Deciphering the Function of Neurexins at Cellular Junctions

J. Troy Littleton, Manzoor A. Bhat, and Hugo J. Bellen

Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030

The mechanisms that allow the nervous system of animals to interpret and respond to their environment requires an astonishing complexity of neuronal connections and signaling pathways. The past decade has seen the emergence of a common theme in the mechanisms mediating these seemingly complex tasks: during evolution, nervous system development has taken components of less complex cells and pathways and modified them to fit the requirements of neuronal signaling. Neurexins were originally identified as a polymorphic family of neuronal-specific type 1 cell surface membrane proteins that were postulated to serve a unique role in specifying synaptic specificity and docking synaptic vesicles at the active zone. However, recent genetic and molecular analyses have provided novel insights that suggest neurexins are evolutionarily conserved and mediate many aspects of cellular function.

Molecular Biology of Neurexins

Neurexins were first identified by Südhof and colleagues as cell surface receptors for α-latrotoxin (19). This component of black widow spider venom is well known for its potent effects at the synapse, causing massive calcium-independent release of neurotransmitters. Neurexins were originally identified as a polymorphic family of neuronal-specific type 1 cell surface membrane proteins that were postulated to serve a unique role in specifying synaptic specificity and docking synaptic vesicles at the active zone. However, recent genetic and molecular analyses have provided novel insights that suggest neurexins are evolutionarily conserved and mediate many aspects of cellular function.

Neurexins were first identified by Südhof and colleagues as cell surface receptors for α-latrotoxin (19). This component of black widow spider venom is well known for its potent effects at the synapse, causing massive calcium-independent release of neurotransmitters. Neurexins were originally identified as a polymorphic family of neuronal-specific type 1 cell surface membrane proteins that were postulated to serve a unique role in specifying synaptic specificity and docking synaptic vesicles at the active zone. However, recent genetic and molecular analyses have provided novel insights that suggest neurexins are evolutionarily conserved and mediate many aspects of cellular function.
Neurexins: A Link Between the Extracellular Environment and Intracellular Signaling Pathways

Both molecular and genetic analysis suggests that neurexins interact with several intracellular protein families of known function. Yeast two-hybrid screening for interacting partners with the rat neurexin intracellular domain identified a ligand that was named CASK (8). The NH2-terminal third of CASK has homology to the calmodulin-binding and autoinhibitory domains of CAMKII. The remaining COOH-terminal region of the protein shares homology with the MAGUK family of proteins and contains a PDZ domain, a Src homology 3 domain, and a COOH-terminal catalytically inactive guanylate kinase domain. CASK is expressed not only in neurons but in many tissues, raising the possibility that additional neurexins may interact with CASK outside the nervous system. CASK interacts with all three vertebrate neurexins, and this binding is abolished by deletion of the last three amino acids of the intracellular COOH-terminal region of neurexin (8). Several subclasses of sequence-specific interactions involving the PDZ domains of MAGUK proteins and the COOH-terminal amino acids of interacting proteins have been defined and include the COOH-terminal sequence EYV[1]VV, which is conserved in vertebrate and Drosophila neurexins and is also present in the erythrocyte integral membrane protein glycophorin C (21). Genetic studies also support an interaction with the MAGUK family of proteins. In Drosophila, the disc large protein (DLG), another MAGUK member, is localized to both synapses and septate junctions (13, 25), and genetic analysis has shown that both DLG and neurexin function in the assembly of septate junctions (2, 26). Interestingly, a new protein with PDZ domains has been recovered in a yeast two-hybrid screen using the Drosophila neurexin intracellular domain as a bait (Bhat, M.A., unpublished data). Thus, members of the PDZ-containing MAGUK family represent a group of intracellular ligands for neurexins. The MAGUK family is concentrated at cellular junc-
ions and involved in clustering ion channels and organizing sites of intercellular communication (for review see reference 20). Most MAGUK proteins contain one to three PDZ domains, a Src homology 3 domain, a protein 4.1 recognition sequence, and a catalytically inactive guanylate kinase domain. Members include the tight junction–associated ZO-1 and ZO-2, the synaptic and septate junction–associated DLG, the postsynaptic PSD95/SAP90, the synaptic chapsyn 110/PSD93, the erythrocyte membrane–associated p55, and the Caenorhabditis elegans LIN-2A cellular junction signaling protein (Fig. 1 B). DLG and PSD95 cluster Shaker-type K+ channels and N-methyl-d-aspartate receptors at synapses via their PDZ domains (10, 11). PSD95 has been shown to bind and localize neuronal nitric oxide synthase to synaptic membranes through an interaction between PDZ domains (3). One missing link in this pathway is how the MAGUK proteins themselves are clustered at sites of contact given their intercellular location. If neurexins were to localize to sites of cell contact via interactions with extracellular or transmembrane ligands, the intracellular domain could then recruit members of the MAGUK family and assist in clustering and organizing ion channels and associated junctional proteins.

One question that arises from these studies is how a neurexin–MAGUK complex is linked to the underlying cell cytoskeleton, a likely requirement for organizing sites of cell contact. One candidate family of proteins performing such a task is the protein 4.1 family. In Drosophila, the protein 4.1 homologue, Coracle (5), along with DLG and neurexin, are localized to septate junctions. In neurexin mutants, protein 4.1 fails to localize to septate junctions, suggesting that the intracellular COOH terminus of Drosophila neurexin is required to localize protein 4.1 (2). This intracellular domain is 68% similar to glycophorin C and contains a conserved 12–amino acid protein 4.1 recognition sequence ([16]; Fig. 1 E). The protein 4.1 recognition sequence, along with surrounding amino acids, is also conserved in vertebrate neurexins and suggests a consensus sequence of $^\text{S/g}^\text{YXXRX}^\text{X/g}^\text{GXYXXE}$ (Fig. 1 E). Another mechanism that may recruit protein 4.1 to this complex is the presence of a distinct protein 4.1 binding sequence identified in p55, and which is conserved in many MAGUK members ([16]; Fig. 1 B). The human DLG homologue has also been shown to interact with protein 4.1 and its homologue ezrin (14, 15), consistent with the MAGUK family binding members of the protein 4.1 family. Thus, an interaction with CASK, which also contains a protein 4.1 binding motif, may provide an independent mechanism for vertebrate neurexins to merge into this pathway and serve as a membrane anchor to the underlying cytoskeleton. The protein 4.1 family is widely expressed in many tissues, including the brain, and includes ezrin, radixin, moesin, talin, neurofibromatous 2/merlin, and protein 4.1 (Fig. 1 C). These proteins are involved in modulating cytoskeletal–membrane interactions, allowing regulation of cell adhesion, shape, and mobility. The erythrocyte complex of glycophorin C and protein 4.1 are required in vivo to localize the MAGUK family member, p55, to the membrane and to maintain cytoskeletal–membrane stability (16). The potential for this pathway to organize sites of cell contact is of general interest, but the finding that the MAGUK protein, DLG, and the protein 4.1 homologue, neurofibromatous 2/merlin, are involved in tumor formation should draw widespread attention. Thus, disrupting junctions required for cell communication and adhesion is a new pathway for cellular overgrowth and tumor formation.

**Extracellular Ligands for Neurexins**

We have reviewed several intracellular protein families suspected to be involved in neurexin’s ability to link the extracellular environment to the cytoskeleton (protein 4.1) and intracellular clustering/signaling proteins (MAGUK). An additional question is whether extracellular ligands interact with neurexins at cellular junctions and maintain or modify neurexin function. Using a recombinant splice-specific Neurexin Iβ as a ligand, a type 1 transmembrane protein family (neuroligins) with homology to esterase proteins (e.g., acetylcholinesterase and carboxylesterase) was identified as a calcium-dependent extracellular ligand (9). Neuroligins (836–848 amino acids, 95 kD) are expressed within neurons and have a large extracellular esterase domain, a single transmembrane domain, and a cytoplasmic tail of ~100 amino acids (Fig. 1 D). The extracellular domain lacks a serine residue at the active site, suggesting that the protein is catalytically inactive, similar to the Drosophila esterase proteins neurotactin and gliotactin. Gliotactin (956 amino acids, 109 kD) is 50% similar to neuroligin and is expressed in glial and epithelial cells, as is Drosophila neurexin (1). Electrophysiological analysis of gliotactin mutants have demonstrated a breakdown of the blood–brain barrier similar to that caused by mutations in neurexin (1). Thus, in vitro studies in mammals suggest a physical interaction between neurexins and the neuroligin/gliotactin family, while in vivo genetic manipulations in Drosophila indicate both proteins function in a similar pathway. This interaction might form a transcellular scaffold allowing neurexins to organize intracellular proteins or may act in a ligand-receptor fashion, modifying neurexin’s interactions with the MAGUK and protein 4.1 families.

Recently, another extracellular ligand, contactin/axonin-1, has been identified that binds hNRX IV/Caspr. This axonally expressed member of the Ig superfamily has been shown to bind glial receptor tyrosine phosphataseζ, an interaction which is thought to play a role in axonal growth (18).

**Summary and Future Prospects**

Recent in vitro and in vivo studies have provided exciting insights suggesting that the neurexin family may function in organizing cellular junctions. This model is supported by the phenotypic analysis of Drosophila neurexin mutants (2). These mutants lack the protein 4.1 binding motif, may provide an independent mechanism for vertebrate neurexins to merge into this pathway and serve as a membrane anchor to the underlying cytoskeleton. The possibility that a distinct neurexin–MAGUK complex might be involved in organizing cellular junctions is supported by the finding that the intracellular domain of the neurexin family may function in organizing cellular junctions. This model is supported by the phenotypic analysis of Drosophila neurexin mutants (2). These mutants lack the ladder-like transcellular septate characteristic of septate junctions. In addition, neurexin is required in glia to form the blood–brain barrier, consistent with an ability of neurexins to form transcellular barriers at cellular junctions. Neurexin also localizes protein 4.1 to sites of cell contact, and neurexin mutations show defects in dorsal closure of the epidermis, a process requiring extensive cellular movements and signaling. Fig. 2 presents a model of a cellular junction incorporating neurexins.
Essential questions remain to be answered concerning the neurexins. Foremost, a precise subcellular location of the known vertebrate neurexins is required to determine if they may also be involved in axonal–glial interactions, similar to that reported for the NRX IV homologue, hNRX IV/Caspr. In addition, it will be important to extend the search for nonneuronal neurexins, as a more complete catalog of the neurexin family is required to begin to determine the variety of roles these proteins might play in cellular junctions. Do neurexins also play a role in the MAGUK’s ability to cluster ion channels at synapses, or is their interaction with members of this protein family restricted to other sites of cell contact? Do extracellular ligands serve as activators or modulators of neurexin’s link to the intracellular environment, and how does the extensive alternative splicing in vertebrate neurexins define or modify these interactions? And finally, what, if any, cellular signals can be relayed through the neurexins? These and other questions should inspire many interesting experiments in the near future, as dissection of the role of neurexins in cell junctions is just beginning.

Received for publication 21 February 1997.

References


Figure 2. Model of neurexin interactions in cellular junctions. (A) Hypothetical junction incorporating a neurexin-gliotactin/neurologin interaction with a link to the underlying cytoskeleton via protein 4.1 and to intracellular signaling/clustering MAGUK proteins. (B) A similar pathway connects the erythrocyte membrane to the cytoskeleton via glycophorin C. (C) MAGUK members have also been shown to cluster ion channels at synapses. It is currently unclear if neurexins exist at synapses and are involved in synapse formation, presynaptic vesicle docking, or postsynaptic channel clustering. Current data favor a role for neurexins in axonal–glial interactions and cellular junctions instead. Neurphilin, Neurexophilin; SV, synaptic vesicle; DLG, discs large protein/PSD95.