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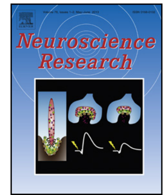

Update article

CAST: Functional scaffold for the integrity of the presynaptic active zone

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ABSTRACT

Cytomatrix at the active zone (CAZ)-associated structural protein (CAST) was first purified from the synaptic junction fraction of biochemically isolated central nervous system, and initially implicated as a critical component of the active zone. Subsequent biochemical analysis of CAST has shown that CAST potentially form a large molecular complex with other CAZ proteins including RIMs, Munc13s, Bassoon, and Piccolo/Aczonin in nerve terminals. Furthermore, recent genetic approaches using animal models such as *C. elegans*, *Drosophila* and mice have revealed that CAST has important functional and organizational roles in the assembly and maintenance of the presynaptic active zone. In this update article, I would like to summarize recent findings that place CAST as a functional scaffold regulating voltage-dependent Ca^{2+} channels and maintaining the integrity of the presynaptic active zone.

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1. Introduction

In nerve terminals, a slightly electron dense region (Gray, 1963; Couteaux and Pécot-Dechavassine, 1970), where neurotransmitter release is tightly regulated in a spatially and temporally coordinated manner (Landis et al., 1988), has been denoted as the active zone (Couteaux and Pécot-Dechavassine, 1970). A plethora of research has demonstrated that synaptic vesicles containing neurotransmitters specifically dock to and fuse with the presynaptic active zone membrane in a Ca^{2+}-dependent manner, resulting in release of neurotransmitter from synaptic vesicles into the synaptic cleft (Südhof and Rizo, 2011). Given the importance of regulation of neurotransmitter release at the active zone, its structure and function have been intensively studied by numerous research groups. However, despite the fact that the active zone was first identified by electron microscopy in the 1960s (Gray, 1963), its molecular composition had been largely unknown until recently (Garner et al., 2000; Südhof, 2012). In 1990s and 2000s, several active zone proteins/gene families, such as RIM1 (Wang et al., 1997), Munc13-1 (Brose et al., 1995), Bassoon (tom Dieck et al., 1998), and Piccolo/Aczonin (Cases-Langhoff et al., 1996; Wang et al., 1999; Fenster et al., 2000), were identified and characterized. Functional studies of these proteins have revealed their involvement in the docking, reloading, and/or priming steps of synaptic vesicles, while deletion of each gene caused no structural abnormality of the active zone in the central nervous system (CNS) (Augustin et al., 1999; Castillo et al., 2002; Schoch et al., 2002, 2006; Altrock et al., 2003). In contrast, in the retina and hair cells, Bassoon supports the structure and function of the ribbon synapses, specialized active zones; in Bassoon mutant mice, the ribbon cannot attach to the presynaptic plasma membrane but rather floats in the cytoplasm of the retina and inner hair cells (Dick et al., 2003; Khimich et al., 2005). Among the active zone proteins, cytomatrix at the active zone (CAZ)-associated structural protein (CAST) is the most recently identified (Ohtsuka et al., 2002). It was identified by a classic biochemical approach (Ohtsuka et al., 2002) and subsequently by the yeast two-hybrid method with a different name ERC2 (Wang et al., 2002). Additionally, using the electron microscopic analysis, ELKS, a protein closely related to CAST, was found as a CAZ protein in the CNS (Deguchi-Tawarada et al., 2004, 2006). The gene encoding ELKS was originally identified as a gene whose 5′-end was fused with the RET tyrosine kinase oncogene in a thyroid carcinoma (Nakata et al., 1999). ELKS was found to play a role in non-neuronal tissues and cells (Ohara-Imaizumi et al., 2005; Lansbergen et al., 2006), and is thus outside the scope of this review; instead, I would like to mainly focus on CAST/ERC2 in the CNS and sensory system. It should be noted that the similarities and differences between CAST and ELKS are described elsewhere in a recent review (Hida and Ohtsuka, 2010).

2. Molecular structure of CAST and its binding partners

CAST is composed of coiled-coil regions and a unique C-terminal motif (IWA) (Ohtsuka et al., 2002; Wang et al., 2002). *C. elegans* and *Drosophila* have one CAST paralog and vertebrates including mice, rats, and humans have at least two family members, CAST and ELKS

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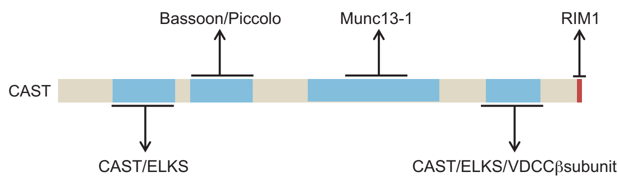

Fig. 1. Molecular structure of CAST and its binding partners. CAST and its family member ELKS directly interact with each other *via* the N- and C-terminal coiled-coil regions. Bassoon and Piccolo share a binding region in CAST. It should be noted that Munc13-1 also directly binds to RIM1, which is implicated in synaptic vesicle priming. Blue and red boxes indicate coiled-coil regions and the IWA motif, respectively.

(Ohtsuka et al., 2002; Wang et al., 2002; Deguchi-Tawarada et al., 2004; Monier et al., 2002; Deken et al., 2005; Wagh et al., 2006). The IWA motif is required for its binding to the PDZ domain of RIM1 (Ohtsuka et al., 2002; Wang et al., 2002), and the coiled-coil regions are required for interactions with Bassoon, Piccolo, Munc13-1, ELKS, and CAST itself (Fig. 1) (Ohtsuka et al., 2002; Wang et al., 2002, 2009; Takao-Rikitsu et al., 2004). It also should be noted that Munc13-1 indirectly binds to CAST *via* its direct binding to RIM1 (Ohtsuka et al., 2002). Therefore, CAST has a potency to interact with all known CAZ proteins and forms a large molecular complex at active zones.

Although the molecular organization of the presynaptic active zone remains elusive, CAST may serve as a platform by

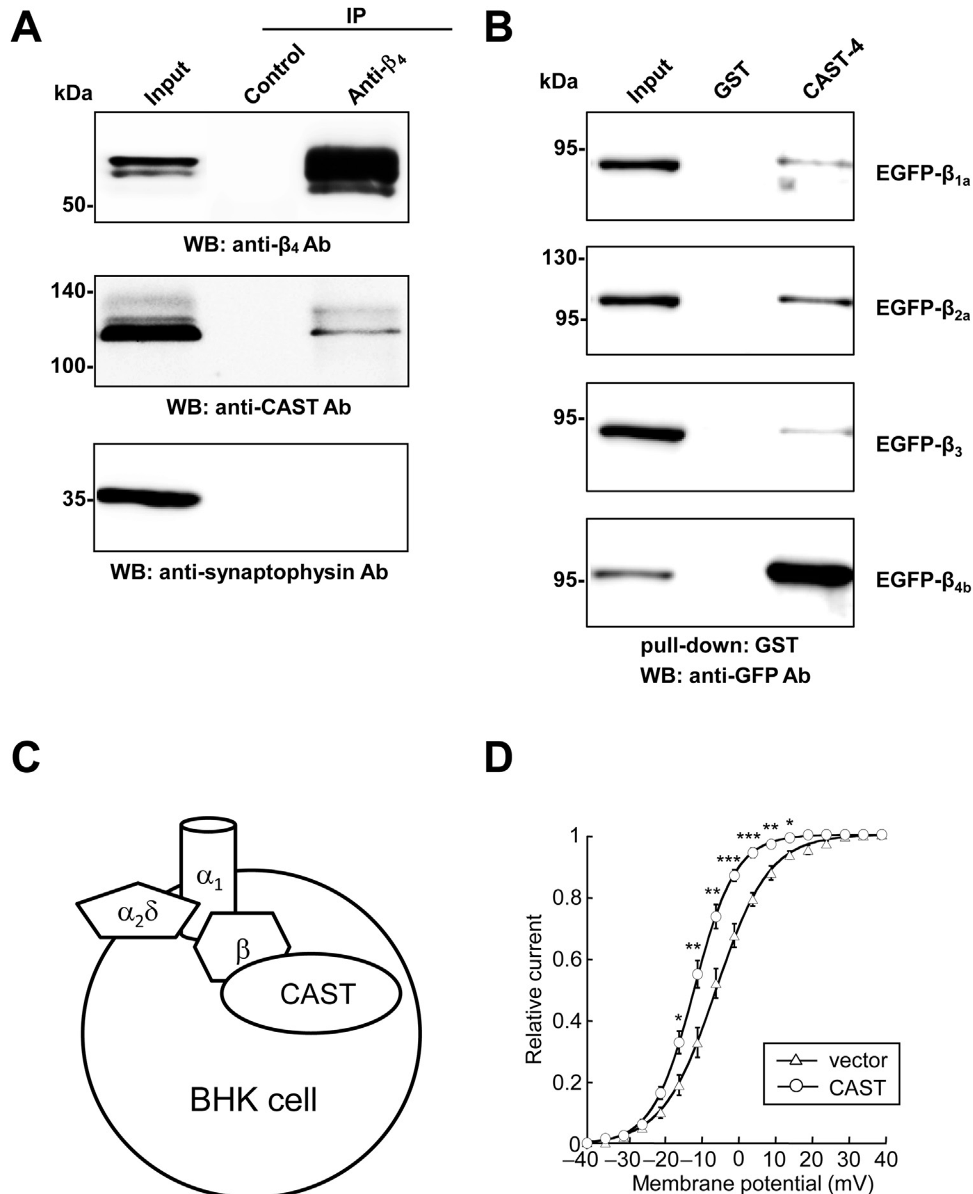

Fig. 2. Physical and functional interactions of CAST with VDCCs. (A) CAST and VDCC interaction *in vivo*. An extract of the mouse brain synaptosomal fraction was subjected to immunoprecipitation with an anti-β_4 antibody. The immunoprecipitate was analyzed by Western blotting using antibodies against the indicated proteins: the β_4 subunit, CAST, and synaptophysin. Ab: antibody; IP: immunoprecipitation; WB: Western blotting. (B) GST-pull down assay of β-subunits (β_{1a}, β_{2a}, β_3, and β_{4b}) with GST-CAST-4. GST-CAST-4-bound glutathione-Sepharose beads were incubated with cell lysates obtained from HEK293 cells expressing EGFP-β subunits. Bound proteins were analyzed by Western blotting using anti-GFP antibody. (C) Strategy using BHK cells. Whole-cell Ba^{2+} currents are measured from BHK cells expressing recombinant P/Q-type VDCCs expressed as $\alpha_1 \cdot \alpha_2/\delta \cdot \beta$ complexes containing the BI-2 variant of $Ca_v2.1$ and the β_{4b}-subunit in the presence or absence of CAST. (D) Effects of CAST on the activation properties of P/Q-type Ca^{2+} channels. Superimposed tail current elicited by repolarization to −60 mV after the 5 ms test pulse from −40 mV to 40 mV with increments of 5 mV, and normalized tail current are plotted against test-pulse potentials. The Boltzmann fit to each plot represents the hyperpolarization shift in the activation properties of P/Q-type Ca^{2+} channels in the presence of CAST. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Data points are means ± SEM.

Source: Adapted from Kiyonaka et al. (2012).

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