Calcium Channels in Retinal Function and Disease

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Abstract

Voltage-gated Ca^{2+} (Ca_V) channels play pivotal roles in regulating gene transcription, neuronal excitability, and neurotransmitter release. In order to meet the spatial and temporal demands of visual signaling, Ca_V channels exhibit unusual properties in the retina compared to their counterparts in other areas of the nervous system. Here, we review current concepts regarding the specific subtypes of Ca_V channels expressed in the retina, their intrinsic properties and forms of modulation, and how their dysregulation could lead to retinal disease.

Keywords: ribbon synapse, photoreceptor, Ca^{2+}, neurotransmitter release, ion channel

1. Introduction

From the initial stages where photoreceptors extract features of the visual scene and through successive and parallel circuits involving retinal interneurons, Ca_V channels play essential roles at nearly all stages of visual processing. The contributions of Ca_V channels to the retinal circuitry have largely been explored using pharmacological modulators. However, the availability of genetic models lacking specific Ca_V channel subtypes, as well as advances in electrophysiological and optical imaging techniques, have

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opened many new avenues for research. In addition, the use of single-cell RNA-seq (scRNA-seq) to illuminate the molecular signature of cell-types in the mouse retina has provided a framework for understanding the expression and function of particular Ca_v subtypes within the retinal circuitry. In this review, we will consider how Ca_v channels have taken on unusual properties and functions that appear to be well-tailored for enabling the retina to carry out complex computational tasks, and how mutations affecting Ca_v channel function may cause vision impairment. Due to space constraints, Ca_v channels will be discussed in the context of a subset of retinal cell-types. For complementary insights on this topic, the reader is referred to a number of excellent reviews (Pangrsic et al 2018, Van Hook et al 2019).

2.0. Molecular diversity of Ca_v channels

Ca_v channels were originally classified by their voltage range of activation, with Ca_v1 and Ca_v2 channels forming the "high-voltage-activated" channels, and Ca_v3 channels forming the "low-voltage-activated" channels. Both types of Ca_v channels are comprised of a pore-forming α_1 subunit, with Ca_v1 and Ca_v2 channels also possessing auxiliary β and $\alpha_2\delta$ subunits (Fig.1A). The α_1 subunit of Ca_v channels contains 4 homologous repeats, each with 6 transmembrane-spanning α-helical domains (S1-S6). Within the S4 segment, a string of positively charged residues plays an important role in voltage-sensing. The S5 and S6 segments contribute to pore-lining regions and are connected by a pore loop containing glutamate residues that determine the high selectivity of Ca_v channels for Ca^{2+} ions (Fig.1A). Ca_v channels were initially named according to the properties of the currents they mediate (*e.g.,* L-type for "long-lasting"). According to current nomenclature, "Ca_v1-3.x" describes the channels according to the identity of their α_1 subunit; "*CACNxxx*" names the genes encoding the various subunits (Fig.1B).

Given this molecular and functional diversity, knowledge of the expression patterns and subcellular localization of Ca_v subtypes in the retina is needed to elucidate the roles of Ca_v channels in visual processing and how they may be derailed in inherited forms of vision loss. However, the current pharmacological modulators do not effectively discriminate between subtypes of Ca_v1 (*e.g.,* $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$) or Ca_v3 channels (*e.g.,* $Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$) (Fig.1B). Moreover, many reports describing the cellular and subcellular localization of Ca_v channels have relied on immunolabelling with antibodies of undocumented specificity. A promising complementary approach is droplet-based scRNA-seq, which allows for analysis of RNA expression in thousands of individual cells at once. This strategy has define, at the molecular level, many known and some newly discovered retinal cell-types. Using one of these scRNA-seq datasets (Macosko et al 2015), we have summarized the expression of Ca_v subunits in a subset of retinal cell-types in Figure 2 and will refer to this resource in subsequent sections.

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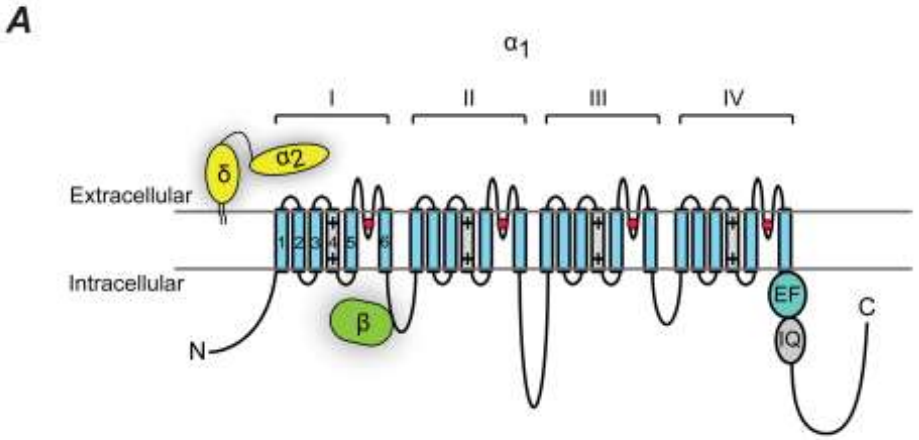

Gene	Protein	α_1 subunit name	Physiology	Pharmacological modulators
CACNA1S	$Ca_v1.1$	α1S	L-type	Dihydropyridines, Phenylalkylamines, Benzothiazepines
CACNA1C	$Ca_v1.2$	α1C		
CACNA1D	$Ca_v1.3$	α1D		
CACNA1F	$Ca_v1.4$	α1F		
CACNA1A	$Ca_v2.1$	α1A	P/Q type	ω-agatoxin IVA
CACNA1B	$Ca_v2.2$	α1B	N-type	ω-conotoxin GVIA
CACNA1E	$Ca_v2.3$	α1E	R-type	SNX-482
CACNA1G	$Ca_v3.1$	α1G	T-type	Mibefradil, ML 218, Z 944, NNC 55-0396
CACNA1H	$Ca_v3.2$	α1H		
CACNA1I	$Ca_v3.3$	α1I		

Figure 1. Molecular composition and nomenclature of Ca_v channels. *A,* The Ca_v α_1 subunit is comprised of 4 homologous domains (I-IV), each containing 6 alpha-helical transmembrane-spanning segments (S) (1-6). Positively charged residues (+) within S4 helices are indicated. Extracellular loop linking S5 and S6 form the pore; red circle indicates conserved glutamate in each domain that contributes to the selectivity filter. The C-terminal domain contains an EF-hand and IQ-domain that are important for Ca^{2+}/CaM-dependent modulation. For Ca_v1 and Ca_v2 channels, auxiliary β and $\alpha_2\delta$ subunits interact with the α_1 subunit at intracellular and extracellular sites, respectively. *B,* Nomenclature for α_1 subunits based on names for human genes and protein classification (Ertel et al 2000). Names were initially based on tissue of origin (*e.g.,* α1S for "skeletal muscle α_1 subunit") and physiological properties (*e.g.*, T-type for "transient" vs. L-type for "long-lasting"). Major classes of pharmacological modulators are indicated.

2.1. Ca_v1 channels

Ca_v1 (L-type) channels are distinguished from other Ca_v channels by their sensitivity to dihydropyridine-based drugs (Fig.1B). In contrast to their primarily postsynaptic functions in most neurons, Ca_v1 channels often play presynaptic roles in the retina. Of the four Ca_v1 subtypes, $Ca_v1.3$ and $Ca_v1.4$ predominate in a number of retinal cell-types such as photoreceptors and bipolar cells (Fig.2A,B). $Ca_v1.3$ and $Ca_v1.4$ exhibit properties that are well-suited for their exocytotic functions at the specialized "ribbon" synapses formed by these cells, which include rapid activation at relatively negative voltages

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and slow inactivation (Pangrsic et al 2018). Ca_v1 channels couple electrical activity to changes in gene transcription in many neurons, but whether they do so in retinal neurons remains to be established.

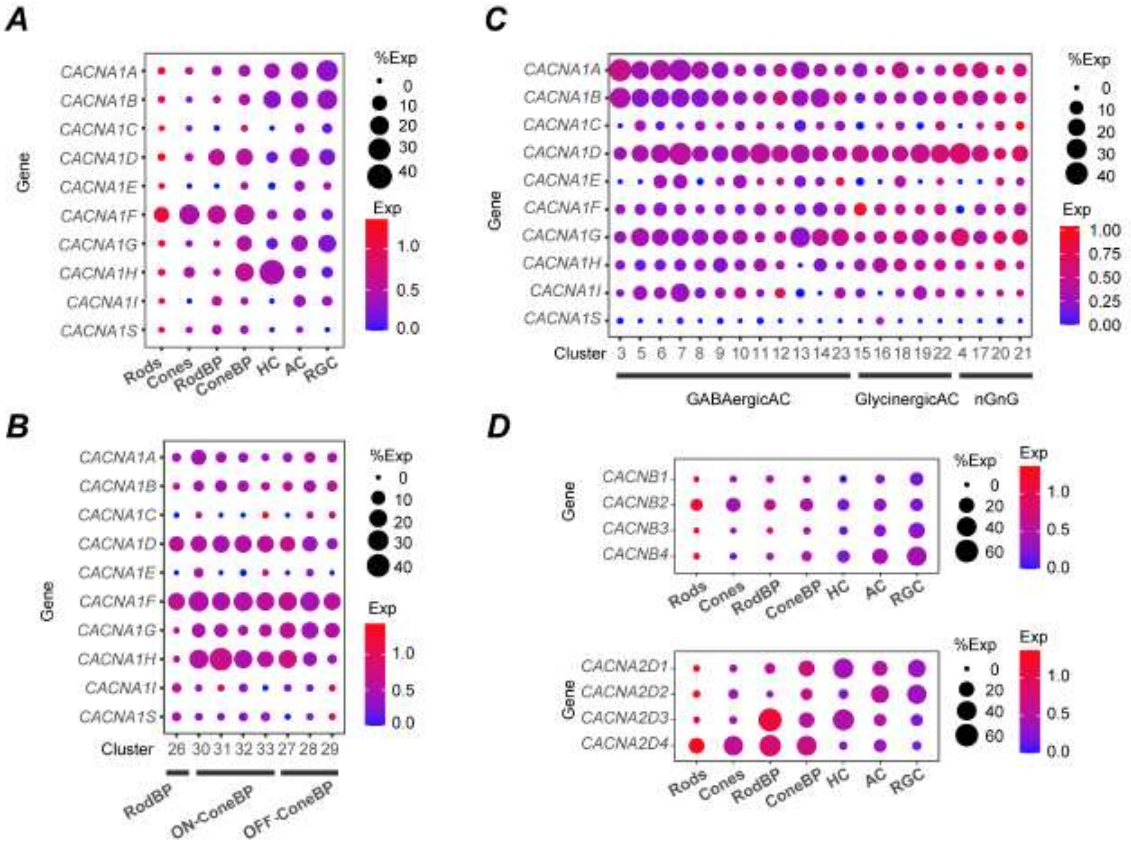

Figure 2. Expression of Ca_v-encoding genes in mouse retinal cell-types. *A-D*, Dot blots illustrating the expression of Ca_v subtypes corresponding to α_1 (*Cacna1x*, *A-C*) or β (*Cacnbx*) and $\alpha_2\delta$ (*Cacna2dx*; *D)* as determined by scRNA-seq. Results are plotted according to cell clusters identified in mouse retina at P14 (Macosko et al 2015). The size and color of the dots reflect the % of cells in which the transcript was detected and the averaged level of that transcript, respectively. Due to limitations in the sensitivity of the scRNA-seq method, as well as the relatively low expression levels of Ca_v genes, the dot size is generally <60%. Because the total number of transcripts in rods is less than that for other cell-types, the resulting expression value in rods appears higher than in other cell classes when scaling to the median value across all classes. For example, the expression of Ca_v genes in rods other than *Cacna1f, Cacnb2,* and *Cacna2d4,* can be considered as not different from background levels. Plots represent: *Cacna1* expression in major retinal cell-types (rods, cones, rod bipolar (BP) cells, cone BP cells, horizontal cells (HC), amacrine cells (AC), and retinal ganglion cells (RGC) (*A*), specific classes of BP cells (*B*), and distinct clusters of amacrine cells (GABA-ergic, glycinergic, and non-GABA-ergic, non-glycinergic, non-glutamatergic (nGnG) (*C)*; and *Cacnb1* and *Cacna2d* expression in the same cell-types as in *A*. In *C*, clusters 3 and 16 exhibit the molecular signature of starburst ACs and AII ACs, respectively.

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2.2. Ca_v2 channels

ω-agatoxin IVA, ω-conotoxin GVIA, and SNX-482 are are highly selective blockers of $Ca_v2.1$ (P/Q-type), $Ca_v2.2$ (N-type), and $Ca_v2.3$ (R-type) channels, respectively (Fig.1B). At many synapses, Ca_v2 channels regulate fast, phasic neurotransmitter release. Molecular determinants in the C-terminal domain (CTD) of Ca_v2, which are not conserved in Ca_v1, have been shown to support the presynaptic localization and function of Ca_v2 channels (Lubbert et al 2017, Maximov & Bezprozvanny 2002). By the same token, the absence of key determinants in the Ca_v2 CTD that are present in Ca_v1 may explain why Ca_v2 channels are excluded from ribbon synapses. Ca_v2 channels are most prominently represented in retinal cell-types other than photoreceptors and bipolar cells (Fig.2A-C), where they work in concert with other ion channels in regulating excitability and neurotransmitter release.

2.3. Ca_v3 channels

Originally classified as "low voltage-activated" channels, Ca_v3 channels activate and inactivate at extremely negative voltages, (~ -50 mV and -30 mV, respectively) (Perez-Reyes 2003). Consequently, Ca_v3 channels are inactivated at voltages near resting potential of most neurons. A period of hyperpolarization permits recovery of these channels from inactivation, and this can trigger rebound burst firing of action potentials (Kim et al 2001). By sc-RNAseq, Ca_v3 channels are expressed at significant levels in most retinal neurons except rods and rod bipolar cells (Fig.2A-C). Compared to major advances in pinpointing the roles of Ca_v3 channels in seizure activity and chronic pain (Perez-Reyes 2003), our understanding of how Ca_v3 channels contribute to retinal function is relatively limited.

2.4. Auxiliary β and $\alpha_2\delta$ subunits

The auxiliary β and $\alpha_2\delta$ subunits are resident components of Ca_v1 and Ca_v2 channel complexes, and critically regulate the biophysical properties, trafficking, and cell-surface expression levels of these channels. The β subunits (β_1- β_4) bind to a site in the cytoplasmic loop connecting domains II and III of the α_1 subunit of Ca_v1 and Ca_v2 channels (Fig.1A). In general, β subunits increase the amplitude of the Ca^{2+} currents (I_{Ca}) mediated by Ca_v1 and Ca_v2 channels due to a higher density of channels in the plasma membrane, an increase in channel open probability, and/or a hyperpolarizing shift in the voltage-dependence of channel activation (Buraei & Yang 2013). The interaction with β protects the α_1 subunit from ubiquitin-dependent proteosomal degradation, thus enhancing the cell-surface levels of Ca_v1 and Ca_v2 channels (Altier et al 2011, Waithe et al 2011). β subunits can also have Ca_v independent functions. For example, β_4 undergoes activity-dependent translocation to the nucleus and regulates gene transcription through interactions with nuclear proteins rather than with the Ca_v channel complex (Subramanyam et al 2009, Tadmouri et al 2012).

$\alpha_2\delta$ subunits ($\alpha_2\delta$-1-4) are comprised of two polypeptides, α_2 and δ, which are encoded by the same gene. The $\alpha_2\delta$ pre-protein is proteolytically processed into α_2 and δ which remain bound together by a disulfide linkage. The mature $\alpha_2\delta$ protein is attached to the plasma membrane via a glycosyl-phosphatidylinositol anchor (Dolphin 2013) (Fig.1A). The most prominent effect of $\alpha_2\delta$ is to increase the cell-surface levels of Ca_v1 and Ca_v2 channels in a manner that requires the presence of $Ca_v\beta$ (Cassidy et al 2014). While in complex with presynaptic Ca_v channels, or possibly independently, $\alpha_2\delta$ may engage in trans-synaptic interactions with proteins that regulate synapse formation and/or stability (Fell et al 2016, Geisler et al 2019, Wang et al 2017).

As is the case in many neurons, most retinal neurons express multiple subtypes of β and $\alpha_2\delta$ (Fig.2D) that differ in terms of their regulation of Ca_v channels as well as their non-canonical functions

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(Dolphin 2016). Studies of the corresponding KO mice would help dissect the retinal functions of specific β and $\alpha_2\delta$ subtypes. However, retinal phenotypes have thus far been described only for mice lacking expression of β_2 or $\alpha_2\delta$-4 (Ball et al 2002, Katiyar et al 2015, Kerov et al 2018, Wang et al 2017, Wycisk et al 2006a).

3. Ca_v channels are differentially expressed in retinal cell-types

Vision begins with the conversion of light into electrical signals by rod and cone photoreceptors, a process that modulates the release of glutamate from their synaptic terminals. The resulting signal is shaped via the activity of horizontal cells, bipolar cells, and amacrine cells prior to integration by the retinal ganglion cells (RGCs) and transmission into higher-order brain regions via RGC axons in the optic nerve (Fig.3A). Within the retinal circuitry, Ca_v channels are functionally specialized and often discretely localized in order to ensure the proper encoding of visual information.

3.3. Rod and cone photoreceptors

The somata of rods and cones lie in the outer nuclear layer (ONL) of the retina and extend axons into the outer plexiform layer (OPL) where they form synapses with bipolar and horizontal cells (Fig. 3A). The terminals of rods (spherules) and cones (pedicles) are characterized by synaptic ribbons that tether and replenish thousands of glutamate-filled synaptic vesicles near the specialized release sites (*i.e.*, active zones) (Matthews & Fuchs 2010). Within these terminals, the opening of Ca_v channels at the depolarized membrane potential of photoreceptors in darkness permits tonic, Ca^{2+}-dependent exocytosis of glutamate into the synaptic cleft. Light-dependent hyperpolarization of the photoreceptor membrane decreases Ca_v-mediated Ca^{2+} influx, thereby suppressing glutamate release (Choi et al 2008, Copenhagen & Jahr 1989, Johnson et al 2007). The decline in synaptic glutamate depolarizes ON bipolar cells and hyperpolarizes OFF bipolar cells by diminishing ligand activation of metabotropic mGluR6 receptors and ionotropic AMPA or kainate receptors, respectively (Connaughton 1995). Based on its sensitivity to dihydropyridine-based drugs (Fig.1B), the Ca_v channel that regulates glutamate release by photoreceptors was classified as the Ca_v1 subtype (Barnes & Hille 1989, Corey et al 1984, Schmitz & Witkovsky 1997, Taylor & Morgans 1998).

3.3.1. $Ca_v1.4$ regulates the maturation and function of rod and cone synapses

Large-scale sequencing of a genetic locus for the X-linked form of congenital stationary night blindness (CSNB2) revealed causative mutations in *CACNA1F*—the gene that is now recognized to encode $Ca_v1.4$ (Bech-Hansen et al 1998, Strom et al 1998). A common feature of the electroretinograms (ERGs) of CSNB2 patients is that b-waves representing transmission from photoreceptors to second-order neurons are significantly reduced while a-waves resulting from light-dependent hyperpolarization of photoreceptors are minimally altered (Zeitz et al 2015). Loss-of-function and null mutations in *Cacna1f* cause similar ERG phenotypes in various mouse strains (Chang et al 2006, Mansergh et al 2005, Regus-Leidig et al 2014) and visual behavior defects in zebrafish (Jia et al 2014).

Among Ca_v-encoding genes, *Cacna1f* is the most prominently expressed in rods and cones in mouse retina (Fig.2A-C). A role for $Ca_v1.4$ in mediating presynaptic Ca^{2+} signals in mouse photoreceptors is further supported by the near elimination of depolarization-evoked Ca^{2+} transients in the OPL (Mansergh et al 2005, Regus-Leidig et al 2014) and the lack of rod I_{Ca} in $Ca_v1.4$ knockout (KO) mice (Maddox et al 2020). $Ca_v1.4$ protein is tightly clustered along the

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base of the synaptic ribbon in rods and cones (Liu et al 2013b, Morgans 2001, Specht et al 2009), and thus is well-positioned to regulate the exocytosis of glutamate.

A notable aspect of the retina of $Ca_v1.4$ KO mice is the complete absence of rod and cone synapses in the OPL (Liu et al 2013b, Zabouri & Haverkamp 2013). Rod and cone synaptogenesis occurs postnatally and involves the formation of elongated ribbons, the coalescence of presynaptic and postsynaptic signaling complexes (Fig.3B), and the invagination into the pedicle or spherule of neurites emanating from two horizontal cells and one ON bipolar cell (Blanks et al 1974, Regus-Leidig et al 2009). The OPL of $Ca_v1.4$ KO mice is devoid of ribbons; bipolar and horizontal cell neurites sprout from the OPL into the ONL of these mice, perhaps due to the absence of presynaptic input (Liu et al 2013b, Raven et al 2008, Regus-Leidig et al 2014, Zabouri & Haverkamp 2013) (Fig.3C). The lack of mature rod and cone synapses in $Ca_v1.4$ KO mice at any time during postnatal development suggests that $Ca_v1.4$ is essential for photoreceptor synaptogenesis (Liu et al 2013b, Zabouri & Haverkamp 2013).

$Ca_v1.4$ could mediate Ca^{2+}-dependent processes, such as neurotransmitter release, which are required for rod and cone synapse formation. An alternative, but not mutually exclusive, possibility is that $Ca_v1.4$ could interact with key scaffolding and other synapse-associated proteins needed to initiate synapse assembly (Fig.3B). To distinguish between these possibilities, our group analyzed rod synapses of mice expressing a non-conducting mutant form of $Ca_v1.4$ (G369i). Unlike in $Ca_v1.4$ KO mice, ribbons and pre- and post-synaptic proteins are present in rods of G369i knock-in mice. Rod bipolar and horizontal cell neurites appose mature spherules in the OPL and ectopically in the ONL, but fail to invaginate into the spherules (Fig.3C) (Maddox et al 2020). Although the cone phenotype of the G369i mice is still under investigation, these results suggest that while dispensable for the molecular organization of rod synapses, $Ca_v1.4$ Ca^{2+} influx is required for their structural maturity and localization in the OPL.

3.1.2. $Ca_v1.3$

A number of studies have reported the expression of *Cacna1d,* which encodes $Ca_v1.3$, in photoreceptors of mouse retina by *in situ* hybridization (Xiao et al 2007) and antibody labelling (Kersten et al). However, scRNA-seq analyses suggest very low representation of *Cacna1d* compared to *Cacna1f* in rods and cones in mouse retina (Macosko et al 2015) (Fig.2A). In addition, $Ca_v1.3$ KO mice exhibit relatively mild retinal phenotypes compared to those of $Ca_v1.4$ KO mice including no significant alterations in ERG b-waves (Busquet et al 2010, Wu et al 2007) but see (Shi et al 2017)). Abnormal ribbon morphology and a reduced density of ribbon synapses was found in the OPL of $Ca_v1.3$ KO retina (Busquet et al 2010, Shi et al 2017), which could result from loss of expression of $Ca_v1.3$ in cell-types other than photoreceptors. For example, *Cacna1d* is expressed in horizontal cells (Fig.2A) which are required for optimal photoreceptor synapse development (Soto et al 2013). Taken together, the current evidence favors $Ca_v1.4$ as the major Ca_v1 subtype that is expressed in rods and cones and which is indispensable for photoreceptor synapse assembly and transmission in the mouse retina.

3.2.1 Auxiliary β_2 and $\alpha_2\delta$-4 subunits are required for rod and cone synaptic transmission

Of all the β and $\alpha_2\delta$ subtypes, β_2 and $\alpha_2\delta$-4 are most prominently expressed among rods and cones (Fig.2D), and likely to be involved in regulating $Ca_v1.4$ in these cells based on multiple lines of evidence. First, mice lacking expression of β_2 or $\alpha_2\delta$-4 exhibit ERG defects indicative of weakened rod and cone transmission (Ball et al 2002, Katiyar et al 2015, Kerov et al 2018, Wang et al 2017). Second, levels of $Ca_v1.4$ protein in the OPL are severely reduced in β_2 KO and $\alpha_2\delta$-4 KO mice (Ball et al 2002, Katiyar et

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al 2015, Kerov et al 2018, Wang et al 2017), consistent with a role for these auxiliary subunits in trafficking Ca_v channels to the cell-surface (Buraei & Yang 2013, Dolphin 2013). Third, β_2 and $\alpha_2\delta$-4 interact with $Ca_v1.4$ in mouse retina based on co-immunoprecipitation and proximity ligation assays (Lee et al 2015).

As is the case in $Ca_v1.4$ KO mice, rod spherules of β_2 KO (Ball et al 2002, Katiyar et al 2015) and $\alpha_2\delta$-4 KO mice (Fig.3C) (Kerov et al 2018, Wang et al 2017) lack ribbons and do not form synaptic specializations with bipolar and horizontal cells. This result is not surprising given the reduced density of $Ca_v1.4$ protein in the OPL of both KO mouse strains (Ball et al 2002, Katiyar et al 2015, Kerov et al 2018, Wang et al 2017). Curiously, synaptic ribbons are spared in cone pedicles of β_2 KO and $\alpha_2\delta$-4 KO mice (Fig.3C) (Katiyar et al 2015, Kerov et al 2018, Wang et al 2017). Perhaps the expression of other β and $\alpha_2\delta$ subtypes in cones, albeit low (Fig.2D), is sufficient to traffic enough $Ca_v1.4$ to support ribbon formation in pedicles of β_2 KO and $\alpha_2\delta$-4 KO mice.

$\alpha_2\delta$-4 has been implicated in the functions of ELFN1, a cell-adhesion molecule that is expressed in rods (Cao et al 2015). It has been proposed that *cis* interactions between $\alpha_2\delta$-4 and ELFN1 in rods enable a *trans* interaction of ELFN1 and mGluR6 (Fig.3B) needed to organize postsynaptic signaling complexes in, and enable synaptic contacts with, rod bipolar cells (Wang et al 2017). In support of this possibility, $\alpha_2\delta$-4 co-immunoprecipitates with ELFN1 when co-transfected in HEK293 cells, and this is disrupted by deletion of the distal part of the ELFN1 ectodomain. However, the isolated ELFN1 ectodomain did not interact with $\alpha_2\delta$-4 in pull-down assays, and the prediction that $\alpha_2\delta$-4 lacking the putative ELFN1 binding sequence should rescue rod synaptogenesis in $\alpha_2\delta$-4 KO mice could not be tested since the mutant $\alpha_2\delta$-4 protein could not be expressed in rods of these mice (Wang et al 2017). Given previous findings that co-immunoprecipitation of $\alpha_2\delta$ proteins in heterologous expression systems can be highly non-specific (Brockhaus et al 2018), and may not accurately reflect protein interactions of $\alpha_2\delta$ at the cell surface (Lana et al 2016), the significance of the interaction of $\alpha_2\delta$-4 and ELFN1 for rod to rod-bipolar connectivity warrants further study.

Despite the normalcy of cone ribbons in $\alpha_2\delta$-4 KO mice, serial block face scanning electron microscopy revealed that cone pedicles lack triadic structure in these mice, most likely due to a failure of postsynaptic horizontal and bipolar neurites to invaginate (Kerov et al 2018)(Fig.3C). Whether resulting from abnormal cone synapse formation and/or maintenance, these structural abnormalities could contribute to the severe defects in cone transmission in $\alpha_2\delta$-4 KO mice (Kerov et al 2018, Wang et al 2017) as well as in humans affected by mutations in the gene encoding $\alpha_2\delta$-4 (*CACNA2D4*) (Ba-Abbad et al 2015, Wycisk et al 2006b).

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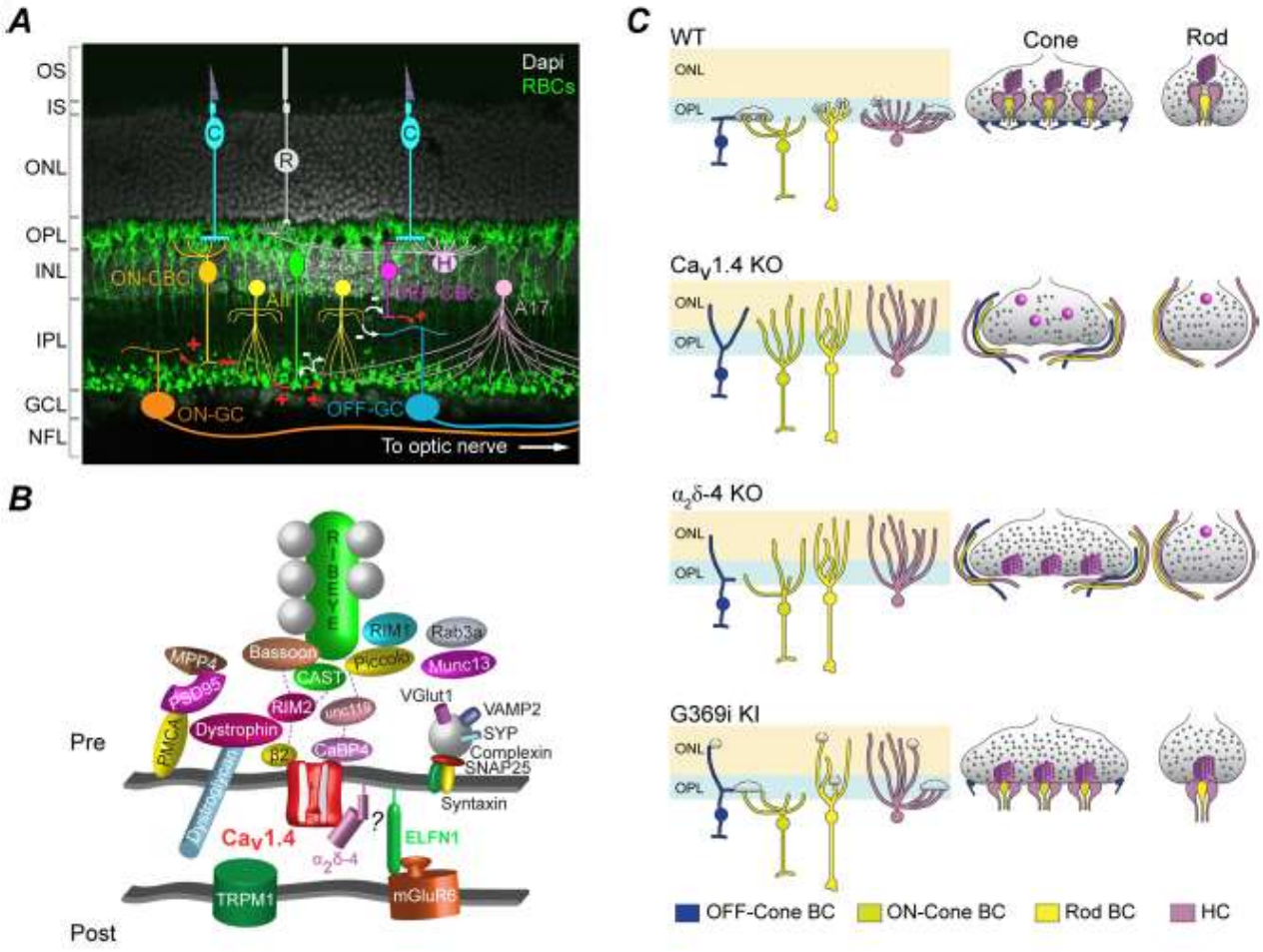

Figure 3. $Ca_v1.4$ channels at photoreceptor synapses. *A,* Cross-section of mouse retina labeled with Dapi and antibodies against protein kinase C to mark nuclei and rod bipolar cells (RBCs), respectively. Overlaid is a schematic showing retinal cell-types and their patterns of connectivity. Outer segment (OS), inner segment (IS), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layers (GCL), nerve fiber layer (NFL), rod (R) and cone (C) photoreceptors, cone bipolar cells (CBC), retinal ganglion cells (GC), horizontal cells (H), and RBCs (green). '+' and '-' indicate excitatory and inhibitory inputs, respectively. Zig-zag line represents gap junction connections. *B*, Schematic of a rod synapse showing relationships of $Ca_v1.4$ with synapse-associated proteins. Dotted lines indicate putative direct or indirect interactions of $Ca_v1.4$ with other synaptic proteins. *C*, Schematic illustrating morphology of WT photoreceptor synapses and defects reported in $Ca_v1.4$ KO, $\alpha_2\delta$–4 KO, and G369i knock-in mouse retina. Modified from (Maddox et al 2020).

3.3 Bipolar cells

Rod (ON) bipolar cells and cone (ON and OFF) bipolar cells receive inputs from rods and cones, respectively, and form glutamatergic ribbon synapses in the inner plexiform layer (IPL) with various partners (Fig.3A). Cone bipolar cells are functionally heterogeneous and release glutamate directly onto the dendrites of RGCs whereas rod bipolar cells form synapses with AII amacrine cells that innervate

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distinct types of cone bipolar cells and RGCs (Euler et al 2014) (Fig.3A). As is the case at photoreceptor synapses, Ca_v1 is the primary Ca_v subtype that regulates transmission at bipolar ribbon synapses. Although their physiological significance is not entirely clear, Ca_v2 and Ca_v3 subtypes are also present to varying degrees in the different classes of cone bipolar cells (Fig.2A,B).

3.3.1. Ca_v1 channels regulate bipolar cell synaptic transmission

Dihydropyridine analogs were used to show that Ca_v1 channels mediate presynaptic I_{Ca} and glutamate release from retinal bipolar cells in rats (Hartveit 1999, Pan 2000, Pan 2001, Protti & Llano 1998) and mice (Satoh et al 1998). In mouse retina, $Ca_v1.4$ and $Ca_v1.3$ are the major Ca_v1 subtypes in most rod and cone bipolar cell-types (Macosko et al 2015) (Fig.2A,B). The compensating effect of either Ca_v1 subtype could explain why depolarization-evoked Ca^{2+} signals are not diminished in the IPL of $Ca_v1.4$ KO mice (Mansergh et al 2005), and that $Ca_v1.3$ KO mice do not show signs of disrupted bipolar cell transmission (Busquet et al 2010).

3.3.2. Ca_v2 and Ca_v3 channels are also expressed in bipolar cells

Based on their unique biophysical properties, Ca_v3 channels have also been detected in electrophysiological recordings of various bipolar cell-types (Hartveit 1999, Pan 2000, Pan 2001, Singer & Diamond 2003). mRNAs corresponding to $Ca_v3.1$ and $Ca_v3.2$ are particularly well-represented in some classes of cone bipolar cells in mouse retina (Macosko et al 2015, Shekhar et al 2016) (Fig.2A,B). In both cone and rod bipolar cells, the transient Ca^{2+} current ($I_{trans,Ca}$) mediated by Ca_v3 is ~4 times larger than that of the Ca_v1-mediated sustained component and contributes significantly to synaptic glutamate release when Ca_v3 inactivation is removed by negative voltages (Cui et al 2012, Pan et al 2001, Singer & Diamond 2003). Although Ca_v3 channels will generally be inactivated at the resting voltage of bipolar cells (~ -40 to -50 mV) (Euler & Masland 2000), bipolar cell terminals receive significant inhibitory feedback from amacrine cells (Eggers & Lukasiewicz 2011). Hyperpolarization resulting from such feedback could relieve inactivation of Ca_v3 channels, thus priming Ca_v3 channels for opening in ways that could augment glutamate release at bipolar synapses.

At first glance, the near complete blockade of the sustained component of I_{Ca} in bipolar cells by Ca_v1 blockers (Hartveit 1999, Pan 2000, Pan 2001, Protti & Llano 1998, Satoh et al 1998) suggests that Ca_v2 channels contribute little to I_{Ca} in these cells. However, *Cacna1a* and *Cacna1b,* which encode $Ca_v2.1$ and $Ca_v2.2$, respectively, are moderately expressed in mouse ON and OFF cone bipolar cells (Fig.2B). Compared to Ca_v1 channels, Ca_v2 channels undergo robust modulation by heterotrimeric G-proteins and Ca^{2+} which underlies short-term synaptic plasticity at non-retinal synapses (Dolphin & Lee 2020). The use of specific fluorescent reporter lines for bipolar cells (Lu et al 2013) could aid in resolving whether Ca_v2 channels similarly contribute to synaptic transmission in ways that could be used to distinguish between cone bipolar cell types.

3.4 Horizontal cells express Ca_v1, Ca_v2, and Ca_v3 channels

Horizontal cells are interneurons that typically respond with graded changes in membrane potential in response to glutamatergic input from photoreceptors (Baylor et al 1971, Kolb 1995b). The primary function of horizontal cells is to mediate lateral inhibition of photoreceptor output, which is critical for shaping the receptive field properties of bipolar cells needed for contrast sensitivity and color discrimination. In all species, there is one horizontal cell-type that forms synaptic contacts with cones and rods via somatodendritic neurites and a laterally extending axon, respectively (Kolb 1995a) (Fig.3A). Each horizontal cell receives synaptic input from numerous rods and cones, and produces a feedback (surround) inhibition that suppresses photoreceptor output or a feedforward inhibition to bipolar cells (Diamond

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2017). The mechanism underlying feedback regulation involves inhibition of photoreceptor Ca_v1 channels and may involve GABA release or proton efflux from horizontal cells, or an ephaptic signal caused by changes in the extracellular resistance within the synaptic cleft (Kramer & Davenport 2015).

Pharmacological evidence supports the existence of Ca_v1, Ca_v2, and Ca_v3 channels in horizontal cells (Feigenspan et al 2020, Liu et al 2013a, Pfeiffer-Linn & Lasater 1996, Picaud et al 1998, Schubert et al 2006, Ueda et al 1992). Ca_v1 channels support spontaneous action potentials in dissociated goldfish horizontal cells (Country et al 2019), but whether this occurs in the intact mammalian retina is less clear. In rat retina, blockade of Ca_v2 channels ($Ca_v2.1$ and $Ca_v2.2$) but not Ca_v1 channels increases Ca^{2+} signals in rods presumably by relieving inhibitory feedback from horizontal cells (Liu et al 2013a). Consistent with prominent levels of $Ca_v2.1$ and $Ca_v2.2$ mRNA (*Cacna1a* and *Cacna1b*, respectively) in mouse horizontal cells (Fig.2A), the corresponding antibodies labeled puncta at the tips of horizontal cell neurites (Liu et al 2013a). $Ca_v2.1$ and $Ca_v2.2$ have opposing actions in regulating release at GABA-ergic synapses (Yamamoto & Kobayashi 2018). Thus, the two Ca_v2 subtypes could differentially regulate GABA release from horizontal cells in ways that fine-tune feedback regulation of photoreceptors over a broad range of light levels.

Although Ca_v3 channels were undetected in initial studies (Liu et al 2013a, Schubert et al 2006), $Ca_v3.2$ mRNA is particularly high in mouse horizontal cells (Fig.2A). ML218, a specific blocker of Ca_v3 channels (Fig.1B), hyperpolarized the membrane and inhibited light responses of adult mouse horizontal cells in retinal slice preparations (Feigenspan et al 2020). Ca_v3-mediated currents undergo a ~50% decline in horizontal cells during the period before and after eye-opening in mice (P12-P13) (Feigenspan et al 2020). Based on studies of $Ca_v1.4$ KO and transgenic coneless mice, it has been proposed that pre-visual signaling between cones and horizontal cells drives the maturation of the latter's dendritic branching and terminal cluster formation (Raven et al 2008, Reese et al 2005). A high density of Ca_v3 channels could facilitate these morphological changes in horizontal cells, as pathological upregulation of Ca_v3 channels is linked to alterations in dendritic branching in some neurons (Niesen & Ge 1999). In the mature retina, Ca_v3 channels could contribute to pathological forms of activity in horizontal cells. For example, retinal remodeling in rd1 mice that occurs as a consequence of photoreceptor degeneration causes spontaneous oscillatory activity in horizontal cells (Haq et al 2014). Considering that they suppress rhythmic oscillations associated with seizures (Cheong & Shin 2014), Ca_v3 blockers may prove useful therapeutically in blunting abnormal retinal activity that could underlie visual symptoms of photoreceptor degeneration in retitinitis pigmentosa (Marc et al 2007).

3.5 Amacrine cells

Of the more than 60 types of amacrine cells that have been identified (Yan et al 2020), most modify information flow from bipolar cells to RGCs via electrical coupling and/or glycinergic or GABA-ergic synapses with bipolar cells, RGCs, and other amacrine cells. These diverse connections negotiate complex inhibitory feedback loops that are crucial for inner retinal computations such as those underlying direction selectivity (Wei 2018). Amacrine cells are broadly characterized according to dendritic morphology and lamination within the IPL. Narrow-field amacrine cells have compact dendritic arbors (<125 μm) and stratify in multiple layers of the IPL whereas wide-field amacrine cells have expansive dendritic arbors (>400 μm) and laminate mainly within one or a few layers of the IPL (Kolb 1995c). The neurites of amacrine cells are specialized for postsynaptic responses and/or presynaptic neurotransmitter release, and often engage in lateral, serial, and reciprocal synapses some of which involve Ca_v channels.

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3.5.1 AII amacrine cells utilize Ca_v1 channels for sustained glycinergic transmission in scotopic vision

Narrow-field AII amacrine cells bifurcate the signal received from rod bipolar cells into ON and OFF pathways via gap junctions on their distal, arboreal dendrites with ON cone bipolar cells, and glycinergic synapses formed between their proximal, lobular appendages with OFF cone bipolar cells or OFF RGCs (Famiglietti & Kolb 1975, Mills & Massey 1991) (Fig.3A). In mouse AII cells, Ca_v currents activate at relatively negative voltages and are sensitive to dihydropyridine antagonists (Balakrishnan et al 2015, Habermann et al 2003). These features are characteristic of $Ca_v1.3$, which was detected in these cells by RT-PCR and scRNA-seq (Habermann et al 2003, Yan et al 2020) (Fig.2C). Ca_v1-mediated Ca^{2+} signals were found in the lobular appendages of AII cells (Habermann et al 2003), and associated with sustained exocytosis that is more typical of ribbon-type synapses than of synapses with purely phasic release properties (Balakrishnan et al 2015). Depolarization-dependent Ca^{2+} signals were rarely observed in the arboreal dendrites of AII cells (Habermann et al 2003), suggesting that $Ca_v1.3$ may be trafficked specifically to glycinergic release sites.

3.5.2. Interplay between big K^+ (BK) channels and Ca_v1 channels in A17 amacrine cells (ACs)

Wide-field A17 cells form hundreds of varicosities along their dendrites, which participate in complex inhibitory microcircuits acting in parallel through reciprocal GABAergic synapses with a rod bipolar terminal (Grimes et al 2010, Kolb 1995c). Ca_v1 seems to be the predominant Ca_v channel expressed in mouse A17 ACs (Chavez et al 2006, Grimes et al 2009), but its role in neurotransmitter release is more complex than in AII ACs. Despite the prominent contribution of Ca_v1 to depolarization-evoked I_{Ca} recorded in the soma and Ca^{2+} signals recorded in the varicosities of A17 ACs (Grimes et al 2009), synaptically evoked GABA release from A17 ACs is mediated by Ca^{2+}-permeable AMPA receptors (CP-AMPARs) rather than Ca_v1 channels (Chavez et al 2006). BK Ca^{2+} activated K^+ channels, which are functionally coupled to Ca_v1 within A17 AC varicosities, suppress synaptic depolarizations and limit the recruitment of Ca_v1 channels (Grimes et al 2009). Under low light levels, BK-mediated suppression of Ca_v1 activation would restrict the amplitude and spread of synaptic depolarizations, thus enhancing feedforward excitatory transmission by rod bipolar cells. Stronger synaptic stimulation of A17 AC varicosities with increasing light intensities is expected to inactivate BK channels, which would enable Ca_v1-dependent contributions to GABA release, thereby expanding the boundaries of surround feedback inhibition (Grimes et al 2009). $Ca_v1.3$ was found to co-immunoprecipitate with BK channels from rat retinal lysates (Grimes et al 2015), suggesting close proximity of these channels within A17 ACs and/or other retinal cell-types. $Ca_v1.3$ interacts with a variety of PDZ-domain containing scaffolding proteins that regulate the clustering of these channels within discrete microdomains (Gregory et al 2011, Jenkins et al 2010, Olson et al 2005). Such protein interactions could ensure compartmentalized signaling by macromolecular complexes of BK, $Ca_v1.3$, and potentially other regulatory molecules within A17 varicosities.

While the profile of A17 cells could not be definitively assigned based on scRNA-seq analysis, most GABA-ergic ACs are characterized by the expression of multiple Ca_v subtypes including $Ca_v1.3$ and Ca_v3 channels (Fig.2C) (Yan et al 2020). Although Ca_v3 channels are often involved in forms of dendritic integration that would run counter to the functional compartmentalization of A17 dendrites (Grimes et al 2015), these channels could play presynaptic roles. For example, at some cortical synapses Ca_v3 channels promote glutamate release but only under conditions that inhibit hyperpolarization activated (HCN) channels that mediate a depolarizing current (Huang et al 2011). HCN channels, which are expressed in amacrine cells (Koizumi et al 2004), could keep Ca_v3 activity in check, much like BK does for $Ca_v1.3$. It is interesting to speculate that BK/$Ca_v1.3$ and HCN/Ca_v3 complexes could be targeted to distinct

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varicosities, thus diversifying reciprocal outputs and augmenting the parallel processing capabilities of each A17 cell.

3.5.2 Ca_v2 channels regulate neurotransmitter release from starburst amacrine cells

Starburst amacrine cells (SACs) are wide-field GABA-ergic amacrine cells and are the only interneurons of the retina that also produce acetylcholine as a neurotransmitter (Brecha et al 1988). SACs transform information received from bipolar inputs into directionally-selective inhibitory inputs to specific subtypes of retinal ganglion cells (Wei 2018). The mechanisms by which SACs compute direction selectivity involve Ca^{2+} signals in the distal dendrites of SACs, where synaptic outputs to RGCs are localized, and are favored by stimulus movement away from the soma (*i.e.*, centrifugal) (Euler et al 2002, Lee & Zhou 2006). Pharmacological evidence suggests that $Ca_v2.1$ and $Ca_v2.2$, but not Ca_v1 or Ca_v3, mediate Ca_v currents in mouse SACs (Kaneda et al 2007) and rabbit (Lee et al 2010), which is generally supported by scRNA-seq (Fig.2C)(Yan et al 2020). Based on the actions of specific Ca_v2 blockers, the release of GABA and acetylcholine from SACs is regulated primarily by $Ca_v2.1$ and $Ca_v2.2$, respectively (Lee et al 2010). The matching of particular Ca_v2 subtypes to the type of neurotransmitter released could result from differences in the proximity of Ca_v2 subtypes to the corresponding vesicle release sites and/or functional coupling to distinct presynaptic proteins that could be involved in exocytosis of GABA and acetylcholine (Dolphin & Lee 2020, Liu et al 2018).

4.0. Modulation of Ca_v channels and retinal function

Ca_v channels are subject to diverse forms of modulation that can alter neuronal excitability and cause short- and long-lasting changes in synaptic strength (Dolphin & Lee 2020). The underlying mechanisms are complex and can involve protein interactions with various Ca_v subunits, as well as alternative splicing and post-translational modifications.

4.1. Retinal Ca_v1 channels exhibit limited Ca^{2+}-dependent inactivation (CDI)

CDI is a negative feedback regulation by incoming Ca^{2+} ions that is characteristic of Ca_v1 and Ca_v2 channels. The mechanism involves calmodulin (CaM), which is pre-associated with a site (IQ-domain) in the CTD of these channels, and is evident as faster decay of I_{Ca} compared to Ba^{2+} currents (I_{Ba}) (Fig.4A). Ca^{2+} binding to CaM initiates conformational changes in the channel protein that favor inactivation; Ba^{2+} binds poorly to CaM and so does not support CDI (Ben-Johny & Yue 2014). While CDI generally causes Ca_v1 channels to inactivate within milliseconds, Ca_v1 channels in the retina can inactivate with a time course on the order of seconds (Barnes & Hille 1989, Corey et al 1984, von Gersdorff & Matthews 1996). Slow CDI of retinal Ca_v1 channels is expected to support sustained neurotransmitter release that is characteristic of ribbon synapses of photoreceptors and bipolar cells (Pangrsic et al 2018) as well as glycinergic synapses formed between AII amacrine cells OFF cone bipolar cells (Balakrishnan et al 2015, Habermann et al 2003).

Why do retinal Ca_v1 channels undergo little CDI? For $Ca_v1.4$, the answer lies within a C-terminal modulatory domain (CTM) that competes with and/or modulates CaM binding to the channel. Via an intramolecular interaction with a proximal region in the CTD, the CTM suppresses CDI and inhibits the voltage dependence of activation (Singh et al 2006, Wahl-Schott et al 2006). In HEK293T cells transfected with $Ca_v1.4$ containing the CTM, I_{Ca} hardly inactivates during a 1-s depolarization, similar to I_{Ba} (Fig.4B).

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However, alternative splicing in the CTD can disrupt the actions of the CTM (Tan et al 2012, Williams et al 2018). One $Ca_v1.4$ splice variant expressed in human retina lacks exon 47, which corresponds to a portion of the CTM ($Ca_v1.4\Delta ex47$) (Haeseleer et al 2016). $Ca_v1.4\Delta ex47$ binds to CaM, and exhibits CDI and a hyperpolarizing shift in voltage-dependent activation compared to $Ca_v1.4$ channels containing exon 47 ($Ca_v1.4$+ex47) (Williams et al 2018) (Fig.4B,C). Although also present in $Ca_v1.3$, the CTM does not nullify CDI of these channels in transfected cells (Singh et al 2008, Singh et al 2006) (Fig.4A). Additional mechanisms could prolong opening of retinal $Ca_v1.3$ channels such as RNA editing of the IQ-domain (Huang et al 2012) and interactions with proteins known to suppress CDI, as described below.

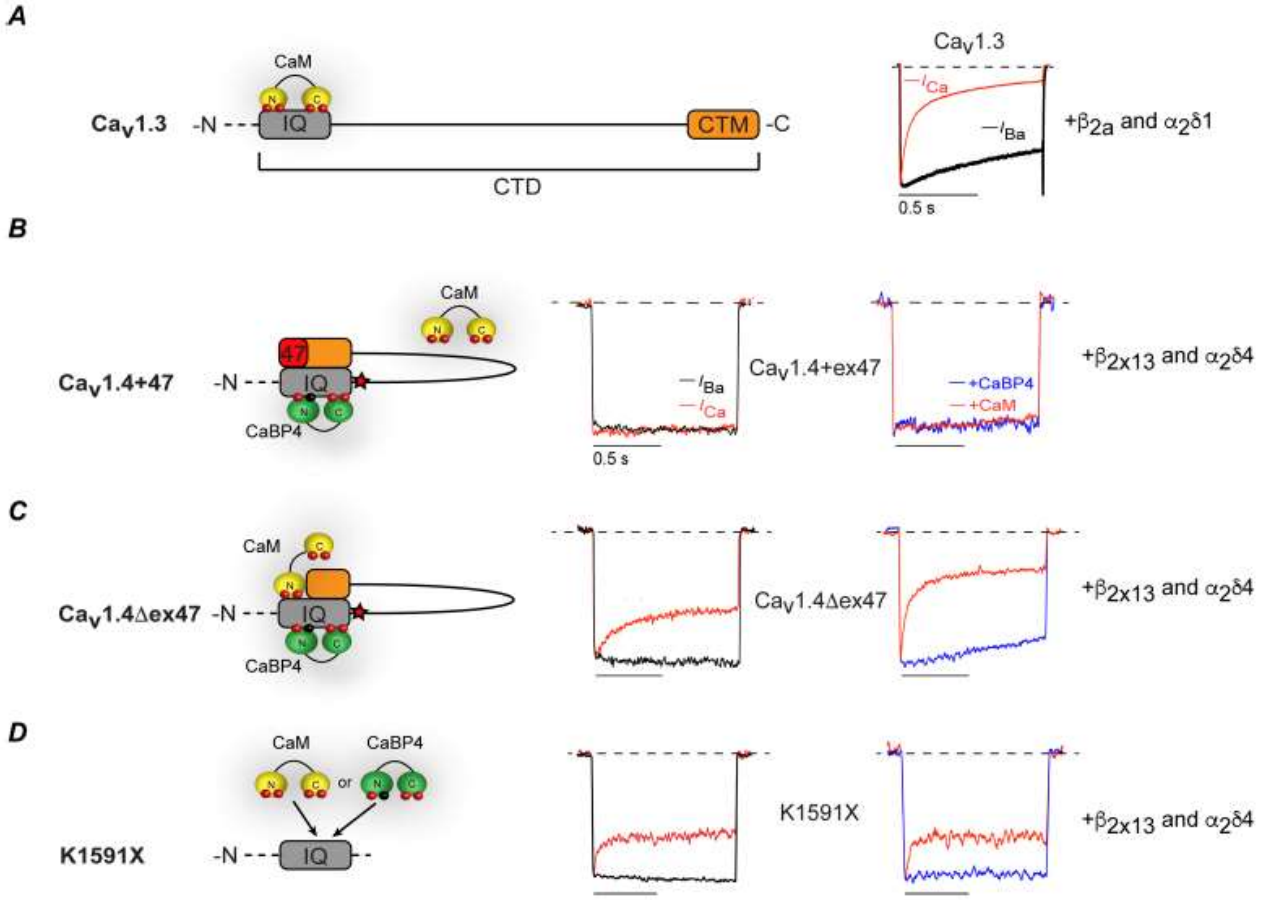

Figure 4. **CaM and CaBP4 modulation of Ca_v1 channels.** Effects of CaM or CaBP4 binding to $Ca_v1.3$ (*A*) or $Ca_v1.4$ (*B-D*) on Ca^{2+}-dependent inactivation (CDI). Left panels show schematics of C-terminal domain (CTD) of each channel, containing IQ-domain and C-terminal modulatory domain (CTM). Right panels show normalized current traces in HEK293T cells expressing $Ca_v1.3$ (A) or $Ca_v1.4$ (B) with the indicated auxiliary subunits. In *B,C*, star indicates site of K1591. *A*, For $Ca_v1.3$, CaM binding to the IQ-domain (IQ) causes CDI, which is evident as faster decay of I_{Ca} compared to I_{Ba} (left traces). *B*, $Ca_v1.4$ channels containing exon 47 ($Ca_v1.4$+ex47) undergo little CDI (I_{Ca} is similar to I_{Ba}, left traces) due to the action of the CTM in displacing CaM from the IQ-domain. CaBP4 binding to the IQ-domain has no effect on CDI in that I_{Ca} for $Ca_v1.4$+ex47 alone (red) is similar to that when co-transfected with CaBP4 (blue, right traces). *C*, CaM binding causes $Ca_v1.4$ channels lacking exon 47 ($Ca_v1.4\Delta ex47$) to undergo strong CDI (left traces). Due to CaBP4 binding, I_{Ca} decays more slowly in cells co-transfected with CaBP4

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compared to cells transfected with $Ca_v1.4\Delta ex47$ (Right traces). *D*, The K1591X mutation eliminates the CTM, which enables CaM binding and CDI (left traces). However, CaBP4 binding prevents CDI, resulting in little inactivation of I_{Ca} (right traces). Modified from (Haeseleer et al 2016, Williams et al 2018, Williams et al 2020).

4.2. CaBPs: modulators of Ca_v channels in a subset of retinal cell-types

CaBPs are a family of CaM-like proteins that are expressed in the brain and retina, and interact with and modulate Ca_v channels. For most Ca_v1 channels, CaBPs compete with CaM for binding to the IQ-domain and thereby suppress CDI (Hardie & Lee 2016). CaBP4 is abundantly localized in photoreceptor synaptic terminals where it associates with $Ca_v1.4$ (Haeseleer et al 2004, Lee et al 2015). While it interacts with the IQ-domain, CaBP4 does not suppress CDI (Fig.4B), but causes a hyperpolarizing shift in activation voltages in $Ca_v1.4$+ex47 (Haeseleer et al 2004, Shaltiel et al 2012). In the absence of a functional CTM, $Ca_v1.4\Delta ex47$ undergoes significantly less CDI when bound to CaBP4 (Fig.4C)(Haeseleer et al 2016). These effects of CaBP4 on activation of $Ca_v1.4$+ex47 and inactivation of $Ca_v1.4\Delta ex47$ likely support prolonged glutamate release at the relatively negative membrane potential of photoreceptors in darkness. CaBP4 KO mice exhibit impairments in rod and cone synapse structure, and greatly diminished ERG b-waves, which are consistent with loss-of function of $Ca_v1.4$ (Haeseleer et al 2004, Liu et al 2013b, Maeda et al 2005).

While CaBP4 is the only CaBP expressed in photoreceptors, additional CaBPs are expressed in other retinal cell-types. CaBP1 is expressed in OFF cone bipolar and amacrine cells, while CaBP2 is expressed in ON cone bipolar cells and a population of OFF cone bipolar cells that do not express CaBP1. Compared to wild-type mice, excitatory synaptic currents are decreased in ON RGCs of CaBP2 KO mice and increased in OFF RGCs of CaBP1 KO mice (Sinha et al 2016). CaBP5 is expressed in rod and cone (ON and OFF) bipolar cells in mouse and primate retina. In CaBP5 KO retina, ON RGCs exhibit ~50% reduction in sensitivity to dim light flashes, which could result from impaired rod bipolar-AII amacrine cell transmission (Rieke et al 2008). Although CaBP1, CaBP2 and CaBP5 all suppress CDI of Ca_v1 channels in heterologous expression systems (Rieke et al 2008, Schrauwen et al 2012, Zhou et al 2004), the direct actions of these CaBPs on Ca_v channel function in bipolar cells remain to be determined. CaBPs can interact with targets other than Ca_v channels, which in addition to impaired regulation of Ca_v Ca^{2+} signals, could contribute to the retinal phenotypes in CaBP KO mice.

4.3 Proton-mediated inhibition of Ca_v1 channels in cones

Because the lumen of synaptic vesicles is acidic, exocytosis of neurotransmitter during heightened periods of neuronal activity can transiently acidify the synaptic cleft. As shown for Ca_v1 channels in other cell-types (Klockner & Isenberg 1994), lowering of pH potently inhibits Ca_v1 channels by causing a positive shift in voltage-dependent activation in rods and cones (Barnes & Bui 1991, Barnes et al 1993, DeVries 2001) as well as bipolar cells (Palmer et al 2003). pH-dependent inhibition of Ca_v1 manifests as a transient component of the $I_{trans,Ca}$ during a step depolarization which recovers with a similar time course as postsynaptic responses and is blunted by maneuvers that prevent presynaptic glutamate release (*i.e.*, substitution of extracellular Ca^{2+} with Ba^{2+}). Along with other evidence, these results suggest that $I_{trans,Ca}$ results from presynaptically released protons (DeVries 2001). *S*imultaneous patch-clamp recordings of Ca_v1 currents and membrane capacitance changes in isolated goldfish bipolar cell terminals

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revealed that pH-dependent inhibition of Ca_v1 reduces exocytosis in ways that may prevent short-term depression of vesicular release (Palmer et al 2003).

As a proposed mechanism whereby horizontal cells produce lateral inhibition in the retina (Kramer & Davenport 2015), feedback inhibition of cone Ca_v1 channels could originate from protons released by various sources in horizontal cells including epithelial Na^+ channels (Vessey et al 2005), vacuolar H^+ pump ATPases (Jouhou et al 2007), and/or Na^+/H^+ exchangers (Grove et al 2019, Warren et al 2016). Single channel recordings of heterologously expressed Ca_v1 channels showed that proton block involves pore-lining glutamate residues that also mediate Ca^{2+} selectivity of these channels (Fig.1A). Proton binding to carboxylate side chains contributed by these residues is thought to compete with Ca^{2+}, thus favoring a low-conductance state and decreased channel open probability (Chen et al 1996). These glutamate residues, and therefore the ability to be inhibited by protons, is conserved in other Ca_v channels that are expressed in bipolar and horizontal cells (Fig.2A,B). Thus, the accumulation of protons in the synaptic cleft could have complex actions on Ca_v channels in the membrane of each cell-type contributing to this triadic synapse.

5.0 Dysregulation of $Ca_v1.4$ and vision disorders

Studies of *CACNA1F* mutations involved in retinal disease have yielded important insights on the structure/function relationships of $Ca_v1.4$. *CACNA1F* is targeted by numerous (>140) mutations that cause vision disorders which, besides CSNB2, include X-linked cone-rod dystrophy (CORDX3) (Jalkanen et al 2006), and Åland eye disease (Jalkanen et al 2007, Vincent et al 2011). Clinical phenotypes linked to *CACNA1F* mutations are heterogeneous and include moderate to severe night blindness, low visual acuity, myopia, nystagmus, and/or strabismus (Hove et al 2016). The variability in these symptoms could result from a complex interplay of how the mutations affect the intrinsic properties of $Ca_v1.4$ and factors such as alternative splicing and protein interactions, which could modify the impact of the mutations on photoreceptor structure and function.

5.1. Mutations that cause retinal disease have diverse effects on $Ca_v1.4$ function

Most disease-causing mutations in *CACNA1F* are expected to cause a loss of channel function (*i.e.,* abolishing or reducing $Ca_v1.4$-mediated Ca^{2+} influx). For example, the mutation L1068P in the pore-forming S5-S6 linker of domain III causes a positive shift in the voltage-dependence of activation and accelerated voltage-dependent inactivation (Hoda et al 2005). By impairing the opening of $Ca_v1.4$ channels, L1068P could limit the levels of glutamate needed to silence ON bipolar cells in darkness, thereby decreasing the gain of the light response.

Some *CACNA1F* mutations cause a gain-of function in channel activity. The I745T mutation in the S6 helix of repeat II causes a major hyperpolarizing shift (~ -30 mV) in the half-maximal voltage of activation as well as slow inactivation (Hemara-Wahanui et al 2005). Males carrying the I745T mutation present with a severe form of CSNB2 characterized by congenital nystagmus, severe nonprogressive impairment of visual acuity, frequent hypermetropia, and in some cases, intellectual disability (Hope et al 2005). Insights into the mechanisms underlying these visual phenotypes have emerged from studies of I745T knock-in mice. In ERGs of these mice (Knoflach et al 2013, Liu et al 2013b, Regus-Leidig et al 2014), b-waves are detectable but strongly reduced, similar to the ERGs of humans bearing the analogous mutation (Hope et al 2005). The strong negative shift in activation voltages of I745T could cause channels to remain open despite light onset, thus limiting the dynamic range of photoreceptor responses.

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5.2 CaBP4 and alternative splicing as modifiers of *CACNA1F* mutations

Because of the complexity of the $Ca_V1.4$ interactome (Fig.3B), a variety of $Ca_V1.4$-interacting proteins could modify the impact of *CACNA1F* mutations on vision. For example, K1591X is a CSNB2 mutation resulting in a premature truncation of the CTD just downstream of the IQ-domain of $Ca_V1.4$ (Fig.4D). Predictably, the mutation results in strong CaM-driven CDI and a hyperpolarizing shift in the voltage-dependence of activation in transfected cells (Singh et al 2006, Williams et al 2018). However, CaBP4 is capable of competing with CaM and preventing CDI when co-expressed with K1591X mutant channels (Williams and Lee, unpublished; Fig.4D). Thus, the pathological consequences of K1591X in is likely to result primarily from Ca^{2+} influx at abnormally negative voltages, rather than increasing CDI.

Alternative splicing is also known to alter the consequences of disease-causing mutations in Ca_V-encoding genes. For example, I745T causes $Ca_V1.4\Delta ex47$ to activate at even more negative voltages and to deactivate with slower kinetics as compared to $Ca_V1.4+ex47$. Moreover, I745T causes a breakdown in the Ca^{2+} selectivity of $Ca_V1.4\Delta ex47$ but not in $Ca_V1.4+ex47$ (Williams et al 2020). Because $Ca_V1.4\Delta ex47$ is expressed in human but not in rodent retina (Haeseleer et al 2016), the I745T knock-in mouse strain (Knoflach et al 2013, Liu et al 2013b, Regus-Leidig et al 2014) might not reflect some of the pathological sequelae of the mutation in the context of $Ca_V1.4\Delta ex47$.

5.3. Do $Ca_V1.4$ channelopathies result from defects in photoreceptor synapse structure and/or function?

A conundrum arising from electrophysiological analysis of the *CACNA1F* mutations and studies of various mutant mouse strains is whether CSNB2 and related disorders might involve alterations in formation and/or maintenance of photoreceptor synapses during development rather than from aberrant function of $Ca_V1.4$ at mature synapses. For example, the CSNB2 mutation W1440X results in the deletion of the entire CTD and loss of $Ca_V1.4$ protein expression in *Xenopus* oocytes and HEK293 cells (Hoda et al 2005). Therefore, W1440X could lead to similar defects in rod and cone synapse formation as are characteristic of $Ca_V1.4$ KO mice (Liu et al 2013b, Regus-Leidig et al 2014, Zabouri & Haverkamp 2013). Similarly, defects in cone synapse structure associated with diminished levels of presynaptic $Ca_V1.4$ channels in $\alpha_2\delta$–4 KO mice (Kerov et al 2018) (Fig.3C) could contribute to stationary or progressive cone dysfunction in individuals with loss-of function mutations in *CACNA2D4* (Ba-Abbad et al 2015, Bacchi et al 2015, Wycisk et al 2006b).

Consistent with the role of CaBP4 in enhancing the activation of $Ca_V1.4$ (Haeseleer et al 2004, Haeseleer et al 2016), *CABP4* mutations cause CSNB2-like phenotypes (Bijveld et al 2013, Hove et al 2016). While a subset of photoreceptor synapses appear normal morphologically, synaptic ribbons are shorter and often localized ectopically with some sprouting of horizontal and bipolar cell neurites in the ONL (Haeseleer et al 2004, Liu et al 2013b, Maeda et al 2005). Similar abnormalities are seen in I745T knock-in mice (Knoflach et al 2015, Liu et al 2013b, Regus-Leidig et al 2014). Thus, either loss-of function or gain-of function in $Ca_V1.4$ may lead to destabilization of photoreceptor synapse structure. In this context, it is noteworthy that decreasing Ca^{2+} levels in photoreceptors with Ca^{2+} chelators or sustained light exposure can disrupt the integrity of synaptic ribbons (Regus-Leidig et al 2010, Spiwoks-Becker et al 2004) and presynaptic clustering $Ca_V1.4$ and RIM2 (Dembla et al 2020).

Summary points

1. $Ca_V1.3$ and $Ca_V1.4$ are the major Ca_V1 subtypes in the retina and play predominantly presynaptic roles. Their unique properties (*e.g.*, rapid activation at relatively negative

voltages and slow inactivation) are well-suited to supporting sustained neurotransmitter release at ribbon and non-ribbon synapses.

2. Ca_v2 channels are expressed primarily in the interneurons of the retina as well as RGCs, where they have been implicated in regulating the release of various neurotransmitters.
3. Ca_v3 channels are prominently expressed in all retinal cell-types except photoreceptors. The contributions of Ca_v3 channels are expected to be evident primarily under hyperpolarizing conditions that relieve their inactivation.
4. Alternative splicing and protein interactions diversify the intrinsic properties of Ca_v channels, as well as the impact of mutations that cause retinal disease.

Future issues:

1. Molecular mechanisms that regulate the localization and function of Ca_v channels in retinal cell-types are largely unknown and yet critical for our understanding of how these channels contribute to visual processing. The use of *in vivo* electroporation and viruses to express recombinant Ca_v channels in a cell-specific manner will help identify determinants within Ca_v channels that enable their trafficking to discrete microdomains and allow them to optimally control processes such as neurotransmitter release.
2. scRNA-seq has revealed a broad cellular distribution of the different Ca_v subtypes in the retina. Defining the retinal functions of Ca_v channels will require new tools such as mouse strains with conditional KO of Ca_v subtypes in specific retinal cell-types. In addition, anti-Ca_v antibodies with greater sensitivity and specificity than those that are currently available will enable methods such as SDS-digested freeze-fracture replica labelling electron microscopy to probe the nanoscale organization of Ca_v channels.
3. In general, the available $Ca_v1.4$ mutant mouse strains exhibit more severe retinal and visual phenotypes than individuals harboring mutations in genes encoding Ca_v subunits or $Ca_v1.4$-interacting proteins. Given the potential for species differences in gene expression patterns and alternative splicing events affecting $Ca_v1.4$, the use of stem-cell derived retinal cell-types or organoid culture systems could provide important insights into the pathophysiology of $Ca_v1.4$ channelopathies in humans, as well as new therapeutic advances.

Disclosure statement

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