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Tomoaki Shirao
Yuko Sekino *Editors*

Drebrin

From Structure and Function to Physiological and Pathological Roles

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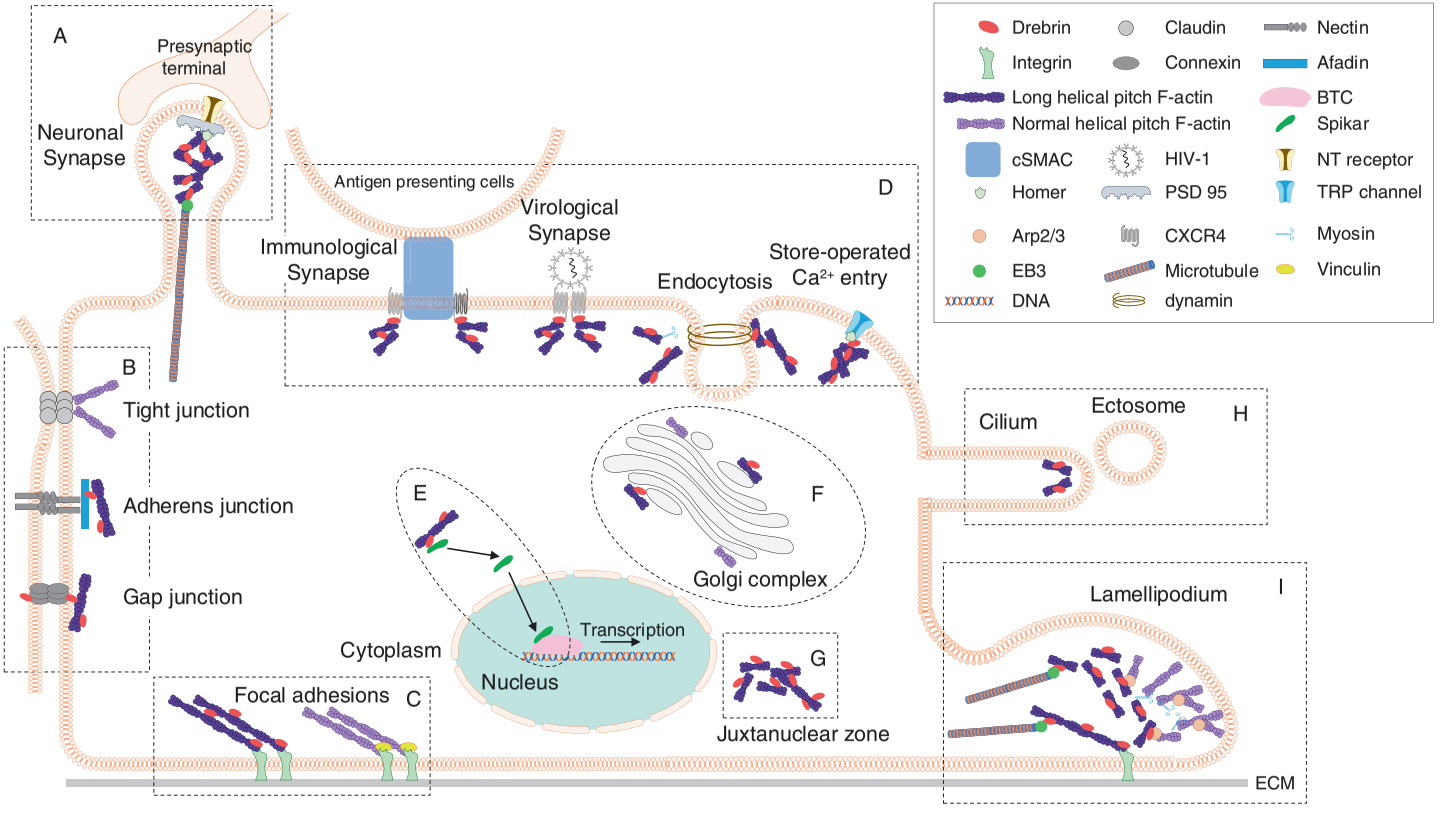

Drebrin forms unique stable F-actin with a long helical crossover and plays a pivotal role in intercellular communication at neuronal synapses, adherens and gap junctions, and immunological and virological synapses. Additionally, drebrin is involved in the cellular mechanisms of cell migration, cell process formation, cancer metastasis, and gene transcription through a transcription co-activator spikar. Drebrin is also found on the Golgi complex, at the juxtanuclear zone, and at a tip of cilia

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Editors

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This book is dedicated to our mentor Dr. Kunihiko Obata whose wise counsel has led us to the discovery and a deeper understanding of drebrin.

Preface

This book consolidates drebrin studies that have accumulated over three decades, since the first identification of drebrin by our group in 1985. Although in the 1980s we could not envision exactly how the study of drebrin would develop, the progress reflected in the chapters presented here was beyond our wildest expectations. This book begins with a general introduction of drebrin from a historical perspective, and then the chapters in the second part provide the molecular characterization of drebrin and drebrin-decorated F-actin. The third and fourth parts discuss its function in the nervous and non-nervous system, respectively.

This review will appeal to researchers who are interested in synapse formation and synaptic plasticity, as well as subcellular local morphogenesis, such as cell protrusion formation, cell migration, intercellular junction formation, and endocytosis. The book will also appeal to researchers who use drebrin as a tool, such as a marker of synaptic function or a disease marker. This book was kept as concise as possible, to be understood by readers from diverse scientific disciplines. Because of the clarity of its presentations, it can also serve as a textbook in graduate courses.

We wish to express our gratitude to the authors who so willingly contributed to this book. We would also like to thank the staff of Springer Japan, in particular Ms. Momoko Asawa and Dr. Yasutaka Okazaki.

Maebashi, Japan — Tomoaki Shirao
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Contents

Part I
History of Drebrin Discovery as an General Introduction

Chapter 1
General Introduction to Drebrin

Tomoaki Shirao and Yuko Sekino

Abstract Drebrin was first discovered by our group as "developmentally regulated brain protein" from the chicken optic tectum. Drebrin is an actin-binding protein, which is classified into two major isoforms produced by alternative splicing from a single *DBN1* gene. The isoform predominantly expressed in the adult brain (drebrin A) is neuron specific, containing a neuron-specific sequence (Ins2) in the middle of the molecule. Drebrin A is highly concentrated in dendritic spines, and its accumulation level is regulated by synaptic activity. In contrast, drebrin E, which lacks Ins2, is found in widespread but not ubiquitous cell types in various tissues. The isoform conversion from drebrin E to drebrin A occurs in parallel with synaptogenesis. Drebrin decorating F-actin is found at the recipient side of cell-cell communication systems, such as gap junctions, adherens junctions, immunological synapses, and neuronal synapses. In addition, it is involved in the cellular mechanisms of cell migration, cell process formation, cancer metastasis, and spermatogenesis. Lack of drebrin leads to the dysfunction of cell-cell communication, resulting in aberrant migration of metastatic cancer cells, aberrant synaptic function in dementia, and rupture of endothelial integrity. Because drebrin forms a unique F-actin with a longer helical crossover, drebrin may create an F-actin platform for molecular assembly and play a pivotal role in intercellular communication.

Keywords Alternative splicing • Cancer • Cell migration • Intercellular communication • Physical property of actin filament • Synaptogenesis • Synaptic plasticity

1.1 Introduction

Drebrin was first discovered by our group as "developmentally regulated brain protein" from the chicken optic tectum in 1985 (Shirao and Obata 1985). In the first 15 years after the discovery, no other groups except us were interested in drebrin,

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which is expressed in the nervous tissue. During these years, we purified chicken and rat drebrins, raised polyclonal and monoclonal antibodies, and cloned *DBN1* cDNAs. Consequently, we have identified major isoforms of drebrin in chicken, rodent, feline, and human expressed in the nervous tissue. We further clarified the genetic and biochemical properties of drebrin, such as actin-binding activity and phosphorylation. The expression of each isoform depends on the developmental stage. Because the isoform predominantly expressed in adult brain (drebrin A) is neuron specific, our later studies were mainly focused on drebrin A (Shirao et al. 2017).

In 1996, we found that drebrin A is highly concentrated in dendritic spines in adult rat brain, forms a complex with actin and myosin, and inhibits the actin-activated ATPase activity of myosin II (Hayashi et al. 1996). Thus, we proposed that drebrin may play a role in the structure-based plasticity of synapses through the actin-linked control of the actomyosin interaction in dendritic spines. In 1999, we successfully showed that exogenously expressed drebrin A specifically elongates dendritic spines of primary cultured neurons (Hayashi and Shirao 1999). This was the first report demonstrating that the manipulation of a single actin-binding protein in a neuron alters spine morphology. After these epoch-making findings, drebrin and the actin cytoskeleton in dendritic spines were thrown into the limelight. Since then we have shown the pivotal roles of drebrin in spine formation (Takahashi et al. 2003; Aoki et al. 2005) and synaptic plasticity (Takahashi et al. 2006; Mizui et al. 2014; Sekino et al. 2006). Nowadays, hundreds of spine-resident proteins have been found, but drebrin is still a key protein in modulating the actin cytoskeleton in dendritic spines (Sekino et al. 2007; Koganezawa et al. 2017).

Actin-binding proteins modulate the characteristics of the actin cytoskeleton and consequently regulate cell structures or produce the motile force of cells. Drebrin isoforms other than drebrin A are widely distributed in nonnervous tissues as well as the nervous tissue, not only in avian (Shirao and Obata 1986) and mammals (Shirao et al. 1994; Peitsch et al. 1999) but also in the soil amoebae (Luna et al. 1997). Furthermore, drebrin has been found at the recipient side of various intercellular communication systems, such as gap junctions, adherens junctions, immunological synapses, and neuronal synapses. This suggests the universal role of drebrin as an actin modulator.

How does drebrin change F-actin structures? Why does drebrin appear at the cell-cell communication sites? More generally, what is the physiological function of drebrin? This chapter will briefly introduce the key discoveries and proposals contributing to elucidating the above questions.

1.2 Historical Orientation

1.2.1 Background of Drebrin Study

The development of the brain is achieved by a combination of several fundamental processes, such as the proliferation and migration of neurons, the directed extension of nerve fibers, and synapse formation. Before 1960 classic morphological

techniques were used for the study of brain development, because morphological structures of the brain dramatically change when each process occurs. In the 1960s and 1970s, developmental studies were accelerated by the progression of new technologies such as the autoradiography using tritiated thymidine. These new methods disclosed in detail the birth date of each neuron, the layer formation, and subsequent maturation in mammalian cerebral and cerebellar cortices and in the chicken optic tectum. However, the molecular mechanism of each process was not yet clarified.

To disclose the molecular mechanism of the brain development, the identification of the master proteins that govern each fundamental process was eagerly pursued. One approach was to select a key function in each developmental process and to look for the protein(s) that mediates that function. Adopting this approach, Edelman and his collaborators developed a specific immunological assay for molecules involved in cell adhesion (Brackenbury et al. 1977) and discovered cell adhesion molecules (CAMs) as key molecules in brain development (Hoffman et al. 1982).

Another approach was based on the conjecture that the master proteins are expressed at limited developmental stages in a restricted region of the brain. Sperry hypothesized the presence of two orthogonal gradients of molecules on retinal ganglion neurons that determine specific connections between retinal and tectal neurons (Sperry 1963), and Nirenberg's group identified an antigen that is distributed in a dorsal-ventral topographic gradient in chick embryo retina by screening a library of monoclonal antibodies in 1981 (Trisler et al. 1981).

1.2.2 Discovery of Drebrin by Proteomics

In January 1982, we started seeking for yet-to-be-discovered master proteins in the developing brain. We surveyed the changes in the proteome of the developing brain using O'Farrell's two-dimensional gel electrophoresis (2DGE) (O'Farrell 1975). The chicken optic tectum was chosen as the target region, because it is a uniform and regularly layered structure that develops correctly on a timetable, as revealed by Cowan and colleagues (LaVail and Cowan 1971a, b). After the electrophoresed gel was stained with Coomassie brilliant blue, 54 proteins were counted (Fig. 1.1). Most of them were found at the beginning (4-day embryo) and remained unchanged until adulthood. There were eight proteins that remarkably changed their staining intensities during embryonic development (Shirao and Obata 1985). These eight proteins were further classified into three groups. The first group was monotonically increasing proteins, including neurofilament proteins and drebrin A (adult-type isoform). The second group was monotonically decreasing proteins. The third group was intensely stained only at embryonic stages and was later named chicken drebrin E1 and E2 isoforms. Note that in mammals there is only one embryonic isoform named drebrin E, while chickens have two embryonic isoforms. The developmental changes in the amount of drebrins in the optic tectum are shown in Fig. 1.2. Drebrin isoforms were found with similar developmental changes in other brain regions. However, the time course of their changes varied from region to region. Even within the optic tectum, developmental changes in drebrin occur earlier in the rostral

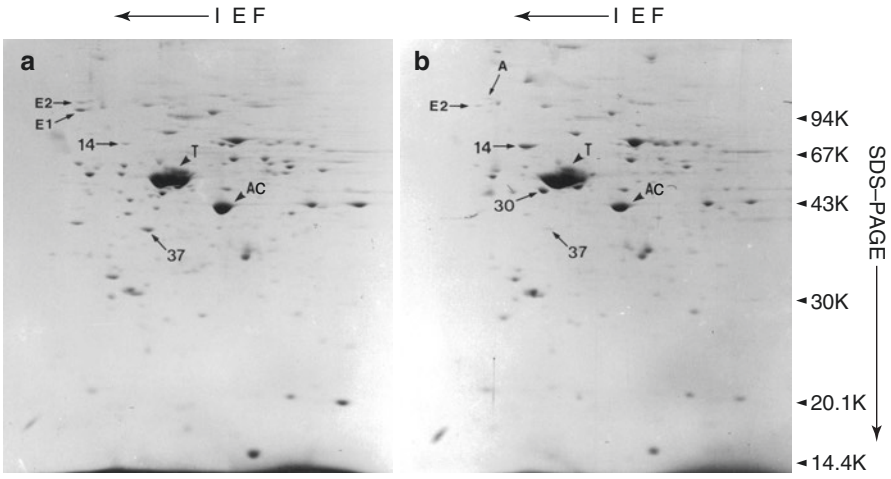

Fig. 1.1 Two-dimensional patterns of proteins of optic tecta. (**a**) Seven-day chick embryo. (**b**) Newly hatched chicken. *A* drebrin A, *E2* drebrin E2, *E1* drebrin E1, *T* tubulin, *Ac* actin. Coomassie Brilliant Blue staining

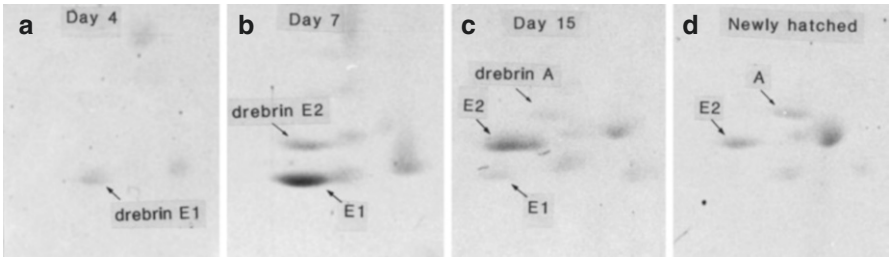

Fig. 1.2 Developmental changes of drebrin isoforms in the chick optic tectum. Panels are regions of interest in two-dimensional gel electrophoresis. (**a**) Day 4. (**b**) Day 7. (**c**) Day 15. (**d**) Newly hatched chick. Coomassie Brilliant Blue staining

portion than in the caudal portion, which corresponds to the rostro-caudal gradient of histological development (LaVail and Cowan 1971a). Together, these results suggest that the changes in drebrin expression are paralleled with brain development, which are explained in detail in Part III of this book.

1.2.3 Purification of Drebrin

In 1985, we succeeded to purify drebrin E1 and E2 from embryonic day (ED) 11 chicken brains (Shirao and Obata 1985). We used the 2DGE assay and found that all drebrins were recovered in the same fractions by various purification methods such as isoelectric precipitation, ammonium sulfate precipitation, and ion-exchange