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A 104-kDa diacylglycerol kinase containing ankyrin-like repeats localizes in the cell nucleus

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ABSTRACT **The cDNA corresponding to a fourth species of diacylglycerol (DG) kinase (EC 2.7.1.107) was isolated from cDNA libraries of rat retina and brain. This cDNA encoded a 929-aa, 104-kDa polypeptide termed DGK-IV. DGK-IV was different from previously identified mammalian DG kinase species, DGK-I, DGK-II, and DGK-III, in that it contained no EF-hand motifs but did contain four ankyrin-like repeats at the carboxyl terminus. These structural features of DGK-IV closely resemble the recently cloned, eye-specific DG kinase of *Drosophila* that is encoded by the retinal degeneration A (*rdgA*) gene. However, DGK-IV was expressed primarily in the thymus and brain with relatively low expression in the eye and intestine. Furthermore, the primary structure of the DGK-IV included a nuclear targeting motif, and immunocytochemical analysis revealed DGK-IV to localize in the nucleus of COS-7 cells transfected with the epitope-tagged cDNA, suggesting an involvement of DGK-IV in intranuclear processes.**

Phosphoinositide (PI) turnover produces two second messengers, diacylglycerol (DG) and inositol trisphosphate, in response to external stimuli (1, 2). DG acts as an activator of several forms of protein kinase C (PKC), whereas inositol trisphosphate mobilizes calcium ions from the endoplasmic reticulum (3, 4). DG is converted to phosphatidic acid by DG kinase. Although this conversion is regarded as the first step of the recycling of PI (5), several reports have recently shown that phosphatidic acid may be involved in the regulation of DNA synthesis; the induction of c-myc, c-fos, and platelet-derived growth factor; and cAMP formation (6, 7). Furthermore, phosphatidic acid-dependent protein phosphorylation occurs in soluble preparations from several organs such as brain, spleen, and testis (6, 8). Therefore, DG kinase may play a role not only in the attenuation of DG, but also in the production of a possible second messenger, phosphatidic acid. The importance of DG kinase in neurologic functions is also indicated by the observation that the activity of this enzyme is lacking in a *Drosophila* retinal degeneration mutant (*rdgA*) (9).

In this study, representing the fourth of a series of our studies, we isolated cDNA for a fourth DG kinase, DGK-IV, from cDNA libraries of rat retina and brain. DGK-IV was distinct from DGK-I, DGK-II and DGK-III in primary structure and expression localization, but closely resembled the recently cloned eye-specific DG kinase of *Drosophila* encoded by the *rdgA* gene (10). These results suggest that DGK-IV is a mammalian homologue of the eye-specific DGK-*rdgA*. Furthermore, immunocytochemical analysis revealed DGK-IV to localize in the nucleus of COS-7 cells transfected with the epitope-tagged cDNA, suggesting that DGK-IV may be involved in intranuclear processes.

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MATERIALS AND METHODS

cDNA Cloning. A rat retinal cDNA library (6×10^5 clones) was screened with mixed cDNA probes encoding the putative catalytic regions of rat DGK-I, DGK-II, and DGK-III under low stringency conditions as described (11–13). One positive clone, pRR1, was obtained that was different from those of the previously cloned DG kinases but partly homologous to them. Furthermore, we isolated three elongated cDNA clones (pRB1, pRB2, and pRB3) by screening a rat brain cDNA library. The cDNA insert of the most elongated clone, pRB1, was sequenced on both strands by the Perkin–Elmer 373 DNA autosequencer.

RNA Extraction and Northern Blot Analysis. Total RNAs (30 μg per lane) from several tissues of adult Wister rats were denatured with formaldehyde and size-separated by agarose gel electrophoresis. The RNA blot was hybridized with a 3′ noncoding sequence (nucleotides 2835–3380) labeled with [^{32}P]dATP as a probe. Conditions for hybridization and washing were as described (12, 13).

Transfection and DG Kinase Activity. Complementary DNAs for a full length of this novel DG kinase, pRB1, and a mutant enzyme lacking about one-third of the carboxyl terminus were subcloned in the expression vector pSRE. After incubating for 3 days, cDNA-transfected COS-7 cells were harvested and lysed by sonication in lysis buffer (13). DG kinase activity was measured by the octyl glucoside mixed-micelle assay and the deoxycholate assay as described (12, 13). 1,2-Dioleoyl-*sn*-glycerol (18:1/18:1 DG) (Sigma), 1-stearoyl-2-linoleoyl-*sn*-glycerol (18:0/18:2 DG) (Biomol, Plymouth Meeting, PA), and 1-stearoyl-2-arachidonoyl-*sn*-glycerol (18:0/20:4 DG) (Biomol) were used as substrates.

***In Situ* Hybridization Histochemistry.** Fresh frozen blocks of brains from adult male rats were sectioned at a 30-μm thickness on a cryostat. The sections were mounted on silane-coated glass slides, fixed with paraformaldehyde, pretreated, and hybridized with [^{35}S]dATP-labeled probe as described (11). After exposure to Hyperfilm-β max (Amersham) for 2–3 weeks, the sections were dipped in Kodak NTB2 emulsion and exposed for 2 months.

Immunocytochemical Localization and Immunoblot in the cDNA-Transfected Cultured Cells. An epitope tag composed of eight amino acids (FLAG marker peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys by Kodak) was fused with DGK-IV by cloning the initiation codon ATG and the subsequent 24-bp FLAG coding sequence to the 5′ end of the coding region of the novel DGK cDNA. FLAG epitope-tagged cDNA was expressed in COS-7 cells using the expression vector pSRE by DEAE/dextran. After 24–36 h, the cells were fixed with 2% paraformaldehyde/0.1% Triton-X 100 and were incubated with a monoclonal antibody (anti-FLAG antibody M2, Kodak)

Abbreviations: PI, phosphoinositide; DG, diacylglycerol; PKC, protein kinase C; PLC, phospholipase C.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. D78588).

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specific for the FLAG marker peptide. Sites of antigen–antibody reaction were visualized using the avidin-biotinylated peroxidase complex system (Vector Laboratories) with diaminobenzidine as a substrate.

Cytoplasmic and nuclear fractions for each transfectant were prepared according to the methods described previously (14, 15). Both fractions were subjected to immunoblotting with anti-FLAG M2 antibody. The immunoreactive band was detected using the enhanced chemiluminescence detection system (Amersham).

RESULTS

By low stringency hybridization, a novel cDNA clone which encoded a putative DG kinase from rat retinal and brain

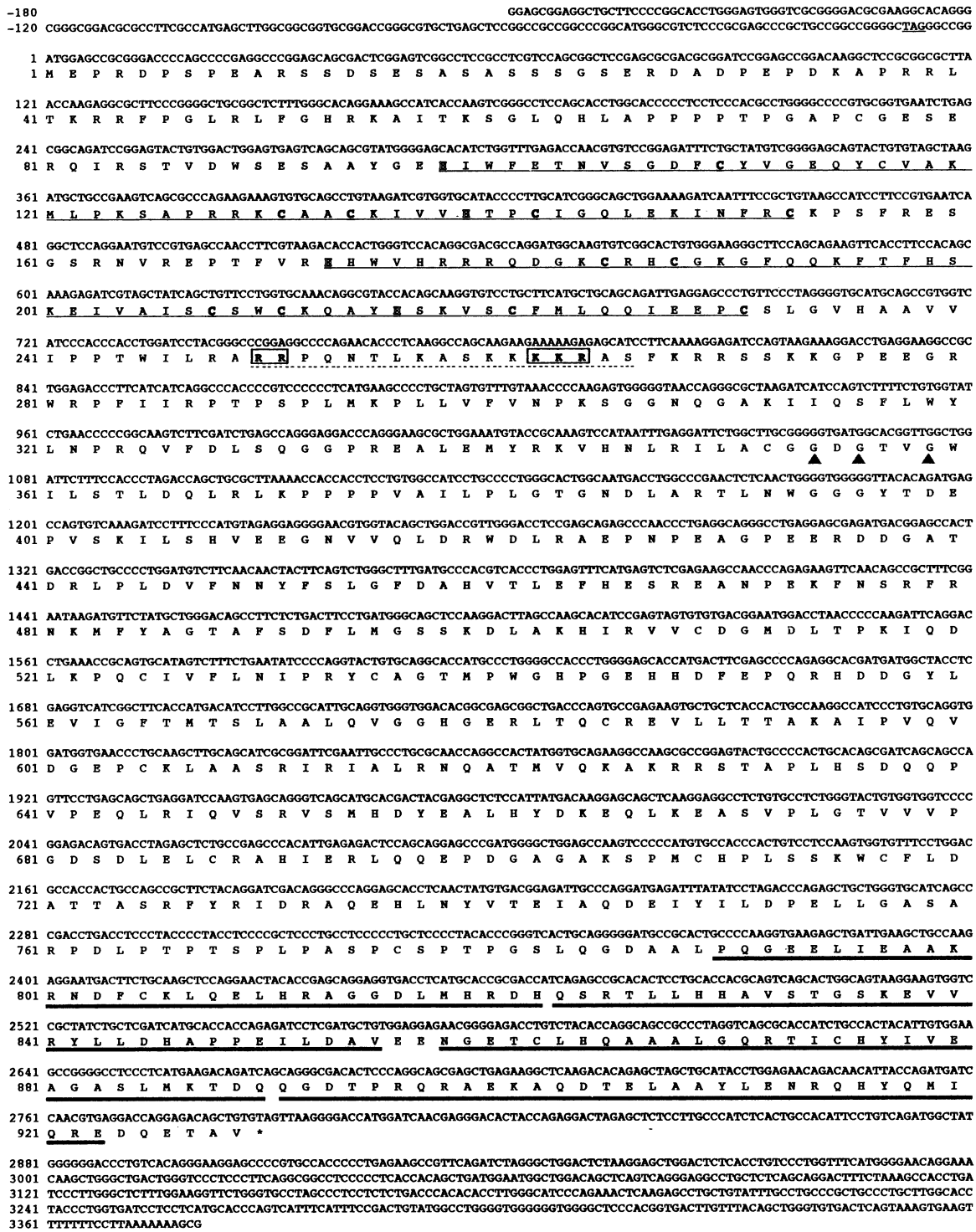

FIG. 1. Nucleotide and deduced primary structure of the composite cDNA for DGK-IV. Nucleotides and amino acids are numbered starting from A of the translation initiation codon ATG and the initiator methionine, respectively. An in-frame stop codon that precedes the translation initiation is underlined at −9. The cysteine-rich zinc finger-like sequences in which conserved cysteine and histidine residues are indicated are underlined. The bipartite nuclear targeting motif in which two clusters of basic amino acids are indicated by boxes is indicated by a dotted line. Glycine residues characteristic of ATP-binding sites found in protein kinases are marked by solid triangles. Four ankyrin-like repeats in the carboxyl region are boldly underlined.

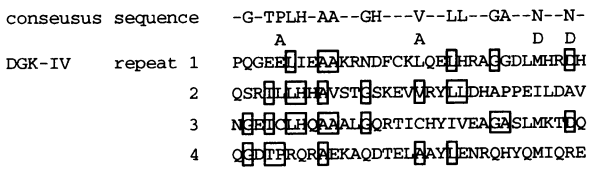

FIG. 2. Comparison of the four ankyrin-like repeats of DGK-IV with the consensus sequence of the erythrocyte ankyrin. Residues identical to the consensus sequence are in boxes.

cDNA libraries was isolated. The composite cDNA contained a 2787-nt open reading frame and a preceding in-frame stop codon at nucleotide −9 (Fig. 1). The deduced amino acid sequence encoded a protein of 929 amino acids with a calculated molecular mass of 104 kDa. This amino acid sequence shared the highly conserved catalytic domain contained in all previously cloned DG kinases. However, in contrast to these DG kinases, it contained no EF-hand motifs in the regulatory domain but did contain four ankyrin-like repeats in an elongated carboxyl-terminal portion (Fig. 2) (16). These structural features resembled those of the recently cloned eye-specific DG kinase of *Drosophila* that is encoded by the *rdgA* gene (Fig. 3) (10). In addition, the putative DG kinase, termed DGK-IV, showed a higher identity (40%) to DGK-*rdgA* than to the other three DG kinase isozymes (31–34%) in its entire chain. These results suggest that DGK-IV is a mammalian homologue of eye-specific DGK-*rdgA*. Furthermore, a bipartite nuclear targeting motif was located next to the second zinc finger-like sequence in the regulatory domain (Figs. 1 and 3). This motif consisted of a cluster of two adjacent basic amino acids separated by 10 amino acids from a second cluster, in which three of the next 5 amino acids were also basic (Fig. 1) (17). This consensus sequence is conserved in many nuclear proteins, such as transcription factors and DNA repair proteins (17, 18).

The ability of DGK-IV to phosphorylate various DGs was measured by expression of DGK-IV cDNA (psreDGK-IV) in COS-7 cells. As a control, pSRE vector was expressed alone. In the octyl glucoside mixed-micelle assay, COS-7 cells transfected with psreDGK-IV showed a kinase activity toward long chain-DG species that was at least 20-fold higher than that of the control (Table 1). Furthermore, similar results were obtained in the deoxycholate assay. The activity of DGK-IV was not affected by the presence (0.02 and 0.1 mM) or absence of calcium ions (5 mM EGTA), as suggested by the lack of EF-hand motifs in the primary structure of DGK-IV in contrast to previously cloned DG kinases.

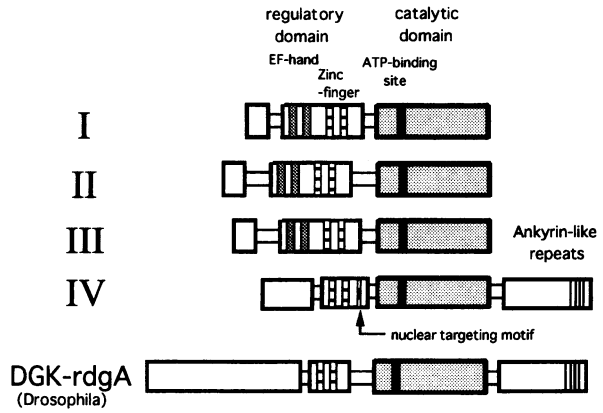

FIG. 3. Schematic representation of DGK-I, DGK-II, DGK-III, DGK-IV, and *Drosophila* DGK-*rdgA*. Homologous regions are indicated by large boxes. EF-hand motifs, cysteine-rich zinc finger-like sequences, putative ATP-binding sites, ankyrin-like repeats, and a nuclear targeting motif are shown.

Table 1. DG kinase activities* in COS-7 cells transfected with pSRE vector alone or psreDGK-IV

Assay	Substrate		
	18:1/18:1 DG	18:0/18:2 DG	18:0/20:4 DG
Octyl glucoside			
pSRE	0.8	0.7	0.7
psreDGK-IV	23.8	23.1	22.5
Deoxycholate			
pSRE	1.8	1.6	1.9
psreDGK-IV	44.7	45.7	48.1

Lysates were assayed for kinase activity toward single DG species in the octyl glucoside mixed-micelle assay and deoxycholate assay. Values represent the means of triplicate determinations. Similar results were obtained in two separate experiments.
*Expressed in nanomoles per minute per milligram.

Northern blot analysis of several rat tissues revealed a dominant band of 3.6 kb mRNA, consistent with an almost full-length DGK-IV cDNA in the thymus and brain, and a faint band of 3.6 kb in the intestine and eye (Fig. 4). Thymus contained two dominant bands, one of 7 kb and one of 3.6 kb, suggesting an alternatively spliced transcript in this tissue.

By *in situ* hybridization histochemical analysis of adult rat brain, mRNA for DGK-IV was expressed intensely in the cerebellum; the hippocampal pyramidal and dentate granular cells; and the olfactory periglomerular, granule, and mitral cells, and moderately in the cerebral cortex and the olfactory tubercle (Fig. 5*a*). In the cerebellar cortex, the hybridization signals were deposited more densely in the Purkinje cell somata than in the granule cells (Fig. 5*b*). In the eye, gene expression was confined to the inner granular layer (Fig. 6).

By the FLAG epitope-tagging method in COS-7 cells, subcellular localization of DGK-IV was examined because it included a nuclear targeting motif. Intense immunoreactivity for the epitope tag was localized homogeneously in the nuclei of COS-7 cells transfected with a full-length DGK-IV cDNA, and weak immunoreactivity appeared in fine dots dispersed throughout the cytoplasm (Fig. 7*c*). Consistent with the immunocytochemical results, this nuclear localization was also shown by Western blotting of the transfected cells, although there was still a certain amount of protein that remained in the cytoplasmic fraction (Fig. 8*a*). To confirm that the nuclear localization of DGK-IV was not a potential artifact of the FLAG tag procedure, we also expressed FLAG-tagged DGK-II (12) in COS-7 cells by the same way as a control. In marked difference from DGK-IV, the immunoreactivity for

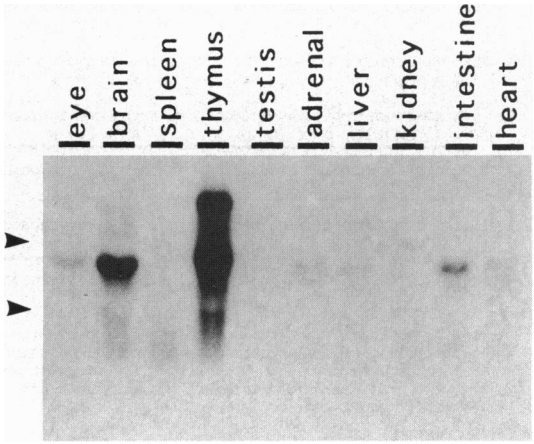

FIG. 4. Northern blot analysis of the DGK-IV mRNA in various rat tissues. Total RNAs (30 μg per lane) from various tissues were electrophoresed and transferred to a nylon membrane. The filter was hybridized with ^{32}P-labeled probe of 3′ noncoding region. Size markers (arrowheads) are 28S and 18S rRNAs.

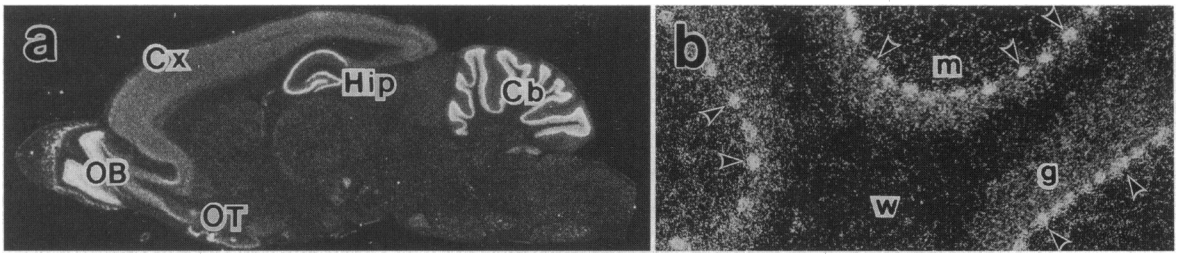

FIG. 5. *In situ* hybridization of DGK-IV mRNA in the adult rat brain. (*a*) Film autoradiographic image of parasagittal section through the caudate-putamen. Note intense expression signals in the cerebellar cortex (Cb), neuronal layers of the olfactory bulb (OB), and the hippocampus (Hip), and moderate signals in the cerebral cortex (Cx) and the olfactory tubercle (OT). (×4.) (*b*) Dark-field photomicrograph of the cerebellum on the emulsion-dipped section at higher magnification. Note intense signals in the Purkinje cells (arrowheads) and moderate signals in the granule cells (g). m, molecular layer; w, white matter. (×90.)

DGK-II appeared as small dots dispersed in the perikaryal cytoplasm but never in the nucleus (Fig. 7*g*). Furthermore, in the COS-7 cells transfected with some other cDNAs studied in our laboratory in this same system, the immunoreactivity did not appear intranuclear but in the cytoplasm (19). These control experiments indicate that the intranuclear immunoreactivity for DGK-IV represents truly the nuclear localization of this DGK-IV but not an artifact by this system.

To further investigate the intranuclear localization of DGK-IV, we removed soluble proteins from the transfected cells by treating them with 0.5% Triton X-100 before fixation. In those cells, moderate immunoreactivity still remained in several intranuclear granules, 3–4 μm in size with faint homogenous immunoreactivity throughout the remaining nuclear region (Fig. 7*d*). In Western blot analysis, a full-length DGK-IV was recovered in soluble and particulate fractions (Fig. 8*b*). These data indicated that DGK-IV was distributed in nuclei in Triton-soluble and Triton-insoluble forms. Then we did some experiments with truncated forms of DGK-IV to identify regions that cause the enzyme to enter nuclei and distribute there. Experiments with a FLAG-tagged, amino-terminal fragment of the enzyme, termed DGK-IVΔC, that contained the nuclear targeting domain but lacked the carboxyl-terminal region showed that this fragment could enter nuclei and bind intensively to Triton-insoluble nuclear components. (Fig. 7*e*). Furthermore, Western blot analysis of DGK-IVΔC revealed that it was mainly present in the particulate fraction (Fig. 8*b*). In contrast, experiments with a FLAG-tagged, carboxyl-terminal fragments of the enzyme, termed C-1/3 and ALR, that lacked the amino-terminal region including the nuclear targeting domain but contained the ankyrin-like repeats, showed that these fragments could enter nuclei and distribute there as Triton-solube components but not as Triton-insoluble components (Figs. 7*f* and 8*b*). These results support the following conclusions. (*i*) The enzyme's amino-terminal region contains both a nuclear targeting domain and a domain that binds to nuclear, Triton-insoluble material. (*ii*) The enzyme's carboxyl-terminal region can enter the nucleus independently of the amino-terminal region, but only in a Triton-soluble form. (*iii*) Both the amino-terminal region and the carboxyl-terminal region of the intact enzyme influence its intranuclear distribution.

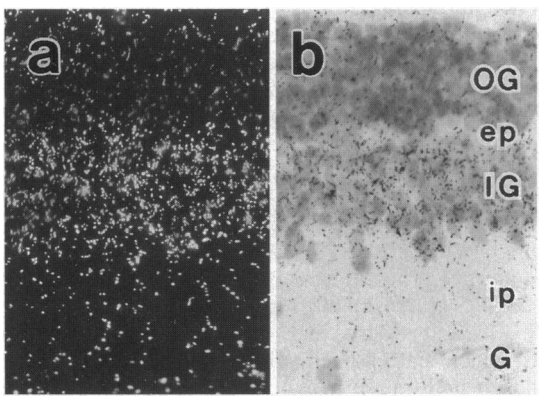

FIG. 6. *In situ* hybridization of DGK-IV mRNA in the rat retina. Dark-field (*a*) and bright-field (*b*) photomicrographs of the retina on the emulsion-dipped section. Note expression signals in the inner granular layer (IG). OG, outer granular layer; ep, external plexiform layer; ip, inner plexiform layer; G, ganglion cell layer. (×270.)

DISCUSSION

The molecular structure of DGK-IV, a novel DG kinase of 104 kDa identified in this study, differs from those of the three previously identified mammalian DG kinase species, DGK-I, DGK-II and DGK-III in three important respects. (*i*) It contains no EF-hand motifs, (*ii*) it contains a nuclear targeting motif, and (*iii*) it contains four ankyrin-like repeats in its carboxyl-terminal portion. These structural features of DGK-IV closely resemble the recently cloned eye-specific DG kinase of *Drosophila* that is encoded by the *rdgA* gene (10), although a nuclear targeting motif is not included in the corresponding region of *Drosophila* DGK-*rdgA*. This resemblance, together with higher identity in the amino acid sequence of DGK-IV to *Drosophila* DGK-*rdgA*, suggests that DGK-IV is the mammalian homologue of the DGK-*rdgA*. However, DGK-IV is expressed more abundantly in the brain and thymus than the eye, and in the eye is confined to the inner granular layer of the retina, which contains bipolar cells, horizontal cells, amacrine cells, and Müller's supporting cells. This localization contrasts with the specific expression of DGK-*rdgA* in photoreceptor cells of the *Drosophila* retina (10) and implies that DGK-IV and DGK-*rdgA* play different roles, though homologous in molecular structure. It is well known that the phototransduction cascade of invertebrates is based on the PI cycle (20), and DGK-*rdgA* clearly plays a role in this cycle. In contrast, vertebrate phototransduction is mediated by the cGMP cascade (21), whereas the role of DGK-IV in the vertebral retina remains to be determined.

Our experiments with cDNA-transfected COS-7 cells showed that FLAG-tagged DGK-IV localized primarily to Triton-soluble nucleoplasm and intranuclear, Triton-insoluble globular structures. What could be the identity of this intranuclear globular material? It is reported that the nucleolar protein, p120, localizes to intranuclear globular material when LOX cells are transfected with its β-galactosidase-tagged cDNA (22). The immunoreactivity of this material closely resembles that of the Triton-insoluble nuclear material to which DGK-IV binds. Therefore, we speculate that DGK-IV may bind to the nucleolus. An immunoelectron microscopic approach might provide useful information with regard to this possibility.

Both nuclear and nucleolar targeting signals have been identified in the structure of p120, and both have been shown

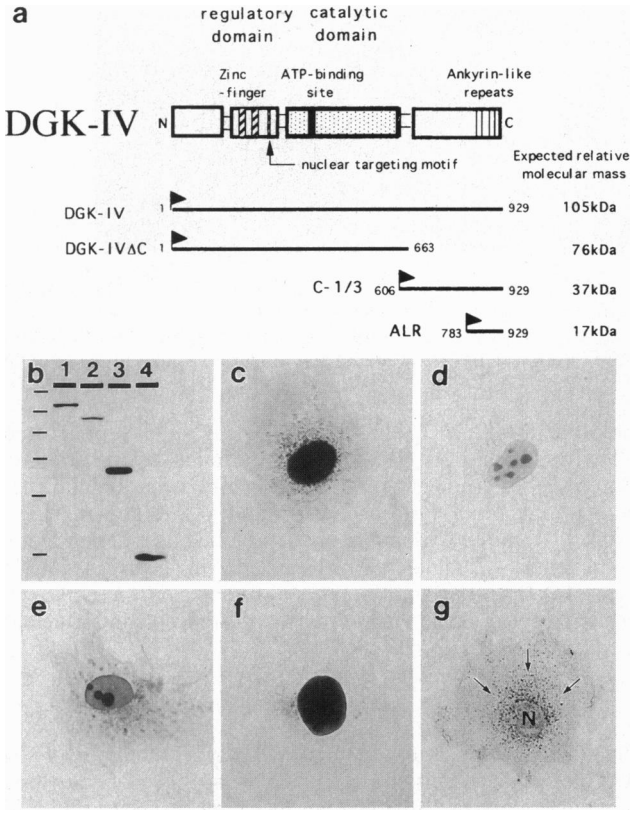

FIG. 7. Subcellular localization of an full-length DGK-IV and its truncated forms in transfected COS-7 cells. (*a*) Schematic representation of the primary structure of DGK-IV and mutants. N and C represent amino and carboxyl termini, respectively. Thick bars represent the region of the expressed protein. Numbers on both sides of the bar indicate the start and end positions of amino acid. The epitope tag (FLAG) marker peptide is indicated by a solid triangle on the left side of the bars. Expected molecular masses of the mutants are also shown on the right side. (*b*) Expression of the full-length DGK-IV and the truncated forms in transfected COS-7 cells. After SDS/7.5% polyacrylamide gel electrophoresis of COS-7 cell lysates (20 μg per lane), the proteins were transferred to a poly(vinylidene difluoride) membrane and analyzed by immunoblotting using anti-FLAG antibody M2 with the chemiluminesence system. Lane 1, DGK-IV; lane 2, DGK-IVΔC; lane 3, C-1/3; lane 4, ALR. Size markers (bars) indicated on the left side are 200, 97.4, 68, 43, 29, and 18.4 kDa from top to bottom. (*c* and *d*) Immunocytochemical staining of the COS-7 cells transfected with the full-length DGK-IV cDNA. Note intense homogenous immunoreactivity in the nucleus. In the transfected COS-7 cells treated with 0.5% Triton X-100 to remove soluble proteins prior to fixation (*d*), moderate immunoreactivity still remains in several intranuclear granules. (×540.) (*e*) Immunocytochemical staining of the COS-7 cells transfected with DGK-IVΔC cDNA. Note intense immunoreactivity in the intranuclear granules and weak to faint immunoreactivity throughout the nucleus. This immunoreactivity was not changed by treatment of the transfected cells with Triton as described above. (×540.) (*f*) Immunocytochemical staining of the COS-7 cells transfected with C-1/3 cDNA. Note intense homogenous immunoreactivity in the nucleus. Similar immunoreactivity was observed in the COS-7 cells transfected with ALR cDNA. No significant immunoreactivity was retained in the nuclei of the transfected cells after Triton pretreatment (data not shown). (×540.) (*g*) Immunocytochemical staining of COS-7 cells transfected with cDNA for FLAG-tagged DGK-II as a control. Note dot-like immunoreactivity dispersed in the perikaryal cytoplasm (arrows) but never in the nucleus (N). (×540.)

to be required for nucleolar localization. DGK-IV contains a nuclear localization signal that is located just downstream of the second zinc finger-like region, and our experiments showed that a FLAG-tagged amino-terminal fragment of DGK-IV, which contains this signal, localized to both the nucleus and putative nucleolar material. However, we could find no obvious nucleolar-targeting signal in the primary structure of DGK-IV. Therefore, the molecular basis for the binding of DGK-IVΔC and DGK-IV to intranuclear, globular material remains to be determined.

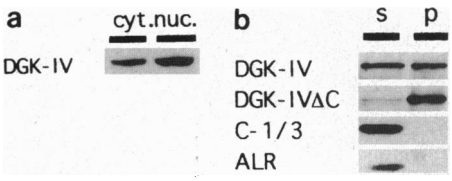

FIG. 8. Immunoblot analysis of a full-length DGK-IV and its truncated forms in transfected COS-7 cells. (*a*) Transfected cells were separated into cytoplasmic (cyt.) and nuclear (nuc.) fractions. Proteins of both fractions derived from 5×10^4 cells were analyzed with an anti-FLAG antibody M2 with the chemiluminescence system. (*b*) Partition of the full-length DGK-IV and the truncated forms in transfected COS-7 cells. Total cell lysates (40 μg) were centrifuged at 100,000 × *g* for 30 min to separate soluble (s) and particulate (p) fractions. The proteins were analyzed as described above.

The carboxyl-terminal region of DGK-IV, which contains the ankyrin-like repeats, clearly influences the intranuclear localization of the enzyme. FLAG-tagged DGK-IV localized to nuclei in both Triton-soluble and Triton-insoluble forms, whereas FLAG-tagged DGK-IVΔC localized to nuclei in a Triton-insoluble form only, and the FLAG-tagged carboxyl-terminal fragments localized only to the Triton-soluble nucleoplasm. The molecular basis of the effect of the carboxyl-terminal region and its ankyrin-like repeats is not clear. Ankyrin-like motifs are included in a number of proteins, such as erythrocyte ankyrin, the transcription factor NFκB, and the GA binding protein GABP-β. They are thought to promote interactions between proteins or within proteins (16). In erythrocyte ankyrin, the motif binds to an integral membrane protein and tubulin, forming a bridge between cytoskeleton and membrane components. In the case of NFκB, on the other hand, the carboxyl-terminal moiety containing the ankyrin-like repeats folds back to mask the region containing the nuclear localization signal. The results that we have obtained to date raise the possibility that the ankyrin-like repeats in DGK-IV may attach the enzyme to Triton-soluble intranuclear components. Furthermore, this may reduce the enzyme's ability to bind to intranuclear Triton-insoluble material. We are currently using the yeast two hybrid system to search for proteins that bind to the ankyrin-like repeats. Furthermore, we are doing experiments with deletion mutants to search for sequences that bind to the presumed nucleolar components.

This study clearly demonstrates that DGK-IV localizes primarily to Triton-soluble nucleoplasm and nuclear, Triton-insoluble components. Furthermore, the study shows that the amino-terminal region of the enzyme, including the nuclear targeting signal, and the carboxyl-terminal region, including the ankyrin-like repeats, influence its intranuclear localization. A previous report has demonstrated that DGK activity is detected in extracted nuclei of two mammalian cell types, mouse 3T3 fibroblasts and rat liver cells, and is associated preferentially to the internal nuclear matrix that contains nucleoli, and less dominantly to the peripheral nuclear matrix or lamina-pore complex (23). It has also been demonstrated that insulin-like growth factor I stimulation causes an increase in nuclear DG as a result of PI turnover activation in the nuclei of 3T3 cells and induces the translocation of PKC into the nucleus, which leads to cell proliferation (24). This suggests that nuclear DG kinase may be involved in regulation of cell replication by attenuation of nuclear DG and subsequent down-regulation of the activity of nuclear PKC in these cells (18). With respect to nuclear localization of PKC, inducible translocation of PKCα, PKCβ, and PKCγ to nucleus has been suggested to occur (25, 26). Sequences resembling the bipartite

nuclear targeting motif are present in the regulatory domains of PKCβ and PKCγ but not of PKCα (27). However, it remains to be elucidated whether these putative nuclear targeting motifs are functionally active. PKCα, on the other hand, appears to contain a cryptic nuclear targeting sequence because deletion of its regulatory domain results in constitutive localization of the enzyme to the nuclear envelop in COS cells (26). It is also suggested that nuclear localization of PKC could be mediated by its interaction with intracellular receptors or binding proteins, because several proteins termed "RACKs" (*r*eceptors for *a*ctivated *C*-*k*inase) have been identified (18, 28).

In addition, activities of several enzyme molecules involved in PI turnover, such as PI kinases and phospholipase C (PLC), have been detected in nuclei of mouse 3T3 fibroblasts and rat liver cells, and differential localization of these enzymes in nuclei has been suggested (23). In 3T3 fibroblasts, phosphatidylinositol 4-kinase is found to be associated exclusively to the peripheral nuclear matrix, whereas phosphatidylinositol 4-phosphate 5-kinase and PLC are found to be associated preferentially to the internal matrix structures. Regarding PLC, the PLCβ isoform has been shown immunocytochemically to localize to nuclei of 3T3 cells (15). Furthermore, the primary structure of PLCβ1 contains bipartite nuclear localization signal sequences (amino acids 467–483, 961–977, and 1055–1071; ref. 29), which appears to mediate its nuclear localization. It is suggested that nuclear PI turnover is involved in control of cell cycle progression (30), although how the nuclear PI cascade could be activated in response to occupancy of specific cell surface receptors and how these enzymes could be targeted to specific intranuclear sites remain to be answered.

Our results raise the possibility that enzymes of PI turnover, including DGK-IV, may be localized to nuclei not only in peripheral cells but also in neurons. This possibility remains to be explored. However, recent studies have suggested that memory formation involves altered gene expression in neurons; repeated, spaced pulses of serotonin cause an increase in cAMP and give rise to translocation of the catalytic subunit of the cAMP-dependent protein kinase to the neuronal nucleus, which induces protein synthesis-dependent, long lasting structural changes in neurons (31). Furthermore, PKC has been shown to be involved in learning and memory as revealed by a gene knock-out study of PKCγ, which is expressed predominantly in central nervous tissue (32, 33). Thus, it is plausible that DGK-IV might be related to the molecular mechanism of memory formation by regulating the activity of the nuclear PKC.

Note. During the revision of this manuscript, human diacylglycerol kinase ζ was reported (34). This kinase was highly homologous to our rat DGK-IV, suggesting that these are homologues between human and rat. Furthermore, the sequences of two additional isoforms were reported (35, 36).

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