SynCAMs Organize Synaptic Membranes Through Heterophilic Adhesion

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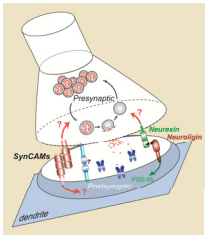
Overview

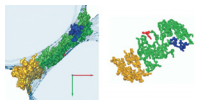
Synapses are asymmetric sites of cell-cell adhesion at which neurons communicate chemically to propagate electrical signals. Synaptic adhesion molecules have been identified, and play roles in synapse stabilization, formation, development and plasticity. Here we describe a novel adhesion complex at central synapses comprised of SynCAMs 1 and 2. SynCAMs 1 and 2 preferentially bind heterophilically *in vitro* and form a stable complex *in vivo*, which can be isolated from synaptic membrane fractions. Additionally, SynCAMs 1 and 2 recruit each other to sites of cell-cell contact in neuronal membranes, suggesting that this complex forms actively during neuronal development. Both components of this synaptic adhesion complex promote synapse organization, and increase synaptic transmission. Together, our studies demonstrate that SynCAM proteins mediate asymmetric synaptic adhesion and organize synapses.



Model of synapse-organizing adhesive interactions. SynCAMs are neuronal surface molecules mediating adhesion at synaptic sites. Trans-synaptic interactions by SynCAMs and other adhesion systems, such as neurexins and neuroligins, are followed by recruitment of synaptic proteins to these interaction sites, causing nascent synapses to develop.

Background

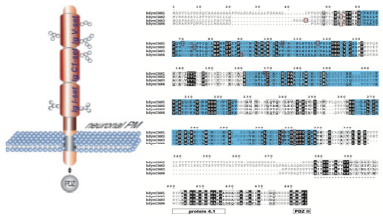
Tight adhesion between the pre-synaptic and post-synaptic neuron is a critical biochemical and morphological feature of synapses in the central nervous system. The adhesion complexes have been visualized using cryo-electron tomography.



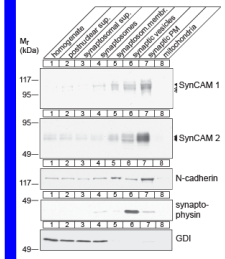
The material in the synaptic cleft. Cryo-EM tomography studies have revealed the first molecular details of the structures in the synaptic cleft (from Lucic et al., 2005).

In recent years, our molecular and cellular understanding of the roles of adhesion receptors in the development of mammalian central synapses has expanded tremendously. Adhesion receptor systems important for synaptic development include the neurexin-neuroligin and EphB-Ephrin asymmetric adhesion system, the SynCAM adhesion molecules, and also the orphan receptors SALM and NGL. All of these proteins influence synapse formation *in vitro* but likely also play important roles in other stages of neuronal and synaptic development.

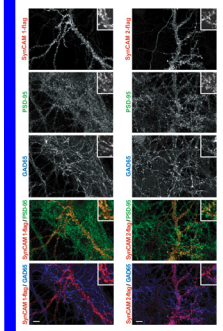
The SynCAM family is comprised of four members and is conserved throughout the vertebrate phylum. SynCAMs have three extracellular Ig-like domains and a short cytoplasmic tail with protein-interaction motifs for PDZ-domain containing scaffolding proteins and the actin cytoskeleton (Biederer, 2006). In the central nervous system, SynCAM 1 is a synaptic adhesion protein with the capacity to induce functional presynaptic terminals in cultured neurons (Biederer et al, 2002). However, the extra- and intracellular protein interactions critical in this process remained unclear. Interestingly, the sequences of the four SynCAM cytoplasmic domains are highly conserved while the extracellular domains diverge. This suggests that the intracellular signalling partners of the SynCAMs might converge, but that each SynCAM might be sensitive to specific adhesive cues. We focused in this study on the extracellular domains of SynCAMs to characterize their biochemical and cell biological properties.



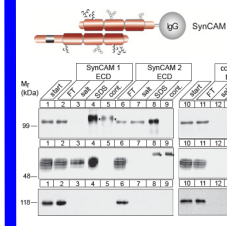
Results



SynCAMs fractionate in synaptic plasma membranes. Equal total protein from the indicated fractions of a subcellular fractionation of rat brain were loaded and analyzed by immunoblotting. Both SynCAMs 1 and 2 enrich strongly in synaptic plasma membranes with the control marker protein N-cadherin. Synaptophysin serves as a marker for synaptic vesicles and GDI as a marker for soluble fractions.

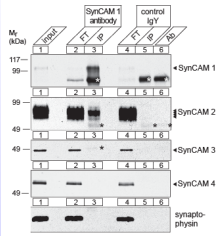


SynCAMs sort to synaptic sites in mature cultured neurons. Hippocampal neurons were transfected with flag-SynCAM constructs and stained at 21 d.i.v. for the synaptic markers PSD-95 and GAD 65. In mature neurons, SynCAMs 1 and 2 are both punctate and prominently co-localize with excitatory synaptic markers and to a lesser extent with inhibitory synaptic markers. SynCAM 2 appears somewhat more punctate/synaptic than SynCAM 1 (not shown).

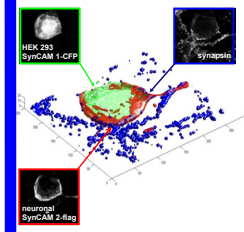


SynCAMs 1 and 2 engage in heterophilic interactions. Affinity chromatography experiments were performed on the recombinantly expressed extracellular domains of SynCAMs 1 and 2. Total brain membranes were solubilized and passed over the extracellular domains of SynCAMs 1 and 2. Bound proteins were eluted sequentially with high salt (800 mM KAc) and sample buffer (2% SDS), and the fractions subjected to immunoblotting. We observed strong and reciprocal binding of SynCAMs 1 and 2, but not to negative control proteins.

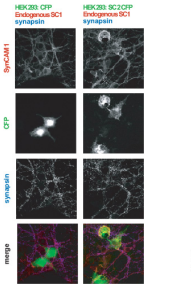
SynCAMs 1 and 2 co-localize at sites of cell-cell contact. HEK 293 cells expressing tagged SynCAM 1 or tagged SynCAM 2 were mixed and analyzed by fluorescence microscopy. SynCAMs 1 and 2 accumulate at sites of cell-cell contact in zipper-like adhesive structures.



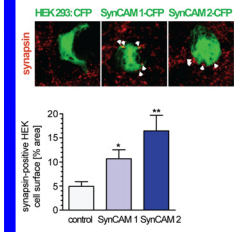
SynCAMs 1 and 2 form a stable complex *in vivo*. Synaptic fractions were purified from rat brain and solubilized. SynCAM 1 immunoprecipitates contain SynCAM 2, but not other SynCAM family members or control proteins, suggesting a stable and specific complex of SynCAMs 1 and 2 is formed *in vivo*. Asterisks mark cross-reactivity from the primary IP antibody.



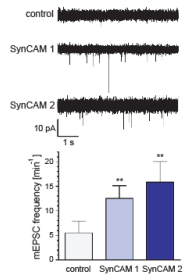
Reciprocal SynCAM recruitment in the co-culture assay. SynCAM 2 was overexpressed in neurons, followed by co-culture with HEK 293 cells expressing the cognate heterophilic partner SynCAM 1. When transfected neurons contact the SynCAM expressing cell, we observed membrane expansion at the sites of contact with enrichment of the heterophilic partner at these sites, as well as the presynaptic marker synapsin.



SynCAM 2 recruits endogenous SynCAM 1 in contacting neurons. HEK 293 cells expressing SynCAM 2-CFP or CFP alone were co-cultured with hippocampal neurons, and then stained for endogenous SynCAM 1 and synapsin. Significant enrichment of SynCAM 1 is seen on the surface of SynCAM 2 cells but not control cells, consistent with SynCAM 2 actively recruiting neuronal SynCAM 1.

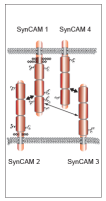


SynCAM 1 and 2 recruit presynaptic proteins. HEK 293 cells expressing CFP-tagged SynCAM 1 or SynCAM 2 were seeded atop dissociated hippocampal cultures at 9 d.i.v. (Biederer and Scheiffele, 2006). These co-cultures were analyzed at 11 d.i.v. by confocal microscopy for localization of the presynaptic vesicle marker synapsin (red) and CFP (green). Expression of SynCAM 1 or SynCAM 2 in HEK 293 cells co-cultured with hippocampal neurons significantly increased the area of synapsin-positive puncta covering the cell surface (arrows).



SynCAMs potentiate excitatory neurotransmission. mEPSCs were measured from hippocampal neurons overexpressing SynCAM 1, SynCAM 2, or untransfected control neurons. Postsynaptic overexpression of SynCAMs 1 or 2 leads to a greater mini-frequency but not mini-amplitude (not shown), consistent with a greater number of active synapses atop the expressing neurons.

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. We have now developed tools to study each of the SynCAM isoforms. Our studies focus on SynCAMs 1 and 2, which we hypothesize to form a synaptic adhesion complex with roles in synapse development and stabilization. We also identified interactions between SynCAMs 3 and 4 (not shown here) in the central nervous system, which play important roles in the myelination of peripheral nerves (Spiegel et al, 2007; Maurel 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 fractionate with synaptic membranes and co-localize in culture with synaptic markers, consistent with their synaptic localization. SynCAM 1/2 complexes can be co-immunoprecipitated from synaptic fractions, suggesting the formation of a stable complex occurs *in vivo*. Additionally, SynCAMs 1 and 2 reciprocally recruit each other at sites of cell-cell contact between neurons and non-neuronal cells, which is likely 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 the other across the synaptic cleft to carry out synaptic function.

This heterophilic interaction of SynCAMs is “pseudo-asymmetric”, in that the extracellular domains have different ligands but the cytoplasmic domains likely share the same effector molecules. The preference for heterophilic interactions by SynCAMs may both organize nascent synapses and specify contacts between distinct populations of neurons. Together, heterophilic interactions of SynCAMs appear sufficient to initiate contact between neuronal membranes in order to mobilize the cytoskeleton and synaptic machinery at these contact sites.

References

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