Cell Calcium 52 (2012) 327–337

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Molecular anatomy and physiology of exocytosis in sensory hair cells

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ARTICLE INFO

Article history:
Received 2 April 2012
Received in revised form 8 May 2012
Accepted 14 May 2012
Available online 6 June 2012

Keywords:
Hair cell
Exocytosis
Synaptic ribbon
Hearing
Calcium
Synaptic protein
Synaptic vesicle
Pool
Ribeye
Active zone
Neurotransmission

ABSTRACT

Hair cells mediate our senses of hearing and balance by synaptic release of glutamate from somatic active zones (AZs). They share conserved mechanisms of exocytosis with neurons and other secretory cells of diverse form and function. Concurrently, AZs of these neuro-epithelial hair cells employ several processes that differ remarkably from those of neuronal synaptic terminals of the brain. Their unique molecular anatomy enables them to better respond to small, graded changes in membrane potential and to produce unsurpassed rates of exocytosis. Here, we focus on the AZs of cochlear inner hair cells (IHCs). As in other hair cells, these AZs are occupied by a cytoplasmic extension of the presynaptic density, called the synaptic ribbon: a specialized protein complex required for normal physiological function. Some proteins found at IHC synapses are uniquely expressed or enriched there, where their disruption can beget deafness in humans and in animal models. Other proteins, essential for regulation of conventional neuronal Ca^{2+}-triggered fusion, are apparently absent from IHCs. Certain common synaptic proteins appear to have extra significance at ribbon-type AZs because of their interactions with unique molecules, their unusual concentrations, or their atypical localization and regulation. We summarize the molecular–anatomical specializations that underlie the unique synaptic physiology of hair cells.

1. Introduction

Auditory hair cells transduce mechanical energy into temporally precise exocytosis at high rates, to represent the fine structure of sound [1–4]. Studies have demonstrated that individual IHC AZs are capable of sustaining exocytosis at several hundreds of vesicles/s without fatigue, which is reflected rigorously in rates of action potentials in individual fibers of the auditory nerve [5–7]. Unlike neurotransmission evoked by all-or-none action potentials invading neuronal presynaptic terminals, in hair cells exocytosis occurs in response to smaller somatic potentials that vary with sound pressure (in the auditory system) or acceleration (in the vestibular system). In response to audible sounds of low intensity, near the threshold of perception, the potential of the IHC may change by only a few mV or even less [8–10]. To encode stimuli continuously with such sensitivity over a large dynamic range, the synapses of sensory hair cells are highly specialized.

Hair cells utilize unique molecular mechanisms for neurotransmitter release. As in neurons, the process of exocytosis is regulated by Ca^{2+} influx at the synapse [11–15]. However, the class of voltage-gated Ca^{2+} channels mediating stimulus-secretion coupling in IHCs (L-type, specifically $Ca_V1.3$ [16–19]) differs from that in neurons (N-, P/Q-, or R-type Ca^{2+} channels [20]). Like most excitatory synapses, sensory hair cells release the chemical neurotransmitter glutamate from synaptic vesicles [21]. However, the unconventional vesicular glutamate transporter-3 (Vglut3) is found in hair cells and absolutely required for hearing [22–24]. In response to Ca^{2+} influx, a hair cell releases neurotransmitter from a pool of docked and primed synaptic vesicles [12,25–27]. However, the synaptic vesicle proteins proposed to be the molecular sensors for synchronous Ca^{2+}-evoked exocytosis in neurons, synaptotagmins 1 and 2, are likely absent from mature IHCs [28–31]. Other proteins involved in the neuronal synaptic vesicle cycle appear to be absent or insignificant in hair cells, for example synapsins and synaptophysins [28,29], synaptogyrin [29], complexins [32], and neuronal SNAREs [33]. Conversely, the putative synaptic vesicle protein otoferlin is specifically required in hair cells but apparently not elsewhere. Disruption of otoferlin results in human deafness [34] due to its special function in the IHC synaptic vesicle cycle and exocytosis [35,36].

A hallmark of hair cell AZ morphology is the presence of a cytoplasmic electron-dense structure called the synaptic ribbon

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http://dx.doi.org/10.1016/j.ceca.2012.05.008

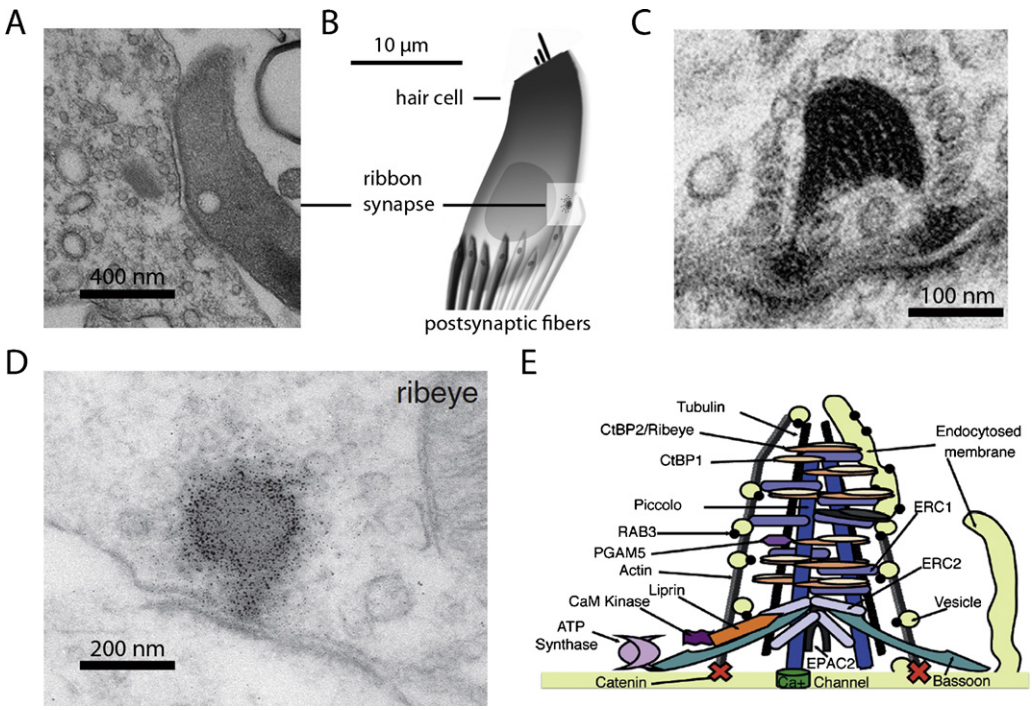

Fig. 1. Hair cell synaptic structure and ribbon-type active zone composition. (A) Transmission electron micrograph of a synaptic contact between a presynaptic cochlear IHC (left) and a postsynaptic bouton of an auditory nerve fiber (right). The electron-dense ovoid structure (the presynaptic ribbon) is surrounded by a halo of neurotransmitter-filled vesicles. Each auditory nerve fiber receives synaptic input from one large ribbon-type AZ. (B) Illustration of a presynaptic IHC contacting several afferent nerve fibers at its basal pole. Each fiber sends action potentials to the brain. The highlighted synapse has a similar orientation as the micrograph in panel A. (C) A synaptic ribbon from a mouse IHC showing a halo of synaptic vesicles and two attachments to the presynaptic membrane density. The ribbon sometimes appears to have an ordered substructure. Panels A and C courtesy of Dietmar Riedel. (D) Immuno-electron microscopic localization of ribeye protein in the cytoplasmically extended, electron-dense cytomatrix at the AZ that characterizes a ribbon-type synapse. Reprinted with permission from the Society for Neuroscience (Copyright 2010) from [29]. Ribeye is thought to be the structural backbone of the ribbon and the primary molecular component. (E) Hypothetical arrangement of synaptic ribbon proteins. Affinity purification of ribbons followed by mass spectroscopy allowed quantification of proteins enriched in retinal ribbons. The ribbon and its tightly associated protein complex is comprised primarily of the novel protein ribeye and proteins found at conventional synapses. Reprinted with permission of the American Chemical Society (Copyright 2011) from [56].

(Fig. 1), which is comprised predominantly of the protein ribeye that is found only at ribbon synapses [37,38]. Bassoon, a ubiquitous vertebrate presynaptic protein, has a unique role at ribbon synapses. It binds to ribeye [39], anchoring the ribbon with its halo of synaptic vesicles to the presynaptic density [25,40]. The ribbon and bassoon improve auditory first-spike latency and enhance synchronous neural encoding of sound onset in mice [25,41,42]. Besides hair cells in the auditory and vestibular systems of the ear and in the lateral line organs of fish, ribbons decorate other synapses that encode small, graded changes in membrane potential and sustain high rates of release for prolonged periods of time (retinal photoreceptors and bipolar cells, and electroreceptors in electric organs of fish), and pinealocytes of the brain. Investigation of ribbon function(s) in each cell type is an active area of research. Hair cell synaptic release is functionally and molecularly different from exocytosis in all other secretory cells, even amongst other ribbon synapses. This overview highlights some molecular biophysical specializations that distinguish sensory hair cells, with a focus on the IHCs of the mammalian cochlea.

2. The ribbon synapse and ribeye

Each IHC ribbon synapse provides strong excitation [7] to a single postsynaptic neuron (Fig. 1A and B) and is thought to represent the sole sensory input to that neuron [43]. The ribbon-type AZ is postulated to have several release sites populated by a number of Ca^{2+} channels, where a synaptic vesicle may dock and comprise part of the readily releasable pool (RRP) of vesicles [12,25,26,44–46]. Also called synaptic rods or dense bodies, synaptic ribbons tether numerous vesicles via fine filaments (Fig. 1C). In addition to the docked vesicle pool, which occupies the space between the ribbon and the presynaptic membrane, the ribbon-tethered vesicle pool is thought to be involved in exocytosis [47,48].

The ribbon is hypothesized to impart special characteristics to exocytosis by regulating the translocation and biochemical fusion-competence of synaptic vesicles. It is thought to stabilize the multiple release sites within its large AZ [25,41,42]. Ribbons may support continuous exocytosis, like a “conveyor belt,” by speeding the delivery of vesicles to release sites [49,50]. They may be involved in simultaneous multi-quantal release by holding vesicles close to each other, promoting their homotypic fusion before exocytosis [49,51], or by promoting the synchronous exocytosis of multiple vesicles docked beneath the ribbon [52]. Conversely, a study from the retina suggested that the ribbon is rather a “timing belt,” slowing vesicle delivery possibly to prevent depletion [53,54]. Perhaps similarly, in the ear of the cat, IHC AZs occupied by larger ribbons were correlated with auditory nerve fibers having lower spontaneous spike rates and relatively low sensitivity to sound when compared with fibers receiving synaptic input from smaller ribbons [55]. Thus, although the ribbon is believed to support high rates of releases, its precise function is unclear and remains a hot topic of investigation. The acute destruction of ribbons in mouse retinal bipolar cells and salamander cone photoreceptors offered another functional interpretation. Immediately after photobleaching of ribeye, exocytosis of the RRP was apparently normal. However, exocytosis was severely attenuated in response to