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Synaptic Ribbon: Conveyor Belt or Safety Belt?

Review

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The synaptic ribbon in neurons that release transmitter via graded potentials has been considered as a conveyor belt that actively moves vesicles toward their release sites. But evidence has accumulated to the contrary, and it now seems plausible that the ribbon serves instead as a safety belt to tether vesicles stably in mutual contact and thus facilitate multivesicular release by compound exocytosis.

Sensory receptors and second-order neurons commonly encode graded input with a graded, nonregenerative membrane potential. By avoiding spikes, the receptor cell can vary synaptic output continuously and thus transfer more information (van Hateren, 1992; de Ruyter van Steveninck and Laughlin, 1996). To finely grade the quantized synaptic output requires that many synaptic vesicles be released at high rates (Laughlin et al., 1987). Accordingly, the photoreceptor, hair cell, and retinal bipolar cell all exocytose several thousand vesicles s^{-1} (Rieke and Schwartz, 1996; Parsons et al., 1994; von Gersdorff and Matthews, 1994). Such performance implies both a large pool of "readily releasable" vesicles and a mechanism for their rapid release.

The active zones at these synapses employ a specialized structure, the "ribbon" or "dense body," which anchors to the presynaptic membrane only nanometers from the clustered, voltage-gated calcium channels. The ribbon tethers 100 or more synaptic vesicles, each by several short filaments, to form an apparently stable depot. This static impression of a depot, based on electron microscopy, seems to be supported by the dynamic observation from total internal reflectance microscopy that vesicles diffuse to "hot spots" on the plasma membrane where they are released preferentially (Zenisek et al., 2000). Each cell employs multiple ribbons, ranging from 10 to 100, and consequently, the number of vesicles tethered to all of the ribbons is on the order of 1000 to 10,000 (von Gersdorff et al., 1996; Lenzi et al., 1999).

These tethered vesicles correspond numerically to the size of the readily releasable pool (von Gersdorff et al., 1996). Vesicles in this pool appear to have undergone all of the priming reactions that are essential for exocytosis, because the whole pool can be released in the absence of ATP hydrolysis (Heidelberger et al., 2002). Thus, the current hypothesis is that the vesicles tethered to all of the ribbons comprise the readily releasable pool.

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Do Vesicles Move Down the Ribbon?

The image of a ribbon fully loaded with readily releasable vesicles suggested an *active* mechanism, some sort of "conveyor belt," to shuttle vesicles downward toward docking/release sites. This idea appeared to provide a mechanism for sustaining high release rates (Parsons et al., 1994). Indeed, the multiple kinetic components of exocytosis could be interpreted as set by the transit time for vesicles from different locations on the ribbon to reach the release sites. Specifically, a small, rapid pool ("ultrafast") matches the number of vesicles pressed up against the presynaptic membrane (Mennerick and Matthews, 1996), and a larger, slower pool ("readily releasable") corresponds to the remaining vesicles tethered to the ribbon (von Gersdorff and Matthews, 1994; von Gersdorff et al., 1996). Similar kinetic components have been reported for auditory hair cells (Moser and Beutner, 2000).

How would the vesicles move along the ribbon? A kinesin polypeptide, Kif3a, was identified on the ribbon by immunostaining (Muresan et al., 1999), suggesting that a motor might transport vesicles along the ribbon. However, microtubules, considered to be the obligatory rails for a kinesin motor, are missing. On the other hand, the ribbon's surface is studded with proteinaceous knobs (Usukura and Yamada, 1987)—which suggest macromolecular "stepping stones" across which the end-feet of motorized filaments might stride in stilt-like fashion (Rao-Mirotznik et al., 1995).

But evidence accumulates against an active conveyor belt. When ATP-γS is substituted for ATP in a patch pipette and dialyzed into an isolated retinal bipolar terminal, there is no effect on the extent or timing of release: stepping the cell to 0mV still releases the whole ready pool of 5000 vesicles within 200 ms (Heidelberger et al., 2002). Since ATP-γS does not support kinesin or other cytoplasmic motors, this result seems to exclude them from a role in discharging either the fast or slow components of vesicles tethered to the ribbon. Furthermore, the readily releasable pool can fuse at 100-fold faster rates: when $[Ca^{2+}]$ is raised instantaneously by flash photolysis in a bipolar terminal, all vesicles exocytose in 1–2 ms (Heidelberger et al., 1994). If vesicles move along the ribbon, the rate would be $>100\ \mu m\ s^{-1}$, much faster than the fastest molecular motor associated with intracellular transport (Gilbert, 2001).

Vesicles might diffuse along the ribbon. To match the rate triggered by flash photolysis, a diffusion constant would need to be at least $0.1 \times 10^{-6}\ cm^2\ s^{-1}$ (Hille, 1992). However, measurements of the actual movement of synaptic vesicles in approaching putative release sites in retinal bipolar cells are consistent with a diffusion constant three orders of magnitude slower (Zenisek et al., 2000). Such slow diffusion cannot account for the maximal measured rates of exocytosis (Heidelberger et al., 1994; Heidelberger, 1998). The situation in a cochlear

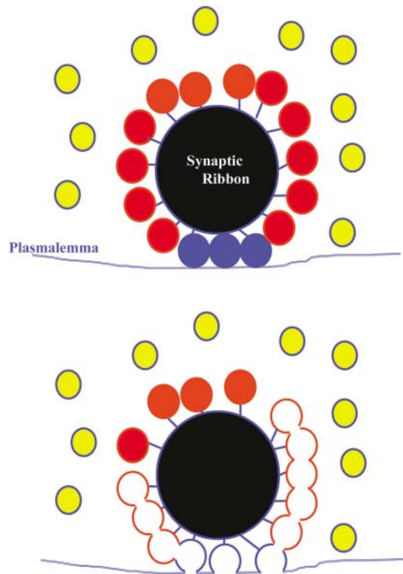

Figure 1. Schematic of Compound Exocytosis at a Ribbon Synapse
(Upper panel) Ribbon holds synaptic vesicles in close proximity to each other near the active zone. Traditional docked vesicles are shown in blue, vesicles tethered to ribbon in red, and cytoplasmic vesicles in yellow. (Lower panel) Compound exocytosis results from the fusion of vesicles tethered to the ribbon with docked vesicles either preceding or following their fusion with the plasmalemma.

inner hair cell is even more challenging, as 27,000 vesicles can fuse in <1 ms (Beutner et al., 2001). This implies that the fusion-competent pool in the hair cell far exceeds the pool of vesicles tethered to the ribbons, so the vesicles would need to move even farther—and faster. Thus, the fast exocytosis of large numbers of vesicles by ribbon synapses cannot be explained by known mechanisms of vesicle movement.

Compound Fusion?

Perhaps vesicles do not move on the ribbon at all. Instead, they might be tethered stably in mutual contact, positioned to fuse serially with each other in a cascading process termed "compound fusion" (Figure 1). Although compound fusion has not been described at synapses, it is a well-established mechanism for focal vesicular release by nonneural secretory cells. For example, blood granulocytes can target and secrete large quantities of degradative enzymes and vasoactive amines via compound exocytosis (Scepek and Lindau, 1993; Lollike et al., 2002). This mechanism has been suggested for ribbon synapses to explain how raising intracellular calcium rapidly by photolytic uncaging can evoke nearly instantaneous exocytosis of the whole releasable pool (Heidelberger et al., 1994; Heidelberger, 1998) and how depolarization can also trigger rapid release of this pool (B.W. Edmonds, F.D. Gregory, and F.E. Schweizer, personal communication).

Some anatomical observations are consistent with compound fusion. Tubular structures near synaptic ribbons are observed in freeze fracture (Hama and Saito, 1977; Hama, 1980) and in reconstructions from ultrathin sections (Rao-Mirotznik et al., 1995; Lenzi et al., 1999, 2002). These structures were interpreted as endosomes and were assumed to represent postrelease coalescence of vesicles that had been released individually. Yet conceivably, these structures represent endocytic recovery of membrane from vesicles released by compound fusion; i.e., the coalescence might represent *exocytosis* rather than endocytosis.

The hypothesis of compound fusion is also consistent with recent recordings postsynaptic to the cochlear inner hair cell. A tiny afferent terminal contacts the base of this cell and receives transmitter from one to two ribbon synapses. Patching this terminal, Glowatzki and Fuchs (2002) observed that the EPSCs were temporally clustered, suggesting that individual quanta tend to be released together. Furthermore, these events varied in amplitude from 40 pA (expected for a single vesicle) to 20-fold larger. The distributions of EPSC intervals and amplitudes are consistent with the coordinated release of multiple quanta. While this does not prove compound fusion, such a mechanism could certainly explain the observation.

Molecular Mechanisms of Compound Exocytosis

Widely accepted models of the events that mediate fast exocytosis involve "heterotypic" fusion between vesicles and plasma membrane. These models require molecular interaction between vesicle v/Q SNAREs and target membrane t/R SNAREs (Sollner and Rothman, 1994; Pevsner et al., 1994). Such simple SNARE models cannot explain compound exocytosis in nonneuronal cell types that use "homotypic" fusion between contiguous vesicles. Homotypic fusion employs interaction between v/Q SNAREs and t/Q SNAREs, both on the vesicles (Hansen et al., 1999; Niemeyer and Schwarz, 2000; Boeddinghaus et al., 2002; Castle et al., 2002). Vesicular t/Q SNAREs have not yet been identified at ribbon synapses (Lenzi and von Gersdorff, 2001), but they are found in significant amounts on vesicles isolated from conventional synapses (Walch-Solimena et al., 1995). Furthermore, vesicular t/Q- and v/R-SNARES interact in the absence of plasmalemma (Otto et al., 1997). Thus, assuming appropriate regulation, known synaptic machinery may be sufficient to mediate compound exocytosis at the ribbon synapse.

Some studies of fast exocytosis suggest that the presynaptic fusion machinery must lie within nanometers of the calcium channels to permit their interaction (Sheng et al., 1994; Rettig et al., 1996). Yet, at ribbon synapses, fast exocytosis certainly occurs for vesicles initially tethered 30–100 nm away from the calcium channels. Furthermore, fusion machinery need not always interact directly with calcium channels, since disruption of their interaction site on the channel (the "synprint" site) reduces neurotransmitter release only ~25% (Mochida et al., 1996; Rettig et al., 1997). At the calyx of Held, an auditory synapse specialized for speed and fidelity, studies with calcium buffers suggest that about half of the vesicles released by fast exocytosis lie 30–300 nm away from any calcium channel cluster (Borst and Sakmann, 1996). Thus, calcium diffusing over hundreds of nanometers apparently does trigger fast exocytosis and could support homotypic fusion and compound exocytosis.

Utility of the Synaptic Ribbon and Compound Fusion

Many junctions in the nervous sytem require synchronized release of numerous synaptic vesicles. Spiking neurons achieve this via a propagated action potential that releases one vesicle at each of many active zones (Korn and Faber, 1991). For example, an action potential at the neuromuscular junction releases ~125 vesicles from ~300 active zones (Miyamoto, 1975), and the calyx of Held releases ~160 vesicles from ~600 active zones (Meyer et al., 2001). This makes transmission reliable, despite the unreliability of release at each active zone, and it makes transmission fast, despite the intrinsic sluggishness of the vesicle fusion cascade (del Castillo and Katz, 1954; Almers, 1994).

Nonspiking neurons in retina and cochlea also require synchronized release of vesicles. But for these cells, hundreds of active zones per synapse are not an option—because of a space constraint. These neurons are precisely organized in two-dimensional arrays: photoreceptor and bipolar cells to map the visual field, and cochlear hair cells to map the tonotopical distribution of auditory frequencies along the basilar membrane. So their synaptic terminals can occupy no more space in two dimensions than their cell bodies. The ribbons seem to address this problem by stably tethering large numbers of vesicles in mutual contact where their synchronized and rapid release might be accomplished by compound exocytosis.

Such multivesicular release might seem unneccessary at a cochlear ribbon synapse, because the postsynaptic axon presents such high resistance that a single vesicle can evoke an action potential (Siegel, 1992; Glowatzki and Fuchs, 2002). However, triggering with one vesicle might introduce temporal jitter—the curse of the auditory system. Multiple vesicles would increase the rate of postsynaptic depolarization ("dV/dt"), thus reducing jitter and improving synaptic fidelity. Multivesicular release should also improve transfer of high-frequency signals by increasing firing probability during the relative refractory period (Geisler, 1998). The 20-fold amplitude variation in synaptic responses suggests that, unlike CNS synapses, postsynaptic receptors at cochlear ribbon synapses are far from saturation (Glowatzki and Fuchs, 2002). Consequently, saturation is not an obstacle to the advantages of multivesicular release. In short, multivesicular release mediated by compound exocytosis at the cochlear ribbon synapse might underlie its unusual fidelity and bandwidth, also important features of ribbon synapses in the retina (DeVries, 2000; Freed, 2000).

Conclusion: Ribbon as a "Safety Belt"

Despite the intuitive appeal of the ribbon as a conveyor belt for actively moving vesicles toward their sites for docking and release, evidence has accumulated to the contrary. It now seems plausible that the ribbon could serve as a safety belt to tether vesicles stably in mutual contact, thus facilitating multivesicular release by compound exocytosis. If compound exocytosis proves to be an important release mechanism at ribbon synapses, some molecular models of synaptic vesicle priming, docking, and fusion would need to be reexamined. Future experiments to test this hypothesis might include release triggered by flash photolyis—visualized ultrastructurally after rapid freezing or imaged in real time by total internal reflectance microscopy.

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References

Almers, W. (1994). Synapses: How fast can you get? Nature *367*, 682–683.

Beutner, D., Voets, T., Neher, E., and Moser, T. (2001). Calcium dependence of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse. Neuron *29*, 681–690.

Boeddinghaus, C., Merz, A.J., Laage, R., and Ungermann, C. (2002). A cycle of Vam7p release from and PtdIns 3-P-dependent rebinding to the yeast vacuole is required for homotypic vacuole fusion. J. Cell Biol. *157*, 79–89.

Borst, J.G., and Sakmann, B. (1996). Calcium influx and transmitter release in a fast CNS synapse. Nature *383*, 431–434.

Castle, J., Guo, Z., and Liu, L. (2002). Function of the t-SNARE SNAP-23 and secretory carrier membrane proteins (SCAMPs) in exocytosis in mast cells. Mol. Immunol. *38*, 16–18.

de Ruyter van Steveninck, R.R., and Laughlin, S.B. (1996). The rate of information transfer at graded-potential synapses. Nature *379*, 642–645.

DeVries, S.H. (2000). Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. Neuron *28*, 847–856.

del Castillo, J., and Katz, B. (1954). Quantal components of the end-plate potential. J. Physiol. *124*, 560–573.

Freed, M.A. (2000). Parallel cone bipolar pathways to a ganglion cell use different rates and amplitudes of quantal excitation. J. Neurosci. *20*, 3956–3963.

Geisler, C.D. (1998). From Sound to Synapse: Physiology of the Mammalian Ear (New York: Oxford University Press).

Gilbert, S.P. (2001). High-performance fungal motors. Nature *414*, 597–598.

Glowatzki, E., and Fuchs, P.A. (2002). Transmitter release at the hair cell ribbon synapse. Nat. Neurosci. *5*, 147–154.

Hama, K. (1980). Fine structure of the afferent synapse and gap junctions on the sensory hair cell in the saccular macula of goldfish: a freeze-fracture study. J. Neurocytol. *9*, 845–860.

Hama, K., and Saito, K. (1977). Fine structure of the afferent synapse of the hair cells in the saccular macula of the goldfish, with special reference to the anastomosing tubules. J. Neurocytol. *6*, 361–373.

Hansen, N.J., Antonin, W., and Edwardson, J.M. (1999). Identification of SNAREs involved in regulated exocytosis in the pancreatic acinar cell. J. Biol. Chem. *274*, 22871–22876.

Heidelberger, R. (1998). Adenosine triphosphate and the late steps in calcium-dependent exocytosis at a ribbon synapse. J. Gen. Physiol. *111*, 225–241.

Heidelberger, R., Heinemann, C., Neher, E., and Matthews, G. (1994). Calcium dependence of the rate of exocytosis in a synaptic terminal. Nature *371*, 513–515.

Heidelberger, R., Sterling, P., and Matthews, G. (2002). Role of ATP in depletion and replenishment of the releasable pool of synaptic vesicles. J. Neurophysiol. *88*, 98–106.

Hille, B. (1992). Ionic Channels of Excitable Membranes, 2nd Edition (Sunderland, MA: Sinauer Associates Inc.).

Korn, H., and Faber, D.S. (1991). Quantal analysis and synaptic efficacy in the CNS. Trends Neurosci. *14*, 439–445.

Laughlin, S.B., Howard, J., and Blakeslee, B. (1987). Synaptic limitations to contrast coding in the retina of the blowfly Calliphora. Proc. R. Soc. Lond. B Biol. Sci. *231*, 437–467.

Lenzi, D., and von Gersdorff, H. (2001). Structure suggests function:

the case for synaptic ribbons as exocytotic nanomachines. Bioessays *23*, 831–840.

Lenzi, D., Runyeon, J.W., Crum, J., Ellisman, M.H., and Roberts, W.M. (1999). Synaptic vesicle populations in saccular hair cells reconstructed by electron tomography. J. Neurosci. *19*, 119–132.

Lenzi, D., Crum, J., Ellisman, M.H., and Roberts, W.M. (2002). Depolarization redistributes synaptic membrane and creates a gradient of vesicles on the synaptic body at a ribbon synapse. Neuron *36*, 649–659.

Lollike, K., Lindau, M., Calafat, J., and Borregaard, N. (2002). Compound exocytosis of granules in human neutrophils. J. Leukoc. Biol. *71*, 973–980.

Mennerick, S., and Matthews, G. (1996). Ultrafast exocytosis elicited by calcium current in synaptic terminals of retinal bipolar neurons. Neuron *17*, 1241–1249.

Meyer, A.C., Neher, E., and Schneggenburger, R. (2001). Estimation of quantal size and number of functional active zones at the calyx of held synapse by nonstationary EPSC variance analysis. J. Neurosci. *21*, 7889–7900.

Miyamoto, M.D. (1975). Binomial analysis of quantal transmitter release at glycerol treated frog neuromuscular junctions. J. Physiol. *250*, 121–142.

Mochida, S., Sheng, Z.H., Baker, C., Kobayashi, H., and Catterall, W.A. (1996). Inhibition of neurotransmission by peptides containing the synaptic protein interaction site of N-type Ca^{2+} channels. Neuron *17*, 781–788.

Moser, T., and Beutner, D. (2000). Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. Proc. Natl. Acad. Sci. USA *97*, 883–888.

Muresan, V., Lyass, A., and Schnapp, B.J. (1999). The kinesin motor KIF3A is a component of the presynaptic ribbon in vertebrate photoreceptors. J. Neurosci. *19*, 1027–1037.

Niemeyer, B.A., and Schwarz, T.L. (2000). SNAP-24, a Drosophila SNAP-25 homologue on granule membranes, is a putative mediator of secretion and granule-granule fusion in salivary glands. J. Cell Sci. *113*, 4055–4064.

Otto, H., Hanson, P.I., and Jahn, R. (1997). Assembly and disassembly of a ternary complex of synaptobrevin, syntaxin, and SNAP-25 in the membrane of synaptic vesicles. Proc. Natl. Acad. Sci. USA *94*, 6197–6201.

Parsons, T.D., Lenzi, D., Almers, W., and Roberts, W.M. (1994). Calcium-triggered exocytosis and endocytosis in an isolated presynaptic cell: capacitance measurements in saccular hair cells. Neuron *13*, 875–883.

Pevsner, J., Hsu, S.C., Braun, J.E., Calakos, N., Ting, A.E., Bennett, M.K., and Scheller, R.H. (1994). Specificity and regulation of a synaptic vesicle docking complex. Neuron *13*, 353–361.

Rao-Mirotznik, R., Harkins, A.B., Buchsbaum, G., and Sterling, P. (1995). Mammalian rod terminal: architecture of a binary synapse. Neuron *14*, 561–569.

Rettig, J., Sheng, Z.H., Kim, D.K., Hodson, C.D., Snutch, T.P., and Catterall, W.A. (1996). Isoform-specific interaction of the alpha1A subunits of brain Ca2+ channels with the presynaptic proteins syntaxin and SNAP-25. Proc. Natl. Acad. Sci. USA *93*, 7363–7368.

Rettig, J., Heinemann, C., Ashery, U., Sheng, Z.H., Yokoyama, C.T., Catterall, W.A., and Neher, E. (1997). Alteration of Ca2+ dependence of neurotransmitter release by disruption of Ca2+ channel/syntaxin interaction. J. Neurosci. *17*, 6647–6656.

Rieke, F., and Schwartz, E.A. (1996). Asynchronous transmitter release: control of exocytosis and endocytosis at the salamander rod synapse. J. Physiol. *493*, 1–8.

Scepek, S., and Lindau, M. (1993). Focal exocytosis by eosinophils–compound exocytosis and cumulative fusion. EMBO J. *12*, 1811–1817.

Sheng, Z.H., Rettig, J., Takahashi, M., and Catterall, W.A. (1994). Identification of a syntaxin-binding site on N-type calcium channels. Neuron *13*, 1303–1313.

Siegel, J.H. (1992). Spontaneous synaptic potentials from afferent terminals in the guinea pig cochlea. Hear. Res. *59*, 85–92.

Sollner, T., and Rothman, J.E. (1994). Neurotransmission: harnessing fusion machinery at the synapse. Trends Neurosci. *17*, 344–348.

Usukura, J., and Yamada, E. (1987). Ultrastructure of the synaptic ribbons in photoreceptor cells of Rana catesbeiana revealed by freeze-etching and freeze-substitution. Cell Tissue Res. *247*, 483–488.

van Hateren, J.H. (1992). A theory of maximizing sensory information. Biol. Cybern. *68*, 23–29.

von Gersdorff, H., and Matthews, G. (1994). Dynamics of synaptic vesicle fusion and membrane retrieval in synaptic terminals. Nature *367*, 735–739.

von Gersdorff, H., Vardi, E., Matthews, G., and Sterling, P. (1996). Evidence that vesicles on the synaptic ribbon of retinal bipolar neurons can be rapidly released. Neuron *16*, 1221–1227.

Walch-Solimena, C., Blasi, J., Edelmann, L., Chapman, E.R., von Mollard, G.F., and Jahn, R. (1995). The t-SNAREs syntaxin 1 and SNAP-25 are present on organelles that participate in synaptic vesicle recycling. J. Cell Biol. *128*, 637–645.

Zenisek, D., Steyer, J.A., and Almers, W. (2000). Transport, capture and exocytosis of single synaptic vesicles at active zones. Nature *406*, 849–854.