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Synaptic Ribbons: Versatile Signal Transducers

Minireview

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Photoreceptors and hair cells, the primary neurons of the retina and cochlea, detect and transmit incoming signals over a staggering dynamic range. From a moonless night at a Zen monastery to the tropical noon of a samba festival, light intensity (brightness) can vary over 10 orders of magnitude and sound pressure levels (loudness) can vary over 6 orders of magnitude. Both types of cells are also precisely tuned to distinguish fine gradations in stimuli that can flicker as fast as 50 Hz in the retina and as fast as 10 kHz in the cochlea of some species (Hudspeth, 2000). This level of performance seems to require synapses that are somehow specialized for rapid and continuous neurotransmitter release. It is thus not surprising that both the cochlea and retina express specialized active zones, with unique ultrastructure, called synaptic ribbons. These enigmatic organelles were first observed with electron microscopy in the early 1950s. Their elaborate architecture (Figure 1B), an electron-dense osmiophilic body or ribbon surrounded by a halo of tethered synaptic vesicles, immediately suggested a specialized function as a compact output site for neurotransmitter release (Lenzi et al., 1999). From early on it was hypothesized that ribbons may facilitate the transmission of the large bandwidths of information associated with sensory perception.

Synaptic ribbons come in a variety of shapes and sizes from large sheets (rod photoreceptors) to small and flattened ellipsoids (bipolar cells) to spheres (frog vestibular hair cells). The meaning of this diversity is unclear, except that it obviously changes the surface area of ribbons. The number of synapses made by a single ribbon can vary. In the retina, a single ribbon may synapse onto two, three, or four distinct postsynaptic processes, while each hair cell ribbon synapses onto just one. Interestingly, conventional active zones also contain an electron-dense structure called a presynaptic "bar" or "dense projection," to which docked vesicles appear to be attached. While the function and composition of these dense "bars" are largely unknown, it is thought they may help stabilize and anchor the docked vesicles. There is also evidence in some species that ribbons are morphologically plastic structures able to dynamically alter their shape and size over a period of hours or days. For example, going from dark- to light-adapted conditions can change the shape of retinal ribbons from sheet-like to spherical (Vollrath and Spiwoks-Becker, 1996). Hair cell ribbons also change shape during development. However, the precise function of such shape alterations and the means by which these changes are regulated remain unclear.

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Besides photoreceptors and hair cells, ribbons are also found in fish electroreceptors and the synaptic terminals of retinal bipolar cells. Ribbons are ubiquitous in vertebrates, but the retina and optic lobes of squid and octopus do not contain ribbons. Evolution has obviously found ways around them. Although there is some dispute regarding their classification, "ribbon-like" active zones are found in the eyes and even neuromuscular junctions of other invertebrates such as *Drosophila* and lobsters. What evolutionary advantages did they confer to vertebrates, and what functional capabilities do ribbon-type synapses have over conventional synapses? Here I briefly review some of the proposed ideas and speculate further on the possible functions of ribbons in the light of recent findings.

Synaptic Ribbons: Precision Machinery for Focal Exocytosis

Presynaptic exocytosis occurs preferentially at active zones where synaptic vesicles release neurotransmitter onto postsynaptic receptors. Since these receptors often have a very low affinity for neurotransmitter, they must be precisely localized just under the synaptic cleft. Consequently, vesicle fusion and the molecular components that trigger and mediate this fusion must all be precisely targeted to active zones. Accordingly, low-affinity glutamate receptors (AMPA-type) are localized just opposite to ribbons (Morigiwa and Vardi, 1999). It also seems that all sine qua non proteins involved in exocytosis at conventional synapses are present at ribbon-type synapses, albeit some in distinct isoforms (Morgans, 2000). Interestingly, some proteins are specifically localized to ribbon-type synapses (e.g., slow-inactivating L-type Ca^{2+} channels), while other components of conventional synapses (i.e., synapsins) have not been found at most ribbons. Some proteins (i.e., bassoon) even appear to be specifically localized to the ribbons of rods and cones in the retina but are absent from bipolar cell ribbons (Brandstätter et al., 2000).

Different Components of Release

In recent years, a variety of studies using a wide range of techniques have investigated the functional characteristics of ribbon synapses. Ribbons are associated with neurons that typically do not fire sodium action potentials. Some ribbon-containing neurons (such as neonatal inner hair cells and a class of bipolar cells) are capable of firing Ca action potentials (Glowatzki and Fuchs, 2000; Matthews, 2000), but for the most part, ribbons transmit signals continuously via graded membrane potential changes. In fact, voltage changes as small as 100 μV can reliably trigger changes in release. Capacitance measurements, vesicle staining FM dyes, and AMPA or NMDA receptor containing "sniffer" cells have all been used to measure exocytosis at ribbon synapses. What has emerged from these studies is the finding that ribbon synapses appear to have release properties distinct from conventional synapses.

Direct measurements of glutamate release via "sniffer" cells have indicated that bipolar cell ribbon synapses are characterized by two distinct components of release (Tachibana, 1999). The first component is small

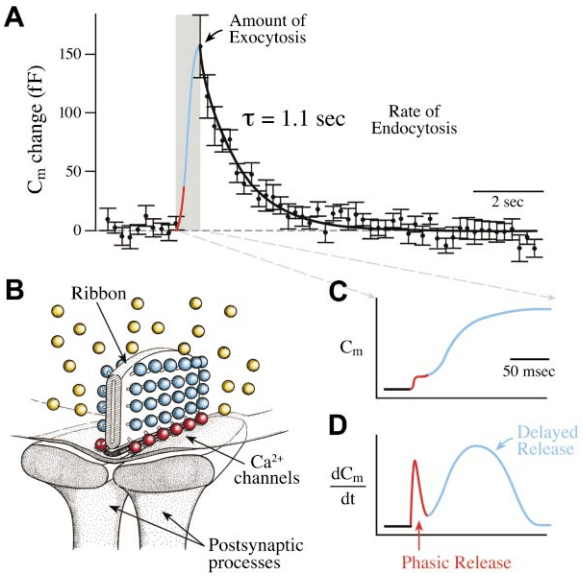

Figure 1. Exocytosis and Endocytosis at a Ribbon-Type Synapse

(A) Average membrane capacitance (C_m) change from isolated goldfish bipolar cell terminals (n = 11). A 250 ms step depolarization from −60 mV to 0 mV was given during the gray bar (on average 12 s after whole-cell break-in). This elicited a Ca current (not shown) and a jump in capacitance (exocytosis) of about 150 fF, which corresponds to the fusion of about 6000 synaptic vesicles. Capacitance then decayed back to baseline with a time constant τ = 1.1 s.

(B) A 3D cross-section of a synaptic ribbon (courtesy of D. Kennedy). Three vesicle pools are apparent: a bottom row of docked vesicles (red), vesicles tethered to the ribbon in upper rows (blue), and vesicles in the cytoplasm (yellow). There are an estimated 5–7 Ca channels per docked vesicle at bipolar cells (Tachibana, 1999).

(C) An enlarged view of the C_m increase during the depolarization (Matthews, 2000). C_m increases in two distinct phases: a rapid phase that may correspond to the docked vesicles (red), and a slower phase that may correspond to the fusion of the remaining vesicles on the ribbon (blue).

(D) The derivative of C_m gives a measure of the rate of exocytosis during the depolarization. A fast phasic component is followed by a slower and delayed component of exocytosis. This biphasic rate of exocytosis corresponds closely to the kinetics of glutamate release measured by AMPA-type currents (Tachibana, 1999).

but very fast, occurring in less than a millisecond after Ca channel opening. The second component occurs with a delay, has slower kinetics, and is much larger in size. It is saturable after about 250 ms of strong depolarization. However, for weak depolarizations, this second component can be quite tonic and prolonged. Accordingly, retinal ganglion cells and auditory nerve fibers, the elements postsynaptic to bipolar and hair cells, have phasic and tonic components to their firing patterns (Freed, 2000; Hudspeth, 2000). Although these two components seem analogous to the phasic and delayed components of release observed at conventional synapses, there are large quantitative differences. Whereas delayed release is a small component (<20%) of the total release at conventional synapses (Chen and Regehr, 1999), it can be 4- to 5-fold greater than phasic release at ribbon synapses. The ability to generate a large component of delayed release thus emerges as a striking difference between ribbon and conventional synapses.

Capacitance measurements, which are exquisitely sensitive to changes in cell surface area, have also been used to determine the release properties of ribbon synapses. Individual synapses vary greatly in their release probabilities, and release probability depends on the size of the readily releasable pool (RRP) of vesicles. An indication that release probability may be high at ribbon synapses is the fact that they exhibit strong paired-pulse depression, which is likely due to short-term vesicle pool depletion. Goldfish bipolar cells, for example, have a saturating capacitance jump of 150 fF, which corresponds to a RRP of about 6000 vesicles (Figure 1A). This RRP is close to the number of vesicles attached in toto to all the ribbons of the terminal, because there are about 55 ribbons per terminal, each with 110 vesicles (Matthews, 2000). Similar to the components of glutamate release described above, high time resolution capacitance measurements have identified two distinct vesicle pools within the RRP. The first component consists of a small ultrafast pool of about 1200 vesicles that is released within the first 5 ms (Figure 1C , red trace). This is followed by a larger and delayed pool of about 4800 vesicles which saturates after about 250 ms (Figure 1C, blue trace). Interestingly, the release properties of mouse inner hair cells are quite different. In hair cells, the ultrafast pool is about 280 vesicles and no subsequent saturation of the capacitance jumps is observed, even for strong depolarizating pulses as long as 1 s (Moser and Beutner, 2000). The number of vesicles released during a 1 s pulse far exceeds those tethered to the ribbons, suggesting that hair cell ribbons may be specialized for more continuous and uninterrupted release than bipolar cell ribbons. Thus, ribbons from different cell types may be fine tuned for different tasks, perhaps by having different isoforms of synaptic proteins (Safieddine and Wenthold, 1999). Compared to the 110 vesicles per bipolar cell ribbon, hippocampal bouton-type conventional synapses have a RRP of about 8–10 vesicles, which corresponds to the coterie of docked vesicles per active zone (Schikorski and Stevens, 1997). Thus, by and large, ribbon active zones seem to be associated with a larger RRP than conventional active zones.

Endocytosis and Ca Channel Locus

The hallmarks of endocytosis at ribbon synapses are speed and completeness. Vesicular membrane is retrieved following a single exponential time constant τ = 1.1 s in bipolar cells (Figure 1A) and τ = 7.5 s in hair cells (Moser and Beutner, 2000), and the capacitance trace returns back to its previous baseline. Recent findings using FM dyes also indicate that a rapid form of endocytosis (τ = 1 s) is present at bouton-type synapses (Pyle et al., 2000). Rapid vesicle recycling thus appears to be present in both ribbon and conventional synapses.

Ca-sensitive fluorescent dyes (Issa and Hudspeth, 1994), as well as more recent immunohistochemical studies (Taylor and Morgans, 1998; Nachman et al., 1999), have demonstrated that Ca channels are anchored and corralled into clusters at sites adjacent to synaptic ribbons. What is the function of this tight clustering of Ca channels at ribbons synapses? One possibility is that by positioning the Ca sensor for exocytosis close to the Ca channel, the synapse is able to detect small changes in Ca concentration and also promote release on rapid time scales. Capacitance measure-

ments have shown that rapid release at bipolar cell synapses requires concentrations of Ca in the range of 100–200 μM (Heidelberger, 1998). More recent measurements of vesicle turnover using FM dyes have suggested that in addition to such rapid release properties, bipolar cell terminals also respond to significantly lower Ca concentrations that can maintain more moderate rates of vesicle turnover for longer time intervals (Neves and Lagnado, 1999). The ensemble of these results suggest that bipolar cell ribbons may be particularly versatile signal transducers capable of mediating both transient and sustained release. A capacity for copious tonic release would be advantageous for a sensory synapse that needs to transmit slow analog-like signals.

Ribbon Function: Vesicle Sieve, Conveyor Belt, or Safety Belt?

The distinct components of release at ribbon synapses may, in fact, be rooted in the intricate architecture of the ribbon. Figure 1B shows a pictorial rendering of a bipolar cell ribbon. A correspondence between the phasic, or first component of release, to the docked vesicles (red vesicles) is apparent, as is one between the second, delayed component of release, to the vesicles attached to the upper rows of the ribbon (blue vesicles). Finally, the vesicles immediately adjacent to the ribbon, but not directly tethered (yellow vesicles), may constitute a reserve pool, which is activated during prolonged depolarizations. Neurotransmitter release during continued stimulation thus displays temporally distinct phases that may correspond to morphologically distinct vesicle pools.

If in fact these vesicle pools are indeed the morphological correlate of the various release components, the obvious question then is how does the ribbon structure contribute to the kinetics of vesicle fusion and release? A number of potential functions for ribbons have been proposed. Recently, Heidelberger (1998) has shown that if ATP is abundant in the terminal's milieu and the Ca stimulus sufficient, the entire pool of 6000 vesicles in bipolar cell terminals can fuse within milliseconds. This remarkable finding suggests that all the vesicles associated with the ribbons may be ready or "primed" for exocytosis. One function of ribbons may thus be to preferentially capture primed vesicles, or even help prime the vesicles themselves. Perhaps ribbons act as a sort of vesicle "Velcro" and only primed vesicles are able to stick to ribbons. Ribbons may thus act as vesicle "sieves" that increase the RRP of vesicles by helping to sort out these "primed" vesicles from the vast reservoir of cytoplasmic vesicles. A further extension of this model is that once a vesicle is captured, an additional function of the ribbon may be as "conveyor-belt" for the active transport of vesicles down their surface toward their docking sites. This idea implies a molecular ATP-driven motor. Accordingly, the experiments of Heidelberger (1998) show that continued release at bipolar cells is dependent on ATP-Mg. Furthermore, a kinesin-type molecular motor has been localized to retinal ribbons (Muresan et al., 1999). Interestingly, increases in Ca influx have been shown to shorten the delay in the second component of release, suggesting that elevated Ca can accelerate the mobilization of vesicles on the ribbon toward their fusion sites on the plasma membrane (Tachibana, 1999). Finally, an additional function of ribbons may be to keep the v-SNAREs of vesicles on the ribbons within "striking distance" of t-SNAREs on the plasma membrane (Zenisek et al., 2000). This would ensure a high probability of release and perhaps allows ribbons to also function as "safety belts" that guarantee continued release, provided Ca channels are open.

Video Taping Vesicles in Action

A potentially direct means of testing these models would be to videotape single synaptic vesicles as they move on ribbons and near the plasma membrane. That strategy seemed like a distant sci-fi dream until the recent experiments of Zenisek et al. (2000). Using evanescent wave fluorescence microscopy, Zenisek et al. (2000) were able to image events occurring within the first 50–100 nm from the plasma membrane by tracking vesicles labeled with FM dyes as they move within this region. Amazingly, single synaptic vesicle transport, docking, and fusion events can be clearly observed. Several findings are noteworthy. First, in close agreement with previous capacitance and glutamate release measurements, the kinetics of exocytosis had a fast component followed by a slower and delayed component. Second, the vast majority of the vesicles in the vicinity of plasma membrane are in a state of continuously colliding and bouncing off the plasma membrane. This free mobility contrasts with conventional active zones where most reserve vesicles appear confined to clusters near active zones where they are presumably bound by synapsin to an actin cytoskeleton. Third, only a small population of "resident" vesicles undergoes fusion following Ca influx, and during repeated rounds of exocytosis, they appear to fuse in more or less the same general location thus defining a "hot spot" for fusion. It will be very interesting to see if these hot spots coincide with the location of ribbons and perhaps to sites of preferential clustering of Ca channels. Interestingly, these videos of vesicles in motion have also shown that, once a vesicle fuses, a new vesicle can rapidly (within 250 ms) move vertically down and occupy that vacant docking site.

Alternative Scenarios

These models for ribbon function are compelling. However, it should also be noted that notwithstanding the recent progress, there is still no direct evidence that ribbons participate in the fusion process. While the second component of release, for example, could be interpreted as a consequence of the mobilization of vesicles from ribbons, other interpretations are possible. For instance, vesicles, which appear uniform via EM, may in fact be biochemically heterogeneous in terms of their sensitivity to Ca, and this may produce distinct components of release. The second component may be mediated not by the vesicles associated with ribbons but by those docked to the plasma membrane at sites distal from Ca channels (Sakaba and Neher, 2001). The slow kinetics of the second component, and its sensitivity to EGTA, a slow Ca buffer, could then reflect the need for Ca to diffuse to and accumulate at the docking site. The ultrafast and, more importantly, single exponential kinetics of exocytosis during flash photolysis of caged Ca would be readily explained by this scenario (Heidelberger, 1998). However, this model would require that the total pool of docked and fusion-competent vesicles on the plasma membrane be somehow fixed at about 6000 vesicles, which seems rather unlikely. Further studies,

perhaps with more intact preparations subject to physiological stimuli, are clearly needed to settle these issues.

The assumption that the saturation in capacitance jumps implies a depletion of a vesicle pool is also subject to debate. First, capacitance measures only net changes in surface area and a constant capacitance could be due to ongoing exocytosis and endocytosis that are exactly compensated. The fast rate of glutamate release, and its saturation within 250 ms (Figure 1C), indicates exocytosis does not overlap temporally with endocytosis for brief stimuli. However, during prolonged stimulation, significant overlap between exo- and endocytosis may be necessary to conserve the area of the synaptic terminal. Second, the saturating 150 fF capacitance jump (Figure 1A) corresponds to an 8%–10% increase in surface area of the terminal. This could cause significant disruption of the plasma membranes ability to support exocytosis. Some forms of synaptic depression may thus result from a transient disruption of active zones following vesicle fusion or from a selective depletion of high release probability vesicles (Burrone and Lagnado, 2000).

There are also many unanswered questions regarding how transmission at ribbon synapses is modulated. The size of the RRP may, for example, increase depending on previous synaptic activity or PKC activation (Tachibana, 1999). Does this correspond to an increase in the size of the ribbon? Or are vesicles not associated with ribbons fusing? In this regard, why are photoreceptor and hair cell ribbons so large compared to bipolar cell ribbons? Do they have larger RRPs? Ultimately, to address these questions it will be necessary to somehow disrupt ribbons and determine whether this blocks vesicle fusion or slows their mobilization rate. It would be fascinating, for example, to prevent vesicles from binding to the ribbons and then check whether capacitance jumps are abolished or reduced. This could be done perhaps by dialyzing peptides into the terminal that specifically bind to ribbons and thereby prevent vesicle attachment. One would then expect to see denuded ribbons and no capacitance jumps. Denuded ribbons have been notoriously difficult to see except in fish electroreceptors (Lenzi et al., 1999). In this regard, experiments using the classic rapid freezing technique of Heuser and Reese could be quite illuminating.

Molecular Composition: RIBEYE

One of the long-standing mysteries of ribbons has been their molecular composition. As discussed previously, ribbon synapses contain many of the proteins found at conventional synapse. But presumably, ribbons must also contain unique components. Recently, after a long and painstaking process of purification, Schmitz et al. (2000) succeeded in cloning a novel protein from synaptic ribbons. RIBEYE is ~120 kDa and is composed of two domains: a unique A domain that is specific for ribbons and a B domain homologous to CtBP2, a transcriptional repressor. While the exact function of RIBEYE remains mysterious, Schmitz et al. (2000) propose an interesting model where ribbons are constructed in a modular fashion by the two subunits of RIBEYE. One subunit may act as an aggregating module, while the other may act as an enzyme. Interestingly, the gene for RIBEYE is not found in the genome of *Drosophila* or *C. elegans*, indicating it may be a vertebrate innovation. RIBEYE also does not seem to be present in hair cells, perhaps indicating yet again that hair cell ribbons may be fundamentally different from retinal ribbons. Given the very different tasks of the retina and cochlea, this may not be too surprising, after all. Clearly, many questions remain regarding RIBEYE function specifically, and the molecular composition of ribbons more generally. However, undoubtedly this tour de force study will open the door for further characterization of the composition and structure of ribbons.

In summary, by a combination of ultrastructural studies and independent measurements of release, evidence has emerged that synaptic ribbons are reservoirs of a large RRP of synaptic vesicles. Furthermore, ribbons appear to function as highly efficient and versatile nanomachines for high-throughput exocytosis, operating in both phasic and tonic modes of release. Obviously, many key questions remain unresolved. However, the recent molecular studies, perhaps coupled to the novel microscopy techniques, may soon allow us to sort out fact from speculation as we unravel the raison d'être of synaptic ribbons.

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