CHAPTER 11

CtBPs as Synaptic Proteins

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Summary

A surprising new aspect of CtBP family proteins arose from the identification of a novel CtBP protein named RIBEYE.[1] RIBEYE, which consists of a unique amino-terminal A-domain and a carboxy-terminal B-domain, largely identical to CtBP2, was discovered not as a nuclear protein but as a major component of synaptic ribbons in mammalian retina.[1] Ribbon synapses are structurally specialized, tonically active chemical synapses, and are present, for example, in the sensory neurons of the retina and the inner ear.[2,3] Recently, we identified also CtBP1, the founder member of the CtBP family,[4] as an active zone component at conventional and ribbon synapses.[5] The discovery of synaptic CtBP family members highlights that CtBP proteins serve more functions than previously envisioned.

Chemical Synapses and Synaptic Transmission

Chemical synapses are highly complex contact sites between neurons specialized for the rapid and efficient transmission of synaptic signals. Ultrastructurally, distinct pre and postsynaptic regions mark the sites of neurotransmitter release and reception (Fig. 1A). In the synaptic terminals, neurotransmitter-filled synaptic vesicles translocate to a specialized region of the presynaptic plasma membrane, the active zone. Here they undergo an ATP-dependent priming step that makes them releasable by exocytosis. Activity-triggered Ca^{2+} influx through voltage-gated Ca^{2+} channels triggers fusion of the primed synaptic vesicles with the plasma membrane and subsequent neurotransmitter release into the synaptic cleft. After exocytosis, the synaptic vesicle membrane is rapidly retrieved by endocytosis, refilled with neurotransmitter and recycled for a new round of the synaptic vesicle cycle.[6] A specialized cytomatrix at the active zone spatially organizes these events in the presynaptic terminal. This cytomatrix at the active zone (CAZ) is an electron-dense cytoskeletal meshwork, which extends into the synaptic terminal where it associates with synaptic vesicles.[7] The mature CAZ is defined by a set of multidomain proteins that harbor several protein-protein or protein-lipid interaction domains. It includes proteins like Munc13-1,[8] RIMs,[9,10] ERC/CAST,[11,12] Piccolo/Aczonin and Bassoon.[13-15] The complete protein composition of the CAZ is not known to date, as it is not known how the CAZ organizes the synaptic vesicle cycle.

Ribbon Synapses

Photoreceptors and bipolar cells in the retina and hair cells in the cochlea transmit light and sound signals, respectively, over a dynamic range of several orders of magnitude in intensity. They continuously adjust their synaptic output to changing inputs thus, optimizing the information transfer. Such a finely graded synaptic output requires the release of several

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CtBP Family Proteins, edited by G. Chinnadurai.

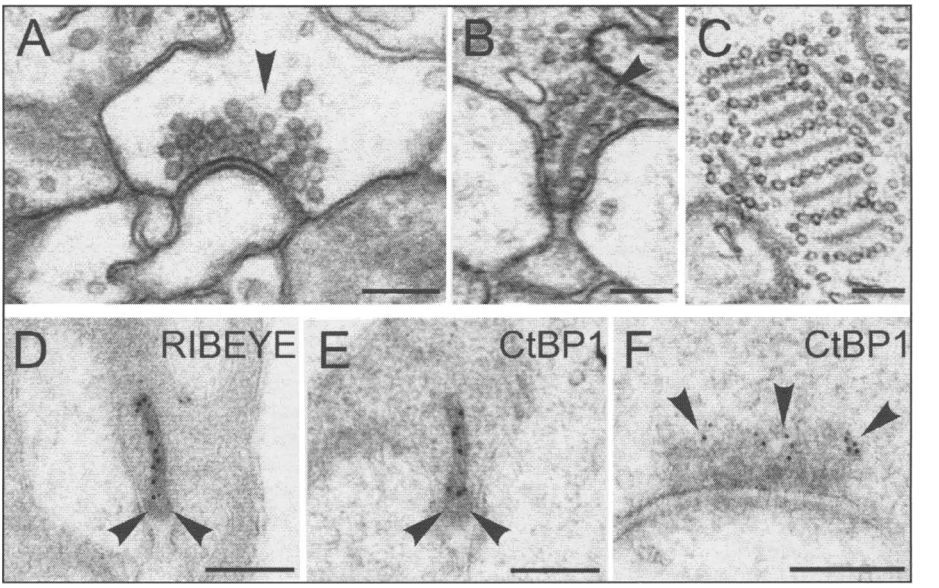

Figure 1. Photoreceptor and Amacrine Cell Synapses in the Retina, and the synaptic expression of RIBEYE and CtBP1. A) Electron micrograph of an amacrine cell synapse. The *arrowhead* points to the accumulation of neurotransmitter-filled synaptic vesicles at the presynaptic active zone. B) Electron micrograph of the ribbon synaptic complex in a rod photoreceptor terminal. The presynaptic ribbon (*arrowhead*) is anchored at its base in the arciform density, it is lined by a row of synaptic vesicles, and it faces two postsynaptic elements. C) In the Bassoon-deficient retina, the presynaptic ribbons, lined by synaptic vesicles, float freely in the cytoplasm of the photoreceptor terminals. D,E) Electron microscopy and postembedding immunogold labeling shows that photoreceptor ribbons are decorated with gold particles (10 nm) for RIBEYE (D) and for CtBP1 (E). Note the absence of gold particles at the base of the ribbons, the region of the arciform density (*arrowheads*). F) Electron micrograph of an amacrine cell synapse postembedding immunogold labeled for CtBP1. The gold particles for CtBP1 are located some distance away from the active zone at the edge of the electrondense CAZ material (*arrowheads*). Scale bars, 0.2 μm. (Fig. 1E,F reproduced from J Cell Biol 2005; 168:825-836, by copyright permission of The Rockefeller University Press.[5])

hundreds to several thousands of vesicles per second.[16] To accomplish this level of performance, these sensory neurons maintain large pools of readily releasable synaptic vesicles, and are equipped with a special type of chemical synapse, the ribbon synapse[17] (Fig. 1B). The presynaptic ribbon constitutes an electron-dense band of large surface area that extends from the site of transmitter release into the presynaptic cytoplasm and tethers hundreds of synaptic vesicles.[18] The synaptic ribbon was thought to be a unique structure specialized to ribbon synapses in sensory organs. An emerging idea, however, is that all chemical synapses are organized according to a common principle in which structural differences correlate with the kinetics of transmitter release.[7] Within this concept, every synapse has dense projections on which vesicles are tethered, and the ribbon is a variation of this common theme. A scaffold of proteins that are just beginning to be identified define and organize the ribbon. One of these proteins is RIBEYE.

The Novel CtBP Protein RIBEYE Is a Component of Synaptic Ribbons

When synaptic ribbons were purified biochemically,[19] a 120 kDa protein named RIBEYE was identified as an integral component unique to these structures[1] (Fig. 1D). Sequence analysis revealed that RIBEYE is a member of the CtBP family with an intriguing domain