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 also found to interact with the synaptic vesicle calcium-binding protein synaptotagmin (7, 19). These studies provided the framework for an attractive model in which α-latrotoxin binds to neurexins, relaying a signal from the outside of the synapse to the synaptic vesicle fusion machinery by activating synaptotagmin. This model also suggested an in vivo correlate that has been propagated throughout the synaptic literature: neurexins may be involved in the formation of the active zone by binding to postsynaptic receptors or extracellular matrix components and subsequently docking synaptic vesicles at these sites through an interaction with synaptotagmin. A second clue to neurexin’s function was the report that they were selectively enriched at synapses (24) and exhibited a striking number of alternatively spliced forms (22). It was proposed that the differential localization of various populations of alternatively spliced neurexins may allow them to function in synaptic targeting (22).

Recent data have challenged these models of neurexin function. These findings include the following observations: (a) Although Neurexin Iα binds α-latrotoxin, their interaction is calcium-dependent. α-Latrotoxin’s effects are calcium independent, and a novel calcium-independent ligand has now been identified as a candidate physiological α-latrotoxin receptor (4, 12). (b) α-Latrotoxin still causes exocytosis in mutant mice lacking Synaptotagmin I (6), raising questions about whether a neurexin–synaptotagmin interaction has a physiological role. (c) Drosophila and Discopyge homologues of neurexins have now been identified, and they are expressed in cells other than neurons and are not found at synapses (2, 19a). In addition, new studies have failed to repeat the reported presynaptic localization of mammalian neurexins (23). Given the challenges to the widely held views on neurexin function, we will review the recent literature and discuss the possible role of these proteins in cell function.

To date, the neurexin family includes three related vertebrate genes (NRX I, II, and III) (23, 24), a Drosophila gene (NRX IV), and its human homologue (hNRX IV) (2), which has recently been shown to be a contactin-associated protein, Caspr (17, 18). A homologue of Neurexin IIIα has also been recently reported in the marine ray, Discopyge ommata, and a second Drosophila neurexin is being characterized (Yuan, L., and B. Ganetzky, personal communication). As shown in Fig. 1 A, the three vertebrate neurexins encode an α-isoform (1,507–1,578 amino acids, 160–220 kD) and a β-isoform (437–471 amino acids). Neurexins have a large extracellular domain, a single transmembrane, and a short cytoplasmic segment (40–55 amino acids). The extracellular portions of the α-isoform contain three EGF repeats, six repeats with low homology to the G domain of laminin A, and an O-linked sugar domain in hNRX IV/Caspr, but not present in Neurexins I, II, or III. EGF and laminin G domains are present in a variety of proteins that are components of the extracellular matrix or involved in cell signaling, including laminin A, agrin, perlecan, and the Drosophila developmental proteins Crumbs and slit, and are thought to function in protein–protein interactions within the extracellular environment. The COOH-terminal intracellular segment of neurexins contains a conserved four–amino acid tail that functions as a recognition sequence for the PDZ domains of membrane-associated guanylate kinase (MAGUK)1 proteins (21). In addition,
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 EY/Y/I/L/V, 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-

Neurexins: A Link Between the Extracellular Environment and Intracellular Signaling Pathways

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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 and junctional–associated human DLG homologue, SAP97, the synaptic chapsyn 110/PSD93, the erythrocyte membrane–associated p55, and the neurexin, are localized to septate junctions. In protein 4.1 homologue, Coracle (5), along with DLG and of general interest, but the finding that the MAGUK proteins are required in vivo to localize the intracellular COOH terminus of glycophorin C and protein 4.1 indicates 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.

Another 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 phosphatase β, 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 mutations (2). These mutants lack the 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.
Figure 2. Model of neurexin interactions in cellular junctions. (A) Hypothetical junction incorporating a neurexin-gliotoxin/neurulin 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 cytoskeleton to the postsynaptic density via glycoprotein 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 clustering. Current data favor a role for neurexins in axonal–glial interactions and cellular junctions instead. Nrx, Neurexin; Nrxp, Neurexophilin; SV, synaptic vesicle; DLG, disc large protein/PSD95.

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.

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