Presynaptic Heterogeneity: Vive la difference

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Since individual synapses of the same neuron may have different molecular composition, an important question in neurobiology is how the properties of individual synapses are established and maintained. Recent technical advances allow assay of activity at individual synapses and investigation of the relationship between function and molecular composition at the synapse.

Neurons are structurally complex cells composed of cellular “compartments,” e.g., dendrites, axons, and synapses. Although most neurons have these compartments in common, individual neurons and the synaptic connections between them differ significantly from each other in structure, functional characteristics, and molecular composition. Differences between synapses are particularly interesting because these are the sites of communication between neurons and differences are likely to have effects on neuronal functioning. There are functional differences between synapses that are well characterized, such as those due to different neurotransmitter type or neurotransmitter receptor. More subtle functional variance between synapses has become the focus of intensive investigation because it is believed that the ability of a synapse to adapt to changing stimuli and undergo functional modification may represent the basis for higher brain functions such as learning and memory (4). Long-term changes in synaptic functioning (e.g., long-term potentiation) can be induced in a specific population of synapses by stimulation of their inputs although unstimulated cells in the same region are unaffected, and there is evidence that individual synapses are differently affected by such stimulation. For instance, cultured hippocampal neurons make synapses that can be potentiated by addition of glutamate. Individual synapses in these cultures have different basal levels of activity and are functionally enhanced to different extents by the induction of this type of long-term potentiation (8). Furthermore, experiments have shown that different boutons of a single neuron can display different functional properties. Synapses made by a single neuron vary in several parameters, including probability of release, extent of facilitation, or depression (6, 9). In fact, synapses made by the same neuron can be facilitated or depressed independently of each other (6).
In addition to these functional differences, individual synapses also vary in their structure. Gross structural differences between specialized synapses have been identified, such as ribbon synapses of the retina that contain an electron-dense organelle presumed to be involved in continuous release of synaptic vesicles. There is also wide variation in structure between conventional synapses within other regions of the central nervous system, e.g., hippocampus or cortex. Ultrastructural studies show differences in overall synaptic size, number of vesicles, vesicle size, synaptic cleft size, and postsynaptic density area at individual hippocampal synapses (5, 11). It has been proposed that these structural differences correlate with variable functional parameters, such as probability of release (5).

Differences in synaptic structure and function may reflect differential molecular expression and/or protein localization. Neurons contain neuron-specific proteins, and, within these cells, such proteins are often restricted to specific compartments. For instance, microtubule-associated protein 2 (MAP2) is a neuronal protein found concentrated in dendrites, synaptosome-associated protein of 25 kDa (SNAP-25) is found primarily in axons, and still other proteins are concentrated in the presynaptic terminal. To date, over 20 presynaptic proteins have been identified that are preferentially located at synaptic terminals. These proteins can be divided into four classes according to subsynaptic localization: 1) intrinsic vesicle proteins, 2) peripheral vesicle proteins, 3) synaptic plasma membrane proteins, and 4) cytosolic proteins (Fig. 1; see Ref. 13). Many of these proteins have multiple isoforms, either homologous genes or splice variants that add even more diversity. For example, there are 12 genes and multiple splice variations encoding synaptotagmin, a protein believed to act as a calcium sensor in the process of vesicle fusion. Although the functions of these and other presynaptic proteins are incompletely characterized, their localization in synapses indicates a role in synaptic processes. It also emphasizes the possibility that the regulated localization/expression of presynaptic proteins may be a source of synaptic functional diversity and adaptation. Differential function could result from the presence, absence, or modification of a protein directly involved in neurotransmitter synthesis, vesicle fusion, or signal transduction. Numerous steps in the process of neurotransmission could be positively or negatively affected by long-term (e.g., transcriptional regulation) or short-term (e.g., protein phosphorylation) modifications of synaptic proteins.

Heterogeneous synaptic protein expression and localization

The expression of presynaptic proteins is not homogeneous in neurons throughout the brain. In situ hybridization studies show that certain mRNAs encoding synaptic proteins and their isoforms are transcribed only in subpopulations of neurons. Some isoforms of a synaptic protein are expressed in most neurons (e.g., synaptotagmins III, IV, VII, VIII, and IX), whereas others are preferentially expressed in neurons in particular brain areas (synaptotagmins I, II, and VI) (15). Similar differences in expression among neurons have been shown for isoforms of synaptic vesicle protein 2 (SV2), synaptophysin, VAMP/synaptobrevin, and synapsins (1). However, the expression of one isoform of a protein in a neuron does not exclude the expression of other isoforms of the same protein in the same neuron. SV2A and SV2B have been localized to the same cell, as have isoforms of synaptotagmin, synaptophysin, and synapsin (1). The combinatorial expression patterns of synaptic proteins in specific neurons suggests that the proteins, and even the different isoforms of these proteins, may have differing roles and that regulated expression of presynaptic proteins may be one means of influencing synaptic function. Further evidence in support of
this hypothesis is the observation that some presynaptic protein mRNA levels are regulated during development or depolarization. For instance, one isoform of SNAP-25 is expressed preferentially in development (SNAP-25a), whereas another isoform (SNAP-25b) is prevalent in mature animals (3). Thus neurons in different states may require different presynaptic proteins.

An obvious consequence of differences in expression between neurons is that molecular composition of synapses among neurons is different. Since similar events must occur at synapses—like release of neurotransmitter in response to neuronal stimulation—molecular differences may seem surprising. These observations support the idea that such differences underlie modulation of basic synaptic function. Studies of synaptic protein expression using immunocytochemistry show the existence of molecular variation between presynaptic terminals. Synaptic protein heterogeneity among synapses has been observed in vivo for synapsin, synaptotagmin, vesicle-associated membrane polypeptide (VAMP), SV2, and synaptophysin, among others (14), and cortical neuron synapses in culture are heterogeneous for SV2, synapsins, and synaptophysin (12). There is no known pattern of associated expression of one isoform of one synaptic protein with an isoform of another synaptic protein, e.g., VAMP2 and SV2B, and the differential distribution of synaptic proteins among synapses has not been correlated so far with other known neuronal characteristics, such as neurotransmitter type.

**Effects of experimental alteration of presynaptic proteins on synaptic function**

Further support for the idea that synaptic protein diversity may reflect variability in synaptic function comes from experiments in which levels of presynaptic proteins are increased or decreased. In fact, such experimental changes in overall levels of presynaptic proteins do affect neurotransmission. When expression of synapsins or synaptophysin is increased by introduction of exogenous protein or DNA encoding these proteins, properties of synaptic function are changed. For instance, when synapsin I is injected into *Xenopus* embryos, an increase in both the frequency and amplitude of spontaneous and evoked synaptic currents is observed (7). Similarly, if the expression/activity of some presynaptic proteins is decreased by inhibition with antibodies, treatment with antisense oligonucleotides, or genetic mutation, synaptic activity is altered. When synapsin I and II are “knocked out” by genetic mutation, mice show synaptic depression in response to repeated stimulation and decreased posttetanic potentiation (10). Similarly, mutation of cysteine string protein in *Drosophila* reduces neurotransmission, as does overexpression of Munc-18/n-Sec1. Interestingly, complete knockout of some synaptic proteins believed to be crucial in neuronal development or basic neurotransmission (synapsin I, synapsin II, synaptophysin) have not proved lethal to the genetically modified animals, although they show effects on synaptic function. Thus these observations reinforce the idea that some synapse-specific proteins may play modulatory rather than fundamental roles at the nerve terminal.

**Correlation of naturally-occurring differences in presynaptic protein localization with functional differences**

The fact that experimental modification of presynaptic proteins in cells influences aspects of neurotransmission suggests that the naturally-occurring differences in presynaptic protein levels at individual synapses may be associated with differences in synaptic activity. Although studies mentioned above show effects of manipulation of presynaptic protein levels in neurons, the question of the significance of endogenous presynaptic protein variation on synaptic functioning is more difficult to address. Recent advances in fluorescent imaging permit monitoring and quantitation of synaptic activity in response to stimulation using a fluorescent styryl dye, FM1-43 (Fig. 2; for review, see Ref. 2). FM1-43 is taken up by neurons during stimulation at sites characterized as synapses by colocalization.
with presynaptic protein immunostaining in mammalian central nervous system neurons and by electron microscopic analysis at frog neuromuscular junctions. Synaptic FM1-43 labeling is releasable on restimulation, demonstrating that, during endocytosis, the dye is taken up by synaptic vesicles that are available again for fusion. Consistent with this, electron microscopic studies of frog neuromuscular junctions show photooxidized FM1-43 in synaptic vesicles (2). Thus the specific localization of FM1-43 in synaptic vesicles allows the visualization of synapses in living neurons and permits evaluation of the extent of synaptic activity at an individual synapse (2). With the use of this method, activity-dependent uptake of FM1-43 has been quantified and compared with immunocytochemistry for presynaptic proteins at individual synapses in primary cultures of rat cortical neurons (Fig. 3). The levels of immunoreactivity for the synaptic vesicle-associated proteins synaptophysin, SV2, and synapsin, correlate with the extent of synaptic activity as detected by FM1-43 uptake. However, not all synaptic proteins show this pattern. Synapsin II immunoreactivity does not correlate with synaptic activity. Thus differences in some endogenous presynaptic proteins at individual synapses do reflect differences in stimulated activity.

**Differential targeting of synaptic vesicle proteins to synapses?**

Molecular synaptic differences may be due to phenotypic differences between neurons, i.e., some neurons may be differentiated in such a way that they do not express “protein A,” and thus synapses do not contain protein A, although other neurons may be of a type which do produce this protein. In this scenario, molecular synaptic heterogeneity would reflect diversity at a neuronal level and not primarily diversity among synapses. Conversely, a neuron may produce...
protein A, but it may be localized at select synapses of this neuron. In this case, heterogeneity would occur at the level of the individual synapse itself.

Recently, we directly tested the idea that different synapses in a single neuron may contain different presynaptic proteins. In these experiments, single neurons were cultured on small islands of permissive substrate in the midst of a nonpermissive growth substrate. This arrangement effectively prevented neurites from growing between islands. The typical single neuron on a substrate island grew neurites in circles on substrate droplets and made synapses after about five days in culture, as detected by immunostaining. When double immunostained for presynaptic proteins, single neurons showed heterogeneous localization of these proteins among synapses, i.e., some synapses contained more of protein A than protein B or vice versa (Fig. 4). In addition, some synapses contained nearly equal immunostaining for both proteins tested. This was the case for several combinations of antibodies, including those against synapsin I/SV2, synapsin I/synaptophysin, and synaptophysin/SV2 (12). These results show that there are molecular differences between synapses within a single neuron and suggests that presynaptic proteins may be targeted to specific synapses in response to local stimuli.

Conclusion

The mechanisms responsible for differential localization of presynaptic proteins at synapses are unknown. The fact that differential expression is seen at synapses of individual neurons argues for an effect of environment in the local vicinity of the synapse. In this view, local interactions between the presynaptic terminal and its target could refine the more general pattern of expression determined by neuronal phenotype. Differential conduction of electrical signals along neurites has been shown to occur and may be imagined to play a role in targeting of presynaptic proteins/vesicles. Alternatively, changes in protein turnover or delivery may depend on interactions at the synapse itself. Although these issues remain to be addressed, it is clear that specific targeting or degradation of presynaptic proteins may play a role in determining the functional properties of single synapses.

References


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