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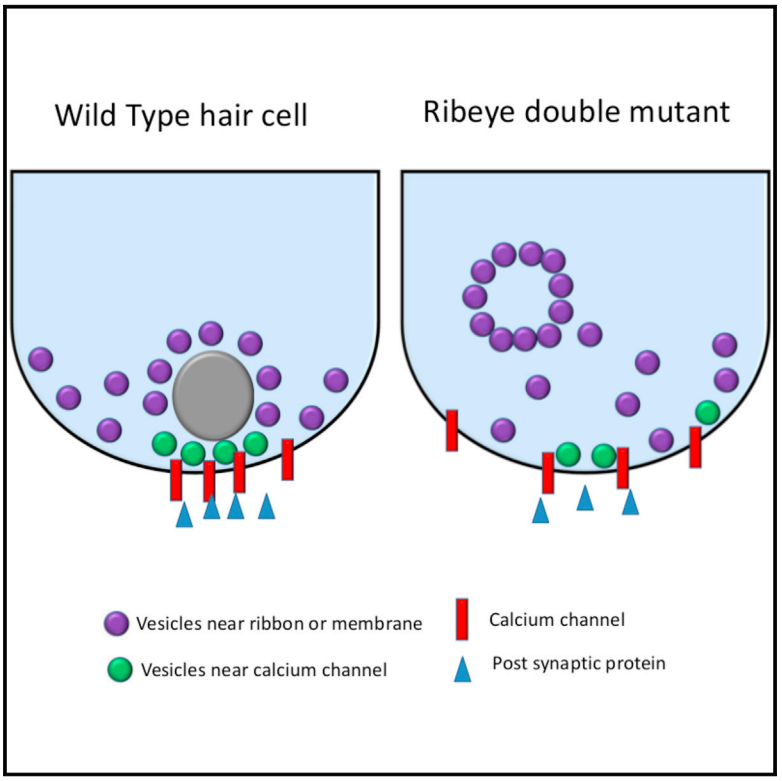
Synaptic Ribbons Require Ribeye for Electron Density, Proper Synaptic Localization, and Recruitment of Calcium Channels

Graphical Abstract



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In Brief

Synaptic ribbons are features of the auditory, vestibular, and visual systems that hold vesicles close to release sites in sensory cells. Lv et al. now find that genetic reduction of Ribeye levels in zebrafish results in the disruption of synaptic ribbon localization and morphology with minor effects on kinetics or levels of vesicle exocytosis.

Highlights

- Ribeye is needed for electron density to form at hair cell synaptic ribbons
- Ribeye mutants have smaller synaptic vesicles and mislocalized ribbons
- Ribeye is required for synaptic ribbon association with calcium channels
- Continuous exocytosis is enhanced in ribeye mutants despite ribbon mislocalization

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Synaptic Ribbons Require Ribeye for Electron Density, Proper Synaptic Localization, and Recruitment of Calcium Channels

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SUMMARY

Synaptic ribbons are structures made largely of the protein Ribeye that hold synaptic vesicles near release sites in non-spiking cells in some sensory systems. Here, we introduce frameshift mutations in the two zebrafish genes encoding for Ribeye and thus remove Ribeye protein from neuromast hair cells. Despite Ribeye depletion, vesicles collect around ribbon-like structures that lack electron density, which we term "ghost ribbons." Ghost ribbons are smaller in size but possess a similar number of smaller vesicles and are poorly localized to synapses and calcium channels. These hair cells exhibit enhanced exocytosis, as measured by capacitance, and recordings from afferent neurons post-synaptic to hair cells show no significant difference in spike rates. Our results suggest that Ribeye makes up most of the synaptic ribbon density in neuromast hair cells and is necessary for proper localization of calcium channels and synaptic ribbons.

INTRODUCTION

Primary sensory cells of the auditory, vestibular, and visual systems encode sensory information as graded changes in voltage that lead to graded changes in glutamate release. These non-spiking cells use synaptic ribbons, which hold a dense array of synaptic vesicles in active zones near release sites. Because these cells exhibit tonic and graded signaling in response to sensory stimuli, it has largely been assumed that the synaptic ribbon is necessary to carry out this task (Matthews and Fuchs, 2010). Indeed, ribbon-synapse-containing cells have been demonstrated to exhibit a sustained phase of exocytosis in response to prolonged stimuli (Lagnado et al., 1996; Parsons et al., 1994; Moser and Beutner, 2000; Edmonds et al., 2004; Bartoletti et al., 2010), and optical studies have revealed that vesicles are immobilized to and move along the ribbon in response to stimuli (Vaithianathan et al., 2016; Zenisek, 2008; Zenisek et al., 2000; Midorikawa et al., 2007).

The most abundant protein in the synaptic ribbon is Ribeye, a protein arising from an alternative start site at the gene encoding for the CtBP2 transcriptional co-repressor (Schmitz et al., 2000). Ribeye can be subdivided into two domains, a ribbon-specific A-domain and a B-domain, which is nearly identical to CtBP2 (Schmitz et al., 2000).

Genetic deletion of mouse Ribeye leads to a loss of synaptic ribbons in the retina, which is accompanied by a reduction in synaptic transmission from bipolar cells without observable changes in the kinetic features of release (Maxeiner et al., 2016), whereas morpholino-oligonucleotide (MO)-driven knockdown of Ribeye expression leads to the mislocalization of calcium channels (Sheets et al., 2011; Lv et al., 2012) and a reduction of spiking rates in afferent neurons postsynaptic to zebrafish hair cells (Sheets et al., 2011).

To better understand the function of Ribeye and the synaptic ribbon, we used genome editing to introduce frameshifting mutations in the A-domain of both zebrafish Ribeye-encoding genes and used light and electron microscopy (EM), whole-cell capacitance measurements, and recordings from afferent neurons to measure the effects on neuromast hair cells. The resultant double-homozygous mutant animals exhibited dramatically reduced Ribeye levels in hair cells, leading to a loss of electron density from hair cell ribbons, shrinkage of ribbon size, mislocalization of ribbon-like structures, and disruption in the association of calcium channels with the ribbon. Despite these changes, the continuous phase of exocytosis is enhanced, and we could not detect differences in afferent neuron response in mutant animals. These results indicate that Ribeye likely makes up the electron-dense portion of the ribbon and recruits calcium channels to release sites in zebrafish hair cells but is not required for vesicle binding, transport, or maintaining continuous release.