SynCAM 1/2 Comprise a Novel Adhesion Complex at CNS Synapses

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Introduction

Synapses are asymmetric sites of cell-cell adhesion at which neurons communicate chemically to propagate electrical signals. Tight adhesion between the pre-synaptic and post-synaptic neuronal membranes is critical for the structural and morphological features of synapses in the central nervous system. Synaptic adhesion molecules control the initial formation and maintenance of synaptic connections, formation, development and potentiation. Here we describe our efforts to characterize a novel adhesion complex at central synapses composed of SynCAMs 1 and 2. SynCAMs 1 and 2 prefer to bind heterophilically in vitro and form a stable complex in vivo which can be isolated from synaptic fractions. Additionally, SynCAMs 1 and 2 can recruit each other to sites of cell-cell contact, suggesting that this complex forms actively during neuronal development.

The material in the synaptic cleft. Cryo-EM tomography studies have revealed the first molecular details of the structures in the synaptic cleft (Biederer and 2006, Structure). What are these complexes and what roles do they play in the formation and stabilization of synapses?

Background

In recent years, our understanding of the roles of adhesion receptors in the formation and development of mammalian central synapses has expanded tremendously (see model above). Adhesion receptor systems identified as important for synaptic development include the neuroligin-neurexin and EphB-Ephrin asymmetric linkages, the synaptic adhesion molecule Necl1 and also the orphan receptors SALM and NGL. All of these proteins influence synapse formation in vitro but likely also play important roles in rats and higher synapses of neuronal and synaptic development.

The SynCAM family is a four-protein family conserved throughout the vertebrate phylum. SynCAMs have three extracellular Ig-like domains which mediate adhesion and a short cytoplasmic tail with protein-interaction motifs for synaptic adapter proteins and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (IAA). The CMS, shows that SynCAM 1 is a synaptic protein with the capacity to induce functional synaptic connections in cultured neurons (Biederer et al., 2002). However, it remains unclear what protein interactions are critical in this process. We have begun to characterize the protein interactions of the SynCAM family and their roles in neuronal development. Interestingly, the sequence of the four SynCAM cytoplasmic domains is highly conserved whereas the extracellular domains diverge. This suggests that the intracellular signaling partners of the SynCAMs might converge, but that each SynCAM might be sensitive to specific adhesive cues. Accordingly, we focused first on the extracellular domains of SynCAMs to characterize their intracellular biochemical and cell biological properties.

Results

Isoform specific SynCAM antibodies. Antibodies were generated against non-conserved regions of each SynCAM, and purified against the corresponding antigen. Immunofluorescence of HEK 293 cells expressing each of the SynCAMs, control artefact, or brain lysate shows that each specific antibody recognizes a similar pattern of bands for overexpressed and endogenous SynCAM.

SynCAMs are modified by N-Glycosylation. Equal fractions of brain lysate and control marker protein N-Cadherin, while SynCAMs 3 and 4 are not (lane 3). Digestion with PNGase F removes all N-linked carbohydrate and reduces SynCAM 1 and 2 and the bound proteins eluted sequentially with high salt (0.5M KCl) and sample buffer containing 0.5% SDS, and the fractions subjected to immunoblotting. We observed strong binding of SynCAMs 1 and 2 but no binding of homophilic interactions or control proteins. Asterisk mark background bands.

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Discussion

SynCAMs are a family of four adhesion molecules expressed strongly during the major period of brain circuit development. Previous work has shown that SynCAM 1 can play an active role in synapse development, similar to activities described for neuroligins, EphB receptors, and NGL-2. We have now developed tools to study each of the SynCAM isoforms. Our studies focus on SynCAMs 1 and 2, which we hypothesize form a synaptic adhesion complex with roles in synapse development and stabilization. We also observed an interaction between SynCAMs 3 and 4 (not shown here) which plays an important role in PNS myelination (Spiegel et al., 2007, Maeru et al., 2007).

In vitro, SynCAMs were first described as homophilic, but we now show that they prefer heterophilic interactions. SynCAMs 1 and 2 both function with synaptic membranes and co-localize in culture with synaptic markers, suggesting a synaptic localization. SynCAM 1/2 complexes can be co-immunoprecipitated from synaptic fractions, suggesting the formation of a stable complex in vivo. Additionally, SynCAMs 1 and 2 are able to reciprocally recruit each other at sites of cell-cell contact between neurons and neuronal cells, which is the interaction necessary for effects in the co-culture assay. From this we conclude that SynCAMs 1 and 2 form an adhesive complex at central synapses, and that the arrival of one isoform might actively recruit neuronal SynCAM 1.

References