

Ribbon Synapses and Visual Processing in the Retina

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vesicle, calcium, active zone, exocytosis, endocytosis, sensory processing

Abstract

The first synapses transmitting visual information contain an unusual organelle, the ribbon, which is involved in the transport and priming of vesicles to be released at the active zone. The ribbon is one of many design features that allow efficient refilling of the active zone, which in turn enables graded changes in membrane potential to be transmitted using a continuous mode of neurotransmitter release. The ribbon also plays a key role in supplying vesicles for rapid and transient bursts of release that signal fast changes, such as the onset of light. We increasingly understand how the physiological properties of ribbon synapses determine basic transformations of the visual signal and, in particular, how the process of refilling the active zone regulates the gain and adaptive properties of the retinal circuit. The molecular basis of ribbon function is, however, far from clear.

WHAT ARE RIBBON SYNAPSES?

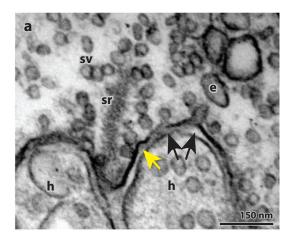
The first synapses that transmit information about vision are set apart from other connections in the brain by an unusual organelle, the ribbon, which holds synaptic vesicles close to the active zone (Schmitz 2009) (Figure 1). Ribbon synapses release the excitatory neurotransmitter glutamate, and they are found in the rod and cone photoreceptors, which convert light into an electrical signal, as well as in the bipolar cells, which transmit this signal to the inner retina (Heidelberger et al. 2005, Masland 2012). Ribbon synapses are not unique to the retina, however: They are also found in light-sensitive neurons in the pineal organ, in mechanosensitive hair cells in the cochlea and vestibular organs, and in the lateral line of fish (Moser et al. 2006a,b; Matthews & Fuchs 2010). It appears that evolution has found the ribbon to be better suited to the transmission of early sensory information than the much more common synapse design found throughout most of the nervous system; the latter lacks a specific structure for storing vesicles immediately behind the active zone. This observation underlies the central question of this review: How do ribbon synapses contribute to the first stages of vision?

A start to answering this question lies in considering the operational differences between conventional and ribbon synapses. Most connections in the central nervous system are silent the vast majority of the time. For instance, pyramidal neurons in the hippocampus fire 1-2-ms spikes at an average rate of once every 2 s. Further, spikes that do arrive at the synaptic terminal are usually ignored—only approximately one in three spikes triggers vesicle fusion (Tarr et al. 2013, Dobrunz & Stevens 1999). Synapses that deliver vesicles at an average rate of only once every 6 s, with high trial-to-trial variability, are not a good start for encoding visual stimuli—one would need an awful lot of them (Abbott & Regehr 2004). Useful vision requires that receptors respond to a quantity that varies continuously and on short time-scales, namely, the intensity of light, which is first encoded by graded changes in membrane potential, rather than spikes (Juusola et al. 1996, Odermatt et al. 2012). The key functional property that allows ribbon synapses to transmit these analog signals is the support of continuous transmitter release, a mode of operation in which the rate of vesicle fusion increases and decreases with depolarizing and hyperpolarizing changes in membrane potential (Dowling & Ripps 1973, Rieke & Schwartz 1996, Lagnado et al. 1996). Ultimately, these analog signals must be converted into a digital format, spikes, for transmission through the optic nerve, but this transformation loses information, a cost that the retina postpones until the visual signal reaches the ganglion cells (Juusola et al. 1996).

To support a continuous mode of transmission, synaptic vesicles must be continuously released when the neuron is depolarized, then retrieved from the surface membrane and recycled back to the active zone (von Gersdorff & Matthews 1994, Lagnado et al. 1996, Neves & Lagnado 1999). If one accepts that structure underlies function, one must suppose that the ribbon plays a key role in this cycle, and the observation that acute destruction of the ribbon with intense light disables continuous transmitter release confirms this supposition (Snellman et al. 2011). Defining the molecular events on the ribbon more precisely has proven difficult (Lenzi & von Gersdorff 2001, LoGiudice & Matthews 2007, Schmitz 2009), but we have made important strides in understanding how the distinctive physiology of these synapses contributes to the processing and transmission of visual information (Jackman et al. 2009, Oesch & Diamond 2011, Snellman et al. 2011, Odermatt et al. 2012, Baden et al. 2013b).

STRUCTURE OF RIBBON SYNAPSES IN THE RETINA

Synaptic ribbons are large proteinaceous structures associated with the presynaptic active zone and are connected to a large number of synaptic vesicles along their entire surface (**Figure 1**a,b). Morphologically, the various types of ribbon synapses differ in the size and shape of both the



b Front view **c** Side view

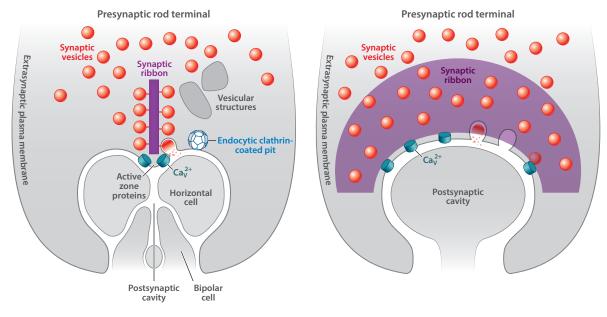


Figure 1

(a) Transmission electron micrograph of a photoreceptor ribbon synapse. The bar-shaped synaptic ribbon (sr) is anchored at the active zone, where exocytosis of synaptic vesicles (yellow arrow) is known to be concentrated (Zenisek et al. 2000). The synaptic ribbon is associated with many synaptic vesicles (sv). In the periactive zone (black arrows), endocytic coated pits are visible as previously shown (Gray & Pease 1971; Wahl et al. 2013). (b,c) Front-view (b) and side-view (c) schematics of the photoreceptor ribbon synapse. Synaptic ribbons appear to be bar shaped in cross-sections (a); lateral views reveal the plate-like, horseshoe-shaped character of these structures. Panel b is modified from a drawing by Wahl et al. (2013), with permission. Abbreviations: Ca_V, voltage-gated calcium channel; h, dendritic tips of postsynaptic horizontal cells; e, larger vesicular structure, possibly a tubular endosome or a cross-sectioned invagination of the presynaptic plasma membrane.

presynaptic compartment and the ribbons themselves (Sterling & Matthews 2005, Schmitz 2009). In electron micrographs of photoreceptor synapses, cross-sections of ribbons usually appear barshaped (Sterling & Matthews 2005, Schmitz 2009) (Figure 1c). Three-dimensional reconstructions reveal a planar, plate-shaped structure that provides a large surface area for vesicle attachment, with a total area of approximately 0.77 μm² in mammalian rods, (Sterling & Matthews 2005; Mercer & Thoreson 2011; Schmitz 2009, 2014). The rod photoreceptor ribbon is ~35 nm thick, several hundred nm high, and can be as long as 1 µm. The anchor point of the ribbon is not the plasma membrane; rather, it is the arciform density located within a small, ridge-like evagination of the presynaptic plasma membrane (Figure 1b). The arciform density is curved, and the ribbon adopts a horseshoe-shaped appearance in lateral views (Figure 1c). The invagination of the presynaptic membrane toward the inside of the presynaptic terminal creates a postsynaptic cavity occupied by the dendritic tips of horizontal and bipolar cells, which are the second-order neurons at the photoreceptor synapse. The tips of these dendritic endings contain different classes of glutamate receptors: metabotropic (mGluR6) glutamate receptors in the case of invaginating rod bipolar cells and ionotropic AMPA receptors and kainate glutamate receptors in the case of horizontal cells (DeVries & Schwartz 1999, DeVries 2000, Wässle 2004, DeVries et al. 2006).

Synaptic vesicles are physically connected along the entire extension of the ribbon. Vesicles in the basal row next to the presynaptic plasma membrane, often called docked vesicles, are the first to be released when calcium channels open, and these vesicles are often collectively termed the readily releasable pool (**Figure 2b**). Many more vesicles are associated with the synaptic ribbon at more distant positions via fine filaments, the nature of which remains mysterious (**Figure 1a**,b). These so-called tethered vesicles correspond physiologically to a reserve pool, which maintains exocytosis on longer timescales (**Figure 2b**). Ribbon-associated vesicles are immobile at rest and do not exchange with cytosolic vesicles at any appreciable rate (LoGiudice et al. 2008, Vaithianathan & Matthews 2014), suggesting that the destiny of a vesicle once attached to the ribbon is to undergo exocytosis.

The number of ribbon-associated vesicles within a presynaptic terminal depends on the total number of ribbons, as well as on the size and shape of the ribbons. For instance, synapses of mammalian rods possess a single ribbon that has \sim 770 attached synaptic vesicles; \sim 130 of these are docked (Sterling & Matthews 2005). In contrast, the active zone at a hippocampal synapse contains approximately 100 vesicles, only a handful of which are docked. Rod photoreceptors in nonmammalian species differ in morphology and do not contain only a single, large active zone. For instance, the particularly large presynaptic terminals of rod photoreceptors in the salamander retina possess 5–7 ribbons per terminal, each of which has \sim 25 docked vesicles and \sim 685 tethered vesicles (Thoreson et al. 2004, Heidelberger et al. 2005, Sterling & Matthews 2005, Thoreson 2007).

Cone terminals usually possess more synaptic ribbons than rod terminals do—10–12 ribbons in most species, and up to \sim 50 in some (Sterling & Matthews 2005). The individual ribbons in cones are smaller than in rods, but their total surface area is larger, allowing cones to have a greater number of ribbon-tethered vesicles (Sterling & Matthews 2005, Heidelberger et al. 2005, Thoreson 2007, Jackman et al. 2009, Schmitz 2009). The larger number of vesicles available to transmit the visual signal in cones allows them to encode light intensity with finer resolution than rods (Rea et al. 2004, Choi et al. 2008).

The ribbons of bipolar cells are bar-shaped to ellipsoid and are smaller but more abundant than those in rod and cone photoreceptors (Sterling & Matthews 2005, Schmitz 2009). A famous example, much studied by physiologists, is that of the rod-driven bipolar cells from the goldfish retina. These cells can be up to 10 μ m in diameter and contain 45–65 small ribbons (**Figure 3***a*). Each ribbon binds ~110 vesicles, of which ~22 vesicles are docked, leading to a grand total of ~6,000 ribbon-bound vesicles, of which ~1,200 are docked (von Gersdorff 2001). These

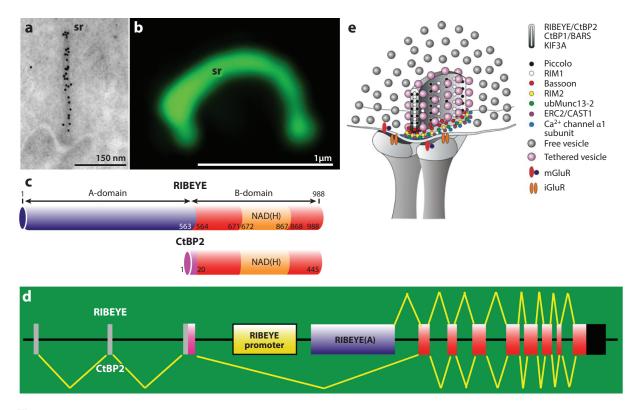
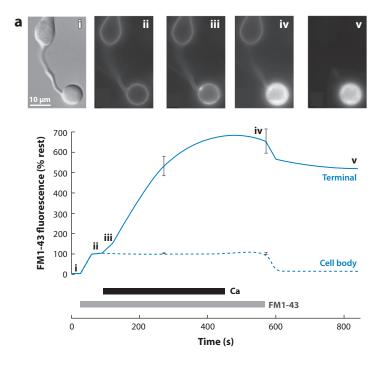


Figure 2

(a) Postembedding immunogold labeling of a synaptic ribbon in a photoreceptor using antibodies against RIBEYE. The synaptic ribbon is strongly positively labeled along its entire length. (Please note that in postembedding protocols, no OsO₄ can be used to enhance membrane contrast; therefore, synaptic vesicles remain largely invisible). (b) Lateral view of a single synaptic ribbon immunolabeled with antibodies against RIBEYE. This image, obtained by superresolution structured illumination fluorescence microscopy (courtesy of S. Wahl), demonstrates the plate-like, horseshoe-shaped character of photoreceptor synaptic ribbons. (c) Domain structure of RIBEYE and CtBP2. RIBEYE consists of a proline-rich A domain and an NAD(H)-binding B domain. The B domain is largely identical to CtBP2 (C-terminal-binding protein 2), except for the unique N-termini. (d) The RIBEYE/CtBP2 gene: The RIBEYE promoter leads to the transcription of the RIBEYE(A) exon, which is then spliced into eight exons that build the RIBEYE B domain. The RIBEYE promoter is active only in neurons that build ribbon synapses. A ubiquitously active promoter (not shown) is responsible for the transcription of the ubiquitously expressed CtBP2 protein. RIBEYE and CtBP2 differ only in their N-terminal regions. (e) Distribution of other proteins at the synaptic ribbon complex (modified image taken from tom Dieck & Brandstätter 2006, with permission). The Piccolo dots shown in the ribbon structures in (e) correspond to Piccolino, a ribbon synapse–specific splice variant of Piccolo with a unique, short C-terminus (Regus-Leidig et al. 2013).

two vesicle pools can also be recognized as the first two kinetically distinct phases of exocytosis by both imaging (Neves & Lagnado 1999) (**Figure 3***b*) and electrophysiology (Mennerick & Matthews 1996).

The terminals of photoreceptors and bipolar cells contain a very large pool of vesicles distributed throughout the cytoplasm in addition to the ribbon, and this pool acts as the reservoir for the continuous mode of exocytosis (Lagnado et al. 1996) (Figure 3). In the mammalian rod terminal, tens of thousands of synaptic vesicles are present in this cytoplasmic pool (Heidelberger et al. 2005, Sterling & Matthews 2005), whereas in goldfish bipolar cells, the number of synaptic vesicles can be as high as several hundred thousand. These numbers are extraordinary when one compares them with a typical synaptic bouton in the mammalian brain, which contains



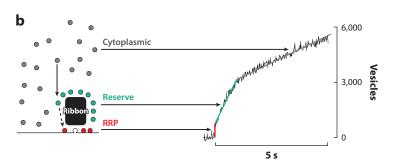


Figure 3

Continuous exocytosis and vesicle pools. (a) A depolarizing bipolar cell isolated from the goldfish retina with a giant synaptic terminal (subpanel i). The fluorescence images in subpanels i-v show the distribution of the membrane dye FM1-43, first added in the absence of calcium (ii) and then during maintained stimulation of exocytosis (iii and iv). When the dye is washed off of the cell surface, the membrane compartments that have been retrieved by endocytosis contain the dye and can be visualized (v). The graph quantifies the increase in fluorescence in the terminal during the experiment and demonstrates that a steady state is achieved when all circulating vesicles have been stained; the combined surface area of these vesicles is equivalent to several times that of the cell surface. Panel modified with permission from Lagnado et al. (1996). (b) Three distinct vesicle pools at the ribbon synapse of bipolar cells. The plot on the right shows the time course of exocytosis in response to a 5-s step depolarization, assayed using FM1-43 to stain exocytosed vesicles. Three distinct phases of release are observed. In the first phase, the rapidly releasable pool (RRP) (red) is released; this group of vesicles is thought to correspond to vesicles docked at the active zone. In the second phase, a group of reserve vesicles (green) is released; this group corresponds numerically with the vesicles attached to the ribbon projecting into the cytoplasm. In the third and slowest phase, continuous vesicle release is supported by a population of vesicles distributed throughout the cytoplasm (gray). Panel modified with permission from Neves & Lagnado (1999).

only \sim 100 vesicles, but the difference between them makes functional sense if ribbon synapses are to support a continuous vesicle cycle through which visual stimuli are encoded. Auditory and vestibular ribbon synapses are covered by some excellent recent reviews and are not considered here (Matthews & Fuchs 2010; Moser et al. 2006a,b; Nouvian et al. 2006).

MOLECULAR ASPECTS OF RIBBON SYNAPSE FUNCTION

The synaptic vesicle cycle is regulated by many specialized proteins, most of which are common to all synapses. Take, for instance, the SNARE [SNAP (soluble NSF attachment protein) receptor] complex, which mediates vesicle fusion with the plasma membrane: In both ribbon and conventional synapses, the vesicle (v)-SNARE on the synaptic vesicle is vesicle-associated membrane protein (VAMP)/synaptobrevin 2, which associates with the target (t)-SNARES on the plasma membrane—syntaxin 1, 2, or 3—and SNAP-25. It also seems likely that the calcium sensor for fusion at all types of synapses is synaptotagmin, a transmembrane protein in the vesicle that has many isoforms. Most conventional synapses contain synaptotagmin I and synaptotagmin II, which are also found in retinal ribbon synapses.

Ribbon synapses do have some notable divergences in molecular composition compared with most others in the brain, however, presumably reflecting their functional specialization for sustained exocytosis. At conventional synapses, movement of the cluster of vesicles behind the active zone is restricted because the vesicles are tethered to the actin cytoskeleton via the vesicle-associated protein synapsin. In contrast, synapsin is absent from ribbon synapses. This molecular difference likely explains the greater mobility of vesicles in ribbon-type terminals compared with that in conventional synapses (Holt et al. 2004). Perhaps the most obvious difference is the ribbon itself, however. The major structural component of the ribbon, and the only specific component identified to date, is RIBEYE (**Figure 2**). Other ribbon-associated proteins, such as piccolo, bassoon, CtBP1 (C-terminal-binding protein 1), and RIMS1 (regulating synaptic membrane exocytosis protein 1), are thought to be involved in tethering synaptic vesicles to the ribbon and/or priming the vesicles for fusion at the active zone (**Figure 2e**). KIF3A, a kinesin-like motor protein, is also attached to retinal ribbons (Muresan et al. 1999). We summarize some key properties of these molecules in the following subsections.

RIBEYE

The major component of the synaptic ribbon, RIBEYE (Schmitz et al. 2000, 2012; Schmitz 2009), is the product of an alternative promoter in the CtBP2 gene and consists of an A-domain that is specific for ribbons and a B-domain that is identical to CtBP2, a transcriptional repressor that in turn is related to 2-hydroxyacid dehydrogenases (Schmitz et al. 2000; von Gersdorff 2001; Piatigorsky 2001; Schmitz 2009, 2014) (Figure 2). CtBP2 is similar to nuclear NADH-regulated corepressor CtBP1 found at the Golgi apparatus (Corda et al. 2006, Chinnadurai 2009). The RIBEYE A-domain contains multiple RIBEYE–RIBEYE interaction sites, suggesting that this protein plays a structural role in the ribbon (Magupalli et al. 2008). In agreement with this hypothesis, overexpression of RIBEYE in COS cells induces spherical synaptic ribbon-like structures (Magupalli et al. 2008), and overexpression of RIBEYE in zebrafish leads to gigantic ribbons (Sheets et al. 2011, 2012, 2014). The B-domain was shown to bind various cytosolic synaptic proteins (Munc119, GCAP2, ArfGAP3) (Alpadi et al. 2008, Venkatesan et al. 2010, Dembla et al. 2014). Therefore, the RIBEYE(B)-domain is most likely positioned at the cytosolic surface of the synaptic ribbons. This domain also has a phospholipid-modifying enzymatic activity that could be relevant in modulating steps in the intense vesicle traffic at the synaptic ribbon complex (Schwarz

et al. 2011). CtBP1 is also present at the synaptic ribbon (tom Dieck et al. 2005), but it appears to be dispensable for synaptic ribbon structure and function (Vaithianathan et al. 2013). At the base of the ribbon, RIBEYE is physically connected to the active zone protein bassoon (tom Dieck et al. 2005, Dembla et al. 2014).

Piccolino, Piccolo, and Bassoon

Piccolo is a large (~550-kDa) multidomain protein belonging to the active zone (Wang et al. 1999, Fenster et al. 2000; for review, see Schoch & Gundelfinger 2006; Gundelfinger & Fejtova 2012; Südhof 2012a, 2012b, 2014). This protein was identified in conventional phasically active brain synapses (Wang et al. 1999, Fenster et al. 2000) and was localized predominantly to the membranedistant portion of synaptic ribbons (tom Dieck et al. 2005, Limbach et al. 2011). Recent analyses demonstrated that the observed piccolo immunoractivity is due to piccolino, a ribbon synapsespecific splice variant of piccolo (Regus-Leidig et al. 2013) that might be important for maintaining the bar-shaped structure of photoreceptor synaptic ribbons (Regus-Leidig et al. 2014). Knockout models are available to further analyze this hypothesis. Related to piccolo/piccolino is the highmolecular weight, multidomain protein bassoon (tom Dieck et al. 1998, Gundelfinger & Fejtova 2012), which is localized to the active zone of conventional synapses and to the base of the synaptic ribbon, where it anchors the ribbons and organizes calcium channels (Dick et al. 2001, 2003; tom Dieck et al. 2005; Frank et al. 2009, 2010; Limbach et al. 2011). At synapses, bassoon binds to RIM-binding proteins (RBPs), which are important regulators of calcium channels at the active zone (Davydova et al. 2014). It is essential for the formation of ribbon synapses in photoreceptors (Dick et al. 2003) and physically interacts with RIBEYE (tom Dieck et al. 2005, Dembla et al. 2014). Bassoon-deficient mice have so-called floating, synaptic ribbons, namely, nonanchored ribbons that are no longer attached to the active zone (tom Dieck et al. 2005).

Rab3-Interacting Molecule Proteins

The multidomain Rab3-interacting molecule (RIM) proteins are well characterized components of active zone proteins in conventional phasically active synapses (Wang et al. 1997; Kaeser et al. 2011; Han et al. 2011; Deng et al. 2011; Südhof 2012a, 2012b, 2013, 2014). RIM proteins are a central hub at the active zone essential for various functions (Südhof 2014). They are important for calcium channel immobilization, synaptic vesicle recruitment, and synaptic vesicle priming (Südhof 2014). They exert their priming function via interaction with Munc13 proteins, such as Munc13-1, and they recruit calcium channels either via direct interaction or an interaction with RIM-binding proteins or the β-subunits of the calcium channels (Hibino et al. 2002; Kiyonaka et al. 2007, 2012). RIM proteins are present in ribbon synapses, although their function is less well characterized (Wang et al. 1997, Schmitz et al. 2000, tom Dieck et al. 2005). RIM2 has been localized to the active zone, whereas RIM1 has been localized to the ribbon body (tom Dieck et al. 2005). In photoreceptor terminals, Munc13-1 is absent, and Munc13-2 appears to take over the priming role (Schmitz et al. 2001, Cooper et al. 2012). CAST1/ERC2, a scaffold protein that interacts with RIM and bassoon, is important for regulating the size of the active zone in photoreceptor synapses, where it could interact with RIM2 (Wang et al. 2002, Takao-Rikitsu et al. 2004, tom Dieck et al. 2012, Ohtsuka 2013). CAST1/ERC2 has also been localized to the body of the ribbon, where it might interact with RIM1 (Ohtsuka 2013). CAST1/ERC2/ELKS2 has been reported to interact with several β-subunits of voltage-gated calcium channels (Kiyonaka et al. 2012, Ohtsuka 2013). In hippocampal synapses, CAST, ELKS, and ERC proteins regulate calcium influx through voltage-gated calcium channels (Liu et al. 2014), but whether they serve a similar function in ribbon synapses is not yet known.

KIF3A

KIF3A is a component of the kinesin II motor complex (Hirokawa 2000) found in the synaptic ribbon complex (Muresan et al. 1999). Although its function at the ribbon has not been established, this protein is worth noting because of its potential to be involved in vesicular transport along the synaptic ribbon, a key aspect of the conveyor belt model in which primed vesicles are actively transported along the ribbon to the release sites at the plasma membrane (Parsons & Sterling 2003, Sterling & Matthews 2005).

L-Type Calcium Channels

A key specialization of ribbon synapses is found in the calcium channels immobilized close to the base of the synaptic ribbon: These are primarily L-type channels (Heidelberger & Matthews 1992, Mennerick & Matthews 1996, Heidelberger et al. 2005, Doering et al. 2007, Thoreson et al. 2013), in contrast to those at the active zone of spiking neurons, which are N-, P/Q-, or R-type channels. Important properties of L-type channels are activation at high voltages, beginning at approximately $-42 \, \mathrm{mV}$, and fast activation and deactivation kinetics that make them well suited for the continuous, fast, and precise regulation of glutamate release.

In photoreceptor ribbon synapses, L-type voltage-gated channels consist of the $Ca_V1.4$ pore-forming $\alpha1$ -subunit ($\alpha1F$), the regulatory $\beta2$ subunit, and the $\alpha2\delta4$ subunit (Ball et al. 2002, 2011; Catterall et al. 2005; Morgans et al. 2005; Wycisk et al. 2006; Buraei & Yang 2010; Striessnig et al. 2010; Catterall 2011; Mercer et al. 2011; Schmitz et al. 2012; Liu et al. 2013). $Ca_V1.4$ -containing calcium channels do not show any calcium-dependent inactivation and show little voltage-dependent inactivation (Singh et al. 2006, Striessnig et al. 2010; Catterall 2011, Shaltiel et al. 2012). Thus, these channels are well suited to support continuous exocytosis at the synapse. The subunit composition of ribbon synapses in the inner retina is more diverse and less well characterized than that of photoreceptor ribbon synapses (Ball et al. 2002, 2011; Specht et al. 2009; Busquet et al. 2010). Furthermore, some bipolar cells also express transient, T-type voltage-gated calcium channels, and these channels may also contribute to signal transmission (Pan et al. 2001, Ma & Pan 2003, Hu et al. 2009). In addition to providing the triggering mechanism for exocytosis, voltage-gated calcium channels probably also have important structural functions in maintaining the normal synaptic architecture (Zabouri & Haverkamp 2013, Liu et al. 2013, Michalakis et al. 2014).

RIBBON SYNAPSES AND RETINAL DISEASE

Photoreceptor synapses are the only synapses that communicate light-induced changes of photoreceptor membrane potentials to the inner retina for further processing. Therefore, not too surprisingly, various diseases are associated with malfunctions and/or mutations of proteins involved in synaptic transmission at photoreceptor synapses. A particular disease focus at the presynaptic side comprises the L-type calcium channels and proteins that affect calcium channel function. Numerous mutations in the $\alpha 1F$ pore-forming subunit of $Ca_V 1.4$, the L-type voltage-gated calcium channel (Ca_V), lead to impaired rod-dependent vision (Hoda et al. 2005, Burtscher et al. 2014). These mutations lead to various forms of congenital stationary night blindness type 2 (CSNB2) in humans. Electroretinogram (ERG) analyses of affected individuals display an abnormal b-wave, which is a sign of abnormal bipolar cell activation, whereas the a-waves of these patients (representing phototransduction) are normal (Boycott et al. 2001, Burtscher et al. 2014). Mutations in RIM1, which is essential for the immobilization and gating of calcium channels via multiple mechanisms, result in cone–rod dystrophy type7 (CORD7) (Johnson et al. 2003, Michaelides et al. 2005). In addition, nyctalopin, a glycophosphatidylinositol (GPI)-anchored protein in the

synaptic cleft of photoreceptor ribbon synapses that interacts with the postsynaptic signal transduction machinery, is prone to malfunctions that cause disease (Bech-Hansen et al. 2000, Pusch et al. 2000, Morgans et al. 2006). Mutations in nyctalopin also cause CSNB (Gregg et al. 2007, Cao et al. 2011). Many mutations of proteins in the mGluR6 or TRPM1-containing postsynaptic transduction machinery are also known to lead to CSNB (Gregg et al. 2007, Nakamura et al. 2010, Cao et al. 2011). Other mutations of postsynaptic signal transduction machinery proteins are covered by recent reviews and thus are not mentioned here (Snellman et al. 2008, Kaur & Nawy 2012, Shen et al. 2012). Note that many spontaneous mutations of photoreceptor synaptic proteins can affect photoreceptor function. For example, a spontaneous mutant of the Ca_V1.4 channel, Nob2, was used to characterize Ca_V1.4 channel function (Chang et al. 2006). Recently discovered spontaneous mutations such as rd8 affect the *crumbs1* gene, which is important for photoreceptor synaptic structure. The rd8 mutant is particularly abundant, and it also occurs in so-called wild-type mouse lines available from commercial vendors (Mehalow et al. 2003, Mattapallil et al. 2012).

CONTINUOUS AND PHASIC MODES OF TRANSMISSION

To understand the role of ribbon synapses in visual processing, one should appreciate that these synapses are capable of two kinetically distinct modes of transmission: a fast, transient mode involving the release of docked vesicles after a period of rest and a slower mode that continues after docked vesicles have been released (**Figure 4**) (von Gersdorff & Matthews 1994, Lagnado et al. 1996, Neves & Lagnado 1999, Rabl et al. 2005, Jackman et al. 2009, Snellman et al. 2011). The rate constants with which docked vesicles can be released are on the order of ~1,000 vesicles/s when the calcium current is activated strongly; this rate is at least as fast as that of phasic release at conventional synapses (Mennerick & Matthews 1996, von Gersdorff et al. 1998, Burrone & Lagnado 2000).

Hyperpolarization of photoreceptors in response to light, and the resulting calcium channel closures, immediately suggests that these cells are capable of continuously releasing transmitter in the dark; otherwise, the light signal would not be transmitted (Dowling & Ripps 1973). This vesicle cycle has been visualized in rod-driven bipolar cells from the goldfish retina, which have unusually large synaptic terminals (Lagnado et al. 1996, Rouze & Schwartz 1998). When the membrane dye FM1-43 is added in the absence of calcium, only the cell surface fluoresces; when the cell is depolarized in the presence of calcium, however, an immediate increase in fluorescence in the terminal occurs as vesicles fuse with the surface and take up the dye (Figure 3a). Continued depolarization causes a gradual accumulation of stained vesicles retrieved from the surface by endocytosis until all vesicles carry the dye. No net change in the surface area of the terminal can be observed over timescales as long as tens of seconds, demonstrating the efficiency of the endocytic mechanisms that maintain the continuous vesicle cycle (Lagnado et al. 1996, Rieke & Schwartz 1996, Rouze & Schwartz 1998). Maintaining continuous vesicle release on these timescales draws on the large cytoplasmic pool of vesicles, and previous studies have demonstrated that vesicles are mobile across the entire presynaptic compartment (Lagnado et al. 1996, Holt et al. 2004, Jackman et al. 2009).

The continuous mode of exocytosis is suited to signaling the intensity and duration of maintained stimuli (Jackman et al. 2009, Odermatt et al. 2012). In some cases, however, ribbon synapses must signal rapid changes such as the onset of light or the frequency of a sound, and they do so by a transient burst of very rapid vesicle fusion (von Gersdorff & Matthews 1994, Mennerick & Matthews 1996, von Gersdorff et al. 1998, Neves & Lagnado 1999, Rabl et al. 2005, Snellman et al. 2009, Oesch & Diamond 2011). An electrophysiological method that allows exocytosis and

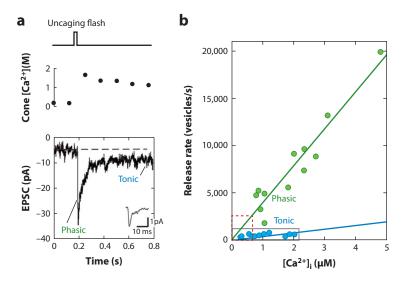


Figure 4

Calcium dependence of vesicle release at a cone ribbon synapse. (a) A rise in the concentration of free calcium imposed on a cone pedicle by a flash of light (top) triggers a postsynaptic current with phasic and tonic components (bottom). (b) The rate of vesicle release displays a linear dependence on free calcium concentration during both the fast initial phase (green) and the slower tonic phase (blue). The dotted red box shows the expected rates for phasic release, and the gray box shows the range over which the tonic component shows a linear dependence on calcium concentration. Figure adapted with permission from Jackman et al. (2009).

endocytosis to be monitored with good temporal resolution is the capacitance technique, which measures the increase in surface area that results from vesicle fusion and the subsequent decrease when the excess membrane is retrieved (von Gersdorff & Matthews 1994, Rizzoli & Betz 2005). Capacitance measurements reveal that vesicles docked to the plasma membrane under the ribbon can be released in a fraction of a millisecond after the calcium channels open (Mennerick & Matthews 1996, Neves & Lagnado 1999, Burrone & Lagnado 2000). When the terminal is strongly depolarized, this rapidly releasable pool (RRP) can be exhausted within a few milliseconds, after which the release rate becomes limited by a process that likely involves the movement of vesicles from the ribbon down to the docking sites on the plasma membrane (Zenisek et al. 2000, Holt et al. 2004, Rizzoli & Betz 2005, Jackman et al. 2009). Synaptic vesicle exocytosis has also been reported to occur at nonribbon sites, especially if ribbon synapses are strongly stimulated (Midorikawa et al. 2007, Zenisek 2008, Chen et al. 2013). Under these conditions, the calcium concentration must be high enough to stimulate release at some distance from the calcium channels under ribbons.

Although sensory neurons transmitting via ribbon synapses have generally been thought to be purely graded, it is now clear that bipolar cells are also capable of producing regenerative voltage signals, either as damped oscillations or as spikes (Baden et al. 2013b). In some cases, these spikes are generated via voltage-sensitive calcium channels, and in others, they are generated via tetrodotoxin (TTX)-sensitive sodium channels. Calcium spikes were first observed in the rod-driven bipolar cell in the goldfish, where the regenerative mechanism is located in the axonal terminal (Kawai et al. 2001, Kawai et al. 2005): The depolarization phase depends on a high density of L-type calcium channels, whereas repolarization is caused by the opening of closely coupled calcium-dependent potassium (BK) channels (Burrone & Lagnado 1997, Protti et al. 2000). At least two types of

bipolar cells in the rat retina can generate sodium spikes upon current injection (Cui & Pan 2008), and one type of ON bipolar cell in the ground squirrel generates sodium spikes triggered by light (Saszik & DeVries 2012). Indeed, most bipolar cells in fish (Zenisek et al. 2001), and about half of those in rats, exhibit clear sodium currents (Pan & Hu 2000).

Recently, a less invasive approach has been used to monitor the activity of bipolar cell ribbon synapses in vivo—multiphoton imaging of calcium reporter proteins localized to synaptic vesicles (Dreosti et al. 2009). This unusually direct method for visualizing the activity of ribbon synapses revealed that most axon terminals generated calcium transients in response to an underlying voltage spike (Baden et al. 2011, Dreosti et al. 2011). These calcium transients were much larger than those associated with graded depolarizations and were observed in a variety of different functional types of bipolar cells, including ON, OFF, transient, and sustained cells. Spikes were also recorded recently in at least three types of mouse bipolar cells using calcium imaging (Baden et al. 2013a). Can a ribbon synapse that transmits information about graded changes in membrane potential also signal the arrival of a spike? The answer is yes. Direct injection of a spike waveform into Mb1 terminals results in a marked increase in membrane capacitance, roughly equivalent to the total number of vesicles docked at the active zone under the ribbon (Palmer 2006). Thus, the fast mode of transmission can be understood as the mechanism by which larger voltage transients in bipolar cells are reliably transmitted to amacrine and ganglion cells in the inner retina.

ACTIVATION OF EXOCYTOSIS BY CALCIUM

The graded responses of photoreceptors and bipolar cells span the voltage range over which calcium channels begin to open, so synaptic transmission is not always active and can be silenced when the neuron is in a hyperpolarized state (Heidelberger et al. 2005, Baden et al. 2011). When the membrane potential varies beyond the threshold of calcium channel activation, however, the amplitude of the calcium current will follow very closely in time (Baden et al. 2011). Because the amplitude of the calcium current just above threshold is a power function of membrane potential, minor changes in voltage cause major changes in the amount of glutamate released (Heidelberger & Matthews 1992, von Gersdorff et al. 1998, Burrone & Lagnado 2000).

Three related aspects of the process by which calcium influx triggers release determine the voltage-dependence of exocytosis: the function relating calcium concentration and release rate, the distance over which calcium entering through channels acts to trigger fusion, and the absolute concentrations required. At most conventional synapses, calcium concentrations of at least 20– 30 μM trigger phasic release by a highly cooperative action (one with a Hill coefficient of 3 or 4), and these concentrations occur only within nanodomains close to calcium channels. In contrast, most published evidence indicates that a large component of release from ribbon synapses in the retina can be driven by calcium signals beginning at submicromolar levels and extending over spatial scales on the order of 1 µm (Burrone et al. 2002, Beaumont et al. 2005). Submicromolar calcium concentrations imposed at the ribbon synapses of goldfish bipolar cells drive release at relatively high rates (Lagnado et al. 1996, Rouze & Schwartz 1998), and similar observations have been made in the ribbon synapse of photoreceptors (Rieke & Schwartz 1996, Thoreson et al. 2004). Thoreson et al. (2004) used flash photolysis of caged calcium in rod photoreceptors to make particularly careful measurements of the relation between calcium concentration and release rate at a ribbon synapse and found "a highly Ca²⁺-sensitive pool of releasable vesicles with a relatively shallow relationship between the rate of exocytosis and [Ca²⁺]i that is nearly linear over a presumed physiological range of intraterminal [Ca²⁺]." In cones, both the phasic and tonic components of release display linear dependence on calcium concentration, beginning from submicromolar concentrations (Jackman et al. 2009).

Current evidence does not rule out the possibility that the fastest phase of exocytosis at bipolar cell synapses also has a linear dependence on calcium concentration. One study concluded that very high levels of calcium act with high cooperativity to trigger release from bipolar cells (Heidelberger et al. 1994), but interpreting these experiments is problematic because calcium transients were imposed on top of basal calcium concentrations known to activate release ($\sim 5~\mu M$). Repeating these measurements when uncaging calcium from resting calcium concentrations that do not trigger any vesicle fusion would be worthwhile.

RECYCLING OF SYNAPTIC VESICLES

Although synaptic terminals containing ribbons tend to contain many vesicles, the long-term maintenance of transmitter release requires that vesicles be recycled within the terminal (LoGiudice & Matthews 2007). At the ribbon synapse of bipolar cells, most if not all of the vesicles fusing with the surface membrane collapse and merge (Llobet et al. 2003), and there is no evidence that vesicles can connect transiently with the surface through a fusion pore that allows the release of transmitter (termed a kiss-and-run interaction). These collapsed vesicles are retrieved by at least two, and possibly three, distinct modes of endocytosis.

Capacitance measurements with comparatively high temporal resolution show that in bipolar cells and hair cells, brief stimuli (those with durations of less than 1 s) are followed by two other kinetically distinct modes of endocytosis. Fast endocytosis occurs in 1 s or less, whereas slow endocytosis occurs on a timescale of 10-20 s (von Gersdorff & Matthews 1994, Neves & Lagnado 1999, Hull & von Gersdorff 2004). Fast and slow modes of retrieval occur through mechanisms that are molecularly distinct: Slow retrieval occurs by the formation of clathrin-coated vesicles from the cell membrane and is similar to the process that internalizes membrane receptors in all types of cells (Jockusch et al. 2005), whereas fast retrieval is independent of clathrin, but the molecular mechanisms of the process remain to be identified (Llobet et al. 2011). In cones, fast endocytosis occurs with time constants as short as 250 ms (Van Hook & Thoreson 2012). The relative importance of fast and slow endocytosis depends on how strongly the synapse is stimulated: In bipolar cells, weak stimuli that result in release of only docked vesicles are followed almost exclusively by fast retrieval, but as more vesicles are released at higher rates, an increasing proportion of them are retrieved by the slower clathrin-dependent process (von Gersdorff & Matthews 1994, Neves & Lagnado 1999). The two mechanisms of vesicle retrieval are also distinguished by their dependence on calcium: Fast endocytosis appears to be triggered by calcium influx at the active zone, whereas slow endocytosis does not appear to depend on calcium directly (Neves et al. 2001).

Electron microscopy demonstrates that chronic and strong stimulation of exocytosis in cones and bipolar cells is followed by bulk retrieval of large chunks of surface membrane; these chunks are equivalent to several vesicles worth of membrane (Matthews & Sterling 2008). This process generates cisternae within the terminal, from which vesicles subsequently bud off (Holt et al. 2003, 2004). Bulk retrieval in bipolar cells appears to be actin dependent, but whether this process is distinct from the fast and/or slow modes of endocytosis observed after brief periods of stimulation remains unclear.

Once vesicles have been retrieved from the cell surface, they must somehow find their way back to the ribbon or active zone. Vesicles in the synaptic terminals of cones and bipolar cells are almost all mobile throughout the presynaptic compartment, moving in a random fashion with an effective diffusion coefficient of approximately 0.05 μ m²/s (Holt et al. 2004, LoGiudice et al. 2008). This degree of mobility is almost an order of magnitude greater than that of vesicles in hippocampal synapses. In the giant synaptic terminals of bipolar cells from goldfish, the

cytoplasmic reservoir contains approximately 300,000 vesicles, so this degree of mobility would result in approximately 900 vesicle collisions/s against the ribbons and in 28,000 collisions/s against the surface membrane (Holt et al. 2004). In comparison, continuous exocytosis can be maintained at rates of 500–1,000 vesicles/s (Lagnado et al. 1996). The random motion of cytoplasmic vesicles therefore seems sufficient to maintain an efficient supply to both the ribbon and the plasma membrane during ongoing activity.

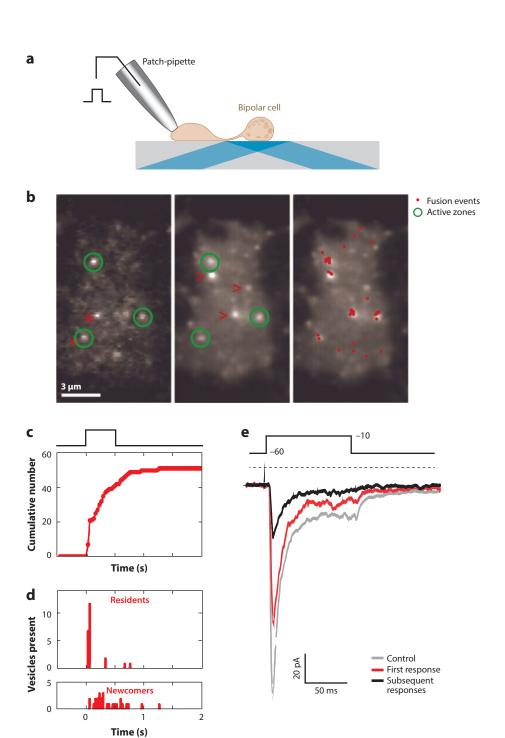
Once depleted, the RRP in fish bipolar cells appears to be refilled in two phases, a fast phase, which has a time constant of a few hundred ms, and a slower phase, with a time constant on the order of tens of seconds (Gomis et al. 1999, Singer & Diamond 2006). The calcium signal in the presynaptic compartment also accelerates the processes that supply vesicles to the RRP (Gomis et al. 1999, Berglund et al. 2002), but the identity of the calcium-sensitive step has not been clearly identified. One can speculate that it might be the attachment of vesicles to the ribbon, their movement down to the active zone, or docking and priming at release sites. The overall picture, however, is one in which calcium accelerates the continuous vesicle cycle by acting at several key points: fusion, retrieval from the membrane surface, and recycling to the active zone.

DOES THE RIBBON ACT AS A CONVEYOR BELT?

The fact that the ribbon holds vesicles immediately behind docking sites at the active zone suggests that it aids this supply function by acting as a trap for vesicles moving about the cytoplasm, similar to a piece of fly paper. A key issue is whether the ribbon then plays a specific role in conveying these vesicles to docking sites. The most direct evidence for the idea that the ribbon regulates the arrival of vesicles at docking sites comes from work using total internal reflection fluorescence microscopy (TIRFM) to image individual vesicles in the synaptic terminals of retinal bipolar cells (**Figure 5**) (Zenisek et al. 2000). This approach demonstrates that active zones of exocytosis coincide with structures that hold vesicles just behind the plasma membrane until stimulation causes the downward transfer of these vesicles. The structures holding these vesicles "in reserve" behind the active zone are most likely ribbons. The time required for transfer and fusion was estimated to be approximately 250 ms, which is consistent with the kinetics of exocytosis

Figure 5

Vesicles, active zones, and ribbons. (a) Individual vesicles containing FM1-43 can be visualized in the terminal of an isolated bipolar cell using total internal reflection fluorescence microscopy (TIRFM). The schematic experimental setup shows a bipolar cell on a cover slip in which a beam of blue light is undergoing total internal reflection to create an evanescent field that will excite fluorophores very close to the surface membrane. A patch-pipette on the cell body allows the cell to be voltage clamped. (b) Single TIRFM image (left) of the membrane; each bright spot is a synaptic vesicle, and green circles mark sites of very rapid vesicle fusion (active zones). TIRFM image averaged over several rounds of stimulation (middle and right); red dots (right) show locations of all fusion events. (c) Plot showing the cumulative number of fusion events as a function of time relative to the depolarizing stimulus. Note the fast and slow phases. (d) Vesicles present in active zones at stimulus onset (residents) are released within 50 ms (top plot), after which newcomers arrive, with an average delay of 250 ms. (e) Plot showing excitatory postsynaptic currents in an AII amacrine cell receiving input from rod-driven bipolar cells in a slice of mouse retina. Under control conditions, (gray) a fast and transient component of release is followed by a slower continuous phase. Responses were then recorded following damage to the ribbon using intense light and a fluorescein-labeled peptide specifically targeted to the ribbon. The first response after damage (red) was reduced in both phases, and subsequent responses were reduced even further. Thus, it appears that the ribbon must be functional to prepare vesicles for exocytosis. Panels a-d adapted with permission from Zenisek et al. (2000). Panel e adapted with permission from Snellman et al. (2011).



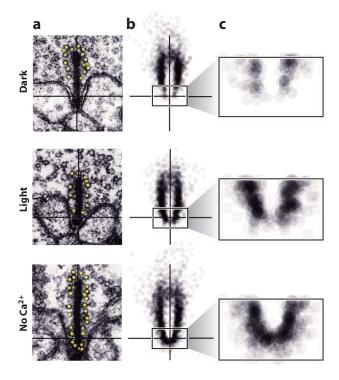


Figure 6

Electron microscopy (EM) of ribbon-associated vesicles. (a) EM images of cone ribbons from a lizard retina fixed in the dark (top), in light (middle), and in a calcium-free saline medium (bottom). (b) The positions of ribbon-associated vesicles from 115 ribbons were mapped and overlaid for each condition (inset rectangles correspond to 200×400 nm areas). (c) Magnified images of the inset areas in panel b. The resultant cloud of vesicles revealed depletion near the base of the ribbon in darkness, when the continuous vesicle cycle is maximally active. Figure adapted with permission from Jackman et al. (2009).

shown in **Figure 3**. Confocal microscopy also demonstrates that while vesicles are actively fusing under the ribbon, new vesicles tend to arrive at the opposite pole (Vaithianathan & Matthews 2014).

Electron microscopy of both cones and bipolar cells also demonstrates that once the RRP is exhausted, the slower continuous component of release is associated with a ribbon that has been depleted of vesicles, indicating that resupply has become rate limiting (**Figure 6**) (Holt et al. 2004, LoGiudice et al. 2008, Jackman et al. 2009). Together, these observations build a strong case for the conveyor-belt model, but a definitive demonstration will require the direct visualization of the ribbon and of vesicle movements toward the active zone. Identification of the molecules forming the filaments that tether vesicles to ribbons and elucidation of the mechanism by which vesicles are moved over the ribbon will also be important.

THE CONTRIBUTION OF RIBBON SYNAPSES TO VISUAL PROCESSING

Synapses play a key role in the computations carried out by neural circuits (Abbott & Regehr 2004), but the following question remains: Why are ribbons specifically used to convey early

sensory information? In the following subsections, we provide some basic examples of how the physiological properties of ribbon synapses contribute to basic tasks of the retinal circuit.

Encoding Changes in Luminance

The gain of retinal responses to changes in luminance is regulated both in the receptors and in the downstream circuitry, allowing vision to operate over a large range of ambient conditions. Ribbon synapses of rod-driven bipolar cells play an important role in gain control over scotopic to mesopic luminance levels, and electrophysiological measurements of excitatory transmission to postsynaptic AII amacrine cells demonstrate that these synapses exhibit strong depression as soon as vesicles are released from the RRP (Dunn & Rieke 2006, 2008). A step increase in light intensity generates a biphasic response: A large initial transient that encodes the time of the change is followed by a sustained response that encodes the new steady-state intensity (Snellman et al. 2008). The amplitude of the phasic response depends on the degree of occupancy of the RRP at the time the stimulus is applied and therefore also depends on the recent history of stimulation. The amplitude of the tonic component depends on the steady-state occupation of the RRP, which in turn depends on the balance between the rate of vesicle release and the rate of refilling (Oesch & Diamond 2011). Phasic and tonic components of release have also been observed in cones, regarding which Jackman et al. (2009) pointed out that "the ribbon behaves like a capacitor, charging with vesicles in light and discharging in a phasic burst at light offset." Thus, phasic release in cones encodes changes in luminance over the photopic range.

Adaptation

The input–output relation of the retina is not fixed; rather, it changes continuously according to the recent history of the stimulus (Wark et al. 2007, Gollisch & Meister 2010). A common change is a decrease in sensitivity to a constant feature of the input, termed adaptation. For instance, many retinal ganglion cells respond strongly just after an increase in the temporal contrast of the stimulus, but this response declines if the input is maintained (Smirnakis et al. 1997). The decrease in gain is maintained while the contrast remains high but recovers with a time constant on the order of a few seconds under low-contrast conditions. Electrophysiological measurements of excitatory synaptic currents in retinal ganglion cells indicate that depression of synaptic transmission from bipolar cells is the major mechanism underlying this form of retinal adaptation (Manookin & Demb 2006, Beaudoin et al. 2007, Jarsky et al. 2011, Benucci et al. 2013), and in vivo measurements of vesicle release using a fluorescent reporter protein provide direct support for this idea (Nikolaev et al. 2013). Synaptic depression can occur in the absence of changes in the presynaptic calcium signal and is caused by depletion of the RRP (Burrone & Lagnado 2000, Chávez et al. 2006, Oesch & Diamond 2009, Jarsky et al. 2011, Ozuysal & Baccus 2012, Nikolaev et al. 2013).

Measurements of retinal ganglion cell output indicate that increases in contrast gain following a return to conditions of low temporal contrast occur on timescales of a few seconds (Smirnakis et al. 1997). Ozuysal & Baccus (2012) found that many adaptive properties of the retina were regulated on similar timescales and therefore suggested that these properties might result from a common mechanism. A strong candidate for this common process is the ribbon-mediated refilling of the RRP, which occurs on timescales that closely match changes in contrast sensitivity (Gomis et al. 1999, Burrone & Lagnado 2000, Silver 2010). Ozuysal & Baccus (2012) were able to build on this connection and construct a biophysical model of signal transmission through the retina that used the kinetics of RRP depletion and recovery measured in bipolar cells to account for several adaptive properties. Their observations suggest that the processes of vesicle movement along the

ribbon and of refilling the ribbon from the cytoplasm might determine the fundamental adaptive properties of the retina that allow coding under a variety of conditions (Ozuysal & Baccus 2012).

Nonlinear Transformations of the Visual Signal

An empirical description of the relation between variations in the intensity of light falling on the retina and the spike rates of ganglion cells can often be obtained using a model comprising two stages: a linear transformation of the input that feeds into a rectifying nonlinearity (Gollisch & Meister 2008). How does this linear-nonlinear model map onto the retinal circuit? Transformations of the visual input are roughly linear through the processes of phototransduction and transmission by the photoreceptor synapses and continue to be so as far down the visual pathway as the cell bodies of bipolar cells (Baccus & Meister 2002, Baden et al. 2011, Schwartz & Rieke 2011). In the next neural compartment in the visual pathway, the synaptic terminals of bipolar cells, however, measurements of vesicle release using sypHy demonstrate strong rectification in the visual signal as it is transmitted (Odermatt et al. 2012, Nikolaev et al. 2013). This behavior is predicted by a model of signal flow through the retina as a reflection of the rectifying relation between the membrane potential and the amplitude of the calcium current (Baden et al. 2014). Thus, voltage-dependent calcium channels in the synaptic terminals of bipolar cells appear to be the molecular basis for the first major nonlinear transformation of the visual signal as it is transmitted through the retina. Several neuromodulators regulate the voltage-dependence of these channels, thereby allowing the visual signal to be gated as it is transmitted to the inner retina (Esposti et al. 2013).

Temporal Filtering

Many bipolar cells transmit the visual signal through multiple terminals located in different strata of the inner plexiform layer, and increasing evidence suggests that these strata form parallel channels that transform the visual signal in different ways. For instance, Asari & Meister (2012) stimulated individual bipolar cells directly while monitoring the effects in multiple ganglion cells and found that different synapses varied in their gain, adaptation, and rectification properties, primarily owing to interactions with amacrine cells. However, increasing evidence suggests that the intrinsic properties of ribbon synapse terminals vary even within a neuron. The most obvious variation is in the size of the presynaptic compartment and the number of ribbons it contains (Connaughton et al. 2004). In a recent study using a combination of in vivo imaging, electrophysiology, and modeling, Baden et al. (2014) found that variations in the size of these terminals also cause the signal in a single neuron to be transformed through different temporal filters as it is transmitted to the inner retina. The mechanistic link between the terminal size and the temporal filters is the calcium signal that controls synaptic transmission: Smaller terminals generate larger and faster calcium transients, increasing the gain of synaptic transmission and allowing higher frequencies to be transmitted. Moreover, diversity in the properties of synaptic transmission extends to timedependent changes in gain: Smaller terminals adapt more completely after an increase in stimulus contrast than larger terminals do, and one can account for this phenomenon by noting that a smaller reserve pool of vesicles limits the refilling of the ribbons within the terminal (Baden et al. 2014). As these variations in synaptic properties expand the number of processing channels that can operate in parallel through the limited number of neurons packed into a given region of the retinal network (Balasubramanian & Sterling 2009, Perge et al. 2009), ribbon synapses may be one element of the different temporal filters through which the visual signal is transmitted (Suh & Baccus 2014).

SUMMARY AND OUTLOOK

In recent decades, the study of ribbon synapses in the retina has advanced on several fronts from structure to molecules to physiology-and increasingly detailed information about how these synapses function has become available. Many design features allow these machines to transmit graded changes in membrane potential using a continuous mode of neurotransmitter release. (a) Terminals tend to be large, containing many thousands of vesicles; (b) the presynaptic compartment typically contains multiple active zones; (c) active zones include a ribbon holding a pool of vesicles adjacent to the fusion sites; (d) vesicles in the cytoplasm are mobile, allowing the efficient resupply of vesicles to ribbon and active zone; (e) vesicle fusion can be triggered by low (submicromolar) levels of calcium; (f) calcium channels at the active zone are usually of the Ltype and display little inactivation; (g) vesicles are efficiently retrieved from the surface membrane by a number of endocytotic mechanisms, and (b) calcium influx accelerates the processes that supply new vesicles for release. The ribbon synapse is also capable of a rapid and transient mode of vesicle release used to signal higher-frequency components of the visual input, and we now appreciate that the processes that supply vesicles to the active zone also determine the efficiency of fast transmission because they determine the number of primed vesicles on which a calcium transient acts.

Important advances have also been made in understanding the role of ribbon synapses in their wider context—visual processing in the retina. Real-time measurements of synapse function obtained by electrophysiology and imaging have allowed researchers to construct quantitative models that help us understand the role that ribbon synapses play in controlling the kinetics, gain, and adaptive properties of the retinal circuit (Jackman et al. 2009, Oesch & Diamond 2011, Ozuysal & Baccus 2012, Baden et al. 2014). The processes that transfer vesicles from the cytoplasm to first the ribbon and then the active zone are fundamental to these models, yet these are the processes about which we have the biggest hole in our understanding. The molecular basis of ribbon function is still, for the most part, a mystery.

SUMMARY POINTS

- 1. The synapses of retinal photoreceptors and bipolar cells contain an unusual organelle, the ribbon, which is involved in the transport and priming of vesicles to be released at the active zone. All ribbon synapses release the excitatory neurotransmitter glutamate.
- 2. The ribbon is a large proteinaceous structure associated with the presynaptic active zone and synaptic vesicles are physically connected along the entire surface.
- 3. The major structural component is the protein Ribeye. Other ribbon-associated proteins, such as Piccolo and Bassoon, CtBP1, and RIM1, are thought to be involved in tethering synaptic vesicles to the ribbon and/or priming the vesicles for fusion at the active zone.
- 4. Ribbon synapses are capable of two kinetically distinct modes of transmission: one is fast and transient, involving the release of docked vesicles after a period of rest, while the second is slower and continues after docked vesicles have been released.
- 5. This continuous mode of exocytosis is suited to signaling the intensity and duration of maintained stimuli. The ribbon also plays a key role in supplying vesicles for rapid and transient burts of release that signal fast changes, such as the onset of light.

- 6. The ribbon is one of a number of design features that allow efficient refilling of the active zone so that changes in light intensity can be continuously signalled. Both clathrin-dependent and clathrin-independent modes of endocytosis act to recycle vesicles after fusion.
- Submicromolar calcium concentrations are sufficient to drive exocytosis from ribbon synapses, and also accelerate the process by which the ribbon becomes refilled with vesicles.
- 8. The processes of vesicle movement along the ribbon and refilling of the ribbon from the cytoplasm determine some of the adaptive properties of the retinal circuit as a whole, thereby allowing coding under a variety of conditions.

FUTURE ISSUES

- 1. The molecular processes that transfer vesicles from the cytoplasm to the ribbon and then to the active zone are yet to be understood.
- 2. What is the molecular composition of the filaments that attach vesicles to the ribbon?
- 3. How do vesicles move on the ribbon, and how does calcium modulate this process?
- 4. What is the function of the Ribeye protein?
- 5. Does the ribbon play a role in the endocytic processes that retrieve vesicles?
- 6. How do neuromodulators act to alter the input-output relation of ribbon synapses?

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