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A Novel, Brain-Specific Mouse Drebrin: cDNA Cloning, Chromosomal Mapping, Genomic Structure, Expression, and Functional Characterization

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Drebrin A, a major neuronal actin-binding protein, regulates the dendritic spine shapes of neurons. Here, we have cloned and characterized a novel mouse cDNA clone encoding a truncated form of drebrin A, named s-drebrin A. Analysis of the genomic organization of the mouse drebrin gene (*Dbn1*), which mapped to the central portion of chromosome 13, revealed that isoforms including s-drebrin A are generated by alternative splicing from a single drebrin gene. The s-drebrin A mRNA was expressed in the brain, but not in non-neuronal tissues. The s-drebrin A expression was barely detected in the embryonic brain, but was upregulated during postnatal development of the brain. Overexpression of GFP-tagged s-drebrin A in fibroblasts showed it to be associated with actin filaments and with changes in actin cytoskeleton organization. These findings suggest that s-drebrin A has a role in spine morphogenesis, possibly by competing the actin-binding activity with drebrin A.

Key Words: actins, cytoskeleton, *Dbn1*, chromosome 13, s-drebrin A

INTRODUCTION

Drebrins are actin-binding proteins found in the central nervous system of various species [reviewed in 1]. Drebrin forms a complex with gelsolin, myosin, and F-actin *in vivo* [2]. Previous *in vitro* studies [3,4] have revealed that drebrin binds to filamentous actin (F-actin) competitively with F-actin-stabilizing proteins such as fascin, α-actinin, and tropomyosin. Overexpression of drebrin in fibroblasts results in actin filament remodeling [5,6]. In addition to its ability to bind to F-actin, drebrin has been shown to interact with profilin, an actin monomer-binding protein that stimulates actin to polymerize, probably via the proline-rich domain of drebrin [7]. These observations indicate a general involvement of drebrin in actin cytoskeleton dynamics.

It has been reported that the expression pattern of drebrin is correlated in time with the changes in the level of physiologically defined plasticity of kitten visual cortex [8]. Moreover, overexpression of drebrin in cultured cortical neurons results in morphological changes of the dendritic spine [9]. Therefore, drebrin is considered to have an important role in the structure-based plasticity of synapses.

Based on their expression pattern and primary structure, drebrins are classified into embryonic (drebrin E) and adult (drebrin A) types [1,10]. Three isoforms of drebrin (E1, E2, and A) have been identified in chicken [11,12]. Drebrins E1 and E2 are mainly expressed in the developing brain and some non-neuronal tissues [13–15]. Compared with E1, E2 has an additional 129-bp insertion (called ins1) in its mRNA [16]. In contrast, drebrin A, which has both ins1 and another additional 138-bp insertion (called ins2), is mainly expressed in mature cortical neurons [13,17]. Analysis of the genomic organization of the chicken drebrin gene revealed that drebrins E1, E2, and A were generated by alternative splicing from a single gene [13]. So far, only two isoforms (drebrins E and A) have been identified and cloned in mammals [1,8,10,18–20]. In addition, the genomic organization of the mammalian drebrin gene has not been clarified.

In our previous work, we noted from immunohistochemical studies of adult rat brain that the labeling pattern of the M2F6 monoclonal antibody [14], which recognizes the carboxy-terminal epitope of drebrin, was similar to the labeling pattern of a polyclonal antibody raised against full-length drebrin [2]. However, the immunostaining pattern of the adult rat brain with DAS1, a polyclonal antibody against the

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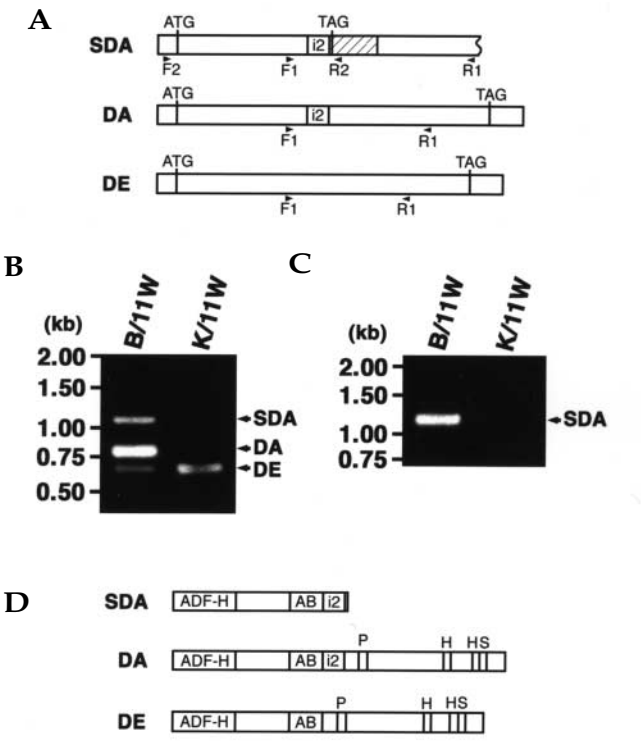

FIG. 1. Novel drebrin isoform. (A) Schematic representation of drebrin isoforms and the primer sets for RT-PCR used to identify s-drebrin A mRNA. The slashed box indicates the s-drebrin A-specific exon. i2 indicates drebrin A-specific exon ins2. Forward (F1 and F2) and reverse (R1 and R2) primers are indicated by arrowheads. SDA, s-drebrin A; DA, drebrin A; DE, drebrin E. (B) Detection of drebrin isoforms by RT-PCR using the F1 and R1 primers. Total RNA from the brain (B/11W) and kidney (K/11W) of 11-week-old mice was analyzed by RT-PCR. cDNA fragments amplified from s-drebrin A, drebrin A, and drebrin E mRNAs are indicated by SDA, DA, and DE, respectively. (C) Amplification of s-drebrin A cDNA by RT-PCR using the F2 and R2 primers. The s-drebrin A cDNA fragment amplified from brain of an 11-week-old mouse is indicated by SDA. (D) Schematic representation of the domain structures of the mouse drebrin isoforms. ADF-H, actin depolymerizing factor homology domain; AB, actin-binding domain; i2, ins2 sequence; P, proline-rich domain; H, Homer ligand motif; S, SH2 ligand motif.

ins2 polypeptide [5], was quite different from the patterns of the above two antibodies (unpublished data). These data raised the possibility that another isoform of drebrin containing the ins2 sequence was expressed in the adult brain.

In this study, we cloned a novel truncated drebrin isoform that contains the ins2 sequence using RT-PCR, and determined the genomic organization and chromosomal location of the mouse drebrin gene (*Dbn1*). To evaluate the functions of the truncated isoform, we then focused on its organic and developmental expression patterns and on its F-actin remodeling activity in fibroblasts.

RESULTS

Identification of a Novel Truncated Isoform of Drebrin

To clone novel drebrin isoforms containing the ins2 sequence, we first analyzed total RNA from adult mouse brain and kidney by RT-PCR using the F1 and R1 primers, which include the ins2 sequence (Fig. 1A). We obtained three bands from the brain RNA, but only one from the kidney (Fig. 1B). Sequence analysis revealed that the upper band was a novel cDNA fragment that, compared with the drebrin A cDNA, contained the ins2 sequence and an additional 319-bp insert (Fig. 1B). We next designed an insert-specific primer, R2 (Fig. 1A), and cloned the 5′-portion of the novel cDNA by RT-PCR using the F2 and R2 primers (Fig. 1C). Because a stop codon was included in-frame at nucleotide 7 of the internal insert, the novel cDNA encoded a truncated form of drebrin A with an additional amino acid, arginine (Fig. 1D). The deduced protein had a calculated molecular weight of 42 kDa, approximately half that of drebrin A (77 kDa) [10]. Because the primary structure of the protein was identical to that of the amino-terminal half of drebrin A and its expression pattern was similar to that of drebrin A, it was named s-drebrin A.

Genomic Structure and Splicing Variants of Mouse *Dbn1*

To determine mouse *Dbn1* structure, we screened a genomic library prepared from TT2 cells [21] using rat drebrin cDNA as a probe and obtained three positive phage clones. The genomic organization of *Dbn1*, except for intron 13 and exon 14, was initially determined by sequencing the phage clones. To determine the complete genomic organization of *Dbn1*, we cloned a genomic DNA fragment from TT2 cells by PCR using the F5 and R5 primers and analyzed its sequence. The sequence data revealed that *Dbn1* was composed of 14 exons (Fig. 2, top lane) and that s-drebrin A mRNA was generated

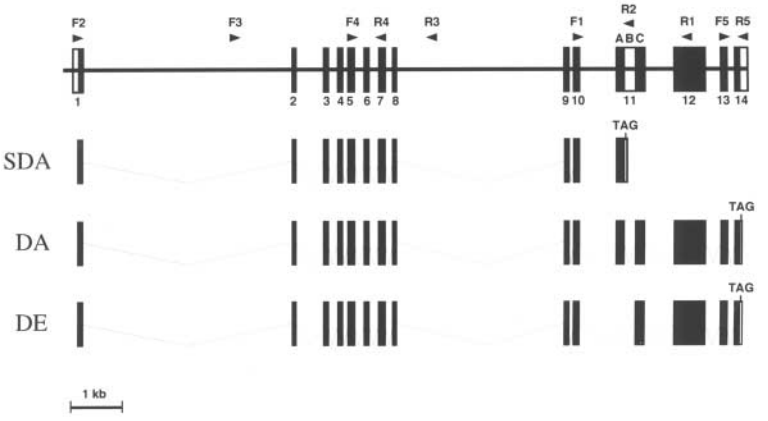

FIG. 2. Genomic organization of mouse *Dbn1* and coding regions for the three drebrin isoforms. The exon and intron structures of the mouse *Dbn1* are shown schematically at the top, and the coding regions for the three drebrin isoforms are shown below. Exons corresponding to open reading frames are indicated by black boxes, and exons corresponding to untranslated regions, by white boxes. Exons 11A, B, and C are included in the s-drebrin A mRNA. An in-frame stop codon is located at nucleotide 7 of exon 11B.

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TABLE 1: Exon-intron sizes and junction sequences of mouse drebrin gene

Exon number and size (bp)		Splicing site 3′- end of exon	Splicing site 5′- end of intron	Intron size (bp)	Splicing site 3′- end of intron	Splicing site 5′- end of next exon
1	204	GCCGACTG	**gt**gagccc	4420	ctcctc**ag**	GGCTCTGT
2	56	GTCAGGAG	**gt**aagaat	582	ttctcc**ag**	AAGGGGGC
3	113	TCAACTGG	**gt**atgtgg	204	cattgc**ag**	GTTGGTGA
4	75	TCTTCCAG	**gt**atgttg	115	tatgga**ag**	GGTGTTGA
5	147	AACCGGTG	**gt**cagtgt	160	ctctgc**ag**	GGTACCAC
6	78	AGGCCAAG	**gt**gggcaa	222	gggtgc**ag**	AAGGAGGA
7	152	GAGCACAG	**gt**aagctc	126	ccttcc**ag**	GAGGAAAV
8	64	CTATCTTT	**gt**aagttc	3513	ttttcc**ag**	GGTGACCA
9	60	AGGTGGAG	**gt**gagagc	97	gccctc**ag**	GAGGCGGC
10	130	CCGACCAG	**gt**agtcct	777	ctcggc**ag**	GTCGTCCG
11	628	AACTCAAG	**gt**aagaga	609	tgccac**ag**	AGGCCCAG
11A	138	CCTCCCAT	**gt**aggtag	319	ctccgc**ag**	GCAGCCAC
11C	171	AACTCAAG	**gt**aagaga	609	tgccac**ag**	AGGCCCAG
12	677	GGACCCAG	**gt**gggaca	300	cccagc**ag**	GCCAGCGA
13	103	GCCTCCAG	**gt**agtatc	162	tctcct**ag**	AAATCGAC
14	256	CTTTTCTC				

Exon and intron sequences are shown in upper- and lowercase letters, respectively. Consensus sequences of splice donor and acceptor are shown in boldface type. Exon 11 contains sequences of A, B and C of exon 11 indicated in Fig. 2.

by the alternative splicing of precursor mRNA from a single *Dbn1* gene. The s-drebrin A mRNA contained exons 11A, B, and C; drebrin A contained exons 11A and C, but not B; and drebrin E contained exon 11C, but neither A nor B (Fig. 2). Although a codon of Cys-367 in drebrin A was located in the splicing site between exons 11A and 11C, the Cys remained in s-drebrin A. All of the splice sites of mouse *Dbn1* obeyed the GT-AG paradigm (Table 1).

S-Drebrin A Is a Brain-Specific Isoform

As a step towards understanding the functional roles of s-drebrin A, we characterized its expression pattern. First, we detected s-drebrin A specific transcripts in various adult mouse tissues by RT-PCR analysis using primers F2 and R2, which specifically amplify s-drebrin A mRNA. The s-drebrin A transcript was expressed in the adult brain, but not in non-neuronal adult tissues (Fig. 3A). In contrast, β-actin mRNA was detected in all RNA samples by RT-PCR using β-actin-specific primers. These results indicated that s-drebrin A was a brain-specific isoform. Next, we examined changes in the expression of s-drebrin A mRNA in the developing brain. RT-PCR using the F1 and R1 primers, which can amplify the three drebrin isoforms, revealed that the expression profile of s-drebrin A was similar to that of drebrin A (Fig. 3B, top). We found that s-drebrin A and drebrin A were barely expressed in the embryonic brain, but were upregulated in the postnatal brain. In contrast, drebrin E was strongly expressed in the embryonic brain and gradually downregulated after birth. The relative amounts of s-drebrin A and drebrin E were reversed between 9 days and 4 weeks after birth (Fig. 3B). The increase in s-drebrin A mRNA in the postnatal brain was confirmed by RT-PCR using the F2 and R2 primers (Fig. 3B, bottom). These results suggest that the s-drebrin A expression may be correlated with brain maturation.

S-Drebrin A Regulates the Actin Cytoskeleton

To understand the functional roles of s-drebrin A, we transfected CHO cells with pGFP-SDA expression vector, which encodes GFP fused with s-drebrin A (GFP-SDA), and compared their actin cytoskeleton structures with those of CHO cells expressing GFP or GFP–drebrin A fusion protein (GFP–DA). Strong expression of GFP–SDA in the cells resulted in changes in actin cytoskeleton structure (Fig. 4, top). We observed thick actin bundles in the transfected cells. These actin bundles were usually curved and sometimes formed a circle. Their thickness changed from place to place. A similar change in actin cytoskeleton structure was observed in the cells expressing GFP–DA (Fig. 4, middle). In contrast, in non-transfected cells (Fig. 4B) and in GFP-expressing cells (Fig. 4, bottom), we observed straight bundles of actin, known as stress fibers, but not the thick, curved actin bundles.

Next, to clarify the association of s-drebrin A with the actin cytoskeleton, we examined the colocalization of s-drebrin A with actin filaments in the transfected cells. Most of the actin filaments stained with rhodamine-phalloidin were colocalized with GFP–SDA and GFP–DA (Fig. 4, top and middle), but not with GFP (Fig. 4, bottom). Similar results were obtained using COS cells (data not shown). These observations suggest that s-drebrin A can regulate the actin cytoskeleton organization and cell morphology by interacting with the actin filaments.

Mapping of the Mouse *Dbn1* to Chromosome 13

To determine the chromosomal location of mouse *Dbn1* by backcross analysis, we first identified an informative restriction fragment length polymorphism (RFLP) between the C57BL/6JEi and SPRET/Ei strains by long PCR [22]. *Pst*I digestion of the long-PCR products (4.98 kb), which include exons 2 to 8 of the mouse *Dbn1*, revealed the RFLP in the parental strains (Fig. 5A). Next, we designed primers F4 and

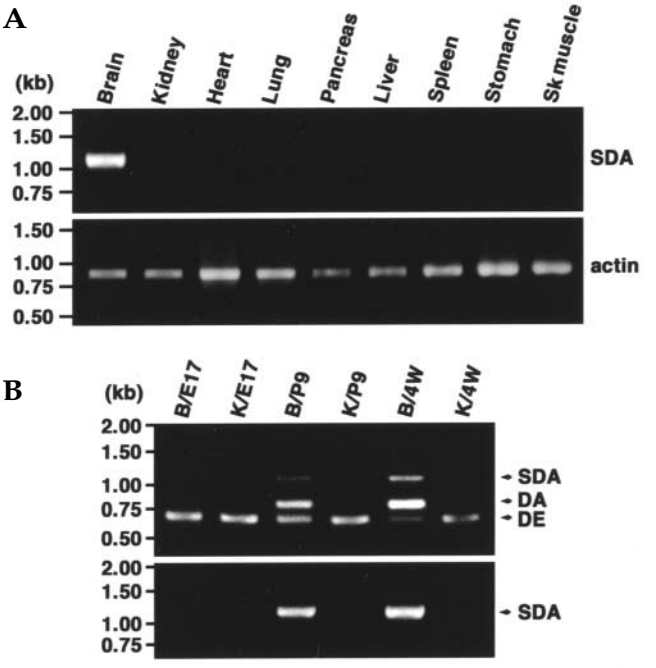

FIG. 3. The s-drebrin A transcript is a brain-specific isoform and its expression in brain is upregulated after birth. (A) Total RNA from the indicated adult mouse tissues was analyzed by RT-PCR using the F2 and R2 primers. The PCR product amplified from s-drebrin A mRNA was detected in brain, but not in non-neuronal tissues (top). As a positive control for RT-PCR, actin cDNAs were amplified by RT-PCR using actin-specific primers (bottom). (B) Total RNA from the brain (B) and kidney (K) of 17-day mouse embryos (E17) and 9-day-old (P9) and 4-week-old (4W) mice were analyzed by RT-PCR using the F1-R1 (top) and F2-R2 primers (bottom). PCR-amplified cDNAs from s-drebrin A, drebrin A, and drebrin E mRNAs are indicated by SDA, DA, and DE, respectively.

R4, which could more efficiently amplify both the C57BL/6JEi and SPRET/Ei alleles. *Pst*I digestion of the 567-bp PCR products yielded two DNA fragments of 360 and 207 bp for the C57BL/6JEi strain and three fragments of 255, 207, and 105 bp for the SPRET/Ei strain. Genomic DNAs from 94 animals of the Jackson BSS backcross were then analyzed to follow the segregation of these polymorphic fragments. The segregation patterns of the RFLPs in the backcross were compared with those of other markers previously typed in the same backcross. The results indicated that mouse *Dbn1* is located on the central portion of chromosome 13, and cosegregates with *D13Mit13*, *D13Mit249*, *Il9*, and *Pitx1* (Figs. 5B and 5C).

DISCUSSION

Here, we have identified a novel, truncated drebrin isoform that contains the ins2 sequence, named s-drebrin A, which is generated by alternative spicing from a single mouse *Dbn1* gene that maps to mouse chromosome 13. Expression of s-drebrin A is brain-specific and increases in parallel with drebrin A during brain maturation. Functional analysis indicates that s-drebrin A is associated with the actin cytoskeleton and regulates actin filament organization.

The nucleotide sequence of s-drebrin A cDNA is identical to that of drebrin A except for an internal 319-bp insertion, which corresponds to exon 11B. Because this insertion includes a stop codon in-frame, s-drebrin A possesses only the N-terminal half of drebrin A, but lacks the C-terminal half. Exon 11A of mouse *Dbn1* corresponds to the ins2 of the chicken drebrin gene [11,13], given that their amino acid sequences are highly conserved (85% identical). In contrast, it remains unclear whether exon 11C corresponds to the ins1 of chicken drebrin or not, as their homology is only 40% and an mRNA lacking exon 11C has not yet been identified. The sequences of three drebrin cDNAs and the genomic organization of mouse *Dbn1* indicate that the insertion or deletion of exons 11A, B, and C generates the heterogeneity of mouse drebrin.

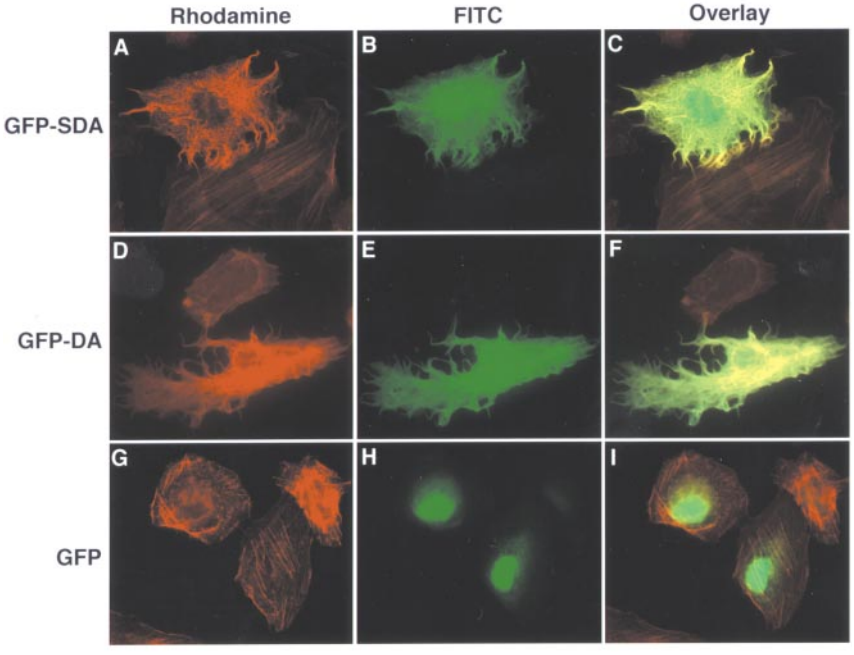

FIG. 4. Association of GFP–SDA with the actin cytoskeleton and morphological changes of fibroblast cells by the overexpression of GFP–SDA. CHO cells transfected with the expression vectors pGFP-SDA (A, B, and C), pGFP-DA (D, E, and F), or pEGFP-C1 (G, H, and I) were stained with rhodamine-phalloidin to label the actin filaments. Actin filaments and the GFP-tagged proteins were visualized using rhodamine (A, D, and G) and FITC (B, E, and H) filter sets, respectively. The rhodamine and FITC images were overlaid (C, F, and I). Note that GFP–SDA and GFP–DA are colocalized with actin filaments.

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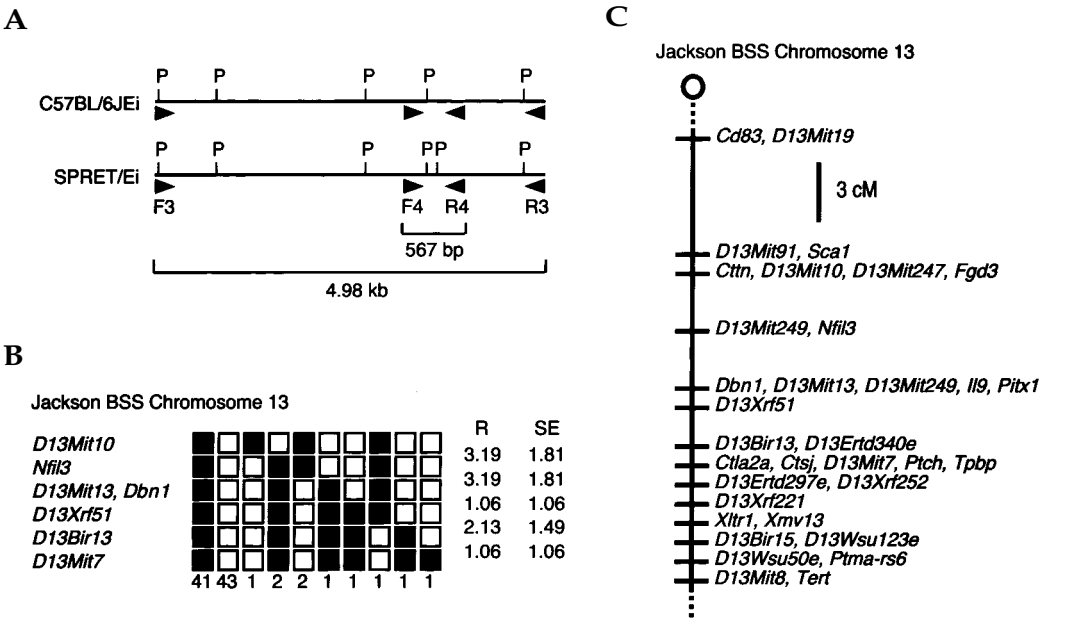

FIG. 5. Genetic mapping of mouse *Dbn1* to chromosome 13. (A) RFLP between the C57BL/6JEi and SPRET/Ei drebrin allele. The RFLP was identified by *Pst*I digestion of long-PCR products (4.98 kb) containing exons 2–8 of mouse *Dbn1*. A PCR-amplified product (567-bp) from 94 backcross animals of the Jackson BSS was analyzed to determine genotypes using this *Pst*I-RFLP. (B) Haplotype from the Jackson BSS backcross showing part of chromosome 13 with loci linked to *Dbn1*. Loci are listed in order with the gene most proximal to the centromere at the top. The black boxes represent the C57BL/6JEi allele and the white boxes the SPRET/Ei allele. The numbers listed at the bottom correspond to the number of offspring inheriting each genotype. The percentage recombination (R) between adjacent loci is given to the right of the figure, with the standard error (SE) for each R. (C) Linkage map of mouse chromosome 13 constructed from the haplotype distribution in (B). The map shows the centromere toward the top. A 3-cM scale bar is shown to the right of the figure. Loci mapping to the same position are listed in alphabetical order. Mouse *Dbn1* was mapped to the middle part of chromosome 13. Raw data from The Jackson Laboratory were obtained from http://www.jax.org/resources/documents/cmdata.

The generation of each alternative splicing variant is regulated in a tissue-specific and developmental stage-dependent manner. Drebrin E is mainly expressed in brain [1,23]. In addition, it is expressed in some non-neuronal tissues in a cell-type-specific manner [13,14,19,24]. In the mouse stomach and kidney, drebrin E is expressed in the acid-secreting cells, where it is localized specifically to the apical plasma membrane [19]. In contrast, drebrin A expression is neuron-specific [1,2,14]. The expression of drebrin E is temporarily dominant in the early stage of brain development, whereas drebrin A is upregulated in the late developmental stage of the brain and seemed to be the dominant form in the adult brain [11,14,20,23]. Therefore, analysis of the expression patterns of s-drebrin A is an important initial step towards understanding its functional roles. Because we do not yet have an antibody that specifically recognizes s-drebrin A, we explored the mRNA expression profiles of s-drebrin A using RT-PCR. Our results demonstrate that s-drebrin A is a brain-specific isoform, although the s-drebrin A expressing cells within the brain remain to be identified. We have also shown that the expression of s-drebrin A is upregulated during postnatal development of the brain. Because drebrin A expression is correlated with synapse formation [23], as assessed by the levels of synaptophysin, a synaptic vesicle membrane protein involved in synapse formation [25–27], the similar expression patterns of s-drebrin A and drebrin A suggest that s-drebrin A expression is also correlated with synapse formation.

Drebrins E and A have been shown to have actin-binding and actin-remodeling activities in fibroblasts [2,4–6]. Moreover, drebrin A functions as a regulator of the spine morphology of neuronal dendrites via actin cytoskeleton reorganization [2,9,28]. To examine whether s-drebrin A can associate with and regulate the actin cytoskeleton, we overexpressed GFP–SDA in fibroblasts. Like that of GFP–DA, most of the GFP–SDA fluorescence was colocalized with actin filaments that were stained with rhodamine-phalloidin, and strong overexpression of GFP–SDA resulted in changes in F-actin structure and cell morphology. These results were consistent with our previous findings that the actin-binding domain and actin-remodeling domain are identical and are located in the central region of drebrin A [6]. Moreover, these results indicate that s-drebrin A is sufficient to associate with F-actin and regulate the actin cytoskeleton, even though it lacks the C-terminal portion of drebrin A.

What is the physiological role of s-drebrin A? Various genes, such as the erbB family and the Homer proteins, regulate their functions by expressing truncated, short isoforms [29–32]. Drebrin A possesses an ADF-H domain [33] and a high-affinity actin-binding domain [6] in its N-terminal

region. In addition, it possesses a profilin-binding portion [7], two Homer ligand-like sequences (PPATF and PPPVF) [34], and a SH2 ligand-like sequence (YNKPP) [35,36] in the C-terminal region. In contrast, s-drebrin A possesses only the N-terminal half of drebrin A. Therefore, one attractive explanation for the physiological significance of s-drebrin A expression is that it may negatively regulate drebrin A function, which is related to the C-terminal region of drebrin A. Although drebrin A is known to be localized to the postsynaptic dendritic spines of mature cortical neurons [2,17], it remains to be determined whether s-drebrin A is localized to dendritic spines. In addition, it will be important to determine the level of s-drebrin A protein in these structures.

The human gene *DBN1* has been mapped to chromosome 5 by spot-blot hybridization using flow-sorted chromosomes [18]. It has been reported that Alzheimer's disease (AD) is a genetically complex disorder involving several genes, and multiple candidates for genetic risk factors have been mapped to human chromosomes 5, 9, and 10 [37]. We have previously reported that the brains of patients with AD show a drastic reduction of drebrin E and A in the dendritic spines of hippocampal neurons [38]. An 81% decrease in drebrin protein was seen in AD brains compared with those of healthy individuals of the same age group [39]. These data prompted us to determine the chromosomal location of mouse *Dbn1* and its proximity to other mapped genes in the mouse. Using the Jackson BSS panel, we demonstrated that mouse *Dbn1* mapped to the central portion of chromosome 13, cosegregating with *D13Mit13*, *D13Mit249*, *Il9*, and *Pitx1*. This result may provide useful information for identifying candidate genes of AD.

MATERIALS AND METHODS

Primers. The primers used in PCR are represented in Figs. 1A, 2, and 5A, and the sequences are as follows: F1, 5′-GGCTGCCATCATTGCCCAGCGGCCTGATAA-3′; R1, 5′-TCTGCCAGGGAGGCCTCAGCACCTGAGGGTGGTGT-3′; F2, 5′-AACTCGAGGCATGGCCGGCGTCAGCTTCAGCG-3′; R2, 5′-AGGGATCCTTACCCCACCCTGCCGAGGCCT-3′; F3, 5′-ATCTCCAGGGCTTGCTGCAGGTGAGGTGTG-3′; R3, 5′-GGCACCCAGCACTTGGGATGCAGTGTCAGG-3′; F4, 5′-TATTGCACCGCCTGCGCCTTCGGGAGGATG-3′; R4, 5′-CCTGAGCCTGGCGTCCAGAGCCTTCTTCCG-3′, F5: 5′-CCTCAACACTTCAGGCTGAACCCAGGGTGC-3′; R5, 5′-CCCTCGAGAAAAGCTGTAAAAGTCAGGCCCTGTGGG-3′; actin-F, 5′-GGACTCCTATGTGGGTGACGAGGCCCAGAG-3′; actin-R, 5′-GGGCCGGACTCATCGTACTCCTGCTTGCTG-3′.

RT-PCR and cDNA cloning. Both cDNA synthesis and PCR amplification were carried out in a single tube using the Superscript One-Step RT-PCR with the Platinum *Taq* system (Gibco BRL). The RT-PCR program consisted of cDNA synthesis at 52°C for 30 minutes and denaturation at 94°C for 2 minutes, followed by 35 cycles of amplification at 94°C for 20 seconds, at 72°C (or 70°C) for 30 seconds, and at 72°C for 1.5 minutes. The total RNAs (3 μg) from several tissues were analyzed by RT-PCR using drebrin-specific primers F1-R1 or F2-R2. The RT-PCR products amplified using the F1 and R1 primers were digested with *Pvu*II and inserted into the *Eco*RV site of the pBluescript II SK vector (pBSK; Toyobo, Japan). The RT-PCR products amplified using the F2 and R2 primers were inserted into the *Xho*I-*Bam*HI sites of pBSK. A pair of mouse β-actin-specific primers was used as a positive control for the RT-PCR.

Analysis of Dbn1 structure. The rat drebrin cDNA [10] was used as a probe to screen a TT2 genomic DNA library inserted into the EMBL3 vector [21]. Of the 10^6 independent phage plaques analyzed, three independent clones were positive. After restriction mapping, the positive clones were subcloned into pBSK. The PCR product that included the region of the mouse *Dbn1* from exon 13 to exon 14 (Fig. 2) was amplified from TT2 cell genomic DNA, and was cloned into pBSK. Full bidirectional sequencing of all *Dbn1* genomic fragments was carried out using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) in an ABI PRISM 377 DNA Sequencer (P. E. Applied Biosystems). Sequence data were analyzed using GENETYX software (Software Development Co., Japan).

Expression vector. A mouse cDNA encoding full-length s-drebrin A was inserted in-frame into the *Xho*I–*Bam*HI sites of the pEGFP-C1 vector (Clontech) to construct the pGFP-SDA vector, which expresses GFP-tagged s-drebrin A. The GFP-DA expression vector was prepared previously in our laboratory [9].

Cell culture and cytochemistry. CHO and COS cells were maintained in Ham's F12 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and in DMEM supplemented with 10% FBS, respectively. The cells were plated in 24-well plates and transfected with expression vectors using the TransFast transfection reagent (Promega). Two days after transfection, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100 in PBS for 15 minutes. The cells were then incubated with rhodamine-phalloidin (Molecular Probes) in PBS containing 3% bovine serum albumin (BSA) for 30 minutes to label the actin filaments. The fluorescence of GFP or rhodamine was observed using a fluorescein isothiocyanate (FITC) or rhodamine filter set, respectively.

Chromosome mapping. The Jackson Laboratory interspecific backcross panel (C57BL/6JEi × SPRET/Ei) F_1 × SPRET/Ei, denoted Jackson BSS [40], was used to determine the chromosomal location of the mouse *Dbn1*. Drebrin-specific primers F3 and R3 were used in long-PCR (95°C for 3 minutes, 30 cycles of 95°C for 35 seconds, 69.5°C for 30 seconds, 72°C for 5 minutes, and 72°C for 10 minutes) to amplify a 4.98-kb fragment from both C57BL/6JEi and SPRET/Ei genomic DNAs (Fig. 5). The long-PCR products were digested with various restriction enzymes including *Pst*I to find a restriction fragment length polymorphism (RFLP) between the C57BL/6JEi and SPRET/Ei *Dbn1* alleles. Genomic DNAs from 94 animals of the Jackson BSS backcross were analyzed by PCR (95°C for 3 minutes, 30 cycles of 95°C for 35 seconds, 71.5°C for 20 seconds, 72°C for 30 seconds, and 72°C for 2 minutes) using the F4 and R4 primers (Fig. 5). The PCR products (567-bp DNA) were digested with *Pst*I and separated by 2% agarose gel electrophoresis. The segregation patterns of the *Dbn1* RFLP were sent to The Jackson Laboratory Mapping Resource to be compared with those of other markers previously typed in the same backcross.

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Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession numbers AB064321 (s-drebrin A) and AB041033 (Dbn1).