Published in final edited form as:

Adv Exp Med Biol. 2012 ; 723: 739–744. doi:10.1007/978-1-4614-0631-0_94.

Trafficking of Presynaptic PMCA Signaling Complexes in Mouse Photoreceptors Requires Cav1.4 α_1 Subunits

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Keywords

Calcium extrusion; Nob2; Photoreceptor; *Cacna1f*; Voltage-operated; PMCA; PMCA1; Ribbon synapse; Retina

94.1 Introduction

The Cav1.4 α_1 subunit gates sustained neurotransmitter release at mammalian retinal ribbon synapses. Gain and/or loss of function mutations in the *Cacna1f* gene that encodes Cav1.4 result in enhanced or suppressed voltage-operated Ca^{2+} influx in heterologously expressing cells (Hemara-Wahani et al. 2005; Hoda et al. 2006; Peloquin et al. 2007). In vivo, *Cacna1f* mutations cause night blindness in mice and humans (Strom et al. 1998; Bech-Hansen et al. 1998; Lodha et al. 2010) by compromising Ca^{2+} -dependent exocytosis in terminals of rod and cone photoreceptors. Accordingly, CSNB2 patients are typically diagnosed by abnormal ERG b-waves and can exhibit reduced acuity, impaired night vision, refractive disorders, strabismus, and nystagmus (Miyake et al. 1986). The *Cacna1fnob2* (*nob2*) mouse model of CSNB2, which lacks ~90% of Cav1.4 transcripts, shows an absence of the b-wave but an essentially normal photopic optokinetic acuity (Doering et al. 2008). In contrast, Cav1.4 knockout (KO; *Cacna1fG305*) mice created by excision of the floxed exon 7 exhibit neither b-waves nor optokinetic responses (Mansergh et al. 2005). Both KO and *nob2* retinas are characterized by “floating” presynaptic ribbons and by extensive outgrowth of postsynaptic bipolar and horizontal dendritic processes into the outer retina (Bayley and Morgans 2007; Specht et al. 2009; Baehr and Frederick 2009). It is unclear, however, whether compromised Ca^{2+} influx in *nob2* rods is associated with impaired development of photoreceptor synapses and/or reorganization of presynaptic Ca^{2+} homeostatic mechanisms. We have therefore investigated the function of Cav 1.4 subunits in organization of the photoreceptor perisynaptic complex that regulates steady-state $[\text{Ca}^{2+}]_i$ and sustained glutamate release in

rods and cones. Plasma membrane Ca^{2+} ATPases (PMCAs) represent a high-affinity Ca^{2+} clearance mechanism that balances sustained influx via L-type channels located in photoreceptor terminals (Križaj and Copenhagen 1998; Križaj et al. 2002) and controls the kinetics of the postsynaptic light response by regulating the rate of clearance of residual $[\text{Ca}^{2+}]_i$ within photoreceptor terminals (Duncan et al. 2006). We report here that PMCA transporters and RIBEYE proteins exhibit a profound mislocalization in photoreceptors from *nob2* retinas. This result suggests that steric interactions mediated through the Cav1.4 peptide and/or Ca^{2+} fluxes gated by the α_{1F} pore are essential for proper trafficking of PMCAs to the synapse and for organization of the active zone in synaptic terminals of mouse photoreceptors.

94.2 Materials and Methods

94.2.1 Animals

C57BL6 wild type and AXB-6/PgnJ animals homozygous for *Cacna1f^{nob2}* were purchased from Jackson laboratories (Bar Harbor, MN). *Basoon* KO mice were a kind gift from Dr. Yong Wang (University of Utah). Animals were maintained in the University animal quarters on a 12 h:12 h light:dark cycle. Animal handling and anesthetic procedures were approved by University Institutional Animal Care committees and conform to NIH guidelines.

94.2.2 Immunohistochemistry

Immunostaining procedures were performed as described previously (Križaj et al. 2002; Mizuno et al. 2010). Polyclonal pan PMCA (used at 1:100), PMCA1 (1:350–1:500), and PMCA2 (1:350) antibodies were purchased from Affinity Bioreagents (Golden, CO). The secondary antibodies utilized were goat antimouse or goat antirabbit IgG (H + L) conjugated to fluorophores (Alexa 488 and Alexa 594 conjugates, Invitrogen), diluted 1:500 or 1:1,000 or goat antimouse Cy3 from Jackson ImmunoResearch at 1:1,000.

94.3 Results

94.3.1 Nob2 Mutation of Cav1.4 Is Associated with Mislocalization of PMCA1 but not PMCA2

Steady-state $[\text{Ca}^{2+}]_i$ in rod and cone terminals is maintained by PMCA1 transporters whereas PMCA2 is mainly localized to rods (Križaj et al. 2002; Duncan et al. 2006). To determine whether compromised expression of L-type channels affects localization of PMCA transporters, we immunostained *nob2* retinas with the 5F10 pan-PMCA antibody that recognizes all four PMCA isoforms (Križaj and Copenhagen 1998) and with PMCA1 and PMCA2-selective antibodies that had been validated in previous studies of the rodent retina (Križaj et al. 2002; Rentería et al. 2005). As shown in Fig. 94.1c, pan PMCA-immunoreactive (ir) puncta were displaced from the outer plexiform layer (OPL) to irregularly spaced juxtannuclear locations across the outer nuclear layer (ONL). Regions of displaced PMCA-ir were typically shadowed by disorganized OPL underneath. Similar displacement into ONL was observed with the PMCA1 antibody (Fig. 94.1g) whereas PMCA2-ir in *nob2* retinas showed no changes from controls (Fig. 94.1k). Few obvious changes were evident in inner retinal expression of *nob2* PMCA1 (Fig. 94.1b, f). While the PMCA1 transporter antibody stained the expected two IPL bands corresponding to b3/b7 cone bipolar cells (e.g., Križaj et al. 2002), PMCA1-ir in *nob2* preparations was not displaced into the inner nuclear layer (INL). Dislocation of PMCA1-ir was paralleled by severe disorganization of RIBEYE-ir (Fig. 94.1n). These results suggest that the Cav1.4 subunit is required for proper expression, sorting, and/or targeting of PMCA1 to

photoreceptor, but not bipolar, synapses. Moreover, PMCA1 but not PMCA2 localization is compromised by the loss of Cav1.4 protein.

Bassoon is a large cytomatrix protein (420 kDa) located at the base of photoreceptor synapses where it may anchor the filamentous network associated with the active zone to the ribbon and the plasma membrane (tom Dick et al. 2003). To determine whether PMCA1 is tethered to the perisynaptic region through bassoon, we analyzed PMCA1 expression in bassoon KO mice. Elimination of Bassoon induced a PMCA1 localization phenotype that was indistinguishable from the improper expression observed in *nob2* retinas. This data suggests that PMCA1 forms the arciform complex together with Cav1.4 and Bassoon.

94.4 Discussion

The data presented in this study shows that proper targeting of synaptic proteins and PMCA1 to photoreceptor terminals requires adequate expression of the Cav1.4 subunit. Loss of Cav1.4 resulted in selective dislocation of the presynaptic machinery at photoreceptor, but not bipolar, ribbon synapses.

It has been suggested that the CSNB2-like phenotype of the *Cacna1f^{nob2}* mouse is caused by diminished production of full-length protein as well as failed targeting of the mutant protein to the plasma membrane (Doering et al. 2008). Our data is consistent with this hypothesis. Displacement of RIBEYE in *nob2* retinas was accompanied by partial, but not complete, dislocation of the key PMCA Ca^{2+} clearance protein from OPL into ONL. Mislocalization of PMCA1-ir into “aggresome-like” puncta to juxtannuclear positions within the *nob2* ONL rods suggests that Cav1.4 subunits are required for tethering the housekeeping PMCA1 mechanism into a multimeric complex, possibly in association with MPP4 and PSD-95 MAGUK proteins that interact with the full-length “b” splice variant of PMCA1 (Križaj et al. 2002; Yang et al. 2007; Aartsen et al. 2009).

It is not clear whether continuous Ca^{2+} influx through “resting” release from ER stores, store-operated, and/or TRP channel conductances (Križaj et al. 2009; Szikra et al. 2009) can partially compensate for the loss of voltage-operated Ca^{2+} influx at *nob2* rod synapses. Although RIBEYE signals also showed prominent dislocation into the *nob2* ONL, the displacement of the ribbon complex in itself was not sufficient to block asynchronous exocytosis in bipolar and hair cells of Bassoon KO mice (Khimich et al. 2005; Midorikawa et al. 2007). In addition to improper targeting and/or anchoring of PMCA1 (Fig. 94.1), *Bassoon*-null retinas exhibit many of the same morphological and physiological features as *Cacna1f* and *Cabp4* mutant/KO retinas, including decreased amplitudes and increased implicit times of the b-wave (Dick et al. 2003; Haeseleer 2008). If Bassoon tethers both Ca^{2+} channels and PMCA transporters into the arciform complex, synchronous vesicle release at photoreceptor ribbon synapses is likely to be governed by local Ca^{2+} microdomains rather than average $[\text{Ca}^{2+}]_i$ levels that are typically measured with fluorescent indicator dyes. Other presynaptic components required for sustained signaling at rod synapses were observed to be displaced in *nob2* retinas in parallel with PMCA1 (D.K. and W.X. in preparation), raising the question of whether such protein agglomerates within the distal ONL represent release-competent proto-synapses. In summary, our study shows that the Cav1.4 α_1 subunit is required for proper targeting of the presynaptic PMCA complex and for tethering of PMCA1 transporters and the Ca^{2+} clearance mechanism into a multimeric presynaptic arciform complex.

Acknowledgments

The work was supported by The National Institutes of Health (EY13870, P30EY014800, EY 12497), The Foundation Fighting Blindness, and the Moran TIGER award. The research was also supported by an unrestricted award from Research to Prevent Blindness to the Moran Eye Institute at the University of Utah.

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Fig. 94.1.

PMCA1 is mislocalized following ablation of Cav1.4 and Bassoon. (a–d) pan-PMCA-ir in wild type, *nob2*, and Bassoon KO retinas shows prominent displacement into the ONL. (e–f) PMCA1-ir. (i–k) PMCA2-ir. (l–n) RIBEYE-ir in *nob2* retinas