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Cadherins: Actin with the Cytoskeleton to Form Synapses

Minireview

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Classic cadherins are calcium-dependent homophilic cell adhesion molecules that are enriched at synapses and thought to function in target recognition and adhesion at synaptic junctions. This brief review highlights evidence that cadherins and their associated catenins play a role in directing the development of pre- and postsynaptic specializations. In particular, the question of whether cadherin regulation of the actin cytoskeleton at discrete contact sites translates into the assembly of synaptic compartments will be explored.

Synapses of the central nervous system (CNS) are highly specialized regions of cell-cell contact designed to rapidly and efficiently relay signals from one neuron to another. To form a synapse, cells must first recognize appropriate targets and then recruit pre- and postsynaptic elements to exquisitely localized microdomains at points of contact (reviewed in Ziv and Garner, 2004). For this reason, synaptically localized transmembrane cell adhesion proteins have long been considered attractive mediators of synapse formation. These protein classes include cadherins, integrins, ephrins-Eph receptors, neurexins-neuroligins, as well as members of the immunoglobulin superfamily including N-CAM, SynCAM, Nectin, Sidekick, and SYG1 (reviewed in Scheiffele, 2003). Cadherins, in particular, have been well studied and are considered attractive candidates for the regulation of synapse form and function. Evidence that cadherins play an important role in establishing synaptic junctions includes observations that cadherins mediate attractive interactions between neurons and their targets (Prakash et al., 2005), that cadherins rapidly accumulate at points of cell-cell contact prior to synaptic differentiation (Jontes et al., 2004; Togashi et al., 2002), and that disruption of cadherin-based contacts inhibits the formation of synapses in primary hippocampal cultures (Bozdagi et al., 2004; Togashi et al., 2002). The intimate cooperation between cadherins and the actin cytoskeleton, together with the emerging view that actin plays a key role during synapse formation (reviewed in Lippman and Dunaevsky, 2005; Zhang and Benson, 2001), further suggests a role for the cadherin complex in the sculpting of the synapse. This minireview explores the contribution of intercellular cadherin interactions in the formation of pre- and postsynaptic specializations in the vertebrate CNS, with particular emphasis on cadherin's regulation of actin dynamics.

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Cadherin Signaling and the Actin Cytoskeleton

Classic cadherins are single-pass transmembrane proteins with five ectodomain repeats that mediate mainly homophilic adhesion. Intracellularly, cadherins interact with a number of proteins that modulate their adhesive properties or activate downstream signaling pathways. Much of what we know about cadherin-based adhesion comes from studies done on epithelial adherens junctions, and to date, it remains unclear how much of cadherin's function is conserved at neuronal contact sites. In epithelial cells, the formation of cadherin-mediated cell-cell junctions is accompanied by a profound remodeling of the actin cytoskeleton. The "core" of cadherin-based adhesion involves cadherin's association with β-catenin, which in turn interacts with α-catenin to tether cadherin to the actin cytoskeleton (Goodwin and Yap, 2004, and references within). Cadherin-catenin complexes can also actively induce actin polymerization in a number of ways. First, cadherins can recruit cortactin and the Arp2/3 actin nucleator complex, thereby delineating specific sites for the assembly of actin filaments (F-actin). Second, formin-1, a member of the Diaphanous/formin-homology protein family that is involved in actin cable formation, is recruited to sites of cell-cell contact by its association with α-catenin (reviewed in Goodwin and Yap, 2004). Cadherins also interact with members of the p120 catenin family of proteins, including p120 catenin, δ-catenin, ARVCF, and p0071, via the juxtamembrane segment of its cytoplasmic domain. p120 and other members of this family can regulate the activity of the Rho family of GTPases, which plays a central role in actin organization (reviewed in Carlisle and Kennedy, 2005; Goodwin and Yap, 2004).

Beyond their role in regulating the actin cytoskeleton, cadherin complexes act as signaling nodes by binding tyrosine kinases and phosphatases, activating PI3-kinase, and interacting with adaptor proteins. In addition, catenins such as β-catenin and δ-catenin act as scaffolding molecules by interacting with proteins containing PDZ (PSD-95/Dlg/ZO1) domains. Finally, by sequestering β-catenin, p120 catenin, and δ-catenin at the membrane, cadherins may act as tonic inhibitors of transcriptional activities mediated by these proteins (Goodwin and Yap, 2004).

Cadherins at the Synapse

Cadherins and their associated catenins have been observed in both pre- and postsynaptic compartments in many neuronal populations in the CNS (Salinas and Price, 2005, and references within). The expression and subcellular distribution of cadherins and catenins varies with the type and developmental stage of a synapse. In mature excitatory synapses of the murine CNS, αN- and β-catenin expression sharply border transmitter release zones, whereas in inhibitory neurons, these catenins are localized throughout the active zone

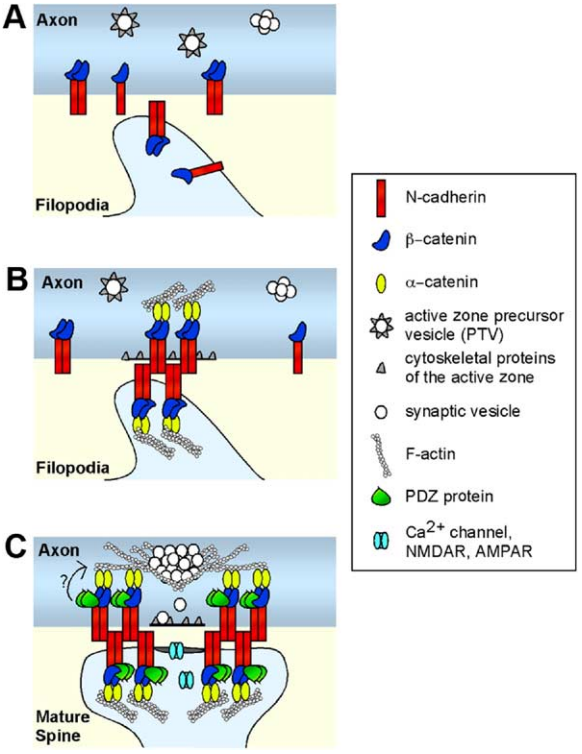

Figure 1. Model for the Development of Excitatory CNS Synapses

(a) Prior to cell-cell contact, cadherin-β-catenin complexes are expressed in a diffuse pattern along the length of filopodia, and in small clusters along the length of axons. Preassembled packets of synaptic vesicle proteins and Piccolo-Bassoon transport vesicles (PTVs) containing N-cadherin and cytoskeletal proteins of the active zone, are translocated along the axon on microtubule motors. **(b)** Following cell-cell contact, cadherins cluster in *cis*, and cadherin-catenin-actin complexes are formed. At early stages of synapse formation, cadherins and catenins are expressed throughout the active zone. **(c)** As synapses mature, β-catenin clusters act as scaffolds to recruit presynaptic PDZ domain-containing proteins to sites of cell-cell contact, thereby mediating the localization of synaptic vesicles to presynaptic compartments. In maturing glutamatergic synapses, cadherins and catenins are excluded from the active zone, and dendritic protrusions mature into short-necked spines. Postsynaptic differentiation is effected by the sequential recruitment of postsynaptic scaffold proteins and receptors to sites of cell-cell contact, and may involve cadherins and associated proteins.

(reviewed in Salinas and Price, 2005; Figure 1). It is possible that this difference is due to the progressive exclusion of one cadherin isoform, N-cadherin, from inhibitory synapses (Benson and Tanaka, 1998). This variability is important to consider when examining and interpreting the role of cadherins in different synaptic populations.

Cadherins and Postsynaptic Differentiation

The first step in the formation of CNS synapses is the stabilization of intercellular contacts generated by filopodial protrusions from dendrites, axons, and axon growth cones that probe the environment for appropriate partners. The dynamics of dendritic filopodia, in particular, have been extensively analyzed. Studies using time-lapse imaging have revealed that the overwhelming majority of contacts made between dendritic filopodia and axons are not stabilized and are retracted within minutes of their formation. However, as contact stabilization and synaptic differentiation proceeds, dendritic filopodia mature into dendritic spines, the postsynaptic structures for the majority of excitatory synapses in the vertebrate CNS (reviewed in Yuste and Bonhoeffer, 2004).

N-cadherin and β-catenin are diffusely distributed along the length of free dendritic filopodia that are not in contact with axons, but rapidly accumulate at filopodia-axon contact sites following target recognition (Figure 1; Jontes et al., 2004; Togashi et al., 2002). The localization of these molecules at filopodia-axon interfaces suggests that they may be involved in mediating contact stabilization, which may trigger spine maturation and synaptic differentiation. In an elegant study by Togashi and colleagues, cadherin-mediated intercellular contacts were inhibited in hippocampal neurons by expressing a dominant-negative N-cadherin that lacked a functional extracellular domain, but competed for intracellular cadherin binding partners (NcadΔE) (Table 1). Inhibition of cadherin contacts prevented spine maturation, and dendritic protrusions tended to retain their long, immature, filopodial morphology. Neurons lacking αN-catenin also exhibited immature filopodial morphologies, which were more dynamic (reviewed in Salinas and Price, 2005). It was thus concluded that contact stabilization, mediated by the formation of cadherin-catenin-actin complexes, is essential for proper spine maturation.

As previously mentioned, cadherins can also modulate the activity and localization of signaling molecules such as cortactin, Arp2/3, formin-1, and the Rho family of GTPases, and although this has yet to be examined, it seems very likely that cadherin's regulation of spine morphology extends beyond its structural association with the actin cyotskeleton. Indeed, cortactin, the Arp2/3 complex (Hering and Sheng, 2003), and Rho GTPases (reviewed in Carlisle and Kennedy, 2005) have been shown to impact spine formation and stability.

Intercellular cadherin interactions are also important in sculpting the postsynaptic compartment. Table 1 outlines the known roles for cadherins, catenins, and actin in the establishment of synaptic junctions and specifically emphasizes the importance of these proteins at different stages of development. Evidence suggests that cadherin-based contacts are important for the appropriate localization of the postsynaptic density marker PSD-95 (Honjo et al., 2000; Togashi et al., 2002) and the kainate receptor subunit glutamate receptor 6 (GluR6) (Coussen et al., 2002). Interestingly, disruption of cadherin interactions in mature cultures only modestly impacts the localization of these postsynaptic proteins, underscoring its importance in synapse formation as opposed to maintenance (Table 1; Bozdagi et al., 2004; Togashi et al., 2002). It is unclear how cadherin-based contacts induce postsynaptic differentiation, however, neither cadherin-catenin-actin complexes (Togashi et al., 2002) nor β-catenin-mediated scaffolding complexes (Bamji et al., 2003) appear to be involved (Table 1). δ-catenin, a neuron-specific catenin that is not part of the classic cadherin-cytoskeleton complex, can form stable complexes with PSD-95 and a number

Table 1. Comparison of Synaptic Phenotypes Resulting from Perturbations in Cadherin-Catenin Proteins and the Actin Cytoskeleton

	Presynaptic Organization	Postsynaptic Organization
N-cadherin	N-cadΔE (4 or 8 + 2 DIV): (1, 2) •Density of presynaptic boutons reduced by >70% •Synaptic vesicles diffusely localized N-cadΔE (21 + 2 DIV): (1) •Density of presynaptic boutons reduced 35%–50% N-cadΔE (0–5 + 10–15 DIV): (2) •Density of presynaptic boutons unaffected •Smaller boutons •mEPSC frequencies significantly reduced	N-cadΔE (10 + 2 DIV): (1) •Abnormal spine morphology •Density of PSD-95 puncta reduced 70% N-cadΔE (21 + 2 DIV): (1) •Density of PSD-95 puncta reduced 50% •Density of GAD puncta reduced 35% N-cadΔE (0–5 + 10–15 DIV): (2) •Density of PSD-95 and GAD puncta unaffected •Intensity of PSD-95 puncta reduced
β-catenin	LOF, β-cateninΔARM, or β-cateninΔPDZ: (3) •Localization of Bassoon puncta unaffected •Synaptic vesicles diffusely localized	LOF, β-cateninΔARM or β-cateninΔPDZ: (3) •Localization of PSD-95 puncta unaffected
α-catenin	LOF: (1) •Synaptic vesicles localize normally •Smaller boutons	LOF: (1) •Abnormal spine morphology
actin cytoskeleton	LatA (5 DIV): (4) •Density of synaptophysin and Bassoon puncta significantly reduced •Smaller boutons •Loss on N-cadherin localization LatA (18 DIV): (4) •Localization of presynaptic proteins unaffected •Loss of N-cadherin localization *Clostridium difficile* toxin B (Rho GTPase inhibitor): (5) •Density of synaptophysin puncta reduced	LatA (5 DIV): (4) •Density of PSD-95 puncta significantly reduced LatA (12 DIV): (4) •Density of PSD-95 puncta reduced 30% LatA (21 DIV): (4) •Density of NMDA & AMPA puncta reduced •Density of GABA clusters unaffected

LOF, loss of function; DIV, days in vitro; LatA, latrunculin A (sequesters actin monomers). 1, Togashi et al., 2002; 2, Bozdagi et al., 2004; 3, Bamji et al., 2003; 4, Zhang and Benson, 2001; 5, Wang et al., 2005.

of excitatory neurotransmitter receptors (Jones et al., 2002). However, the role of δ-catenin in postsynaptic development has yet to be determined.

Zhang and Benson have shown that F-actin plays an important role in synapse assembly, but may not be as important for maintenance. Sequestering actin monomers with latrunculin A in young cultures severely affected the localization of postsynaptic proteins compared to more modest effects in older cultures (Table 1; Zhang and Benson, 2001). In accordance with this, F-actin levels are specifically elevated in developing synapses (Zhang and Benson, 2002). It would be of great interest to determine the molecular changes that occur during synaptic maturation that result in the variability in both F-actin levels and significance.

Cadherins and Presynaptic Differentiation

Assembly of pre- and postsynaptic zones is now known to occur via fundamentally different mechanisms. While postsynaptic development occurs in a gradual manner, presynaptic zones are rapidly assembled from preassembled packets of presynaptic proteins that translocate along the axon in transport vesicles and are "trapped" at points of cell-cell contact (reviewed in Ziv and Garner, 2004). At least two main classes of transport vesicles have been described thus far: (1) large dense-core vesicles referred to as Piccolo-Bassoon transport vesicles (PTVs) that transport N-cadherin as well as many proteins associated with the active zone, and (2) small pleiomorphic vesicles that translocate along the axon in clusters and transport many of the characteristic synaptic vesicle molecules (reviewed in Ziv and Garner, 2004).

Increasing evidence suggests that the cadherin complex plays an important role in localizing synaptic vesicles (SVs) at nascent synapses (see Table 1). This was first observed in *Drosophila* N-cadherin missense mutants, which exhibited an overabundance of SVs at photoreceptor-interneuron synapses (Iwai et al., 2002). Additional support for this was observed in primary hippocampal cultures, where inhibition of N-cadherin function prevented the accumulation of SVs at sites of cell-cell contact (Table 1; Bozdagi et al., 2004; Togashi et al., 2002). Inhibition of N-cadherin function also prevented the accumulation of β-catenin at sites of cell-cell contact (Togashi et al., 2002), and it is now known that cadherin's effects on vesicle localization are largely mediated by β-catenin. Indeed, SVs were diffusely localized along the axon in cultured neurons lacking β-catenin, and EM analyses revealed a reduction in the number of SVs associated with synapses in β-catenin conditional knockout mice (Table 1; Bamji et al., 2003).

SV clusters are surrounded by a barrier of actin filaments (Sankaranarayanan et al., 2003). Although both cadherins and β-catenin play a major role in localizing vesicles, the cadherin-catenin-actin linkage is not responsible for this function. In hippocampal neurons, loss of αN-catenin (Togashi et al., 2002) or disruption of α-N-catenin-β-catenin interactions (Bamji et al., 2003) did not inhibit the clustering of SVs. Instead, analyses of known protein interaction domains of β-catenin revealed that the armadillo repeats involved in cadherin binding and the C-terminal PDZ binding motif are essential for SV localization (Bamji et al., 2003; see Table 1). These data suggests that intercellular cadherin interactions may provide β-catenin-rich nucleation sites for recruitment of synaptic PDZ proteins and other scaffold proteins, which in turn "trap" preassembled packets of SVs at contact sites (Figure 1). This does not rule out the

possibility that cadherin-β-catenin complexes localize SVs by regulating actin polymerization at discrete sites. Indeed, β-catenin may interact with and localize PDZ domain-containing protein(s) that in turn impact the actin cytoskeleton, for example, PDZ domain-containing Rho guanine nucleotide exchange factors (GEFs) (Fukuhara et al., 1999).

The localization of SVs is highly dependent on F-actin and cadherin in young, but not mature synapses (Bozdagi et al., 2004; Sankaranarayanan et al., 2003; Zhang and Benson, 2001). However, cadherins and F-actin continue to play a significant role at mature synapses. Increasing evidence suggests that neuronal activity regulates the distribution of cadherins, catenins (Salinas and Price, 2005), and F-actin (Colicos et al., 2001), which in turn impacts synapse function and remodeling.

Despite evidence indicating that cadherin function is essential for the formation of young synapses, cadherin interactions are not sufficient to induce synaptic differentiation (Sara et al., 2005; Scheiffele et al., 2000). This is in contrast to other adhesion systems such as syn-CAM (Biederer et al., 2002; Sara et al., 2005) and neurexin-neuroligin-1 (Biederer et al., 2002; Nam and Chen, 2005; Sara et al., 2005; Scheiffele et al., 2000), which have been shown to induce synapse formation in heterologous systems, though not in primary neuronal cultures (Sara et al., 2005). It is likely that many of these synaptic cell adhesion systems act in concert to coordinate the development of synaptic junctions. Indeed, it has been shown that β-catenin plays a large role in localizing neuroligin-1 to putative postsynaptic sites via its association with the synaptic scaffolding molecule, S-SCAM (Iida et al., 2004).

Our understanding of how synapses are assembled and the players involved in this highly complicated process remains incomplete. Although it is clear that cadherins and their associated catenins are involved in some aspects of synapse assembly, there are still a number of key questions that need to be addressed. For example, what aspects of cadherin function at synapses are dependent on its regulation of actin dynamics? How do cadherins communicate with other cell adhesion systems to regulate synapse specificity and the formation of synaptic junctions? How does regulation of cadherin interactions modulate synapse form and function in mature synapses? Answers to these questions will provide a foundation for understanding the molecular mechanisms by which cadherins, in conjunction with other cell adhesion systems, assemble, remodel, and even eliminate synaptic junctions.

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