Identification of a Novel *Drosophila* Protein Kinase Highly Homologous to Protein Kinase N (PKN)

Nozomi Ueno,*'† Isao Oishi,* Shin Sugiyama,‡ Yasuyoshi Nishida,‡ Yasuhiro Minami,*'¹ and Hirohei Yamamura*

*Department of Biochemistry, Kobe University School of Medicine, 7-5-1 Kusunoki-chou, Chuo-ku, Kobe 650, Japan; †1st Department of Surgery, Kobe University School of Medicine, 7-5-1 Kusunoki-chou, Chuo-ku, Kobe 650, Japan; and ‡Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

Received January 14, 1997

Academic Press

We identified a novel *Drosophila* gene, Dpkn (*Dro*sophila protein kinase related to PKN), encoding a putative protein serine/threonine kinase. Although the cDNA obtained was incomplete at its 5'-terminal region, the deduced amino acid sequence of its kinase domain exhibits a high degree of similarity to protein kinase N (PKN), which has a kinase domain related to protein kinase C (PKC) and leucine zipper-like sequences in the amino terminal region. Expression of Dpkn was observed throughout Drosophila development, although its expression level decreased at later stages of embryogenesis. The expression of Dpkn is first detected in the newly formed mesodermal cell layer and is then restricted to the developing somatic musculature, indicating a possible role of Dpkn in the development of somatic muscles in Drosophila. © 1997

The protein kinase C (PKC) family of serine-threonine protein kinases are activated by calcium, diacylglycerol, and phorbol esters, and play important roles in regulating a variety of cellular functions, including developmental processes (1, 2, 3, 4). Members of the PKC family have diverse expression profiles in vertebrates; some are widely expressed in different tissues, while others have more restricted expression patterns (3, 4, 5, 6). Thus far, three PKC genes in the fruit fly $Drosophila\ melanogaster$ have been identified (7, 8, 9). They include DPKC53E, a homologue of the mammalian PKC α (8), DPKC98E, a homologue of the mammalian PKC α (9), and eye-PKC, a mammalian PKC α homologue expressed exclusively in photoreceptor cells of the visual system (9).

A gene encoding a novel serine/threonine protein kinase, PKN, whose kinase domain is related to those of members of the PKC family, has recently been cloned from *Xenopus*, rat and human (10, 11). Interestingly,

```
1 GGTCGCGGCCACTTTGGCAACGTGATTCTGTCCCAATTGCGAAGCAACAACCAGTACTAC 60
  1 \ \underline{G} \ R \ \underline{G} \ H \ F \ \underline{G} \ K \ V \ I \ L \ S \ Q \ L \ R \ S \ N \ N \ Q \ Y \ Y
 61 GCTATTAAGGCACTGAAGAAGGGAGACATCATTGCCCGCGACGAAGTGGAGTCCCTGCTT 120
 21 A I K A L K K G D I I A R D E V E S L L
121 AGCGAAAAGCGTATCTTCGAGGTGGCCAACGCCATCCGCTCCTTAGTTAACTTG 180
 41 SEKRIFEVANAMRHPFLVNL
181 TATTCGTGCTTCCAGACTGAGCAACACGTATGCTTTGTGATGGAATACGCTGCTGCCGGA 240
 \texttt{61} \ \ \texttt{Y} \ \ \texttt{S} \ \ \texttt{C} \ \ \texttt{F} \ \ \texttt{Q} \ \ \texttt{T} \ \ \texttt{E} \ \ \texttt{Q} \ \ \texttt{H} \ \ \texttt{V} \ \ \texttt{C} \ \ \texttt{F} \ \ \texttt{V} \ \ \texttt{M} \ \ \texttt{E} \ \ \texttt{Y} \ \ \texttt{A} \ \ \texttt{G} \ \ \texttt{G} 
241 GATTTGATGATGCACATCCACACGGACGTGTTCCTAGAGCCGAGAGCCGTTTTCTACGCC 300
81 D L M M H I H T D V F L E P R A V F Y A 100
301 GCTTGTGTGGTTCTGGGCCTGCAGTACCTGCACGAGAACAAGATCATCTACCGCGACCTG 360
101 A C V V L G L Q Y L H E N K I I Y R D L 120
361 AACCTGCACAATTTCCTTTTGCACACGGAAGGATATGTGAAAATTGCGGACTTTCGTTTG 420
121 K L D N L L L D T E G Y V K I A D F G L 140
421 TGCAAGGACGCCATGGCCTTTGGTGATCGCACGGGCACTTTCTGTGGTACGCCCGAGTTT 480
141 CKEGMGFGDRTGTFCGTPEF160
481 CTGGCACCGGAAGTGCTCACGGAAACTTCGTACACACGAGCTGTGGATTGGTGGGGCTTG 540
161 LAPEVLTETSYTRAVDWWGL 180
541 GETETETTEATETTTEAGATETTGETTGETEAGTCCCCATTCCCTGGTGACGATGAGGAG 600
181 G V L I F E M L V G E S P F P G D D E E 200
601 GAAGTATTCGATTCAATTGTCAACGATGAGGTGCGCTATCCGCCGCTTCCTGTCGCTGGAG 660
201 E V F D S I V N D E V R Y P R F L S L E 220
661 CCCATAGCCGTGATGCGTAGGCGTTTTTGCGCAAGAATCCAGAGACGTCTCGGATCTTCG 720
221 A I A V M R R L L R K N P E R R L G S S 240
721 GAACGGGATGCGGAGGATGTTAAGAAACAGGCATTCTTCCGGTCAATTGTGTGGGGATGAC 780
241 E R D A E D V K K Q A F F R S I V W D D 260
781 CTGCTCCTGCGAAAGGTTAAACCACCATTTGTGCCGACAATTAACCACTTGGAGGATGTG 840
261 L L L R K V K P P F V P T I N H L E D V 280
841 TCAAACTTTGACGAGGAGTTCACGTCGGAGAAGGCTCAGCTTACGCCACCGAAGAGCCGC 900
281 SNFDEEFTSEKAQLTPPKSR 300
901 GACACTIGA 909
```

FIG. 1. Nucleotide sequence and deduced amino acid sequence of the kinase domain of Dpkn gene product. The putative ATP binding motifs [GlyXGlyXXGly-----(AX)K] are underlined.

¹ To whom correspondence should be addressed. Fax: ++81-78-371-8734. E-mail: minami@icluna.kobe-u.ac.jp.

Abbreviations used: PKC, protein kinase C; PKN, protein kinase N; Dpkn, *Drosophila* protein kinase related to PKN.

$\begin{array}{cccc} \text{Dpkn} & & 1 \\ \text{Dpkc} & & 1 \\ \text{FKC1} \text{ (S.pombe)} & & 1 \\ \text{CaFKC1} \text{ (C. albicans)} & & 1 \\ \text{human FKC } \beta \cdot 1 & & 1 \\ \text{human FKC } \theta & & 1 \\ \text{human FKN} & & 1 \\ \end{array}$	10	20 GRCHFCKVIL GRCSFCKVIL GRCSFCKVML GRCSFCKVML GRCSFCKVFL GRCSFCKVFL GRCHFCKVIL	30 SOLRSINOYY ABREGSEELLY AELKSEKOLY ABSTRITSKIC SERKGIDELY AEFKKINOFF SEFRPSEELF	AIKALKKSDI AIKILKKIJU AIKVLKKEFI AIKVLKKEFI AVKILKKIJU AIKALKKIJU AIKALKKSDI	IARDEVESILI IQUIDVECIM LENDEVESIK VENDEAESIK IQUIDVECIM IMUDIVECIM VARDEVESIM	55 55 55 55 55 55			10	20	30	40	57	
Dpkn 51 Dpkc 51 EKC1 (S.pombe) 51 CaPKC1 (C.albicans) 51 human FKC \(\textit{\textit{B}} \cdot \) 51 human FKC \(\textit{\textit{B}} \cdot \) 51	60 SEKRIFEVAN IPKRVLALGE SEKRVFLVAN SEKRVFLITAN VERRVLALPG VERRVLALPG VERRVLSDAW	70 AMRHPFLANL KPFTPFLANL REPHPFLANL KEMPFLINL KPPFLIOLHS EHPFLITMFC	80 YESTOTEOHV HESTOTETRI HESTOTETRI HESTOTETRI CFOIMERLYF TFOIKENIFF	90 CFVMEYAAGG FFVMEYVAGG YFVMEYTISGG YFVMEYTISGG VMEYVAGEDL VMEYLAGGDL	100 DEMMHIHIDV DLIFOTOFF DIMIHIOFEQ DEMHIQKIRE MYHIQXVERE MYHIQXVERE MYHIQSCHKE	100 100 100 100 100 100	Dokn Xenopus FKN rat FKN human FKN consensus	1 1 1 1		CRCHFCKVIL CRCHFCKVIL CRCHFCKVIL CRCHFCKVIL	SOLRSINOVY SEYKETGELF SEFFESCELF SEFFPSCELF SE GELF	IGENALANIA IGENALANIA IGENALANIA IGENALANIA IGENALANIA	TARDEVESILI TARDEVESILI VARDEVESILM VARDEVESILM ARDEVESIL	
Dpkn 101 Dpkc 101 PKC1 (S.pombe) 101 CaFKC1 (C.albicans) 101 human FKC β-1 101	110 FLEPRAVFYA KFKEPVAVFYA KFKRAQFYA FTAKRAKFYA KEPHAVFYAA	SACHPFLVNIL 120 ACVVLGLQYL AAEIAAGIFF AEVCIALKYF CEVILGIKYF EIAIGIFFIQ	FOCEQUEENV 130 HENKILLYRDL LHTFIKGILY HINGILYRDL HINGIVYRDL SKGITYRDLK	CFVMEYSAGG 140 KLONILLIDTE RDLKLONVLL KLONILLISPD KLONILLITIK LDNVMLOSEG	DIMINIHSEV 150 GYVKIADFGI, DADGHVKIAD GHVKVADYGI, GHIKIGDYGI, HIKIADFGMC	150 150 150 150 150	Dokn Xenopus PKN rat PKN human PKN consensus	51 51 51 51	SERRIFEVAN CERRVEVAVS CERRIFAAVT CERR V	AMRHEPIANIL DASHPPILSIL RAGHPPIANIL SAGHPPIANIL A HPFIL L	YSCFOIFORN LICEPOPADSV FOCFOIPPHV FOCFOIPPHV GCFOIF V	CFVMEYAAG CFVMEYSAAG CFVMEYSAAG CFVM Y AQG	DEMMHEREV DEMMENSEV DEMMENSEV DEMMENSEV D M HITHS V	10 10 10
human FKC θ 101 human FKN 101 Dokn 151 Doke 151 PKC (S. pombe) 151 CaPKCI (C. albicians) 151 human FKC β - 1 151	DISRATFYAA FSEPRALFYS 160 CKECMGFGIR FCMCKENIVG CKEIMMENII CKEIMMENII CKEIMMESTI KENIWDGVITI	ETHICLOFTH ACWLCLOFT 170 TGIFCGIPEF DKPIKIFCGI TATECGIPEF ISTFCGIPEF KIFCGIPEF	SKGTVYRDLK HEHKTVYRDL 180 LAPEVLIETS PDYLAPETIL MAPETILECQ MAPETVACKA APETIAYOPY	LDVILLIONG KLINILLIOTE 190 YIRAVIWGL YOPYFTCKSV YIRSVIWAF YIRSVIWAF CKSVDWAFG	HIKLADFOLC GYVKTADFOL 200 GYLIFFMIN'G DWAYGYLLY GYLIFYMILC GYLIFYMILC VILIPMILAGO	150 150 200 200 200 200 200 200	Dokn Xenopus PKN rat PKN human PKN consensus	101 101 101 101	FLEPRAVEYA FSOSRAMEYA FSOSRAMEYS FSOSRALEYS FS RA FY	ACWLGLOYL ACWLGLOFL ACWLGLOFL ACWLGLOFL ACW LGLQ FL	HENKLIYRDI HSRNIVYRDI HEHKIVYRDI HEHKIVYRDI H IVYRII	KUNLLIDE KUNLLIDE KUNLLIDE KUNLLIDE KUNLLIDE	GYVKIADFGI, GYVKIADFGI, GYVKIADFGI, GYVKIADFGI, GYVK AD GI,	15 15 15 15
Numan HC θ 151 Numan HN 151 Numan HN 151 Doka 201 Doka 201 PKC1 (S. pombe) 201 CaPKC1 (C. albicans) 201	KENNLEDAKT CKECMGYCER 210 ESPFREDISE EMINCOPPFD OSPFREDISE OSPFREDISE	NIFCGIPDYI TSIFCGIPEF 220 EVFDSIMIDE GEDEEFLFAA ELIFDAILSDE DIFNAIRNDE	APELLICORY LAPEVLIDIS 230 VRYPRFISLE ITIDHNVSYPK PLYPIHMPRD VKYPINLSRO	NHSVDWSFG VIRAVIWWCL 240 ALAVMRRIJR SLSKEAKEAC SVSILQQLIT TVIVIQALIT	VILYBMLIGO GWLLYFMLVG 250 KNPERRICSS KCFLIFFIKOP RDPKKRLGSC KDPSORLGSC	200 200 250 250 250 250 250	Dokn Xenopus PKN rat PKN human PKN consensus	151 151 151 151	CKREMESTR CKREMESTR CKREMEKER CKREMEKER CKREME DR	TGIFOGIFEF TSIFOGIFEF TSIFOGIFEF TSIFOGIFEF	LAPEVLITETS LAPEVLITEAS LAPEVLITEIS LAPEVLITES LAPEVLITES 230	YIRAVDWAL YIRAVDWAL YIRAVDWAL YIRAVDWAG YIRAVDWAG	GVLIFFMING GVLIVEMING GVFLYEMING GV YEM VG	20 20 20 20
human PKC β-1 201 human PKC θ 201 human PKN 201 Dokn 251 251 Doke 251	APFECEDEDE SPINICOLEE ESPEPCIDEE 260 ERDAEDVIKKO NKRLCCCSSC	LFQSIMEHOV LFHSIRMDNP EVEDSIVNDE 270 AFFRSIVALID EEDVRIHPFS	AYPKSMSKEA FYPRWLEKEA VRYPRFLSAE 280 LLLRKVKPPF BRIDWEKIEN	VAICKGLMIK KDLLVKLFVR AIGIMRRLLR 290 VPTINHLEDV REVOPPFKPK	HPCKRLGOGP EPEKRLGVRG RNPERRLGSS 300 SNFDEEFISE IKHRKMCPTL	250 250 250 250 300 300	Dokn Xencopus PKN rat PKN human PKN consensus	201 201 201 201 201	ESPFECIDER ESPFECIDER ESPFECIDER ESPFECIDER ESPFECIDER	EMPOSTANDE EMPOSTANDE EMPOSTANDE EMPOSTANDE EMPOSTANDE	VRYPRPLSLE VRYPRPLSAE VRYPRPLSAE VRYPRPL AE	ALAVMRRILR ALALMRRILR ALGEMRRILR ALGEMRRILR AL EMRRILR	KNPTRRLGSS RNPTRRLGAG RNPTRRLGST RNPTRRLGSS RNPTRRLG	25 25 25 25
FKC1 (S. pombe) 251 CAPKC1 (C. albicans) 251 Inmen FKC β-1 251 Inmen FKN 251 Inmen FKN 251 Dokn 301	ENDAEDMITH PKDAEEIMEH BGERDIKEHA DIROHPLFRE BRDAEDWKKQ 310 KAOLTPPKSR	PFFSNIMDD PYFHDVNFTD FFRYIDWEKL INWEELERKE PFFRILGMEA 320 DT	IYHKRIOPPY VLIKRIPAFY ERKEIQPPYK IDPPFRPKVK LLARRLPPPF	IPSLINSPITAT IPEWOSEHDY PKACCRUAEN SPITICSNITIK VPILISCRITIV	KYFDEFFIRE SNFDKEFISE FDRFFIRHPP EFINEKPRLS SNFDEFFICE	300 300 300 300 300	Dokn Xenopus PKN rat PKN humen PKN consensus	251 251 251 251 251	260 ERDAEDVIKO ERDAEDVIKO ERDAEDVIKO ERDAEDVIKO ERDAEDVIKO	270 APPRILMED PERILDADA PERILDADA PERILDADA PETEL A	280 ILLRKVKPPF ILSRRLPPPF LLARRLPPPF LLARRLPPPF LL R <i>RLP</i> PPF	290 VETTINHLEDV TPCVKEPHDI VPTLSCRIDV VPTLSCRIDV P G D	300 SAFDEEFISE SAFDEEFISE SAFDEEFISE SAFDEEFISE SAFD EFT E	30 30 30 30
Doke 301 PKC1 (S.pombe) 301 CaPKC1 (C.albicans) 301 human PKC $β$ -I 301 human PKC $θ$ 301	TSSSHOFTRK LEVLTPVNST TPRLTPVETV VLTPPDQEVT FADRALINSM	QT. LIF. LIF. RN. DQ.					Dokn Xenopus PKN rat PKN human PKN consensus	301 301 301 301	310 KAQLIPPKSR GPELIPPKEP APPLSPPRDA APPLSPPRDA P L PPR	320 DT RL RP R				

FIG. 2. (A) Alignment of Dpkn kinase domain with other kinase domains from members of the PKC family. Conserved residues among these kinases are shaded. (B) Alignment of Dpkn kinase domain with the previously reported *Xenopus*, rat, and human PKNs. Conserved residues among these kinases are shaded.

it has been shown that PKN is a target of Rho, a Raslike small guanosine triphosphatase (GTPase), implicated in cytoskeletal responses to extracellular signals (12, 13), and it is activated by the binding of the active GTP-bound form of Rho (12).

Here we report a novel Drosophila gene, Dpkn, possessing a putative kinase domain exhibiting a striking homology with that of PKN. We also show the unique expression pattern of the Dpkn gene during embryogenesis of the fly.

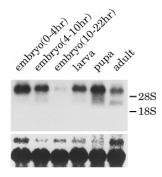
EXPERIMENTAL PROCEDURES

DNA amplification and sequencing. For PCR, degenerated primers were designed to hybridize to nucleotides coding two well conserved kinase subdomains VI B (HRDL) and IX (DVWSYG). The primer sequences were 5'-CCGCGAATTCATCCAC(A/C)G(A/C/G/T)GA(C/T)(C/T)T-3' and 5'-CCGCAAGCTTGCC(A/G)(A/T)A(A/G)(C/G)ACCA(C/G)AC(A/G)TC-3' (restriction sites for EcoRI and HindIII are underlined). 100ng of genomic DNA was used as a template in 100μ l PCR. The first 10 PCR cycles were 1.5min at 94° C, 2min at 55° C to 50° C (decreased 0.5° C per cycle), and 2.25min at 73° C. In

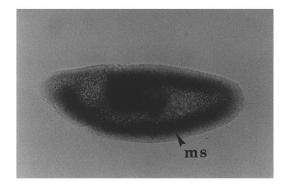
the subsequent 20 cycles, samples were denatured 1.5min at 94° C, incubated 2min at 50° C and heated 2.25min at 73° C. Amplified DNA fragments of the expected size (about 200bp) were digested with EcoRI and HindIII, purified on 2% agarose gel and cloned into the EcoRI/HindIII sites of the Bluescript vector (pBS, Stratagene).

Isolation of cDNA clones. An imaginal disc cDNA library (14) was screened using probes (0.2kb EcoRI-HindIII fragment from pBS-Dpkn) radiolabeled by random priming. Probes (3×10^6 cpm/ml) were hybridized to the plasmid DNA immobilized on nitrocellulose membrane filters (Schleicher & Schuell) for 12hr at 65°C in 1× hybridization buffer (1M NaCl, 50mM Tris-HCl [pH8.0], 10mM EDTA, 0.1% [v/v] SDS), 1× Denhardt's reagent, and 100 μ g/ml denatured salmon sperm DNA, and washed twice for 30 min at 65°C in 0.1× SSC, 0.1% [v/v] SDS. Clones were isolated and the longest 1.6kb clone (pNB40-Dpkn) was subjected for further analyses.

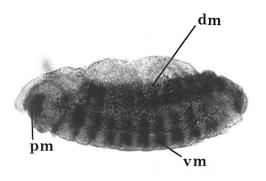
DNA sequencing and analysis. Sequencing was performed by the dideoxynucleotide chain termination method using the Thermo Sequenase core sequencing kit (Amersham) and a SQ5500 DNA sequencer (HITACHI). The final sequence was confirmed from both strands. Sequence analyses, comparison, and subsequent sequence alignment were performed using Genbank and EMBL databases through the BLASTN programs as well as the DNASIS program (Hitachi Software Engineering Co., Ltd.).



a.



D.



c.

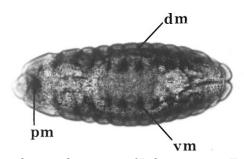


FIG. 3. (A) Developmental expression of Dpkn transcripts. Total RNA was prepared from *Drosophila* at various stages of development, separated by 1% agarose formaldehyde gels, transferred onto nylon membranes, and hybridized with radiolabeled probe for Dpkn as described in Experimental Procedures. The filters were stained with methylene blue to show total RNA [see panels indicating 18S and 28S ribosomal RNAs (control), stained with methylene blue]. Amount of RNA loaded in each lane was also normalized by rehybridization with labeled probe for rp49 (data not shown). (B) Localization-

Northern blot analysis. Total RNA from embryo, larva, pupa and adult flies were prepared by using ISOGEN (WAKO). For RNA blot analysis, $5\mu g$ of total RNA was electrophoresed on 1% agarose formaldehyde gels, and transferred onto nylon membranes. The probe DNA was prepared from pBS-Dpkn by digestion with EcoRI and HindIII, and labeled with $[\alpha^{-32}P]dCTP$ (Amersham, 3000Ci/mmol) using the Multiprime labeling kit (Amersham) and hybridized as described previously (15). Specific activity was $\sim 1\times 10^6$ cpm/ng for all the probe DNAs.

In situ hybridization of whole mount embryos. In situ hybridization to whole mount embryos using digoxigenin-labeled RNA probes was performed as described (16) with minor modifications (17). Single strand antisense or sense RNA probes were synthesized *in vitro* using T7 or T3 RNA polymerases, DIG RNA Labeling Mix (Boehringer Mannheim), and the pBS-Dpkn as a template following the manufacturer's recommended protocol.

RESULTS AND DISCUSSION

Cloning of Dpkn cDNAs. Using degenerate oligonucleotide primers to well-conserved stretches of amino acids in protein kinases, we PCR-amplified *Drosophila* genomic DNA. After subcloning of amplified products, several were found to encode kinase domains and among them one encoded kinase subdomains related to those of members of the PKC family. Using this amplified region as a probe, we screened a cDNA library from *Drosophila* imaginal discs (see Experimental Procedures) and the cDNA clone with the longest insert (~1.6 kb) was sequenced.

One of the frames of the Dpkn cDNA encoded an amino acid sequence of a typical protein kinase domain, including a putative ATP binding motif (Fig.1). The amino acid residues conserved within the serine/threonine protein kinase family are found in Dpkn, indicating that Dpkn is a member of this family (18, 19). Comparative sequence analysis revealed that the kinase domain of Dpkn (\sim 300 amino acids) is highly homologous to the corresponding domains of the protein kinase C family (Fig.2A). The highest homology (\sim 80%) was seen between the kinase domain of Dpkn and that of a recently identified novel protein kinase, designated PKN, from *Xenopus*, rat and human (Fig.2B, 10, 11).

It has been shown that PKN is a target of Rho, a Ras-like small GTPase and that the Rho-binding site is localized within the N-terminal portion of PKN, that has been assumed to be a regulatory domain of PKN (12). Thus, the entire sequence of Dpkn is required to elucidate whether Dpkn is indeed a *Drosophila* homologue of mammalian PKNs.

of Dpkn transcripts in embryos detected by whole mount *in situ* hybridization. (a) Lateral view of embryo at gastrulation (stage 9). Expression is restricted to mesodermal cell layers. (b) Lateral view of developing embryo (stage 14). Expression of Dpkn was retained in the developing somatic muscular system throughout embryogenesis. (c) Ventral view of developing embryo (stage 14-15). Anterior to left in all embryos. Dorsal to the top in a and b. ms, mesoderm; pm, pharyngeal musculature; dm, dorsal musculature; vm, ventral musculature.

Expression of Dpkn. To characterize the expression pattern of the Dpkn gene, we first performed Northern blot analysis with RNA samples from embryos (0-4hr, 4-10hr, 10-22hr), larvae, pupae, and adult flies. The 1.6 kb fragment of the Dpkn cDNA was used as a probe. Dnrk probe detected a major band of about 7 kb in size (Fig.3A). As shown in Fig.3A, Dpkn was expressed throughout *Drosophila* development, yet its expression level decreased at later stages of embryogenesis (Fig.3A).

To determine the tissue specificity of Dpkn transcripts during embryogenesis, we performed *in situ* hybridization on whole-mount embryos (see Experimental Procedures). Distinct expression of Dpkn was detected primarily in the mesodermal layer (Fig.3B, panel a.). Expression of Dpkn was then restricted to the somatic musculature (Fig.3, panels b. & c.). This expression appeared to be sustained in a subset of the muscular cell lineage throughout the remainder of embryogenesis (data not shown). In this regard, it is important to note that mammalian PKNs identified thus far are expressed rather ubiquitously, although a higher degree of expression is detected in heart and skeletal muscle (11). These results indicated that Dpkn may play a role in the development and function of somatic muscles in *Drosophila*. A functional characterization of Dpkn awaits isolation of mutations in the Dpkn gene. The existence of such mutants will unravel the possible roles of Dpkn in the development and function of somatic muscles in Drosophila.

ACKNOWLEDGMENTS

This work was supported by the grants provided by Nippon Boehringer Ingelheim Co., Ltd., Kawanishi Pharma Research Institute, Yamanouchi Foundation for Research on Metabolic Disorders, Uehara Foundation, and Kato Memorial Bioscience Foundation.

REFERENCES

- 1. Nishizuka, Y. (1992) Science 258, 607-614.
- 2. Dekker, L. V., and Parker, P. J. (1992) TIBS 19, 73-77.
- 3. Nishizuka, Y. (1995) FASEB J. 9, 484-496.
- 4. Hardie, G., and Hanks, S. (1995) The Protein Kinase FactsBook. Vol. I, pp. 80–88, Academic Press.
- Ohno, S., Kawasaki, H., Imajoh, S., Suzuki, K., Inagaki, M., Yokokura, H., Sakoh, T., and Hidaka, H. (1987) 325, 161–166.
- Baier, G., Telford, D., Giampa, L., Coggeshall, K. M., Baier-Bitterlich, G., Isakov, N., and Altman, A. (1993) 268, 4997-5004.
- Hardie, G., and Hanks, S. (1995) The Protein Kinase FactsBook, Vol. I, pp. 89–90, Academic Press.
- 8. Rosenthal, A., Rhee, L., Yadegari, R., Paro, R., Ullrich, A., and Goeddel, D. V. (1987) *EMBO J.* **6**, 433–441.
- Schaeffer, E., Smith, D., Mardon, G., Quinn, W., and Zuker, C. (1989) Cell 57, 403-412.
- Mukai, H., Mori, K., Takanaga, H., Kitagawa, M., Shibata, H., Shimakawa, M., Miyahara, M., and Ono, Y. (1995) *Biochim. Bio-phys. Acta* 1261, 296–300.
- Mukai, H., and Ono, Y. (1994) Biochem. Biophys. Res. Commun. 199, 897–904.
- Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morii, N., Mukai, H., Ono, Y., Kakizuka, A., and Narumiya, S. (1996) Science 271, 645–648.
- Amano, M., Mukai, H., Ono, Y., Chihara, K., Matsui, T., Hamajima, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 271, 648–650.
- Brown, N. H., and Kafatos, F. C. (1988) J. Mol. Biol. 203, 425– 437
- 15. Minami, Y., Oishi, I., Liu, Z-J., Nakagawa, S., Miyazaki, T., and Taniguchi, T. (1994) *J. Immunol.* **152**, 5680–5690.
- 16. Tautz, D., and Pfeifle, C. (1989) Chromosoma 98, 81-85.
- 17. Kobayashi, S., Saito, H., and Okada, M. (1994) *Dev. Growth Differ.* **36**, 629–632.
- Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42-52.
- Hanks, S. K., and Quinn, A. M. (1991) Methods Enzymol. 200, 38–62.