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Calcium Channels in Retinal Function and Disease

Brittany Williams,[1] J. Wesley Maddox,[2] and Amy Lee[2]

[1]Department of Cell Biology & Physiology, Carolina Institute for Developmental Disabilities, and Neuroscience Center, University of North Carolina, Chapel Hill, North Carolina, USA

[2]Department of Neuroscience, University of Texas, Austin, Texas, USA; email: amy.lee1@austin.utexas.edu

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Abstract

Voltage-gated Ca^{2+} (Ca_v) channels play pivotal roles in regulating gene transcription, neuronal excitability, and neurotransmitter release. 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. In this article, 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.

1. INTRODUCTION

From the initial stages, where photoreceptors extract features of the visual scene, and through successive and parallel circuits involving retinal interneurons, voltage-gated Ca^{2+} (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, has opened many new avenues for research. In addition, the use of single-cell RNA-sequencing (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 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 are discussed in the context of a subset of retinal cell types. For complementary insights on this topic, the reader is referred to several excellent reviews (see Pangrsic et al. 2018, Van Hook et al. 2019).

2. MOLECULAR DIVERSITY OF CA_V CHANNELS

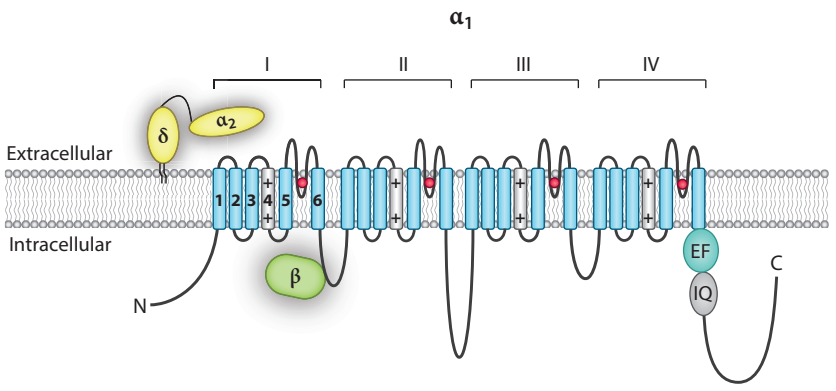
Ca_v channels were originally classified by their voltage range of activation, with Ca_v1 and Ca_v2 channels forming the high-voltage-activated channels and Ca_v3 channels forming the low-voltage-activated channels. Both types of Ca_v channels are composed of a pore-forming α_1 subunit, with Ca_v1 and Ca_v2 channels also possessing auxiliary β and $\alpha_2\delta$ subunits (**Figure 1*a***). The α_1 subunit of Ca_v channels contains four homologous repeats, each with six 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 (**Figure 1*a***). Ca_v channels were initially named according to the properties of the currents that 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 (**Figure 1*b***).

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 among subtypes of Ca_v1 (e.g., $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$) or Ca_v3 channels (e.g., $Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$) (**Figure 1*b***). 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 defined, at the molecular level, many known and some newly discovered retinal cell types. Using one of these scRNA-seq data sets (Macosko et al. 2015), we summarize the expression of Ca_v subunits in a subset of retinal cell types in **Figure 2**; we refer to this resource in subsequent sections.

2.1. Ca_v1 Channels

Ca_v1 (L-type) channels are distinguished from other Ca_v channels by their sensitivity to dihydropyridine-based drugs (**Figure 1*b***). 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 several retinal cell types such as photoreceptors and

a



b

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-agatoxinIVA, ω-conotoxin GVIA, SNX-482
CACNA1B	$Ca_v2.2$	α1B	N-type	
CACNA1E	$Ca_v2.3$	α1E	R-type	
CACNA1G	$Ca_v3.1$	α1G	T-type	ABT 639, Mibefradil, ML 218, Z 944, NNC 55-0396
CACNA1H	$Ca_v3.2$	α1H		L-Ascorbic acid, ABT 639, Mibefradil, ML 218, Z 944, NNC 55-0396, (2R/S)-6-PNG
CACNA1I	$Ca_v3.3$	α1I		Mibefradil, ML 218, Z 944, NNC 55-0396

Figure 1

Molecular composition and nomenclature of voltage-gated Ca^{2+} (Ca_v) channels. (*a*) The Ca_v α_1 subunit is composed of four homologous domains (I–IV), each containing six α-helical transmembrane-spanning segments (S1–S6). Positively charged residues (+) within S4 helices are indicated. The extracellular loop linking S5 and S6 forms the pore; the 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+}/calmodulin (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 versus L-type for long-lasting). Major classes of pharmacological modulators are indicated. Panel *a* adapted with permission from Haeseleer et al. (2016).

bipolar cells (**Figure 2*a*,*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; these properties include rapid activation at relatively negative voltages 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.

2.2. Ca_v2 Channels

ω-Agatoxin IVA, ω-conotoxin GVIA, and SNX-482 are highly selective blockers of $Ca_v2.1$ (P/Q-type), $Ca_v2.2$ (N-type), and $Ca_v2.3$ (R-type) channels, respectively (**Figure 1*b***). 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

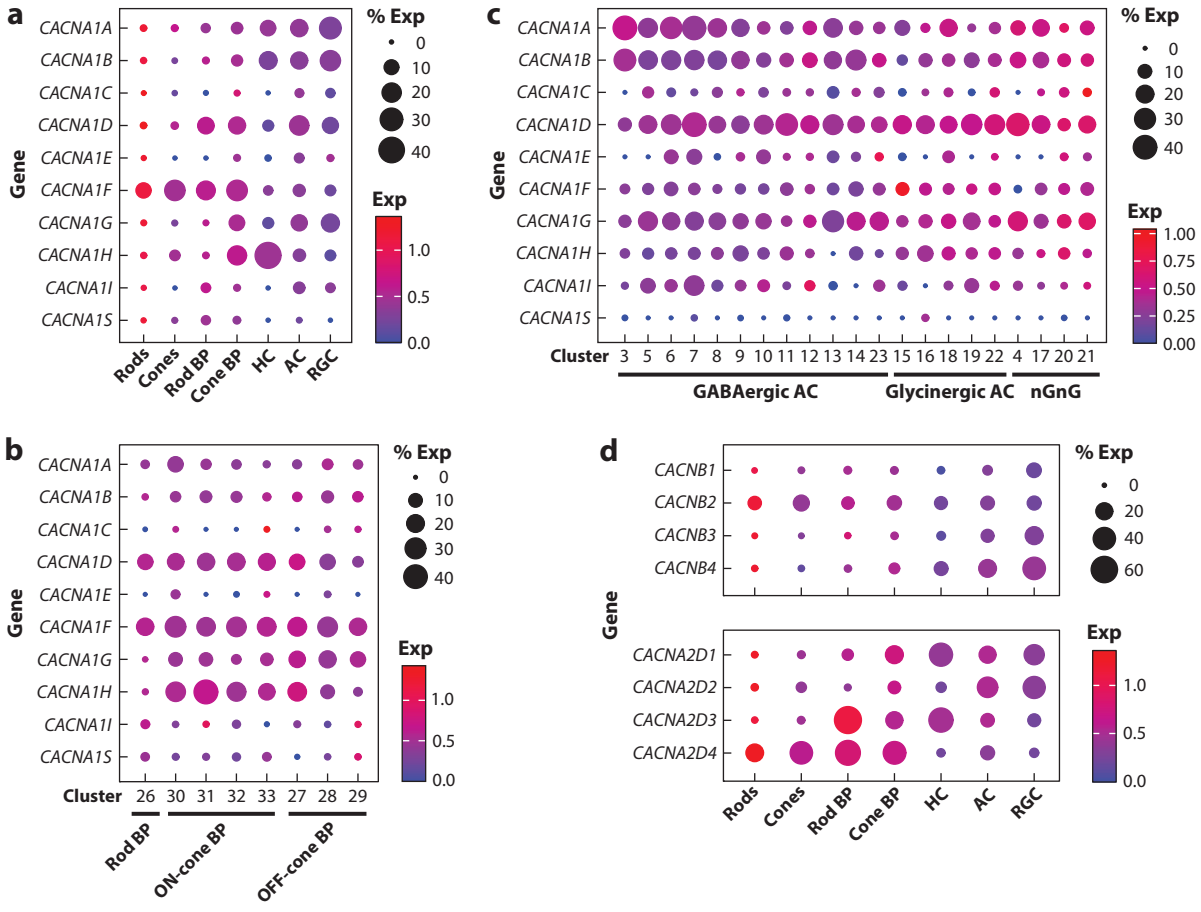


Figure 2

Expression of voltage-gated Ca^{2+} (Ca_v)-encoding genes in mouse retinal cell types. (*a–d*) Dot blots illustrating the Ca_v subtype percentage of expression (% Exp) corresponding to (*a–c*) α_1 (*Cacna1x*) or (*d*) β (*Cacnbx*) and $\alpha_2\delta$ (*Cacna2dx*), as determined by single-cell RNA-sequencing (scRNA-seq). Results are plotted according to cell clusters identified in the mouse retina at P14 (Macosko et al. 2015). The size and color of the dots reflect the percentage 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 that of 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 no different from background levels. Plots represent (*a*) *Cacna1* expression in major retinal cell types [rods, cones, rod bipolar (BP) cells, cone BP cells, horizontal cells (HCs), amacrine cells (ACs), and retinal ganglion cells (RGCs)]; (*b*) specific classes of BP cells; and (*c*) distinct clusters of ACs [GABA-ergic, glycinergic, non-GABA-ergic, nonglycinergic, nonglutamatergic (nGnG)], as well as *Cacnb1* and *Cacna2d* expression in the same cell types as in panel *a*. In panel *c*, clusters 3 and 16 exhibit the molecular signature of starburst ACs and AII ACs, respectively.

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 (**Figure 2*a–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 (approximately –50 mV and –30 mV, respectively) (Perez-Reyes 2003). Consequently, Ca_v3 channels are inactivated at voltages near the 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). As shown by scRNA-seq, Ca_v3 channels are expressed at significant levels in most retinal neurons except rods and rod bipolar cells (**Figure 2*a–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 (**Figure 1*a***). 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 composed of two polypeptides, α_2 and δ, which are encoded by the same gene. The $\alpha_2\delta$ preprotein 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) (**Figure 1*a***). 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 $\alpha_2\delta$ (**Figure 2*d***) that differ in terms of their regulation of Ca_v channels, as well as in their noncanonical functions (Dolphin 2016). Studies of the corresponding knockout (KO) mice would help dissect the retinal functions of specific β and $\alpha_2\delta$ subtypes. However, retinal phenotypes have to date 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 (**Figure 3*a***). Within the retinal circuitry, Ca_v channels are functionally specialized and often discretely localized to ensure the proper encoding of visual information.

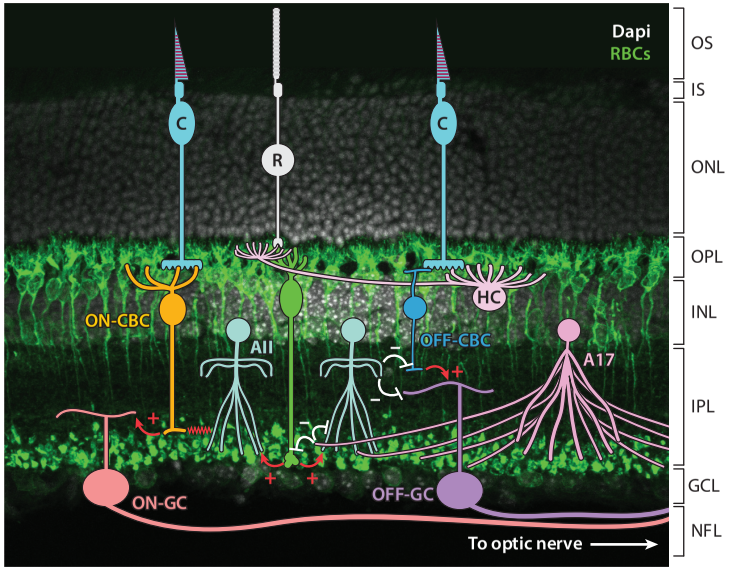
3.1. Rod and Cone Photoreceptors

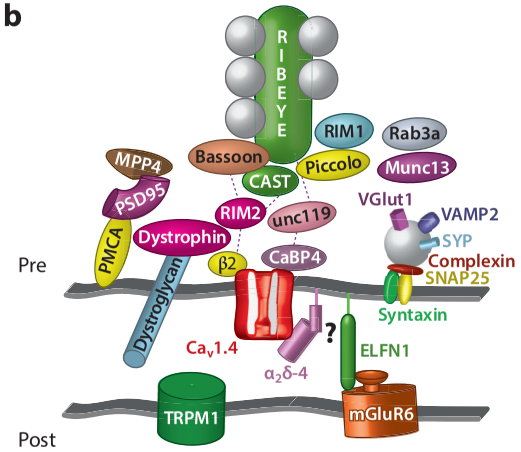
The somata of rods and cones lie in the outer nuclear layer (ONL) of the retina and extend axons into the outer plexiform layer (OPL), where they form synapses with bipolar and horizontal cells (**Figure 3*a***). The terminals of rods (spherules) and cones (pedicles) are characterized by synaptic ribbons 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 (**Figure 1*b***), 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).

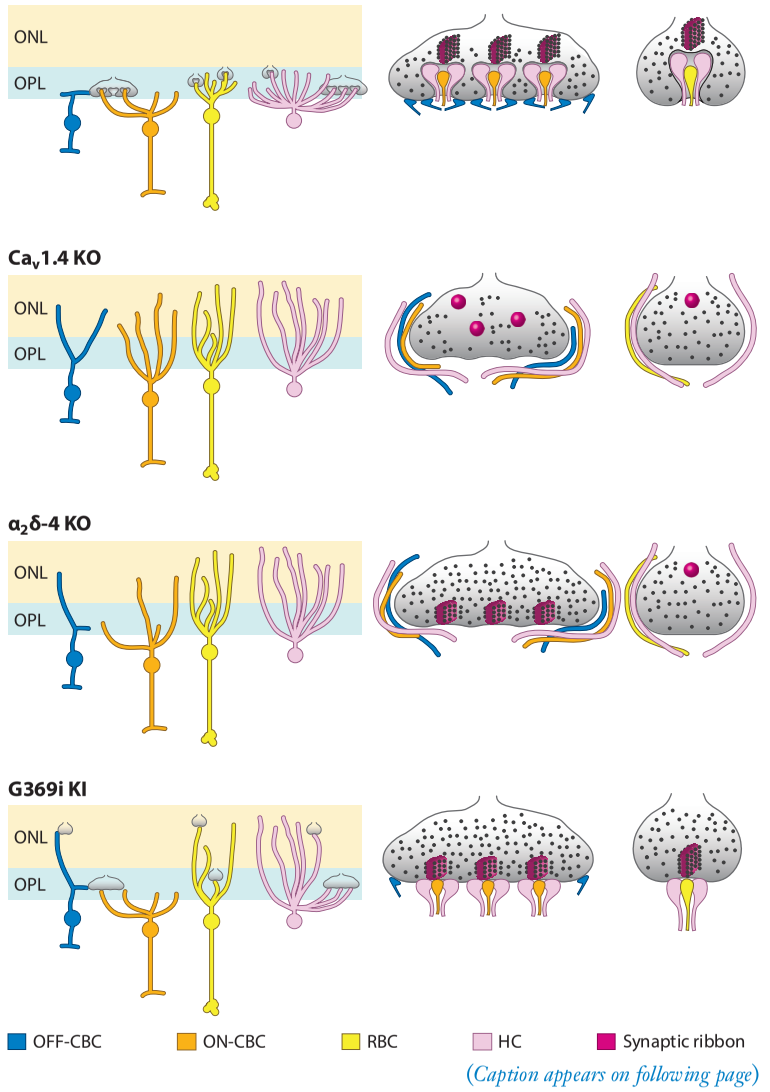
3.1.1. $Ca_v1.4$ regulates the maturation and function of rod and cone synapses. Large-scale sequencing of a genetic locus for the X-linked form of congenital stationary night blindness type 2 (CSNB2) revealed causative mutations in *CACNA1F*—the gene that is now recognized as encoding $Ca_v1.4$ (Bech-Hansen 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 the mouse retina (**Figure 2*a–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 $Ca_v1.4$ KO mice (Maddox et al. 2020). $Ca_v1.4$ protein is tightly clustered along the 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 (**Figure 3*b***), 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) (**Figure 3*c***). The lack of mature rod and cone synapses in $Ca_v1.4$ KO mice at







(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

$Ca_v1.4$ channels at photoreceptor synapses. (*a*) Cross-section of mouse retina labeled with Dapi and antibodies against protein kinase C to mark nuclei and RBCs, respectively. Overlaid is a schematic showing retinal cell types and their patterns of connectivity. RBCs are shown in green. + and – indicate excitatory and inhibitory inputs, respectively. The 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 the morphology of WT photoreceptor terminals and synaptic ribbons (*magenta*) and the defects reported in $Ca_v1.4$ KO, $\alpha_2\delta$–4 KO, and G369i knockin mouse retina. Figure adapted from Maddox et al. (2020). Abbreviations: C, cone; Ca_v, voltage-gated Ca^{2+}; CBC, cone bipolar cell; GC, ganglion cell; GCL, ganglion cell layer; HC, horizontal cell; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segment; KO, knockout; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; R, rod; RBC, rod bipolar cell; WT, wild type.

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 (**Figure 3*b***). To distinguish between these possibilities, our group analyzed rod synapses of mice expressing a nonconducting mutant form of $Ca_v1.4$ (G369i). Unlike in $Ca_v1.4$ KO mice, ribbons and pre- and postsynaptic proteins are present in rods of G369i knockin 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 (Maddox et al. 2020) (**Figure 3*c***). 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. $Ca_v1.3$. Several studies have reported the expression of *Cacna1d*, which encodes $Ca_v1.3$, in photoreceptors of the mouse retina using in situ hybridization (Xiao et al. 2007) and antibody labeling (Kersten et al. 2010). However, scRNA-seq analyses suggest very low representation of *Cacna1d* compared to *Cacna1f* in rods and cones in the mouse retina (Macosko et al. 2015) (**Figure 2*a***). 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 the $Ca_v1.3$ 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 (**Figure 2*a***), 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 that is indispensable for photoreceptor synapse assembly and transmission in the mouse retina.

3.1.3. 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 (**Figure 2*d***), and multiple lines of evidence suggest that they are likely to be involved in regulating $Ca_v1.4$ in these cells. 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 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 the mouse retina based on coimmunoprecipitation 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 (Kerov et al. 2018, Wang et al. 2017) (**Figure 3*c***) 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 (Katiyar et al. 2015, Kerov et al. 2018, Wang et al. 2017) (**Figure 3*c***). Perhaps the expression of other β and $\alpha_2\delta$ subtypes in cones, albeit low (**Figure 2*d***), 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 between ELFN1 and mGluR6 (**Figure 3*b***) 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 coimmunoprecipitates with ELFN1 when cotransfected in HEK293 cells, and this process 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 the rods of these mice (Wang et al. 2017). Given previous findings that coimmunoprecipitation of $\alpha_2\delta$ proteins in heterologous expression systems can be highly nonspecific (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) (**Figure 3*c***). Whether resulting from abnormal cone synapse formation, abnormal maintenance, or both, 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).

3.2. 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 (**Figure 3*a***). 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 distinct types of cone bipolar cells and RGCs (Euler et al. 2014) (**Figure 3*a***). 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 (**Figure 2*a*,*b***).

3.2.1. 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, 2001; Protti & Llano 1998) and mice (Satoh et al. 1998). In the 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) (**Figure 2*a*,*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 why $Ca_v1.3$ KO mice do not show signs of disrupted bipolar cell transmission (Busquet et al. 2010).

3.2.2. Ca_v2 and Ca_v3 channels are also expressed in bipolar cells. Ca_v3 channels have unique biophysical properties, and they have also been detected in electrophysiological recordings of various bipolar cell types (Hartveit 1999; Pan 2000, 2001; Singer & Diamond 2003). Messenger RNAs (mRNAs) corresponding to $Ca_v3.1$ and $Ca_v3.2$ are particularly well represented in some classes of cone bipolar cells in the mouse retina (Macosko et al. 2015, Shekhar et al. 2016) (**Figure 2*a,b***). In both cone and rod bipolar cells, the transient Ca^{2+} current ($I_{trans,Ca}$) mediated by Ca_v3 is approximately four 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 (approximately –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, 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 (**Figure 2*b***). Compared to Ca_v1 channels, Ca_v2 channels undergo robust modulation by heterotrimeric G proteins and Ca^{2+}; this modulation underlies short-term synaptic plasticity at nonretinal 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.

3.3. 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) (**Figure 3*a***). 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 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 the 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 (**Figure 2*a***), 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 (**Figure 2*a***). ML218, a specific blocker of Ca_v3 channels (**Figure 1*b***), 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 an approximately 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 previsual 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).

3.4. 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.

3.4.1. 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 and OFF cone bipolar cells or OFF RGCs (Famiglietti & Kolb 1975, Mills & Massey 1991) (**Figure 3*a***). 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 reverse transcription–polymerase chain reaction (RT-PCR) and scRNA-seq (Habermann et al. 2003, Yan et al. 2020) (**Figure 2*c***). 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.

3.4.2. Interplay between big K^+ channels and Ca_v1 channels in A17 amacrine cells. 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 amacrine cells (Chavez et al. 2006, Grimes et al. 2009), but its role in neurotransmitter release is more complex than in AII amacrine cells. 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 amacrine cells (Grimes et al. 2009), synaptically evoked GABA release from A17 amacrine cells is mediated by Ca^{2+}-permeable AMPA receptors rather than Ca_v1 channels (Chavez et al. 2006). Big K^+ (BK) Ca^{2+} activated K^+ channels, which are functionally coupled to Ca_v1 within A17 amacrine cell 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 amacrine cell 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 coimmunoprecipitate with BK channels from rat retinal lysates (Grimes et al. 2015), suggesting close proximity of these channels within A17 amacrine cells 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 amacrine cells are characterized by the expression of multiple Ca_v subtypes, including $Ca_v1.3$ and Ca_v3 channels (Yan et al. 2020) (**Figure 2*c***). 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 varicosities, thus diversifying reciprocal outputs and augmenting the parallel processing capabilities of each A17 cell.

3.4.3. 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 RGCs (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 (Kaneda et al. 2007) and rabbit (Lee et al. 2010) SACs, which is generally supported by scRNA-seq (Yan et al. 2020) (**Figure 2*c***). 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. 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 either short-term or 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

Ca^{2+}-dependent inactivation (CDI) is 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 preassociated with a site (the IQ domain) in the CTD of these channels and is evident as faster decay of I_{Ca} compared to Ba^{2+} currents (I_{Ba}) (**Figure 4*a***). 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 and 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} slightly inactivates during a 1-sec depolarization, similar to I_{Ba} (**Figure 4*b***). 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) (**Figure 4*b*,*c***). Although it is also present in $Ca_v1.3$, the CTM does not nullify CDI of these channels in transfected cells (Singh et al. 2006, 2008) (**Figure 4*a***). Additional mechanisms, such as RNA editing of the IQ domain (Huang et al. 2012) and interactions with proteins known to suppress CDI, could prolong opening of retinal $Ca_v1.3$ channels, as described below.

4.2. CaBPs: Modulators of Ca_v Channels in a Subset of Retinal Cell Types

CaBPs are members of 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

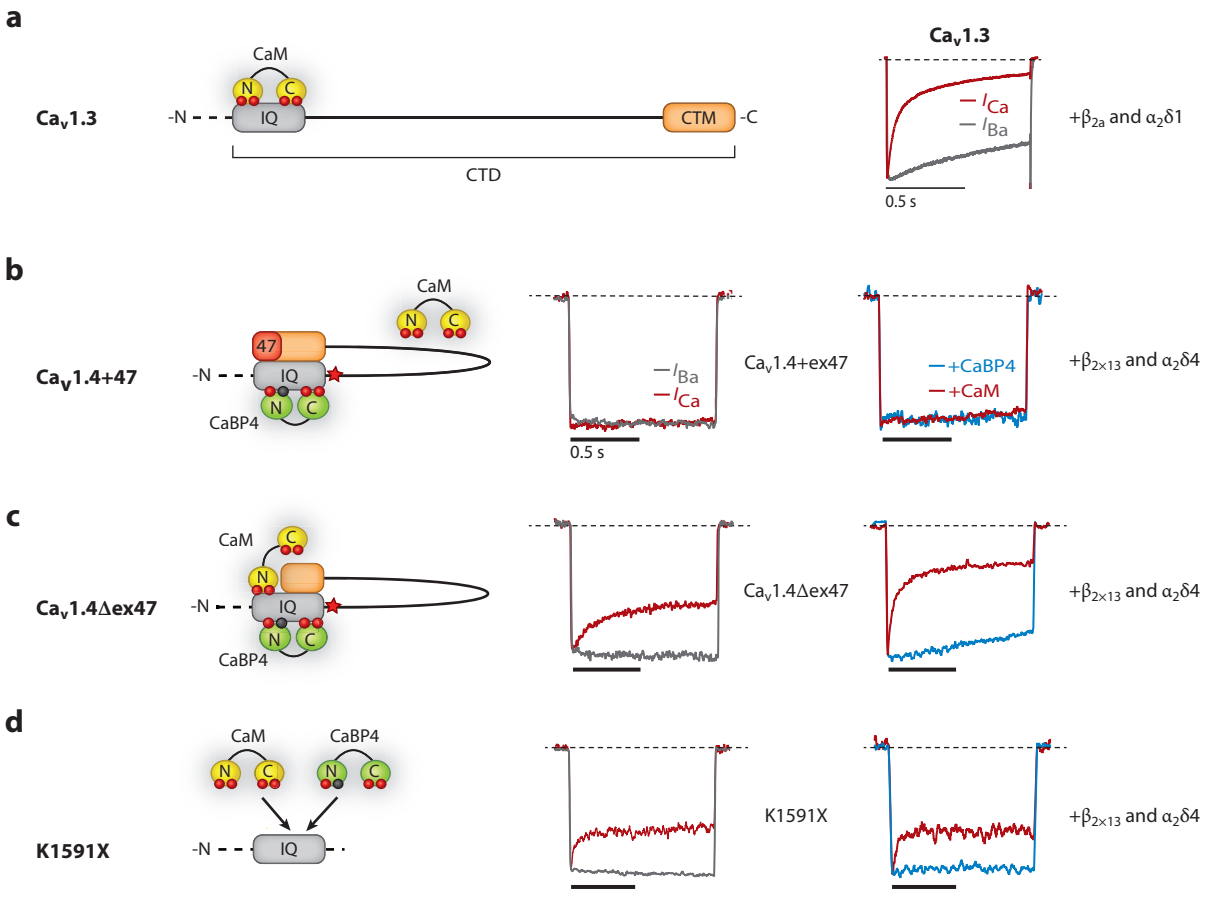


Figure 4

Calmodulin (CaM) and CaBP4 modulation of voltage-gated Ca^{2+} (Ca_v) 1 channels. Effects of CaM or CaBP4 binding to (*a*) $Ca_v1.3$ or (*b–d*) $Ca_v1.4$ on Ca^{2+}-dependent inactivation (CDI) are shown. Left panels show the schematics of the C-terminal domain (CTD) of each channel, containing an IQ domain and C-terminal modulatory domain (CTM). Right panels show normalized current traces in HEK293T cells expressing (*a*) $Ca_v1.3$ or (*b*) $Ca_v1.4$ with the indicated auxiliary subunits. In panels *b* and *c*, the star indicates the site of K1591. (*a*) For $Ca_v1.3$, CaM binding to the IQ domain 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 I_{Ca} when $Ca_v1.4$+ex47 is cotransfected with CaBP4 (*blue*, *right traces*). (*c*) CaM binding causes $Ca_v1.4$ channels lacking exon 47 ($Ca_v1.4$Δex47) to undergo strong CDI (*left traces*). Due to CaBP4 binding, I_{Ca} decays more slowly in cells cotransfected with CaBP4 compared to cells transfected with $Ca_v1.4$Δex47 (*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*). Panels *b* and *d* adapted from Williams et al. (2018); panel *c* adapted from Haeseleer et al. (2016).

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 (**Figure 4*b***), but instead 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, $Ca_v1.4$Δex47 undergoes significantly less CDI when bound to CaBP4 compared to channels

containing a functional CTM (Haeseleer et al. 2016) (**Figure 4*c***). 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, both of which are consistent with loss of function of $Ca_v1.4$ (Haeseleer et al. 2004, Liu et al. 2013b, Maeda et al. 2005).

CaBP4 is the only CaBP expressed in photoreceptors, but 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 the mouse and primate retina. In the CaBP5 KO retina, ON RGCs exhibit approximately 50% reduction in sensitivity to dim light flashes compared to WT, 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 in 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 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).

In one proposed mechanism whereby horizontal cells produce lateral inhibition in the retina (Kramer & Davenport 2015), feedback inhibition of cone Ca_v1 channels originates 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 (**Figure 1*a***). 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 probability of the channel being open (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 (**Figure 2*a*,*b***). Thus, the accumulation of protons in the synaptic cleft could have complex effects on Ca_v channels in the membrane of each cell type contributing to this triadic synapse.

5. DYSREGULATION OF $CA_V1.4$ AND VISION DISORDERS

Studies of *CACNA1F* mutations involved in retinal disease have yielded important insights into 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 strabismus (Hove et al. 2016). The variability in these symptoms could result from a complex interplay between 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 (approximately –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 knockin 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.

5.2. CaBP4 and Alternative Splicing as Modifiers of *CACNA1F* Mutations

Because of the complexity of the $Ca_v1.4$ interactome (**Figure 3*b***), 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 $Ca_v1.4$ (**Figure 4*d***). 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 coexpressed with K1591X mutant channels (B. Williams and A. Lee, unpublished manuscript) (**Figure 4*d***). Thus, the pathological consequences of K1591X are likely to result primarily from Ca^{2+} influx at abnormally negative voltages, rather than from 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 the human but not in the rodent retina (Haeseleer

et al. 2016), the I745T knockin 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 alterations resulting 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 defects in rod and cone synapse formation similar to those that 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) (**Figure 3*c***) 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, 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 knockin mice (Knoflach et al. 2015, Liu et al. 2013b, Regus-Leidig et al. 2014). Thus, both 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 nonribbon synapses.
2. Ca_v2 channels are expressed primarily in the interneurons of the retina, as well as in 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 labeling 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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