Chapter 2
Molecular Cloning of Drebrin: Progress and Perspectives

Nobuhiko Kojima

Abstract Chicken drebrin isoforms were first identified in the optic tectum of developing brain. Although the time course of protein expression was different in each drebrin isoform, the similarity between their protein structures was suggested by biochemical analysis of purified protein. To determine their protein structures, the cloning of drebrin cDNAs was conducted. Comparison between the cDNA sequences shows that all drebrin cDNAs are identical except that the internal insertion sequences are present or absent in their sequences. Chicken drebrin are now classified into three isoforms, namely, drebrins E1, E2, and A. Genomic cloning demonstrated that the three isoforms are generated by an alternative splicing of individual exons encoding the insertion sequences from single drebrin gene. The mechanism should be precisely regulated in cell-type-specific and developmental stage-specific fashion. Drebrin protein, which is well conserved in various vertebrate species, although mammalian drebrin has only two isoforms, namely, drebrin E and drebrin A, is different from chicken drebrin that has three isoforms. Drebrin belongs to an actin-depolymerizing factor homology (ADF-H) domain protein family. Besides the ADF-H domain, drebrin has other domains, including the actin-binding domain and Homer-binding motifs. Diversity of protein isoform and multiple domains of drebrin could interact differentially with the actin cytoskeleton and other intracellular proteins and regulate diverse cellular processes.

Keywords Neural development • cDNA cloning • Alternative splicing • Homology Protein family • Actin-binding • ADF-H domain

2.1 Introduction

The organization of the nervous system is accomplished by the events which are precisely and genetically programmed during neural development. These events include neurogenesis, cell migration, axon guidance, and synapse formation. These

N. Kojima
Faculty of Life Sciences, Toyo University, Itakura-machi, Ora-gun, Gunma 374-093, Japan
e-mail: kojima033@toyo.jp

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events are likely to depend on developmental stage-dependent and cell-type-specific expression of key molecules. We demonstrated that drebrin is one of these molecules and may contribute in part to the molecular mechanisms of neural development.

Chicken drebrin isoforms were first identified as three developmentally regulated proteins (S5, S6, and S54, later renamed as E1, E2, and A) in chicken developing optic tectum by two-dimensional gel electrophoresis (Shirao and Obata 1985). Peptide mapping of partial digested proteins by a protease revealed that the digestion pattern of these proteins resembles among drebrins E1, E2, and A, suggesting that these three proteins are structurally related with each other.

Drebrin-like immunoreactive proteins exist in rat brain (Shirao et al. 1989). The proteins also showed heterogeneity, and their expression is developmentally regulated. Their independent changes in expression during neural development suggest that each play a unique role at a particular developmental stage. The distinct functions should be caused by their distinct molecular structures and developmental stage- and cell-type-specific control of expression of these proteins. To clarify their structural relationship and biological functions, the structural differences among these drebrin proteins, as well as their genomic organization, should be clarified. Cloning of drebrin cDNAs and gene revealed that the structures of these three proteins were identical except for the presence or absence of the internal sequences, and these are derived from a single gene by alternative splicing mechanism (Kojima et al. 1988, 1993). Interspecies comparison of the deduced amino acid sequences of drebrin shows a high degree of similarity, although the number of isoforms is different between chicken and mammals (Kojima et al. 1993). This suggests that functions of drebrin are important beyond species. We describe here a progress in 30 years and perspectives through the molecular cloning of drebrin.

2.2 cDNA Structure of Each Drebrin

Three isoforms of drebrin have been first identified in chicken developing optic tectum as S5, S6, and S54 of those spots are developmentally changed in their protein contents in two-dimensional gel electrophoresis (Shirao and Obata 1985). Peptide mapping using *Staphylococcus aureus* V8 protease, which selectively cleaves peptide bonds on the carboxyl side of glutamate (and in some case aspartate) residues, revealed that the digestion pattern of these proteins resembles between S5, S6, and S54, suggesting that these three proteins are structurally related with each other. Thereafter we renamed these proteins S5, S6, and S54 to drebrins E1, E2, and A, respectively.

To determine the protein structure of them, we tried to isolate the cDNA clones encoding drebrin mRNA using anti-drebrin antibody. For this purpose, we screened a lambda gt11 cDNA library prepared from chick 10-day-old embryo mRNAs. The lambda gt11 phage produces stable fusion proteins of β-galactosidase and the products of mRNAs having the same orientation and reading frame as lacZ. Thus, only

1/6 clones containing the desired sequence will express the protein. Also, incomplete cDNA clone missing important epitopes would not be recognized by the antibodies. We successfully obtained one cDNA clone (Dcw1) from a lambda gt11 chick 10-day-old embryo cDNA library by plaque hybridization with anti-drebrin antiserum (Shirao et al. 1988). The fusion protein of Dcw1 reacted with only three out of five individual monoclonal antibodies, and the length of inserted cDNA of Dcw1 was about half of that of drebrin mRNA detected by Northern blot analysis, indicating that the Dcw1 does not cover the entire cDNA for drebrin. To isolate the cDNA clones that cover the full-length drebrin sequence, Dcw1 was employed as a probe to rescreen the same cDNA library. Screening using cDNA probe instead of antibodies allows us to get the cDNA clones carrying the drebrin sequences, even though they are not inserted in the vector with the same orientation and in-flame with lacZ's reading frame. Consequently, 74 positive clones in which the cDNA inserts were strongly hybridized with the Dcw1 probe (Kojima et al. 1988). They were divided into two groups by restriction mapping. Nucleotide sequences of cDNA of Dcw6 and Dcw17, each of which was longest in each group, were separately determined (Fig. 2.1a). They were identical except that an internal 129-bp sequence, named Ins1, was present in Dcw17 and absent in Dcw6. To determine the drebrin isoform to which each cDNA clone corresponded, we raised antiserum (named anti-pep1) against the synthetic polypeptide pep1 that was the last 25 amino acid sequence of deduced Ins1. Immunoblot analysis showed that the anti-pep1 antiserum specifically reacted with drebrins E2 and A, but not drebrin E1 (Kojima et al. 1988), suggesting that drebrins E2 and A have Ins1 sequence, whereas E1 does not. Using a 80-bp sequence within Ins1 as a probe, we further isolated another group of cDNA (Dcb21) encoding the drebrin mRNA from the newly hatched chicken brain cDNA library (Kojima et al. 1993). Dcb21 had another internal 138-bp sequence, named Ins2, in the 5' direction of immediately upstream from Ins1, and the remaining part of the sequence was identical to Dcw17 (Fig. 2.1a). Calculated molecular weights of the proteins encoded in the full-length sequences of the three cDNAs Dcw6, Dcw17, and Dcb21 are 62,165, 66,553, and 71,532, respectively. Although they are about two thirds of the molecular weights estimated by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblot analysis showed that protein generated by in vitro transcription of each full-length cDNA, Dcw6, Dcw17, and Dcb21, and translation of their mRNAs in a rabbit reticulocyte lysate co-migrates with drebrins E1, E2, and A, respectively (Kojima et al. 1993), indicating that each of the three cDNAs corresponds to drebrins E1, E2, and A (Fig. 2.1a). The discrepancy between the molecular weights of drebrins predicted from the cDNA sequences and the apparent molecular weights estimated by SDS-PAGE seems to result from the anomalous migration of drebrins on SDS polyacrylamide gel, i.e., drebrins do not bind a constant weight ratio of SDS due to the unusual features of their amino acid sequences, such as a paucity of hydrophobic regions and an abundance of negatively charged amino acid residues. We raised antisera against various synthetic peptides, which covered a part of the deduced amino acid sequence of Ins2 from rat drebrin A cDNA (Shirao et al. 1992) described below, and two of them, namely DAS1 and DAS2, specifically reacted

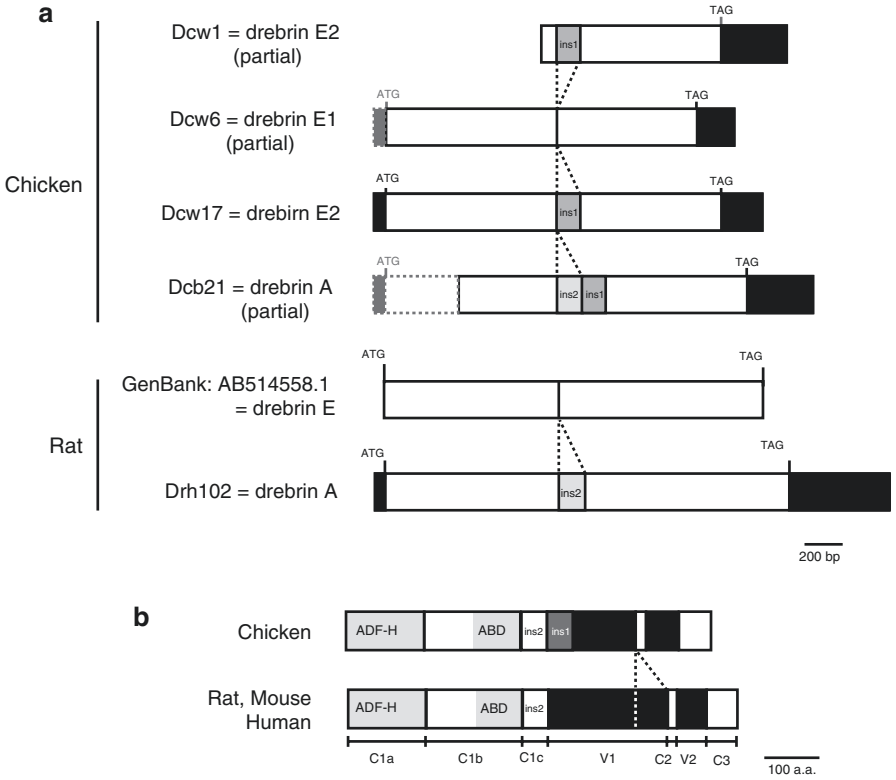

Fig. 2.1 Structure of drebrin cDNAs and domain structure of the deduced drebrin proteins. (**a**) Alignment of cDNA sequences of chicken and rat drebrin. The open rectangles and the black rectangles indicate coding regions and noncoding regions, respectively. The putative initiation codon (ATG) and the stop codon (TAG) are indicated. The shaded rectangles indicate the insertion sequences (Ins1 and Ins2). (**b**) Comparison of the protein structure of chicken drebrin A (top) and mammalian drebrin A (bottom). A diagram of the conserved (C1a, C1b, C1c, C2, and C3) and variable (V1 and V2) domains in the deduced amino acid sequence is indicated. ADF-H actin-depolymerizing factor homology. *ABD* actin-binding domain

with only drebrin A (Aoki et al. 2005). This result is consistent with the above conclusion that Dcw6, Dcw17, and Dcb21 carry the cDNA corresponding to drebrins E1, E2, and A, respectively.

Immunoblot and immunohistochemical analyses of rat tissues using an anti-drebrin antibody (mAb M2F6) raised against chicken drebrins suggest that drebrin also exists in mammalian brain (Shirao et al. 1989). Immunoblot analysis indicates that at least two distinct protein bands react to mAb M2F6 in rat brain. Their apparent molecular weights are slightly higher (approx. 130 and 140 kDa) than those of chicken drebrins (approx. 95, 100 and 110 kDa), and their isoelectric points (pI = 4.5) are similar. As in chicken the expression of each drebrin isoforms is developmentally changed in rat (Shirao et al. 1989). To obtain the rat drebrin cDNA, we constructed

Fig. 2.2 Comparison of amino acid sequence between species. Identical and substitutable amino acids compared among various species are marked with *yellow* and *green*, respectively. Ins1-H, Ins1-homology amino acid sequence

rat hippocampal cDNA library and screened the positive cDNA clones using chicken drebrin cDNA Dcw17 (encoding chicken drebrin E2) as a probe. We isolated the cDNA clone Drh102 encoding the full length of drebrin mRNA including the homologous sequence of chicken Ins2 (Shirao et al. 1992) and later a non-published cDNA variant (GenBank: AB514558.1) encoding another drebrin mRNA that lacks Ins2 in its sequence (Fig. 2.1a). Ins2 sequence is composed of 46 amino acids and exhibits 85% identity between chicken and rat (Fig. 2.2), suggesting that the rat cDNAs with and without the Ins2-homologous insertion sequence are corresponding to rat drebrin A and drebrin E, respectively. Unlike Ins2 sequence, there is no drebrin cDNA that lacks Ins1 sequence in its sequence. However, the alignment of the sequence just behind the Ins2 sequence shows some similarity to the chicken Ins1 sequence. Although identity is low (44%), the sequence comparison including substitutable amino acids in addition of identical amino acids shows the higher similarity between chicken and mammals (81%) (Fig. 2.2). We further tried to isolate another cDNA variant which does not have Ins1 in its sequence by reverse transcription-polymerase chain reaction (RT-PCR) of mouse brain mRNAs, but we have not isolated such mouse version of drebrin E1. Instead, besides the two isoforms drebrin E and drebrin A in rodent, we isolated the truncated form of drebrin, named by s-drebrin A, which lacks C-terminal half of drebrin A (Jin et al. 2002), as described below.

Interspecies comparison of the deduced amino acid sequences of drebrin between chicken and rodent shows a high degree of similarity (Fig. 2.1b; see also Fig. 1.3 in Chap. 1) (Kojima et al. 1993), suggesting that biological functions of drebrin are important beyond species. In particular, the homology of the N-terminal half (named domain C1) and two short regions in the C-terminal region (domains C2 and C3) is greater than 85%. These regions are also conserved in human (Toda et al. 1993). The secondary structure prediction by the method of Kyte and Doolittle (1982) suggests that the large homologous region domain C1 can be subdivided into three. The most N-terminal domain C1a is relatively hydrophobic and has potential to form repeating stretches of β-sheet that are separated by short stretches of α-helix. This region

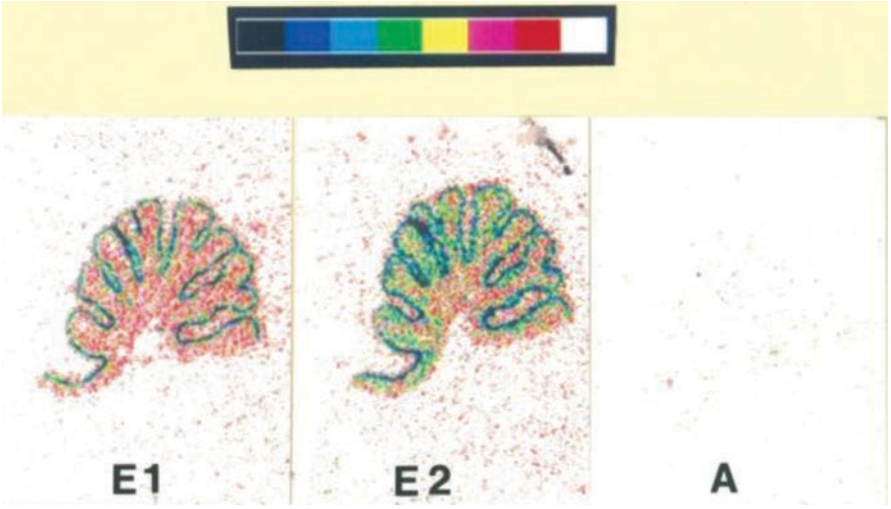

Fig. 2.3 Isoform-specific distribution of drebrin in the developing chicken cerebellum. Chicken drebrin E1 probe (GCAGCCATAGGACAGACTCCCCCAGCCCCA) hybridized strongly to the external granular layer, but not to the internal granular layer. Chicken drebrin E2 probe (GCAGCCATAGGACAGGCAGCCAGTCCGACT) hybridized to both the external and internal granular layers. There was no significant hybridization with the A probe (CGTCTGCACTGTCCTTTCATAAAGACAGCT). (Adopted from Shirao et al. 1990)

is followed by a hydrophilic, elongated α-helical region (domain C1b). Ins2 sequence is included in domain C1c, in which β-turns are concentrated. A repeat of seven to nine serine residues within a middle part of Ins2 is conserved between chicken and mammals. This highly conserved domain C1 may contribute to expression and regulation of biological function of drebrin. By analogy with other proteins, domain C1a may organize into a relatively stable β-sheet and form a globular core, named by actin-depolymerizing factor homology (ADF-H) domain (Lappalainen et al. 1998; Poukkula et al. 2011). The systematic analysis using various fragments of drebrin cDNA reveals that 85-amino acid sequence within the domain C1b (also called as coiled-coil domain) contributes to the binding to and remodeling of F-actin (Hayashi et al. 1999; Grintsevich et al. 2010). In contrast to the homologous domains, the sequence of the C-terminal half is diverged and shows low homology among species. This variable region is separated into two domains V1 and V2 by a short conserved domain C2. The domain V1 has a potential to form repeating stretches of α-helix with several β-turns. Although a repeat of ten proline residues observed in this domain of rat drebrin does not exist in that of chicken drebrin, and positioning of proline residue in domain V1 is not identical, proline-rich feature is retained among species. Proline residue generally facilitates the formation of protein secondary structure elements such as turns and destabilizes α-helix and β-sheet conformation and serves a site for protein-protein interactions. Mammoto et al. reported that a proline-rich region of this domain may contribute to the interaction with other proteins such as actin-binding protein profilin (Mammoto et al. 1998). The sequence of Ins1 in chicken is included in this domain. Although these conserved domains in

the C-terminal region do not exhibit domain structure, a binding motif (PPXXF or PXXF) for Homer (Tu et al. 1998), a scaffolding protein of metabotropic glutamate receptors, exists in domain C2 of both chicken and rodents, and in domain C3 of rodents but not of chicken, suggesting that the conserved domains in the C-terminal region serve a function in interaction with intracellular proteins other than F-actin.

The structural difference between the adult and embryonic isoforms is a 46-amino acid sequence (Ins2). This sequence is absent from embryonic isoforms of drebrin (drebrin E in mammals and drebrins E1 and E2 in chicken) but present in drebrin A and s-drebrin A. As we mentioned previously, rat drebrin E is more similar to chicken drebrin E2 than drebrin E1 because drebrin E has Ins1 homologous sequence (1ns1-H in Fig. 2.2). Then does not rat need the other drebrin isoform that is similar to chicken drebrin E1?

In order to analyze the isoform-specific distribution of drebrins E1 and E2 in the chicken brain, we produced the 30-mer oligonucleotide probes corresponding to the joining region of each drebrin mRNA and successfully identified the localization of each drebrin mRNA in embryonic day 16 cerebellum by in situ hybridization (Shirao et al. 1990). Chicken drebrin E1-specific probe hybridized strongly to the external granular layer, but not to the internal granular layer. Chicken drebrin E2-specific probe hybridized to both the external and internal granular layers. There was no significant hybridization with the drebrin A-specific probe in the developing cerebellum (Fig. 2.3). Thus, the drebrin E1 is expressed in newly generated neurons but not in migrating neurons, and drebrin E2 is expressed in migrating and neurite growing neurons in addition to newly generated neurons, suggesting that drebrin E1 plays a part of drebrin E2 roles. In other words, drebrin E1 is not necessary for optic tectum development, and this might be a reason why rat does not need the other drebrin isoform similar to chicken drebrin E1 in the brain.

The drebrin isoforms exhibit differences in (1) the time course of their expression during neuronal development (Shirao et al. 1989), (2) the type of cells or tissues expressing drebrin (Luna et al. 1997; Keon et al. 2000; Peitsch et al. 1999; Shirao and Obata 1986; Shirao et al. 1987, 1994), and (3) their intracellular distribution (Shirao and Obata 1986; Shirao et al. 1987; Aoki et al. 2005; Sasaki et al. 1996; Song et al. 2008). We have, therefore, hypothesized that drebrin E and drebrin A are functionally different in the brain. To know the genomic relationship between drebrin isoforms, we next isolated and analyzed the drebrin gene.

2.3 Drebrin Isoforms Produced by Alternative Splicing

Ribonuclease protection assay is a highly sensitive technique for detecting and quantifying the specific mRNA in RNA samples. Using this assay with a radioisotope-labeled antisense-stranded cRNA probe of the drebrin fragment containing the insertion sequences, we revealed that stage- and tissue-specific expression of each drebrin isoform is regulated at the transcriptional level (Kojima et al. 1993). Drebrin E is widely expressed in the embryonic brain and other various non-neuronal tissues including the intestine, heart, and skeletal muscle. On the other hand, drebrin A is expressed in a neural tissue-specific fashion and is predominant in the postnatal brain.

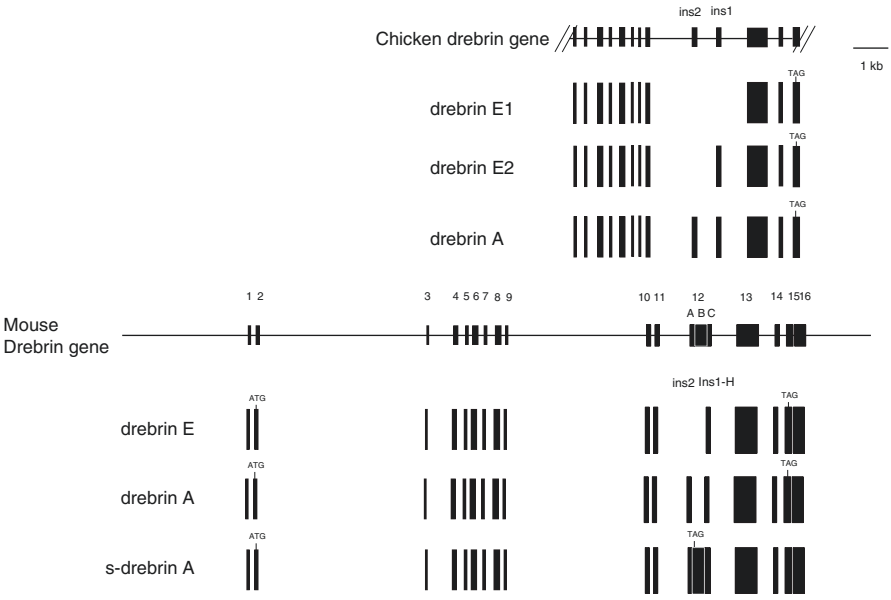

Fig. 2.4 Structural organization of drebrin gene. The genomic region is partial in chicken and covered full-length transcripts in mouse. Exons are indicated by rectangles in each genome. The selected exons in each drebrin mRNA isoform are indicated. The putative initiation codon (ATG) and the stop codon (TAG) are also indicated. See text for details

To clarify the genetic correlation of each drebrin isoform, a drebrin genomic clone was isolated from the chicken and mouse genomic library by drebrin cDNA as a probe (Kojima et al. 1993, 2010; Jin et al. 2002). The exact location of the exons was confirmed through comparison with the cDNA sequence in partial chicken drebrin gene and mouse drebrin gene covering full-length transcripts (Fig. 2.4). The exon boundaries in cDNA sequence are well conserved between chicken and mouse, that is, the cDNA sequence for domain C1a is located in exons 2 to 5; the sequence for domain C1b is in exons 6 to 11; the sequence for domain C1c (Ins2) is in exon 12A[1]; the sequences for domains V1, C2, and a half part of C3 are in exons 12C to 13; and the sequence for the remaining half part of C3 is in exons 14 to 15. Striking feature is that the sequences of Ins2 and Ins1 (Ins1-homology in mouse) are independently encoded in the individual exons of chicken genome. This fact demonstrates that the mechanism for generating drebrin isoform mRNAs from a single drebrin gene is likely to be according to a common mechanism for an alternative splicing that is the process by which the exons of primary transcripts (pre-mRNAs) from genes can be spliced in different combinations to produce structurally and functionally distinct mRNA and protein variants (Roy et al. 2013). Unidentified trans-acting factor(s) expressed in a tissue-specific and a stage-dependent manner

[1] Exon 12 was referred to as exon 11 in the original reports published before the discovery of the initial (5' terminal) exon (Jin et al. 2003; Kojima et al. 2010).

should regulate the alternative splice site selection of the drebrin gene. Similarly, in the mouse drebrin gene, Ins2 sequence is encoded in the 5'-terminal region of exon 12 (exon12A). Ins1-H sequence is encoded in the 3'-terminal region of exon 12 (exon 12C). Further, sequence analysis in mouse drebrin gene has shown that the 319-nucleotide sequence in the middle region of exon 12 (exon 12B) is excluded as an intron in drebrin E and drebrin A but included as an exon in s-drebrin A (Fig. 2.4). The fact that there is no significant sequence homology of exon 12B in the intron located between exons encoding Ins2 and Ins1 in chicken genome may suggest the difference in isoform constitution between chicken and rodent, that is, the existence of s-drebrin A and the absence of drebrin E1 in rodent. Because of the existence of a stop codon located in-frame at the nucleotide 7 in exon 12B, s-drebrin A possesses only the N-terminal half of drebrin A (including Ins2) but lacks the C-terminal half. We confirmed the actin-remodeling activity of this isoform in s-drebrin A-transfected fibroblasts (Jin et al. 2002). We, however, have not yet known the protein level and cellular distribution of s-drebrin A in the brain as a comparison with those of drebrin A.

2.4 Protein Family to Which Drebrin Belongs

As described above, drebrin protein is well conserved in chicken and mammals including human with a slight variation in isoform constitution. What is the origin of drebrin protein? The current knowledge for the ancestor protein and homologous family proteins of drebrin is overviewed below.

First reported protein which shows a significant similarity to drebrin is SH3P7 (Sparks et al. 1996). SH3P7 (also known as Abp1) is an actin-binding protein identified as a SH3 domain-containing protein (Sparks et al. 1996). The N-terminal half of SH3P7 is highly homologous to the N-terminal region of drebrin, and the SH3 domain of SH3P7 is similar to that of another actin-binding protein cortactin (Kessels et al. 2000). SH3P7 is located primarily in dendrites and co-localized with drebrin in dendritic spines (Yamazaki et al. 2001). The N-terminal region of drebrin and SH3P7 is now known as the ADF-H domain (Lappalainen et al. 1998; Poukkula et al. 2011). The ADF-H domain is an actin-binding module, which is present in members of a protein family that consists of five phylogenetically distinct classes: ADF/cofilins, twinfilins, coactosins, glia maturation factors (GMFs), and drebrin/Abp1s (Fig. 2.5) (Poukkula et al. 2011). Based on the structural homology, it has been proposed that these five classes of ADF-H domain proteins have evolved from a common ancestral protein. Although ADF-H domains between different protein classes share less than 20% sequence identity at the amino acid level, the predicted secondary structure is well conserved throughout the entire family. It is likely that all ADF-H domains have a similar three-dimensional fold composed of five internal β-strands surrounded by at least four α-helices (Poukkula et al. 2011). Such conserved three-dimensional structures of ADF-H domain proteins appear to promote cytoskeletal dynamics by interacting with actin. However, differences in

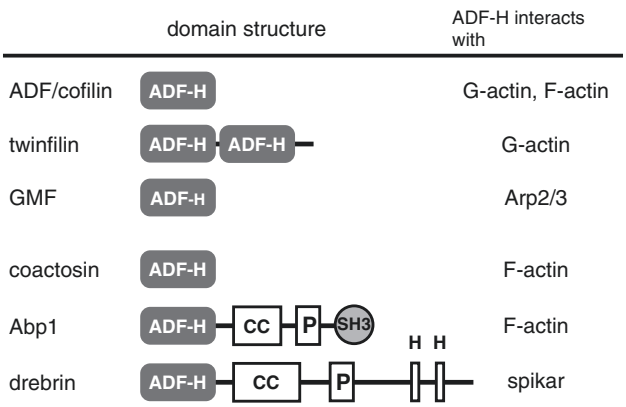

Fig. 2.5 A diagram comparing the domain structures of the ADF-H domain proteins and their ability of ADF-H domain to bind other proteins. ADF/cofilins, glia maturation factor (GMF), and coactosin consist of single ADF-H domain. Twinfilin is composed of two tandem repeats of ADF-H domain. Abp1 and drebrin are multidomain proteins that have single ADF-H domain in addition of coiled-coil domain (CC), proline-rich domain (P), SH3 domain (SH3), and Homer-binding motifs (H). (Modified from Poukkula et al. 2011)

the chemical properties of surface-exposed residues and small structural variations are responsible for the diversity in domain architectures and biochemical functions between distinct classes of ADF-H domain proteins. Of the ADF-H domain proteins, twinfilin interacts with G-actin, and ADF/cofilin binds both G-actin and F-actin, whereas drebrin/Apb1 and coactosin bind F-actin but not G-actin. GMF does not bind actin but interacts directly with Arp2/3 complex, a key regulator of F-actin nucleation. The binding of drebrin to F-actin may not be achieved via the ADF-H domain but via the actin-binding domain located downstream of ADF-H domain (Hayashi et al. 1999; Grintsevich et al. 2010). Therefore, drebrin ADF-H domain is unlikely to make a significant contribution to actin binding. We have recently discovered novel drebrin partner, named spikar, which directly interacts with drebrin ADF-H domain using yeast two-hybrid system (Yamazaki et al. 2014). Thus, ADF-H domain of drebrin is likely to have a distinct role from other members of ADF-H domain proteins.

2.5 Conclusions and Perspectives

A class of ADF-H domain protein family, drebrin/Abp1 shows higher homology with each member. After drebrin was first identified in vertebrate (Shirao and Obata 1985), Abp1 was identified as an actin-interacting protein in budding yeast (Drubin et al. 1988). Later Abp1 was also found in mammals (Sparks et al. 1996; Kessels et al. 2000; Yamazaki et al. 2001). Drebrin is predominantly expressed in the brain (Shirao and Obata 1985). Drebrin A is a neuron-specific isoform and enriched in

dendritic spines, whereas drebrin E (E1 and E2 in chicken) is expressed predominantly in the embryonic brain and in a wide range of non-neuronal tissues. All the isoforms are generated from a single gene by alternative splicing. Unidentified trans-acting factor(s) expressed in a tissue-specific and a stage-dependent manner should regulate the alternative splice site selection of the drebrin gene. Although in vitro study does not show the difference in F-actin-binding activity between two isoforms (Ishikawa et al. 1994), in vivo interaction of F-actin and drebrin E might not be stronger than that of F-actin and drebrin A (Aoki et al. 2005; Kojima et al. 2010). Recently our studies using drebrin A-specific knockout mice also indicate fundamentally different biological activities between drebrin E and drebrin A (Kojima et al. 2010, 2016). It is important to note how the internal insertion sequence Ins2 influences the biological activities of drebrin. Since Ins2 has several unique features in its amino acid sequence (i.e., a long stretch of serine residue and repeats of β-turn), the insertion of Ins2 into drebrin may result in a change of the tertiary structure by phosphorylation or by binding with certain specific proteins. Also, it will be important to elucidate whether its activity is complemented by other functionally similar proteins, since mice homozygous for null mutation of drebrin does not show overt abnormalities in many aspects of brain anatomy and animal behavior.

References

Aoki C et al (2005) Drebrin A is a postsynaptic protein that localizes in vivo to the submembranous surface of dendritic sites forming excitatory synapses. J Comp Neurol 483:383–402

Drubin DG et al (1988) Yeast actin-binding proteins: evidence for a role in morphogenesis. J Cell Biol 107(Pt 2):2551–2561

Grintsevich EE et al (2010) Mapping of drebrin binding site on F-actin. J Mol Biol 398:542–554

Hayashi K et al (1999) Domain analysis of the actin-binding and actin-remodeling activities of drebrin. Exp Cell Res 253:673–680

Ishikawa R et al (1994) Drebrin, a development-associated brain protein from rat embryo, causes the dissociation of tropomyosin from actin filaments. J Biol Chem 269:29928–29933

Jin M et al (2002) A novel, brain-specific mouse drebrin: cDNA cloning, chromosomal mapping, genomic structure, expression, and functional characterization. Genomics 79:686–692

Keon BH et al (2000) Isoform specific expression of the neuronal F-actin binding protein, drebrin, in specialized cells of stomach and kidney epithelia. J Cell Sci 113(Pt 2):325–336

Kessels MM, Engqvist-Goldstein AEY, Drubin DG (2000) Association of Mouse Actin-binding Protein 1 (mAbp1/SH3P7), an Src Kinase Target, with Dynamic Regions of the Cortical Actin Cytoskeleton in Response to Rac1 Activation. Mol Biol Cell 11:393–412

Kojima N et al (1988) Nucleotide sequences of two embryonic drebrins, developmentally regulated brain proteins, and developmental change in their mRNAs. Brain Res 464:207–215

Kojima N et al (1993) Molecular cloning of a developmentally regulated brain protein, chicken drebrin A and its expression by alternative splicing of the drebrin gene. Brain Res Mol Brain Res 19:101–114

Kojima N et al (2010) Genetic disruption of the alternative splicing of drebrin gene impairs context-dependent fear learning in adulthood. Neuroscience 165:138–150

Kojima N et al (2016) Drebrin A regulates hippocampal LTP and hippocampus-dependent fear learning in adult mice. Neuroscience 324:218–226

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157:105–132
Lappalainen P et al (1998) The ADF homology (ADF-H) domain: a highly exploited actin-binding module. Mol Biol Cell 9:1951–1959
Luna EJ, Pestonjamasp KN, Cheney RE, Strassel CP, Lu TH, Chia CP, Hitt AL, Fechheimer M, Furthmayr H, Mooseker MS (1997) Actin-binding membrane proteins identified by F-actin blot overlays. Soc Gen Physiol Ser 52:3–18
Mammoto A et al (1998) Interactions of drebrin and gephyrin with profilin. Biochem Biophys Res Commun 243:86–89
Peitsch WK, Grund C, Kuhn C, Schnölzer M, Spring H, Schmelz M, Franke WW (1999) Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells. Eur J Cell Biol 78:767–778
Poukkula M et al (2011) Actin-depolymerizing factor homology domain: a conserved fold performing diverse roles in cytoskeletal dynamics. Cytoskeleton 68:471–490
Roy B et al (2013) Review: alternative Splicing (AS) of genes as an approach for generating protein complexity. Curr Genomics 14:182–194
Sasaki Y, Hayashi K, Shirao T, Ishikawa R, Kohama K (1996) Inhibition by drebrin of the actin-bundling activity of brain fascin, a protein localized in filopodia of growth cones. J Neurochem 66:980–988
Shirao T, Obata K (1985) Two acidic proteins associated with brain development in chick embryo. J Neurochem 44:1210–1216
Shirao T, Obata K (1986) Immunochemical homology of 3 developmentally regulated brain proteins and their developmental change in neuronal distribution. Brain Res 394:233–244
Shirao T, Inoue HK, Kano Y, Obata K (1987) Localization of a developmentally regulated neuron-specific protein S54 in dendrites as revealed by immunoelectron microscopy. Brain Res 413:374–378
Shirao T et al (1988) Molecular cloning of a cDNA for the developmentally regulated brain protein, drebrin. Brain Res 464:71–74
Shirao T et al (1989) Two forms of drebrins, developmentally regulated brain proteins, in rat. Proceedings of the Japan Academy 65:169–172
Shirao T et al (1990) Expression of three drebrin isoforms in the developing nervous system. Neurosci Res Suppl 13:S106–S111
Shirao T et al (1992) Cloning of drebrin A and induction of neurite-like processes in drebrin-transfected cells. Neuroreport 3:109–112
Shirao T, Hayashi K, Ishikawa R, Isa K, Asada H, Ikeda K, Uyemura K (1994) Formation of thick, curving bundles of actin by drebrin A expressed in fibroblasts. Exp Cell Res 215:145–153
Song M et al (2008) Expression of drebrin E in migrating neuroblasts in adult rat brain: coincidence between drebrin E disappearance from cell body and cessation of migration. Neuroscience 152:670–682
Sparks AB et al (1996) Cloning of ligand targets: systematic isolation of SH3 domain-containing proteins. Nat Biotechnol 14:741–744
Toda M et al (1993) Molecular cloning of cDNA encoding human drebrin E and chromosomal mapping of its gene. Biochem Biophys Res Commun 196:468–472
Tu JC et al (1998) Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. Neuron 21:717–726
Yamazaki H et al (2001) Molecular cloning and dendritic localization of rat SH3P7. Eur J Neurosci 14:998–1008
Yamazaki H et al (2014) Spikar, a novel drebrin-binding protein, regulates the formation and stabilization of dendritic spines. J Neurochem 128:507–522