Ile	Thr	Pro	Pro	Asp	Glu	Leu	Ser	Cys	Pro	Leu	Leu	Val	Phe	Val	Asn	Pro	Lys
421	AGC	GGC	GGT	CGC	CAG	GGG	GAT	CGC	ATC	CTG	CGG	AAG	TTC	CAG	TAC	ATG	CTC	AAT	CCG	CGC
141	Ser	Gly	Gly	Arg	Gln	Gly	Asp	Arg	Ile	Leu	Arg	Lys	Phe	Gln	Tyr	Met	Leu	Asn	Pro	Arg
481	CAG	GTG	TAC	GAC	CTG	TCC	AAG	GGC	GGG	CCC	AAG	GAG	GGA	CTC	ACA	CTC	TTC	AAG	GAC	CTG
161	Gln	Val	Tyr	Asp	Leu	Ser	Lys	Gly	Gly	Pro	Lys	Glu	Gly	Leu	Thr	Leu	Phe	Lys	Asp	Leu
541	CCT	CGC	TTC	AGG	GTC	ATT	TGC	TGT	GGC	GGC	GAC	GGC	ACC	GTC	GGC	TGG	GTT	TTG	GAA	GCC
181	Pro	Arg	Phe	Arg	Val	Ile	Cvs	Cvs	Glv	Glv	Asp	Glv	Thr	Val	Glv	Tro	Val	Leu	Glu	Ala
		V																		
601	ATG	GAC	TCC	ATA	GAG	TTG	GCC	AGC	CAG	CCG	GCC	ATC	GGA	GTG	ATT	CCC	CTG	GGA	ACG	GGC
201	Met	Asp	Ser	Ile	Glu	Leu	Ala	Ser	Gln	Pro	Ala	Ile	Gly	Val	Ile	Pro	Leu	Gly	Thr	Gly
661	AAC	GAC	CTG	GCT	CGC	TGT	CTC	CGC	TGG	GGC	GGT	GGA	TAC	GAG	GGC	GAA	AAC	ATC	CCC	AAA
221	Asn	Asp	Leu	Ala	Arg	Cys	Leu	Arg	Trp	Gly	Gly	Gly	Tyr	Glu	Gly	Glu	Asn	Ile	Pro	Lys
721	CTG	ATG	GAC	AAG	TTT	CGA	AGA	GCC	TCC	ACC	GTC	ATG	CTG	GAC	CGC	TGG	AGC	ATC	GAG	GTG
241	Leu	Met	Aan	Lva	Phe	Arg	Arg	Ala	Ser	Thr	Val	Met	Leu	Asp	Arg	Tro	Ser	Ile	Glu	Val
7.01	100		1.00	000	020	200	030	020	2000	002	000	220	Vama	200	omo	020	mcc	220	2000	CNC
261	MCC	AAT	MCT	Dro	LAC	AGC	Dan	Age	Mot	Arra	Bro	Tare	Ual	mbr	Lou	Lie	Cor	Ann	Mot	Gln
201	1111	MBII	1111	FIO	n10	Der	wab	Nop	Mec	MIG	FIO	рув	var	1111	Deu	110	Der	ABII	Met	GIII
841	AAG	GTG	ATT	GAG	CTG	TCG	CAA	AGT	GTT	GTG	GTC	GAC	AAA	TCG	CTG	ATG	GAA	CGC	TTC	GAG
281	Lys	Val	Ile	Glu	Leu	Ser	Gln	Ser	Val	Val	Val	Asp	Lys	Ser	Leu	Met	Glu	Arg	Phe	Glu
901	GAA	ATC	CAA	CGG	CAG	AGC	AAG	CAG	GTG	GCC	ACG	TCC	ATG	GGC	ACG	GCG	GCC	TCA	AGC	ACA
301	Glu	Ile	Gln	Arg	Gln	Ser	Lys	Gln	Val	Ala	Thr	Ser	Met	Gly	Thr	Ala	Ala	Ser	Ser	Thr
961	TCG	ATT	ATG	ATG	GCC	AGC	AAG	ACG	GAA	ACG	GAA	ATG	GAA	ACG	ATG	GCC	ACG	ATG	GAG	TTC
321	Ser	Ile	Met	Met	Ala	Ser	Lys	Thr	Glu	Thr	Glu	Met	Glu	Thr	Met	Ala	Thr	Met	Glu	Phe
1021	000	NOC	200	200	200	200	200	220	101	200	200	200	200	220	NCC	გოო	mcc	200	mc.c	ACG
341	Gly	Ser	Ser	The	The	The	The	Agn	Ana	The	The	Thr	The	LAVE	Ser	TIe	Ser	Met	Ser	Thr
241	GTÀ	Set	Det				-	nell	nid					. Lys	Set	**e	961	rie L	061	****
1081	TTC	GAG	ACG	CAG	TGC	CTG	CAA	CAG	ACC	CTG	CGG	ACA	GCG	ACG	AGC	AGC	AGC	AGC	AGC	AAC
361	Phe	Glu	Thr	Gln	Cys	Leu	Gln	Gln	Thr	Leu	Arg	Thr	Ala	Thr	Ser	Ser	Ser	Ser	Ser	Asn
1141	ACG	AGC	AGC	GGC	AGT	CCG	TGC	AAC	GGC	AAC	CAG	GAT	GCG	GAA	ACG	GAA	GTA	GAT	TCC	CAT
381	Thr	Ser	Ser	Gly	Ser	Pro	Cys	Asn	Gly	Asn	Gln	Asp	Ala	Glu	Thr	Glu	Val	Asp	Ser	His

FIG. 1. Structure of the Drosophila DGK gene: Restriction map of the Drosophila DGK genomic region, genomic phages no. 24 and no. 40, and the composite cDNA. Restriction endonuclease sites: B, BamHI; E, EcoRI; and X, Xba I. The nine introns shown interspersed in the composite cDNA sequence (lines) were located by comparison with the corresponding genomic sequence (data not shown). The three transcripts below the composite cDNA correspond to the cDNAs no. 1A, no. 2A, and no. 26.

concluded that clone no. 40 represents the genomic sequence highly homologous to the porcine DGK gene. Furthermore, we screened the *Drosophila* adult whole cDNA library with the no. 40 genomic fragment under high-stringency conditions, resulting in the isolation of six cDNA clones. We

1201	GCC	GCT	GCC	GCT	GCC	GAT	GIC	CGG	GAG	AAG	TCC	GTG	CCC	AGG	AGG	TCG	GGT	GAA	ACG	GAA
401	Ala	Ala	Ala	Ala	Ala	Asp	Val	Arg	Glu	Lys	Ser	Val	Pro	Arg	Arg	Ser	Gly	Glu	Thr	Glu
1261	AAG	CAA	TCA	CTT	GAG	ACG	CTG	CTG	CTG	CAG	CAC	AAA	CAG	CAG	ATG	CAA	CAA	CAG	CAG	CAG
421	Lys	Gln	Ser	Leu	Glu	Thr	Leu	Leu	Leu	Gln	His	Lys	Gln	Gln	Met	Gln	Gln	Gln	Gln	Gln
								mor	0.00	0.05	0.00			0.05	0.07	101	0.01	100	001	0000
1321	CAA	CAG	CAG	CAA	GGA	GTC	ACA	TCA	CTA	GCT	GTC	GAG	GAA	GCT	GCA	ACA	GCA	ACG	CCA	GTC
441	GIn	GIn	GIn	Gin	GIY	Val	Thr	Ser	Lieu	Ala	vai	GIU	GIU	ALA	Ala	Thr	ALA	Thr	Pro	val
1381	GGG	AGT	AAT	CAA	AGC	GAT	AAT	AGT	AGC	CAG	CGC	AAT	AAG	CAA	AAC	AAC	ATC	CTT	AAA	CAG
461	Gly	Ser	Asn	Gln	Ser	Asp	Asn	Ser	Ser	Gln	Arg	Asn	Lys	Gln	Asn	Asn	Ile	Leu	Lys	Gln
1441	CAA	ATT	ACA	TTG	TCA	TTG	GAC	TTG	TCC	GAC	CAT	GAG	GAC	GAG	CCC	AAG	GAC	GAT	GGC	GGT
481	Gln	Ile	Thr	Leu	Ser	Leu	Asp	Leu	Ser	Asp	His	Glu	Asp	Glu	Pro	Lys	Asp	Asp	Gly	Gly
1501	GGC	GCT	GGC	GAT	GGT	ACC	AAG	AGC	AAC	GGC	AAC	AGC	ATT	CCG	GCA	ACA	CCA	GCC	ACA	CCA
501	Gly	Ala	Gly	Asp	Gly	Thr	Lys	Ser	Asn	Gly	Asn	Ser	Ile	Pro	Ala	Thr	Pro	Ala	Thr	Pro
1561	ATC	ACA	CCC	ACC	ACT	CCG	AAT	GCC	GCA	TCA	AGT	GTG	CTG	CAG	CAA	CAA	CAG	CAA	CAG	CAT
521	Ile	Thr	Pro	Thr	Thr	Pro	Asn	Ala	Ala	Ser	Ser	Val	Leu	Gln	Gln	Gln	Gln	Gln	Gln	His
							~ ~					-						-	100	0700
1621	CTC	CAG	TTC	GAG	CAG	CAA	CAG	AAG	CCC	ATC	AAA	GTG	CAA	TCC	GAC	AAG	GAC	TGC	ACG	GIG
541	Leu	GIN	Phe	GIU	GIN	GIN	GIN	гуз	PIO	TTe	гуя	Val	GIU	V	Asp	гуа	Asp	CAR	1111	Yai
1681	CCC	TAC	AAC	ATA	ATC	AAC	AAT	TAT	TTC	TCC	GTC	GGC	GTG	GAC	GCC	GCC	ATC	TGC	GTC	AAG
561	Pro	Tyr	Asn	Ile	Ile	Asn	Asn	Tyr	Phe	Ser	Val	Gly	Val	Asp	Ala	Ala	Ile	Cys	Val	Lys
1741	TTC	CAC	CTG	GAG	CGT	GAG	AAG	AAC	CCG	CAC	AAA	TTC	AAC	AGC	CGC	ATG	AAG	AAC	AAG	CTG
581	Phe	His	Leu	Glu	Arg	Glu	Lys	Asn	Pro	His	Lys	Phe	Asn	Ser	Arg	Met	Lys	Asn	Lys	Leu
1801	TGG	TAC	TTC	GAG	TAT	GCA	ACA	TCC	GAG	ACA	TTT	GCG	GCA	TCC	TGC	AAG	AAT	CTC	CAC	GAG
601	Trp	Tyr	Phe	Glu	Tyr	Ala	Thr	Ser	Glu	Thr	Phe	Ala	Ala	Ser	Cys	Lys	Asn	Leu	His	Glu
1861	AGC	ATC	GAG	ATT	GTG	TGC	GAT	GGC	GTG	GCC	CTG	GAC	TTG	GCC	AAC	GGA	CCC	CAC	TTG	CAG
621	Ser	Ile	Glu	Ile	Val	Cys	Asp	Gly	Val	Ala	Leu	Asp	Leu	Ala	Asn	Gly	Pro	His	Leu	Gln
1921	GGA	GTG	GCT	CTG	CTTC	AAC	ATC	CCG	TAC	ACA	CAC	GGC	GGT	TCC	AAC	CTG	TGG	GGC	GAG	CAC
641	Glv	Val	Ala	Leu	Leu	Asn	Ile	Pro	Tvr	Thr	His	Glv	Glv	Ser	Asn	Leu	Trp	Gly	Glu	His
1001	CTC	mom	ChC	222	000	200	000	220	ACT	000	000	0000	TTC	COC	AAG.	AGT	AAG	222	CTTA	cac
661	Leu	Ser	Gln	LVS	Arg	TIE	Arg	LVS	Ser	Ala	Glv	Pro	Phe	Glv	LVS	Ser	LVS	LVS	Leu	Arg
2041	000	000	CAC	220	CAG	mmc	TCC	CCC	ACC	ACC	TTC	007	TOC	CTC.	GAC	CTC	TOG	GTG	GCC	ATT
681	Ala	GIV	Aan	LVA	GIU	Phe	Ser	Ala	Thr	Ser	Phe	Aan	Ser	Val	Asp	Leu	Ser	Val	Ala	Ile
001		V	mmm	003	010	000	000	2000	030	002	2.000	000	000	020	ANC	mac	OTO	CAC	ATC	000
2101	CAG	GAC	Dho	GGA	GAT	200	Ton	TIC	GAG	GIA	TIO	Clar	Lou	GAG	AAC	Cure	Len	Hig	Met	Glv
701	GTH	MSD	Pile	GTÀ	ASP	MTY	Lieu	176	oru	Var	770	GTA	Deu	Gru	Rom	~ 30	2004			200
2161	CAG	GTG	AGG	ACT	GGA	CTT	CGC	GCG	TCG	GGA	CGC	CGC	CTG	GCC	CAG	TGC	AGC	GAA	Wal	ATC
721	GIN	var	Arg	Thr	GTA	Leu	Arg	ALA	Ser	GTÀ	Arg	Arg	THAM	Ald	GTH	Cys	DEL	GTU	AGT	116
2221	ATC	AAG	ACG	AAG	AAA	ACA	TTT	CCC	ATG	CAG	ATA	GAT	GGC	GAG	CCC	TGG	ATG	CAG	ATG	CCC
741	Ile	Lys	Thr	Lys	Lys	Thr	Phe	Pro	Met	Gln	Ile	Asp	GLY	Glu	Pro	Trp	Met	GIN	Met	Pro
2281	TGC	ACG	ATC	AAG	GTG	ACC	CAC	AAG	AAC	CAG	GTG	CCC	ATG	CTG	ATG	GCA	CCG	CGG	TCG	GAG
761	Cys	Thr	Ile	Lys	Val	Thr	His	Lys	Asn	Gln	Val	Pro	Met	Leu	Met	Ala	Pro	Arg	Ser	Glu
2341	AAG	GGA	CGC	GGA	TTT	TTC	AAC	CTG	CTG	TGC	AGC	TGA	TTC	AGGC	GCAC	GCA'	rcga	GCGA	CTTC	ATGG
781	Lys	Gly	Arg	Gly	Phe	Phe	Asn	Leu	Leu	Cys	Ser	sto	p							
2408	GCC	GGAG	IGCC	CAGG	AGTO	GCC	GATG	AGCT	GGAT	GATG	ACTT	TCGG	ATCG	TCCC	TAGA	AGCA	CCAC	CGTC	CACA	ACAT
2487	AGC	CTAG	CGTC	AATA	GCTT	TAGC	CCAG	ICCA	CCCG	CGAT	CGCA	TCGA	ATTG	GTTA	ACCC	TAGG	TTAA	GGCT	TCCC	TAT
2566	GAT	ATGA	TATG	ATAT	GATA'	IGAT.	ATGA'	TATG	TATA	GTGT.	AGAT	CTGC	TATA	TGTG	TATG	TATT	CTTG	TATG	CATG	TATG
2645	TCG	AGTG	AGTT	ACAG	AGCT	PTAT.	ATTT	AGTG	TTAG	ACTA	GCCA	AATA	TACA	ACCG	AGTC	AAGT	CCAA	AAAA	AAAA	AAAA

FIG. 2. Nucleotide and deduced amino acid sequences of the composite cDNA derived from the cDNAs no. 1A, no. 2A, and no. 26. A single open reading frame of 2373 nucleotides encodes a protein of 791 amino acids. An <u>AATATA</u> potential polyadenylylation signal is located at nucleotide position 2688–2693. The in-frame termination codon that precedes the translation initiation site is boxed in position -144. The two regions homologous to the porcine DGK are light shaded. The stretches of single amino acid residues are indicated by underlines (threonine and serine residues) and double underlines (glutamine residues). Intron positions are marked by arrows. Comparison of nucleotide sequences between DGK cDNAs (Oregon R *Drosophila* strains) and genome (Canton S *Drosophila* strain) revealed 16 differences in the coding region. Eight of them are silent and do not result in amino acid changes (C \rightarrow T at position 660; T \rightarrow C at 1515; A \rightarrow G at 1608; C \rightarrow T at 1647; T \rightarrow C at 1959; C \rightarrow T at 1980; A \rightarrow G at 2214; G \rightarrow A at 2274). Six of them are nucleotide substitutions accompanied by amino acid changes (G \rightarrow T at 1004, Gln \rightarrow His; A \rightarrow G at 1163, Asn \rightarrow Ser; A \rightarrow T at 1298, Gln \rightarrow Leu; G \rightarrow T at 1504, Ala \rightarrow Ser; C \rightarrow T at 1556, Thr \rightarrow Ile; G \rightarrow A at 2021, Gly \rightarrow Asp). Two insertions are found in the genome, CTG (Leu) between 1287 and 1288; CAACAGCAGCAG (Gln-Gln-Gln-Gln) between 1317 and 1318. The latter insertion increases the number of residues in the first glutamine repeat from 12 to 16.

sequenced these cDNA clones and about 9 kb of the corresponding genomic region except for a part of the sixth intron. The two clones having the largest cDNA inserts, phage no. 1A and no. 2A, were mapped in relation to the genomic region (Fig. 1). This transcript is found to correspond to 10 exons. The sequences at the intron borders conform strictly to the consensus sequences of splice donor and acceptor sites (20) (data not shown). These two cDNA clones were adjacent to each other at the EcoRI site in the genomic region but did not overlap. To obtain an overlapping clone, cDNA clone no. 26 was amplified from $poly(A)^+$ RNA of an adult fly with polymerase chain reaction using specific primers based on the sequence of these two clones. The cDNA sequence derived from the three overlapping clones was shown to contain a single open reading frame that encodes 791 amino acids (Fig. 2). The sequence surrounding the putative start codon, ACACATG, satisfies the proposed Drosophila trans-

lation initiation sequence, ${}^{C}_{A}AA^{A}_{C}ATG$ (21).

Structure of the Drosophila DGK Gene. An alignment of the Drosophila homologue and the porcine DGK gene is shown in Fig. 3a. These genes share a similar carboxyl-terminal domain, which is thought to be the catalytic domain of DGK. However, the most striking feature of the Drosophila DGK protein is that the homologous sequences are divided into two clusters. The first homologous region is located closer to the amino-terminal region of the Drosophila sequence (residues 74-267; Fig. 3b), and the second is at the carboxyl-terminal region (residues 555-790; Fig. 3c), the latter being located at a comparable position in the *Drosophila* and porcine DGKs. Drosophila DGK protein has an ≈50% amino acid identity with the porcine homologue in each of the two homologous regions. The homology of these regions is even more striking if one takes into account the fact that many of the differences between the Drosophila and porcine proteins represent conservative amino acid changes. In this case, the similarities of the first and second domains would be about 76% and 72%, respectively. The characteristic amino-terminal domain of the porcine DGK, which contains E-F hand and cysteine-rich zinc finger motifs, does not exist in the Drosophila DGK. There are several stretches of repeated single amino acid residues in the central domain that do not align with the porcine DGK.

Mapping of the *Drosophila* DGK Gene. To determine the cytogenetic location of the DGK gene, a 9-kb digoxigeninlabeled *Xba* I genomic fragment of clone no. 40 was used as a probe for *in situ* hybridization to polytene chromosomes of larval salivary glands. This probe hybridized to a single site that was identified as position 43F1 on chromosome 2 (data not shown). This genetic locus is different from that of *rdgA* (*rdgA* maps at position 8A4-C6 on chromosome X).

Northern Blot Analysis. Using DGK cDNA clone no. 17B (nucleotides 671–2189), which contained no poly(A) tract, we measured the size and level of mRNA for DGK in various developmental stages (data not shown). Northern blot analysis revealed the existence of two equally abundant transcripts of ≈ 6 and 3.5 kb in adult head. Only the 6-kb mRNA could be detected in the embryo and only the 3.5-kb mRNA was found in the adult body. No expression could be observed during larval stages. This developmental profile shows that the expression of this DGK mRNA is regulated in a tissue- and stage-specific manner. The sequencing analysis of all seven cDNA clones did not detect nucleotide differences that might have resulted from an alternative splicing. A genomic fragment located downstream of the polyadenylylation site of the composite cDNA was used as a probe for Northern blot analysis; it hybridized only to the 6-kb transcript (data not shown), suggesting that the length of 3'untranslated region may be responsible for the difference in size between the two transcripts.

Tissue Localization of the DGK mRNA. The spatial localization of the DGK transcripts was investigated more precisely by *in situ* hybridization to adult tissue sections. Singlestranded antisense RNA probes were used, with sense strands as a negative control. The gene was expressed abundantly in the adult head (Fig. 4a), where the highest hybridization signal was in the retina and in the brain cortex. In contrast to the cortex, the central neuropil region showed only background hybridization. In adult horizontal sections, strong hybridizations were also observed in the indirect flight muscles and in the jump muscles (Fig. 4c).

Whole-mount in situ hybridization to stage 14 embryos (10:20-11:20) revealed that the DGK transcript exists predominantly in several groups of cells in the procephalic region and the central nervous system. In the procephalic region, strong signals were detected on both sides of the dorsolateral foregut wall (Fig. 5a). This expression continues to the hatching stage. At the stage 17, these cells were adjacent to the dorsal pharyngeal musculature (Fig. 5b). The location corresponds to the larval photoreceptor organ described by Bolwig (22) in *Musca*. Furthermore, a strong hybridization was also detected in three different regions of



FIG. 3. Homology between the *Drosophila* and porcine DGKs. (a) Schematic comparison between the *Drosophila* and porcine DGKs. The dark shaded boxes indicate homologous regions. The extent of amino acid identity and total conservation between the two regions of homology are indicated. Glutamine repeat (*Drosophila*), E-F hand (porcine), and cysteine-rich zinc-finger motif (porcine) are marked. a.a., amino acids. (b and c) Detailed alignment of the *Drosophila* and porcine DGK sequences. Vertical lines indicate identical amino acids and single dots represent conservatively changed amino acids. Conservative substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A, and G; N, D, E, and Q; H, R, and K; M, I, L, and V; and F, Y, and W.

each segment of the ventral nerve cord (Fig. 5c). These signals were observed only at stage 14 and disappeared later, unlike the expression in the procephalic region. By stage 14, anterior and posterior commissures become clearly separated from each other, resulting in the appearance of the ladder-like central nervous system. The strongest signals could be observed in three or four midline cells located ventral to each posterior commissure at this stage (Fig. 5d). These cells are distinct from midline precursors, because midline precursors are located between and within the transverse commissures. It is not clear whether these cells correspond to the ventral unpaired median (VUM) neurons known as the identified pioneer neurons (23). In addition, the DGK mRNA was also expressed abundantly in a bilateral pair of cells located on the most lateral border of the ventral cord, at the entry point of the axon tracts from sensory organs (Fig. 5e). Other signals were observed just lateral to the longitudinal connectives, at the cross-point of the anterior commissures, but they were weaker than the expression in the cells described above (Fig. 5d).

DISCUSSION

The Drosophila DGK homologue and the porcine DGK gene product share homologous amino acid sequences at the



FIG. 4. In situ hybridization of the DGK gene to tissue sections. Cryostat sections of adult *Drosophila* were hybridized with singlestranded digoxigenin-labeled antisense RNA. (a and b) Photographs of adult head section with antisense-stranded probe (a), and with the corresponding sense strand (b). The identified regions of the head section are brain (b), lamina (la), lobula (lo), lobula plate (lop), medulla (m), and retina (r). (c and d) Horizontal section of adult flies hybridized with antisense (c) and sense-stranded probe (d). The identified regions of the thorax section are jump muscle (tergal depressor of the trochanter muscle) (t) and indirect flight muscle (i). (Scale bars, 200 μ m.)

carboxyl-terminal domain. This is consistent with the idea that the region is necessary for the DGK catalytic activity. Furthermore, this region is divided into two in Drosophila. This suggests that each part may be responsible for different functions necessary for the catalytic activities. In the homologous regions, significant similarity was detected at several blocks: residues 132-162, 188-199, 214-237, 560-577, 585-609, 639-659, and 743-774. These sequences may represent functionally important domains. The porcine and human DGKs contain two putative ATP-binding sites, one of which is within the first cysteine-rich zinc-finger motif. The Drosophila DGK has only one putative ATP-binding site in the carboxyl-terminal region, and lysine (Lys) in the sequence Gly-Xaa-Gly-Xaa-Xaa-Gly- - - - Lys is replaced by serine (Ser) at residue 249. At present it is not established whether this ATP-binding motif constitutes a true ATP-binding site. In fact, the activity of porcine DGK is not affected by the amino acid substitution of Lys for arginine (Arg) when assayed after transfection of COS cells with cDNA expression vectors (H.



FIG. 5. Whole-mount in situ hybridization to embryos. Cells expressing the DGK mRNA were localized in whole-mount embryos by using a digoxigenin-labeled probe. Antisense probe was used in all photographs. (a and b) Dorsal views of whole-mount in situ hybridization of a stage 14 embryo (a) and a stage 17 embryo (b). Anterior is up. Arrowheads indicate hybridization signals in the procephalic region. (c) Ventrolateral view of a stage 14 embryo. Anterior is left. The strong expression could be observed in three different classes of cells of each segment in the ventral nerve cord. (d and e) Higher magnification of the central nervous system of a stage 14 embryo at different ventrodorsal levels. (d) Arrow marks the midline cells that abundantly express the DGK mRNA. Arrowhead indicates weaker signals located on either lateral side of anterior commissures, closely associated with the longitudinal connectives. (e) Arrow indicates a bilateral pair of cells located on the most lateral border of the ventral cord. (Scale bars, 50 μ m.)

Kanoh, personal communication). These facts suggest that the ATP-binding site of DGK protein may have properties different from those of binding sites found in other protein kinases and ATPases.

In situ hybridization of the DGK probe to adult head cryosections shows that the DGK gene is abundantly expressed in the retina. In stage 14-17 embryos, the DGK mRNA is abundantly expressed in the larval photoreceptor organ. The studies on visual mutant no receptor potential A (norpA) have revealed that phosphatidylinositol metabolism plays a central role in phototransduction in the Drosophila eye (24–27). The expression of the DGK gene in early embryonic and adult photoreceptors raises the possibility that this DGK protein may phosphorylate the DG that is released by the action of photoreceptor-specific PI-PLC (the product of the *norpA* gene) in response to light. It is necessary as a next step to analyze the possible visual function of this DGK homologue by its ectopic photoreceptor-specific expression using P-element-mediated germ-line transformation. The DGK activity is drastically reduced in the head in the retinal degeneration mutant, rdgA. This seems to be contradictory to the expression of this DGK homologue in the adult head. But the biochemical studies on mammalian DGK revealed that estimation of the DGK activity depends upon the assay conditions. The defect of DGK activity in the head of rdgA was originally demonstrated by Inoue et al. (11). If the DGK homologue reported here corresponds to an isoform whose activity can be detected only by the more refined assay method, the above contradiction disappears. It is therefore possible that rdgA encodes another DGK isozyme expressed in the retina. In fact, we have isolated another Drosophila DGK homologue, which is expressed in the adult eye and is mapped to the cytogenetic position 8BC (rdgA locus), using the most conserved region of this DGK gene as a probe (unpublished results). Thus, we consider that there are multiple isoforms of DGK, and that the DGK homologue we reported here is the DGK isozyme other than rdgA.

Strong expression was observed in three groups of cells of each segment of the ventral nerve cord in stage 14 embryos. These data show the expression pattern of phosphatidylinositol-metabolizing enzyme during the embryonic development of the central nervous system. This suggests that this DGK homologue may function during nervous system development. To clarify the roles of the DGK in the embryonic stage, it is important to know whether the cells in the three clusters where the DGK mRNA is abundantly expressed are the identified neurons or glia that have been previously characterized in detail. We tried to recognize the several identified neurons, using antibodies that detected fushi tarazu (ftz), even skipped (eve), and engrailed (en) gene products, but it is rather difficult to establish the identity of DGKexpressing cells, because of the treatment of the embryo with proteinase K in whole-mount in situ hybridization. Specific antibody against the DGK homologue will be necessary for such an experiment and is expected to be a useful tool to characterize the expression pattern of the embryonic nervous system more clearly.

To investigate further the biological function of the DGK gene, in particular the physiological role of the DG-PKC cascade, it is essential to isolate a mutant of the DGK gene and analyze its phenotype. Furthermore, it may be possible to transform *Drosophila* with a DNA sequence coding for the DGK protein under the control of tissue-specific promoters or enhancers for artificial expression of the DGK activity in ectopic cells. If ectopically tissue-specific expression of the DGK gene alters cellular DG metabolism, the examination of such a transformant for phenotype in physiology, morphology, and behavior will provide a powerful means to genetically dissect the role of the DG-PKC cascade.

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