Functional effects of Cx50 mutations associated with congenital cataracts

Clio Rubinos,1 Krista Villone,1 Pallavi V. Mhaske,2 Thomas W. White,2 and Miduturu Srinivas1

1Department of Biological and Vision Sciences and the Graduate Center for Vision Research, State University of New York College of Optometry, New York, New York; and 2Department of Physiology and Biophysics, State University of New York, Stony Brook, New York

Submitted 9 April 2013; accepted in final form 2 September 2013

Rubinos C, Villone K, Mhaske PV, White TW, Srinivas M. Functional effects of Cx50 mutations associated with congenital cataracts. Am J Physiol Cell Physiol 306: C212–C220, 2014. First published September 4, 2013; doi:10.1152/ajpcell.00098.2013.—Mutations in connexin50 (Cx50) cause dominant cataracts in both humans and mice. The exact mechanisms by which mutations cause these variable phenotypes are poorly understood. We have examined the functional properties of gap junctions made by three Cx50 mutations, V44E, D47N, and V79L, expressed in mammalian cell lines. V44E trafficked to the plasma membrane properly and formed gap junctional plaques. However, the mutant did not form functional gap junctions when expressed alone, or with wild-type (WT) Cx46 and Cx50, indicating that V44E is a dominant negative inhibitor of WT connexin function. In contrast, D47N subunits did not localize to junctional plaques or form functional homotypic gap junctions; however, mixed expression of D47N and WT subunits of either Cx50 or Cx46 resulted in functional intercellular channels, with high levels of coupling. Single-channel studies indicated that D47N formed heteromeric channels with WT Cx46 with unique properties. Unlike either V44E or D47N, V79L formed functional homotypic intercellular channels. However, the mutation caused an alteration in voltage gating and a dramatic reduction in the single-channel open probability, resulting in much lower levels of conductance in cells expressing V79L alone, or together with WT connexin subunits. Thus, each mutation produced distinct changes in the properties of junctional coupling. V44E failed to form intercellular channels in any configuration, D47N formed only heteromeric channels with WT connexins, and V79L formed homotypic and heteromeric channels with altered properties. These results suggest that unique interactions between mutant and wild-type lens connexins might underlie the development of various cataract phenotypes in humans.

gap junction; cataract; lens; intercellular communication; heteromers; connexin

INTERCELLULAR COMMUNICATION mediated by gap junctions (GJ) plays important roles in a number of tissues, including the lens. These channels are formed by the association of two hemichannels that are located in the plasma membranes of adjacent cells. Each hemichannel is a hexamer composed of proteins called connexins (Cx). Each connxin includes cytoplasmic amino and carboxy termini as well as four transmembrane domains linked by a single cytoplasmic loop and two extracellular domains, E1 and E2 (47). Gap junction channels may be formed by the docking of two hemichannels containing a single connxin subtype (i.e., homotypic junctions), by association of two connexons each containing a different connxin subtype (i.e., heterotypic), or by combination of hemichannels each containing multiple connxin subtypes (i.e., heteromeric junctions) (47).

The lens expresses three connxin family members (Cx43, Cx46, and Cx50) in distinct spatial and overlapping patterns. Cx43 is exclusively expressed in the epithelium (7, 25), whereas Cx46 is present only in fiber cells, where its expression coincides with fiber cell differentiation (29). Cx50 is present in both epithelial cells and fibers (8, 32, 44, 45). The expression of connexins in epithelium and fibers is important for both postnatal lens development and lens transparency. Targeted deletions of genes encoding Cx46 and Cx50 lead to cataracts in mice (15, 32, 46). Deletion of Cx46 in mice results in dense nuclear cataracts coupled with precipitation of crystallins and proteolysis (14, 15), a phenotype that is due to a disruption of the lens circulation (12, 13, 21, 23). Deletion of Cx50 in mice results in smaller nuclear cataracts, along with microphthalmia (32, 43, 46). In addition, mutations within the Cx46 and Cx50 gene loci have been linked to a variety of dominant cataract phenotypes in humans and mice (1, 2, 10, 19, 33, 49, 50). To date, more than 20 different mutations in the Cx46 and Cx50 genes have been identified that lead to different cataract phenotypes.

Characterization of the functional effects of individual mutations has provided important insights into understanding the roles of intercellular communication in the lens. The majority of mutations studied to date do not form functional intercellular channels when expressed by themselves, often due to aberrant trafficking to the plasma membrane or retention in the endoplasmic reticulum (ER) (1–3, 6, 9, 27, 28, 38, 40, 48, 49, 52). When coexpressed with wild-type (WT) lens connexins in a 1:1 ratio, many of these loss-of-function mutations inhibit coupling provided by WT Cx46 and/or Cx50, a finding that possibly explains the dominant pattern of inheritance. Other loss-of-function mutations do not affect Cx46 or Cx50 coupling magnitude in a dominant manner (1, 9, 52); such loss-of-function mutants are assumed to form functional heteromeric channels with WT Cx46 or Cx50 subunits with altered gating and possibly permeability. Other effects of mutations that have been reported include activation of intracellular stress responses and formation of aberrant hemichannels (6, 24, 38).

In this study, we examined the properties of three different Cx50 missense mutations, V44E, D47N, and V79L, which are associated with congenital cataracts in humans (1, 10, 39). V44 and D47 are localized in the first extracellular loop (E1) of connexins, which is known to line the pore and to be involved in channel gating (17, 18, 37, 41), whereas V79 is located at the border of E1 and the second transmembrane domain (TM2). The V44E missense mutation causes whole lens cataracts, whereas the D47N and V79L mutations cause pulverulent nuclear opacities and “full moon” with Y-suture opacities, respectively. We demonstrate that each of the three mutations produces distinct changes in the properties of junctional coupling when expressed alone or in combination with WT lens
connexins. The results provide a molecular basis for the formation of various cataract phenotypes in human patients with these Cx50 mutations.1

1 This article is the topic of an Editorial Focus by James E. Hall (15a).

**Fig. 1.** Coupling levels in cells expressing wild-type (WT) connexin50 (Cx50), V44E, D47N, or V79L. Bar graphs show the junctional conductance ($G_j$) values in N2A cells transfected with wild-type and mutant Cx50 cDNA. $G_j$ values in cells expressing V44E ($n = 18$) or D47N ($n = 19$) were extremely low, and not higher than those found in untransfected parental cells, indicating that they do not form functional intercellular channels. V79L formed functional gap junction channels ($n = 15$), but $G_j$ values in cells expressing cells were much lower than those expressing WT Cx50 ($n = 32$). Each bar represents the mean ± SE.

**Fig. 2.** Cx50 mutant subunits differ in their ability to localize to the plasma membrane. Transiently transfected HeLa cells expressing WT Cx50 (A), V44E (B), D47N (C), and V79L (D) proteins were immunostained and examined by fluorescence microscopy. Merged images of Cy3 (red) staining of connexins and DAPI staining of cell nuclei (blue) were taken at ×40 magnification. D47N failed to correctly localize to cell membrane appositions, whereas V44E and V79L formed gap junction plaques at areas of cell-cell contact similar to WT Cx50.
kHz and sampled at 1–2 kHz. Each cell of a pair was initially held at a common holding potential of 0 mV. To evaluate junctional coupling, 200-ms hyperpolarizing pulses from the holding potential of 0 mV to −20 mV were applied to one cell to establish a transjunctional voltage gradient ($V_j$), and junctional current was measured in the second cell (held at 0 mV). To evaluate the voltage dependence of the junctional conductance, 7- to 10-s hyperpolarizing or depolarizing pulses were applied every 30 s from the holding potential of 0 mV to various test potentials (between −100 and 100 mV). The instantaneous and steady-state levels of junctional currents were measured at the beginning and at the end of each $V_j$ pulse. Steady-state junctional conductance ($G_{j,ss}$) was normalized relative to the instantaneous current, and these values were plotted as a function of $V_j$. Single-channel currents were investigated in weakly coupled cell pairs (1 or 2 channels) without the use of uncoupling agents by applying −20 mV pulses to one cell of a pair. Gating events were recognized as simultaneously occurring events of equal amplitude and opposite polarity in current traces for both cells in the pair. All-point amplitude histograms were used to separate the mean and variance of the baseline and open-channel current. Data were acquired with pCLAMP9.2 software (Molecular Devices); analysis was performed with pCLAMP9.2 and ORIGIN 6.0 software (Microcal Software, Northampton, MA).

Immunocytochemistry. HeLa cells were plated on 22-mm square coverslips and grown to 50% confluence then transiently transfected (Microcal Software, Northampton, MA). After overnight incubation, cells were fixed with 2% paraformaldehyde in PBS, blocked with 5% BSA in PBS with 0.1% Triton X-100 and 0.02% NaN3. Cells were stained with a 1% polyclonal anti-Cx50 antibody followed by incubation with an anti-Cy3 goat anti-rabbit secondary antibody (Jackson ImmunoResearch). Images were photographed at 40 magnification, and areas of cell-cell contact were examined for the presence of gap junctional plaques.

Fig. 3. The V44E point mutation inhibits coupling provided by WT connexins in a dominant negative fashion. V44E does not form functional heteromeric or heterotypic gap junction channels with WT Cx50 (A) or WT Cx46 (B). Junctional conductance was measured in N2A cell pairs transfected with WT connexins alone or in combination with V44E at a 1:1 ratio. Cell pairs expressing WT Cx50 or WT Cx46 subunits alone formed functional gap junctions. In contrast, cells transfected with both WT and V44E cDNA failed to form functional gap junction channels. Similarly, pairing of V44E expressing cells with those expressing WT connexins did not result in the formation of functional heterotypic intercellular junctions. Each bar represents the mean ± SE from 9–12 cell pairs.

Fig. 4. Lack of dominant negative inhibition of WT connexin channel function by D47N. D47N forms functional heteromeric but not heterotypic gap junction channels with WT Cx50 (A) or WT Cx46 (B). Cells cotransfected with both WT and mutant Cx50 cDNA formed channels that displayed $G_j$ values that were not significantly different from homotypic Cx50 channels ($n = 13$). Similarly, the coexpression of Cx46 and D47N subunits did not significantly alter junctional conductance ($n = 18$). Heterotypic pairing of D47N with WT connexins failed to form functional channels as levels of conductance were not higher than that of background levels seen in untransfected N2A cells. Each bar represents the mean ± SE from 5–18 cell pairs.

Cells were viewed and photographed on an Olympus BX51 microscope using an Optronics MagnaFire digital camera. Gap junctional plaque formation was quantified by immunofluorescent microscopy. Images were photographed at ×40 magnification, and areas of cell-cell contact were examined for the presence of gap junctional plaques.

RESULTS

V79L, but not V44E and D47N, form functional gap junction channels. To determine whether V44E, D47N, and V79L formed functional gap junction channels, we measured gap junctional conductance ($G_j$) in N2A cell pairs transfected with mutant and WT Cx50 cDNAs (Fig. 1). $G_j$ was measured by applying brief voltage pulses to −20 mV from a holding potential of 0 mV to one cell of a pair. As expected, cell pairs expressing WT Cx50 were robustly coupled ($G_j \sim 38.5 \pm 4$ nS; $n = 32$). In contrast, no detectable coupling was found between cell pairs expressing homotypic channels of V44E ($n = 18$). Similarly, N2A cells expressing D47N were not coupled ($n = 19$), confirming previous results in the Xenopus oocyte expression system (1). Cells transfected with V79L were found to have levels of coupling ($G_j \sim 2.8 \pm 0.9$ nS; $n = 26$) that were significantly ($P < 0.05$) higher than in those found in nontransfected parental cells ($G_j \sim 0.31 \pm 0.08$; $n = 30$). However, $G_j$ values in V79L-expressing cells were markedly lower than in those expressing Cx50 WT (Fig. 1).

The absence of coupling in V44E- and D47N-expressing cells might be explained by aberrant trafficking or a reduced capacity to form gap junctional plaques as previously shown for a number of Cx50 mutants. To determine whether V44E
and D47N subunits fail to traffic to the plasma membrane, mutant subunits were expressed in transiently transfected HeLa cells. Immunofluorescent images of HeLa cells expressing WT Cx50 and mutant subunits stained with anti-Cx50 antibody are shown in Fig. 2. V44E subunits localized to the plasma membrane similar to WT Cx50 subunits, specifically to appositional regions between adjacent cells, indicating that they form gap junctions. Similarly, expression of V79L was found at regions of cell-cell contact, confirming that it forms functional intercellular junctions (Fig. 2). In contrast, D47N was unable to localize to sites of cell-cell apposition, instead appearing to accumulate in subcellular compartments surrounding the nucleus, as previously reported (1).

**V44E inhibits coupling provided by Cx46 and Cx50 in a dominant negative fashion.** The V44E mutation causes total lens opacification (10), a phenotype that is similar to that found in Cx46 and Cx50 double-knockout mice. To obtain further insight into the mechanism by which the V44E mutation produces whole cataracts, we determined whether the mutant subunit inhibited the coupling provided by Cx46 and Cx50. Junctional conductances in N2A cells transfected with a mixture of V44E and WT Cx46 or WT Cx50 cDNAs in a 1:1 ratio are shown in Fig. 3. Coexpression of V44E with WT Cx50 caused a 95% decrease in the junctional conductance (2.4 ± 1 nS for V44E + Cx50 vs. 43 ± 3.2 nS for WT Cx50; Fig. 3A). Similarly, cells expressing both Cx46 and V44E subunits displayed a mean conductance of 1.4 nS (n = 12), a significant decrease in Gj compared with those expressing homotypic Cx46 channels (Fig. 3B). In addition, V44E did not form heterotypic channels with either Cx46 (n = 10) or Cx50 (n = 9) (Fig. 3). These results indicate that V44E mutant failed to form functional homotypic or heterotypic gap junctions and strongly inhibited coupling provided by Cx50 and Cx46 in a dominant negative manner.

**D47N forms functional heteromeric channels with Cx46.** The ability of D47N subunits to form heterotypic and heteromeric channels with WT connexins is summarized in Fig. 4. Mean conductance of heterotypic D47N/Cx46 (n = 5) or D47N/Cx50 (n = 8) was not much higher than those found in untransfected cells (Fig. 4), indicating that D47N does not form heterotypic channels with WT subunits. However, in contrast to V44E, D47N did not inhibit the conductance of WT channels. Cells transfected with both WT Cx50 and D47N cDNA formed channels that displayed Gj values that were not significantly different from homotypic Cx50 channels (Fig. 4A). Similarly, coupling in cells expressing heteromeric Cx46 and D47N gap junctions (n = 18) was not reduced in magnitude to those expressing homotypic Cx46 (Fig. 4B). To determine whether D47N forms heteromeric gap junctions with WT connexins, we examined properties of single channels in cotransfected cells (Fig. 5). Figure 5A shows examples of unitary currents of homotypic Cx46 and heteromeric D47N and WT Cx46 obtained in response to voltage ramps from −100 mV to +100 mV. The unitary conductance for the D47N:Cx46 mixture, measured as the slope conductance at 0 mV (~230 pS), is considerably larger than that of WT Cx46 (~140 pS) and similar to the single-channel conductance of Cx50 homotypic channels (~220 pS) (16, 34). A second example of a heteromeric Cx46:D47N gap junction is illustrated in Fig. 5B where single-channel recordings at a Vj of 40 mV were obtained from a cell pair containing two or three active channels. All-points amplitude histograms show peaks at 9.5 pA and 17 pA, corresponding to the opening of one or both channels in the cell pair, indicating a unitary conductance of ~240 pS.
Thus, expression of D47N causes a shift in the single-channel properties of WT Cx46. Because D47N does not form heterotypic or homotypic junctions, the shift in unitary conductance to higher values is direct evidence for the formation of heteromeric gap junctions. We also examined the properties of heteromeric channels formed by D47N subunits and Cx50 subunits. Although large-conductance channels were found in cotransfected cells, we could not distinguish heteromeric channels from those formed by WT Cx50 due to their similar unitary conductances (data not shown).

V79L markedly alters $V_j$ gating and reduces channel open probability. As shown in Fig. 1, cells expressing the V79L mutation had levels of coupling that were higher than background, suggesting that it forms functional channels. Therefore, we characterized the voltage gating and single-channel properties of homotypic, heterotypic, and heteromeric gap junction channels formed by V79L to obtain an understanding of the putative mechanism by which the mutation produces opacities. Because coupling levels in a majority of cell pairs were too low, we examined the voltage gating properties of V79L junctional currents only in a select number of cell pairs ($n = 4$) in which $G_j$ values ranged between 3 and 5 nS. The voltage gating properties of homotypic Cx50 WT or V79L gap junctions are illustrated in Fig. 6. Junctional currents were measured in response to $V_j$ pulses between −100 and +100 mV in 20-mV increments applied from a holding potential of 0 mV. In Cx50-expressing cell pairs, the junctional current declined from initial maximal values to steady-state values at transjunctional voltages exceeding ±20 mV (Fig. 6A). Conductance declined steeply with $V_j$ to a residual conductance ($G_{\text{min}}$) that is ~20% of the maximum conductance as previously described (5, 34, 36, 51). The voltage at which the conductance is half-maximal ($V_{1/2}$) was −38 mV for pulses of negative polarity and 37 mV for positive polarity. In contrast, homotypic V79L junctional currents exhibited a weak dependence on transjunctional voltage (Fig. 6B), suggesting that the mutation altered voltage gating ($V_{1/2}$ values were −79 mV and 82 mV at negative and positive voltages, respectively).

N2A cells transfected with V79L formed functional heterotypic channels when paired with cells expressing WT Cx46 or WT Cx50. Heterotypic channels formed by WT hemichannels and V79L hemichannels exhibited highly asymmetric voltage gating properties. Representative junctional currents of heterotypic Cx50/V79L in response to voltage pulses applied to
V79L-expressing cells are illustrated in Fig. 6C. Application of negative voltage pulses relative to the V79L-expressing cell caused a decline in the junctional current, whereas positive voltage pulses to the V79L side caused an increase in the junctional current. The steady-state $G_i\cdot V_j$ relation shows the decline of $G_i$ to near zero for voltages relatively negative on the V79L side and the increase in $G_i$ for voltages relatively positive to the V79L side. $G_i$ is not at maximum when $V_j = 0$ ($\sim 0.2$), suggesting that V79L channels are likely to be closed in the absence of a $V_j$ gradient. Similar results were obtained when V79L was paired with WT Cx46 (data not shown).

To determine the effect of the V79L mutation on single-channel open probability ($P_o$), we examined the single-channel properties of channels formed by the mutation. Figure 7A shows recordings of single homotypic gap junction channels in cells expressing WT Cx50 or V79L at a $V_j$ of $-20$ mV. WT Cx50 channels are predominantly open at this transjunctional voltage as previously described ($P_o \sim 0.98$). In contrast, V79L channels appeared to reside predominantly in the fully closed state ($P_o \sim 0.21$ for the recording in Fig. 7A). Transitions to the open state were accomplished by frequent brief chirps that could be ascribed to incomplete opening transitions that varied in duration and amplitude. Such transitions between open and closed states occurred throughout the voltage range of $\pm 100$ mV as shown in Fig. 7B. The gating behavior of V79L channels is in marked contrast to that of WT Cx50 channels, which exhibited transitions between the open and subconductance states and were typically open in the voltage range of $-30$ to $+30$ mV (representative current trace in Fig. 7B). These results indicate that channels formed by the V79L missense mutation are closed even in the absence of a trans-junctional voltage gradient.

In contrast to the effects on channel open probability, the V79L mutation does not appear to cause a reduction in unitary conductance of the fully open state (Fig. 7B). Single-channel current-voltage ($I$-$V$) relations of homotypic Cx50 and V79L obtained in response to voltage ramps from $-100$ mV to $+100$ mV are shown in Fig. 7B. The single-channel conductances of the fully open state of WT Cx50 and V79L gap junction channels obtained by linear regression of the single-channel $I$-$V$ relationships were $215 \pm 4$ pS and $230 \pm 15$ pS, respectively.

V79L also formed functional heteromeric channels with both Cx46 and Cx50. However, $G_i$ values in cells expressing a mixture of V79L and WT lens connexins in a 1:1 ratio were much lower than those expressing Cx46 or Cx50 homotypic channels (Fig. 8A). Junctional conductance of heteromeric channels was reduced by 67% and 78%, on average, compared with homotypic channels formed by WT Cx50 and Cx46, respectively. The decrease in $G_i$ values can be explained by a strong reduction in channel open probability of mixed channels. Figure 8B shows single-channel recordings obtained from cell pairs expressing WT Cx50 alone or a mixture of V79L and Cx50 at a 1:1 ratio at a transjunctional voltage of $-20$ mV. Cx50 homotypic channels were predominantly open at this voltage ($P_o \sim 0.98$). In contrast, single channels in cells expressing V79L and Cx50 mixture showed frequent transitions to a fully closed state. In the example shown, $P_o$ was reduced from 0.98 to 0.43 (see all-points histograms). Large-conductance channels typical of WT

---

**Fig. 7.** The V79L mutation causes a strong reduction in open probability. *A:* single-channel recording of junctional current from cell pairs expressing V79L or WT Cx50 at a $V_j$ of $-20$ mV. WT Cx50 homotypic channels are predominantly open at this voltage. In contrast, V79L channels appeared to reside predominantly in the fully closed state with occasional transitions to one or more open states, indicating a marked reduction in the channel open probability. These transitions between open and fully closed states were brief and often varied in duration and amplitude. The closed state (c) in both current traces is indicated by a dotted line. *B:* single-channel current-voltage relationships from cell pairs expressing WT Cx50 and V79L cells. Slope conductances (dashed line) in WT Cx50 and V79L cells are 220 pS and 234 pS, respectively. Note that V79L channels show very few transitions to the fully open state throughout the voltage range of $-100$ mV to $+100$ mV.
C218  DIFFERENTIAL EFFECTS OF Cx50 MUTATIONS UNDERLYING CATARACTS

V44E is a dominant negative inhibitor of WT connexin function. The V44E mutant was unable to form functional homotypic channels, and it acted in a dominant negative fashion to reduce the conductance of both wild-type channels. The valine at position 44 in the TM1/E1 border is conserved in the connexin family of proteins. Along with other hydrophobic residues at the TM1/E1 border including the conserved tryptophan at position 45, the valine forms part of a hydrophobic core, which participates in intraprotomer interactions believed to be important for the stabilization of the connexin structure (20). Thus, substitution of the valine with a charged residue is expected to interfere with connexin oligomerization, resulting in a loss of function. The effects of V44E are similar to those of the cataract-associated Cx50 mutation W45S (38), and other connexin point mutations of hydrophobic residues in the TM1/E1 border linked to deafness (26, 42). Many of these mutations fail to form functional homotypic or heteromeric gap junction channels and produce dominant inhibition of wild-type conductances.

The severe reduction of Cx46 and Cx50 conductance caused by V44E provides an explanation for the phenotype of whole cataracts seen in humans carrying this mutation. Gap junctions formed by Cx46 and Cx50 are integral components of the lens circulating current, which is important for maintenance of transparency (11, 22, 23). This current is primarily carried by Na+ and enters the lens along the extracellular spaces between cells. After crossing the fiber cell membranes in the lens interior, it flows towards the surface through a gap junction-mediated pathway (11, 22, 23). The high concentration of gap junction channels at the equator allows the intracellular current to be directed to the surface where equatorial Na/K pumps transport the sodium out of the lens (11, 22, 23). The circulating ionic current generates water flow, transport of essential solutes and Ca2+. In the absence of Cx46 gap junctions, which provides the majority of coupling in inner fiber cells, Ca2+ is known to accumulate in the lens center, leading to activation of proteases and ultimately cleavage of crystallins (4, 12). Thus, the marked reduction of coupling provided by WT connexins, particularly that of Cx46, caused by V44E might lead to formation of whole cataracts by initiating a similar cascade of events in the human lens.

D47N forms functional heteromeric channels. D47N subunits alone failed to form functional intercellular channels or target to gap junctions in transfected HeLa cells. However, they were able to form functional gap junctions when coexpressed with wild-type lens fiber connexins with levels of electrical coupling that were comparable to those recorded from homotypic wild-type channels. By measuring single-channel conductances, we provided direct evidence for heteromerization, at least between mutant D47N subunits and WT Cx46. In coexpressing cells, unitary conductances ranged from 220 pS to 240 pS, values close to those found for gap junction channels formed by WT Cx50 and much higher than those formed by WT Cx46 channels (16, 34). These results indicate that D47N subunits can interact with WT Cx46 to form mixed channels with unique properties.

The absence of dominant negative effects of D47N on WT channel conductance is also seen with other Cx50 mutations that cause cataracts in mice and humans (e.g., S50P and D47A) (2, 9, 52). The dominant cataract phenotype observed with these mutations is therefore not caused by a deficiency in ionic coupling but is due to alternative mechanisms. One possibility

Cx50 were observed in cells coexpressing Cx50 WT and V79L subunits. In this particular example, the amplitude histogram showed a peak at 4.1 pA corresponding to a fully closed state resulting in a Po of 0.43. Amplitude histograms are shown to the right of each current trace. The closed state (c) is indicated by a dotted line.

DISCUSSION

In this study, we characterized the electrophysiological properties of three Cx50 mutations, V44E, D47N, and V79L, that cause cataracts in humans. V44E and V79L formed gap junctional plaques similar to WT Cx50, suggesting that they were able to oligomerize and traffic to the plasma membrane properly. In contrast, D47N subunits did not show plasma membrane localization in a consistent fashion, as described previously (1). Functional studies indicated that each of the three mutations produced unique changes in the properties of junctional coupling when expressed alone or in combination with WT lens connexins.

Fig. 8. V79L forms heteromeric channels with WT lens connexins. A: Gj values in cells expressing a mixture of V79L and WT lens connexins were much lower than those expressing Cx46 or Cx50 homotypic channels. Gj values of heteromeric channels were reduced by 67% and 78%, on average, compared with homotypic channels formed by WT Cx50 and Cx46, respectively. B: recordings of junctional current of heteromeric channels formed by V79L and WT Cx50 at a Vj of 20 mV. Cx50 homotypic channels were predominantly open at this voltage [open probability (Po) ~ 0.98]. In contrast, single channels in cells expressing V79L and Cx50 mixture showed frequent transitions to a fully closed state resulting in a Po of 0.43. Amplitude histograms are shown to the right of each current trace. The closed state (c) is indicated by a dotted line.

AJP-Cell Physiol • doi:10.1152/ajpcell.00098.2013 • www.ajpcell.org

Downloaded from http://ajpcell.physiology.org/ by 10.220.33.2 on August 26, 2017
is that opacities are formed by the activation of a stress response caused by the retention of mutant subunits in the ER. Accumulation of mutant subunits in the ER and altered degradation of cataract-associated Cx50 mutants have been reported previously (6). Alternately, heteromeric gap junction channels formed by D47N and wild-type subunits might exhibit alterations in permeability to signaling molecules essential for lens homeostasis, a hypothesis that was proposed to explain the effects of S50P based on studies on lenses from mice carrying the mutation. The aspartic acid at position 47 in E1 is highly conserved and pore-lining according to the 3.5 Å Cx26 crystal structure (20). Thus it is likely that substitution of the negatively charged aspartic acid with the neutral asparagine will cause significant changes in permeation of signaling molecules. Our results indicate that coexpression of D47N leads to a dramatic shift in the single-channel properties of WT Cx46. Whether these large-conductance channels have an altered permeability to signaling molecules remains to be investigated.

**V79L forms functional channels with altered properties.**

Unlike most of the cataract-associated Cx50 mutations characterized to date, the V79L mutation formed functional homotypic channels. However, channels formed by V79L showed gating properties that were dramatically different from WT Cx50 channels. At low transjunctional voltage gradients, typically expected between adjacent lens fibers, homotypic channels made of V79L resided predominantly in the closed state, leading to a strong reduction in the single-channel open probability. The reduction in $P_o$ essentially translated into a $-85\sim 90\%$ reduction in the magnitude of ionic current compared with WT Cx50 (Fig. 1). The drastic change in gating was also seen in single-channel recordings of heteromeric channels made by V79L and WT connexins and led to a reduction 67% and 78%, on average, of the junctional conductance of heterotypic WT Cx50 and Cx46, respectively. The strong reduction in the levels of Cx46 and Cx50 coupling by V79L would be expected to disrupt the lens circulation, promoting the formation of dominant opacities.

The valine at position 79 at the E1/TM2 border is not highly conserved among the connexin family of proteins. Other connexin isoforms contain an alanine (e.g., Cx26, Cx46, Cx31) or a serine (Cx32). Although residues in TM2 are known to be involved in voltage gating of connexins, the effect of residues in the E1/TM2 border on gating, conductance, and permeability is not known. Our results indicate that the V79L mutation in Cx50 appeared to alter channel structure, leading to strong effects on voltage gating. These effects can be explained by considering the existence of two distinct voltage-dependent gating mechanisms that close Cx50 gap junction channels at positive and negative voltages. One mechanism closes Cx50 gap junction channels to a subconductance state in response to voltage pulses of relative positive polarity and underlies the decline of steady-state $G_j$ to a residual conductance with increasing $V_j$ (5, 34, 36, 51). In V79L gap junction channels, this mechanism is either abolished or shifted to voltages beyond the range examined in this study because gating events between open and subconductance states were rarely observed in single-channel recordings (Fig. 7). The other mechanism that closes Cx50 gap junction channels, termed loop gating, is characterized by transitions to the fully closed state and is seen in response to voltage pulses of relative negativity in each hemichannel of a gap junction channel. In WT Cx50, the negative gate is open at low $V_j$ but closes at large $V_j$ values (exceeding $-80$ mV) (36). The V79L mutation appeared to cause a large positive shift in the conductance-voltage relation of this form of gating, as indicated by the increase in the incidence of full closing transitions at low $V_j$ and a much reduced $P_o$ at $V_j = 0$. The closure of the loop gate of V79L hemichannels explains the decrease towards near zero at $V_j$ values relatively negative to the V79L side seen in $G_{j,ss}$-$V_j$ relations of heterotypic gap junctions in which V79L hemichannels were paired with WT Cx50 hemichannels. The apparent absence of $V_j$-induced closure of homotypic V79L junctional currents can similarly be explained by the positive shift in the voltage dependence of the loop gate of each component hemichannel (30, 31).

Additional studies on unopposed hemichannels are necessary to confirm the effect of V79L on loop gating.

In conclusion, our results indicate that Cx50 mutations produce different effects on gap junction channel function. These distinct effects, which are produced by the unique interaction of individual mutant subunits with WT connexin subunits, likely modulate intercellular communication in the lens in different ways and possibly contribute to the formation of various cataract phenotypes attributed to Cx50 mutations. A better understanding of the interactions between WT and mutant subunits may lead to the identification of newer approaches to cataract prevention.

**REFERENCES**

DIFFERENTIAL EFFECTS OF Cx50 MUTATIONS UNDERLYING CATARACTS


