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Review article

CAST: Its molecular structure and phosphorylation-dependent regulation of presynaptic plasticity

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ABSTRACT

Our brain functions rely on sophisticated communication between synapses in the nervous system. Most synapses utilize a specialized submembranous structure, the so-called 'active zone', for the efficient transmission of chemical signals. The presynaptic active zone plays pivotal roles in the precise regulation of neurotransmitter release from the nerve terminals in a temporally and spatially coordinated manner. During the last two decades, several active zone-specific proteins have been isolated and characterized, including Bassoon, Piccolo/Aczonin, RIM, Munc13-1, ELKS, and CAST. The CAST/ELKS family is capable of potent direct interactions with other active zone proteins, forming a large protein complex that seems to be a molecular basis for electron density in the presynaptic active zone. The molecular details of the integrity of the active zone been well studied, however, we are just beginning to understanding its physiological significance in higher brain functions such as learning and memory, emotion, and consciousness. Focusing on the CAST/ELKS protein family, this review describes their biochemical properties, physiological functions in brain areas such as the hippocampus, and the significance of CAST phosphorylation in presynaptic short-term plasticity.

Contents

1. Introduction

Nerve terminals contain specialized organelles, synaptic vesicles, which are filled with neurotransmitters such as glutamate, γ-aminobutyric acid, acetylcholine, and glycine. Precise regulation of synaptic vesicle cycling in nerve terminals is crucial for our higher brain functions such as learning and memory, emotion, cognition, and consciousness. At the subcellular level, presynaptic active zones are where synaptic vesicle cycling occurs, and thus presynaptic active zones are the principal sites for the docking, priming, and fusion of synaptic vesicles in the neurotransmitter release process (Landis et al., 1988; dhof, 1995, 2012;). The active zone was identified by electron microscopy in the 1960s (Gray, 1963; Couteaux and Pecot-Dechavassine, 1970), but its exact

Abbreviations: ASD, (autistic spectrum disorders); CAST, (cytomatrix at the active zone-associated structural protein); Cdk5, (cyclin-dependent kinase 5); EPSP, (excitatory postsynaptic potential); KO, (knock-out); LAR, (leukocyte common antigen-related); LAR-RPTP, (LAR family receptor protein phosphatase); LTP, (long-term potentiation); p-CAST, (phosphorylated-CAST); PKA, (protein kinase A); Rab6IP2, (Rab6-interacting protein 2); RRP, (readily-releasable pool); SCGN, (superior cervical ganglion neuron); SNP, (single nucleotide polymorphism); VDCC, (voltage-dependent Ca2+ channel).

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molecular composition remained largely unknown until the 1990s. The active zone is evolutionally conserved between worms, flies, mice, and humans, showing its diversity in the structure (Burns and Augustine, 1995; Zhai and Bellen, 2004; Ackermann et al., 2015). For example, the first identified active zone protein in vertebrates was Munc13-1, as a rat homologue of *C. elegans* UNC13 (Brose et al., 1995). Next, Südhof's group identified RIM1 as a binding partner for small GTP-binding protein Rab3A (Wang et al., 1997). In addition to these prominent active zone proteins, Bassoon and Piccolo/Aczonin were isolated and characterized (Cases-Langhoff et al., 1996; tom Dieck et al., 1998; Fenster et al., 2000). CAST and ELKS were isolated by our and Südhof's groups independently. We isolated CAST from rat brain by classic biochemical approaches while Südhof's group isolated CAST/ERC2 using the yeast two hybrid-method (Ohtsuka et al., 2002; Wang et al., 2002). In this review, we provide a brief summary of the molecular structures of the CAST/ELKS protein family. We then discuss their phosphorylation and regulation of neurotransmitter release and synaptic functions such as presynaptic short-term synaptic plasticity.

2. Molecular structure of CAST and ELKS and the mode of their complex formations at the presynaptic active zone

CAST (*C*ytomatrix at the *A*ctive zone –associated *ST*ructural protein) is a 120 kDa protein first purified from rat brain (Ohtsuka et al., 2002), and subsequently isolated by yeast two-hybrid screening as the RIM-binding protein, ERC2 (Wang et al., 2002). ELKS was originally identified as the product of a gene translocated to a different chromosome in a thyroid carcinoma (Nakata et al., 1999). Subsequently, we found by immune-electron microscopy that ELKS is localized at the presynaptic active zone (Deguchi-Tawarada et al., 2004). CAST is mainly expressed in the brain whereas ELKS is ubiquitously expressed (Fig. 1) (Hida and Ohtsuka, 2010). CAST and ELKS have relatively high homology (~70% amino acid identity), and presumably form hetero- and/or homo-oligomers through their N- and C-terminal coiled-coil regions (Deguchi-Tawarada et al., 2004). They contain several coiled-coil domains over the entire sequence and a unique C-terminal amino acid IWA sequence. (Ohtsuka et al., 2002; Wang et al., 2002; Deguchi-Tawarada et al., 2004). The IWA sequence is required for a specific interaction with the PDZ domain of RIM1 (Ohtsuka et al., 2002; Wang et al., 2002; Deken et al., 2005; Lu et al., 2005; Hida and Ohtsuka, 2010). Disruption of this interaction causes mislocalization of RIM1 in nerve terminals, but has no effect on the localization of CAST (Ohtsuka et al., 2002). This suggests that CAST serves as an anchoring protein for RIM1 at the presynaptic active zone. Accordingly, in CAST/ERC2 knock-out (KO) mice, the level of soluble RIM1 is slightly increased to ~27% (Kaeser et al., 2009). Thus, CAST may control the stability or homeostasis of RIM1 at the presynaptic active zone.

In addition to this C-terminal region, CAST has many binding regions for the other active zone proteins (Fig. 1) (Takao-Rikitsu et al., 2004; Hida and Ohtsuka, 2010). Bassoon, Piccolo and Munc13-1 bind directly to the middle coiled-coil region of CAST (Takao-Rikitsu et al., 2004; Wang et al., 2009; Hida and Ohtsuka, 2010). In contrast to the CAST-RIM1 interaction, disruption of the binding between CAST and Bassoon has no effects on the synaptic localization of CAST and Bassoon in nerve terminals (Takao-Rikitsu et al., 2004). However, the disruption does significantly inhibit neurotransmission in cultured superior cervical ganglion neurons (SCGNs), accessed by recording excitatory postsynaptic potentials (EPSPs) (Takao-Rikitsu et al., 2004). Thus, CAST-Bassoon binding is essential for the physiological functions of these proteins in the brain.

The physiological significance of CAST-Munc13-1 binding has also not yet been investigated. However, as shown in Fig. 1, bMunc13-2 has been reported to bind the C-terminal region of ELKS, but not CAST (Kawabe et al., 2017). Indeed, ELKS recruits bMunc13-2 to presynapses in cultured hippocampal and cortical neurons, which controls basal synaptic vesicle priming and short-term plasticity. Therefore, the distinct interactions of different Munc13 isoforms (here, bMunc13-2) with ELKS seem to be a key determinant of the heterogeneity of presynaptic active zones (Kawabe et al., 2017). Some of the significance of these protein–protein interactions among active zone proteins is still unclear. However, it seems likely that the CAST/ELKS protein family serves as a molecular hub or platform at presynaptic active zones to allow other active zone proteins to execute their physiological functions in transmitter release processes such as the docking, priming, fusion/exocytosis, and/or endocytosis of synaptic vesicles.

In addition to these active zone proteins, CAST and ELKS have other interaction partners such as liprins, Rab6 small GTP-binding protein, LNX, and voltage-dependent Ca^{2+} channels (VDCCs) (Figs. 1 and 2). Vertebrates have at least four liprin α family members (liprin α1–4), while *Drosophila* and *C. elegans* have one homologue each, liprin α and Syd-2, respectively (Zhen and Jin, 1999; Dai et al., 2006). A genetic screen to find synaptic deficient mutants in *C. elegans* first identified Syd-2 (Zhen and Jin, 1999). In the Syd-2 mutant animals, the active zone length became longer than that of the wild types. Liprin α has some binding partners such as leukocyte common antigen-related (LAR), GIT1, CASK, RIM1, and CAST/ELKS (Schoch et al., 2002; Ko et al., 2003a,b; Olsen et al., 2005; Stryker and Johnson, 2007). CAST and liprin binding has been shown to be involved in synapse maturation at least in primary cultured hippocampal neurons (Ko et al., 2003a). Together with the morphological deficit in Syd-2 mutants, it seems that mammalian liprins have the same and/or similar functions in active zone formation. However, at least to our current knowledge, there have been no reports demonstrating that the active zones in liprin KO mice show morphological abnormalities such as increased length. Technically, it may be difficult to evaluate the phenotype since liprin members could compensate for each other in KO mice.

Rab6 is a member of the small GTP-binding protein Rab family and is known to regulate various kinds of intracellular membrane trafficking such as retrograde transport from the Golgi apparatus to the endoplasmic reticulum and from the Golgi to the endosome in cellular transport (Grosshans et al., 2006). ELKS was re-identified as Rab6-interacting protein 2 (Rab6IP2) by the Goud group and shown to function in endosome to Golgi transport (Monier et al., 2002). Moreover, by interacting with Rab6 and LL5β, ELKS regulates the dynamics of microtubule stability at the plus end (Lansbergen et al., 2006). ELKS is also enriched in the cortical plasma membrane, to where Rab6 targets secretory vesicles (Grigoriev et al., 2007). In non-neuronal cells such as fibroblasts ELKS regulates the reorganization of microtubules and Rab6-mediated vesicle exocytosis within the cellular space. However, it is largely unknown whether, and how, ELKS and Rab6 interactions coordinate vesicle traffic in nerve terminals, especially presynaptic active zones, in neural polarity and/or synaptic plasticity.

Another important binding partner for CAST is VDCCs (Figs. 1 and 2). VDCCs such as the N-, P/Q-, R-, and L-types play essential roles in neurotransmitter release at active zones (Takahashi and Momiyama, 1993; Wheeler et al., 1994; Catterall, 1998; Wu et al., 1999). The pore-forming α_1-subunit and the auxiliary α_2/δ-, β-, and γ-subunits form heteromultimeric protein complexes as VDCCs (Ertel et al., 2000; Catterall et al., 2005). Among the auxiliary subunits, the β-subunits (β_1–β_4) interact with the α_1-subunit in the cytoplasm to augment functional channel trafficking to the plasma membrane (Mori et al., 1991; Bichet et al., 2000), and to control multiple kinetic properties (Lacerda et al., 1991; Varadi et al., 1991). CAST then directly interacts with the β-subunits (β_1–β_4), and, remarkably, CAST has a higher binding