SNARE proteins in retinal synaptic vesicle release

Maggie Huang[1, 2], Shuzo Sugita[1, 2]
[1]Faculty of Medicine, Department of Physiology, University of Toronto
[2]Division of Fundamental Neurobiology, Krembil Brain Institute, University Health Network

Introduction

The first step for vision is light transduction by photoreceptors. In contrast to conventional neurons, photoreceptors are depolarized by default and continuously release glutamate; therefore, photoreceptor synapses must be able to release massive amounts of glutamate to sustain vision. Neurotransmitter release requires vesicle fusion; called exocytosis. Exocytosis is driven by the continuous assembly and disassembly of the SNARE complex. Traditionally, the SNARE complex consists of the core proteins syntaxin-1, SNAP-25, and synaptobrevin-2 and other regulatory proteins such as Munc18/stxbp1. This large release is likely made possible by the specialized synapses found in photoreceptors: photoreceptors are comprised of morphologically and functionally distinct ribbon synapses which contain a ribbon structure tethering the vesicles to be fused. Non-conventional photoreceptor synapses have SNARE complexes containing syntaxin-3 and SNAP-23 rather than the isoforms found in conventional synapses. Elucidating the role of these SNARE proteins and SNARE complex mediators will be important in understanding photoreceptor synapses, particularly because synaptic transmission impairment often precedes retinal degeneration which is the leading cause of vision loss worldwide. We hypothesize non-traditional SNARE proteins such as syntaxin-3 and SNAP-23 along with traditional mediators such as stxbp1 play a role in photoreceptor neurotransmitter release.

Results

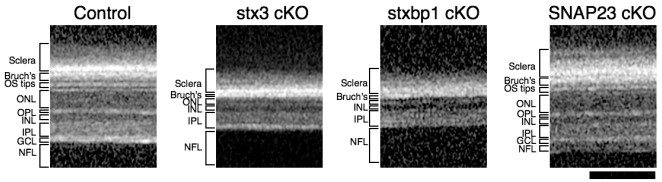


Figure 1: Decrease in retinal thickness of stx3 and stxbp1 cKO retinae observed in vivo by optical coherence tomography. This study was performed on a system of three conditional knockout (cKO mice) where the protein of interest was specifically removed from the photoreceptors. Optical coherence tomography (OCT) scans from control, syntaxin-3, stxbp1, and SNAP-23 cKO retinae. Syntaxin-3 and stxbp1 cKO retina are dramatically thinned. Scale bar = 200µm.

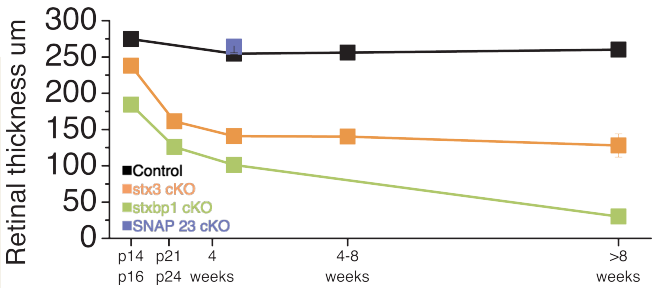
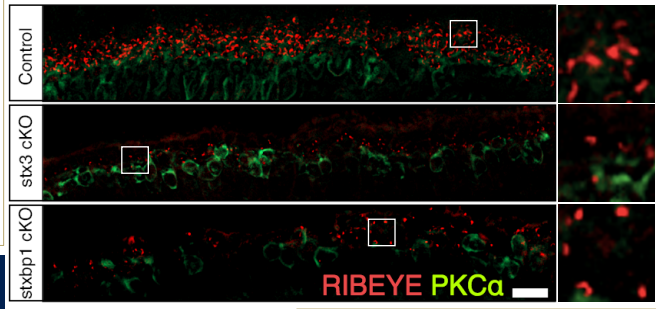


Figure 2: In vivo tracking of average retinal thickness 1.5mm away from optic nerve over time. Longitudinal tracking of retinal thickness in live mice by OCT find syntaxin-3 cKO retinal thickness stabilizes between 4-8 weeks of age while stxbp1 cKO retina continue to degenerate.



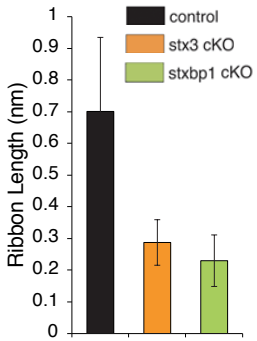


Figure 3: Decreased and shorter to ribbons in syntaxin-3 and stxbp1 cKO retina. RIBEYE staining for the structural ribbon protein (red) and PKCα staining for rod bipolar cells (green) in control, stx3 cKO and stxbp1 cKO retina (top); ribbons are neatly localized presynaptically in control retinae. Ribbons stray from the synaptic boundary and are decreased in number, appear shorter and more punctate in cKO retinas. Quantification of ribbon length by electron micrograph analysis (left); shorter ribbons are observed in syntaxin-3 cKO and stxbp1 cKO retina.

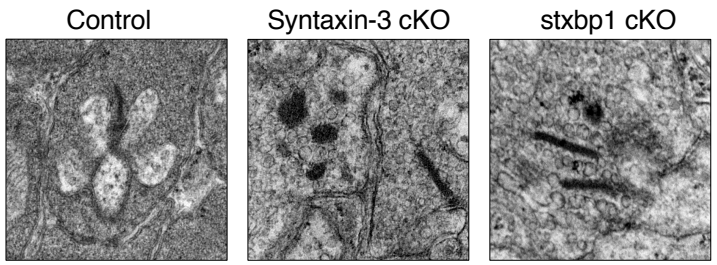


Figure 4: Electron micrographs of control, stx3 cKO, and stxbp1 cKO photoreceptor terminals. Photoreceptor terminal with invaginated post-synapses (lighter regions) and single ribbon clearly observed in control retina. Syntaxin-3 cKO and stxbp1 cKO photoreceptor terminals lack defined post-synaptic connections. Presence of abnormal duplicated and rounded ribbons also observed in both syntaxin-3 and stxbp1 cKO retina.

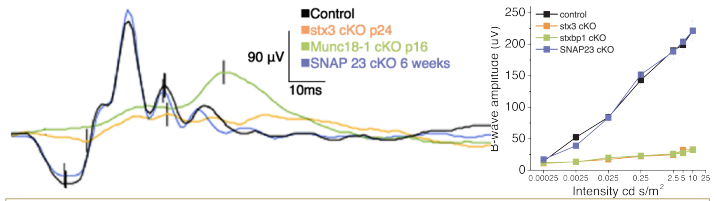


Figure 5: Electroretinogram recordings shows decline in functional photoreceptor response to light in stx3 cKO and stxbp1 cKO retinas. Representative electroretinogram trace (left) and average b-wave amplitude across light intensities (right). Electroretinogram b-wave is indicative of photoreceptor synaptic transmission; b-wave is severely attenuated and asynchronized in both syntaxin-3 and stxbp1 cKO retina.

Summary of Findings

Syntaxin-3 and **stxbp1** are critically important to retinal synaptic transmission. Loss of these proteins leads to

- Progressively decreased retinal thickness
- Impaired synaptic structure and shorter synaptic ribbons
- Attenuated response to light

Acknowledgements

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