Figure S1. Current-voltage relations of L108 and E109C hemichannels expressed in *Xenopus* oocytes. Current-voltage traces obtained from a holding potential of 0 mV followed by membrane polarizations applied sequentially in 10 mV increments from 50 mV to −90 mV. Recordings were performed in bath solution containing 100 mM CsMes, 10 mM Hepes, and 1.8 mM MgCl₂, pH 7.6. In L108C hemichannels, voltage-dependent current relaxations observed with depolarizations more negative than −30 mV represent closure of both loop- and V_j-gates. The N2E substitution reverses the polarity of V_j-gating from closure with negative polarizations to closure with positive polarizations (Oh et al., 2000, 2004). The polarity of loop-gate closure is not changed. The open state is strongly favored at potentials ± 20 mV. The slight increase in outward currents observed at 20 and 30 mV in the N2EL08C and at 50 mV in N2E E109C is most likely due to the transient activation of an unidentified endogenous oocyte channel during the course of the recording.

Figure S2. Extracellular Mg²⁺ stabilizes the loop-gate closed conformation of endogenous Cx38 hemichannels. (A) A segment of a continuous current trace evoked by a train of alternating 20-s voltage polarizations from −10 mV (15 s duration) to −70 mV (5 s duration). The observed current relaxations represent loop-gating. The portion of record marked by the black bar was obtained in CsMES bath solution containing 0.2 mM MgCl₂. The green bar demarcates the time during which the concentration of MgCl₂ was increased to 1.8 mM. The blue bar indicates the time of subsequent wash with bath solution containing 0.2 MgCl₂. The increase in extracellular [Mg²⁺] causes a marked reduction in Cx38 currents. (B) Plots of normalized fitted currents at steady-state of loop-gate closure at −70 mV in 0.2 Mg²⁺ (initial, black trace; wash, blue trace) and 1.8 mM Mg²⁺ (green trace). Current relaxations resulting from loop-gate closure reach steady-state much faster in the presence of higher Mg²⁺ concentrations. The result suggests that Mg²⁺ stabilizes the loop-gate closed state by interactions with one or more unidentified sites.
**Figure S3.** Extracellular Cd\(^{2+}\) stabilizes the loop-gate closed conformation of endogenous Cx38 hemichannels. (A) A segment of a continuous current trace evoked by a train of alternating 20-s voltage polarizations from −10 mV (15 s duration) to −70 mV (5 s duration) recorded in a bath solution containing 0.2 mM MgCl\(_2\). Relaxation of currents at −70 mV corresponds to loop-gate closure. The fast upward going deflections are transient capacitive currents unrelated to Cx38 hemichannels. The time of perfusion with 20 µM Cd\(^{2+}\) is depicted by the red bar. Peak inward currents (downward deflections) are substantially reduced by Cd\(^{2+}\) and do not fully recover when Cd\(^{2+}\) is removed by washing with bath solution containing 0.2 mM Mg\(^{2+}\). (B) Plots of normalized fitted currents at steady-state of loop-gate closure at −70 mV in 0.2 Mg\(^{2+}\) (initial, black trace; 20 µM Cd\(^{2+}\) added, red trace; wash, blue trace). Current relaxations resulting from loop-gate closure reach steady-state much faster in the presence of 20 µM Cd\(^{2+}\). The results suggest that Cd\(^{2+}\) stabilizes the loop-gate closed state by high-affinity interactions with one or more unidentified sites.

**Figure S4.** Extracellular Cd\(^{2+}\) alters \(V_g\)-gating of endogenous Cx38 hemichannels. (A) Current trace evoked by a train of alternating 20-s voltage polarizations from 10 mV (15 s duration) to 50 mV (5 s duration) recorded in a bath solution containing 0.2 mM MgCl\(_2\). Relaxation of currents at 50 mV corresponds to \(V_g\)-gate closure. The time of perfusion with 20 µM Cd\(^{2+}\) is depicted by the red bar. Peak currents are substantially reduced by Cd\(^{2+}\) and do not fully recover when Cd\(^{2+}\) is removed by washing with bath solution containing 0.2 mM Mg\(^{2+}\) (blue bar). (B) Continuation of current trace shown in A. 20 µM Cd\(^{2+}\) is reapplied for time duration depicted by the red bar. The blue bar indicates the time of wash with 0.2 Mg\(^{2+}\). (C) Plots of normalized fitted currents at steady-state of \(V_g\)-gate closure at 50 mV illustrated in A (initial, black; 20 µM Cd\(^{2+}\), red; wash, blue). (D) Plots of normalized fitted currents at steady-state of \(V_g\)-gate closure at 50 mV illustrated in B (first wash, black; 20 µM Cd\(^{2+}\), red; second wash, blue). Current relaxations resulting from \(V_g\)-gate closure reach steady-state more slowly in the presence of 20 µM Cd\(^{2+}\) and recover to the state observed initially after washing (currents shown in A). There is no difference in the time course of current relaxation with the second Cd\(^{2+}\) perfusion and wash (B). The slowing of current relaxations (C) and lack of change in time course of current relaxations (D) are inconsistent with an action of Cd\(^{2+}\) that stabilizes the \(V_g\)-closed state. Interpretation of the changes in the time course of current relaxations associated with \(V_g\)-gating are complicated by the mechanism of \(V_g\)-gating, which requires only movement of an individual connexin subunit. Further experiments are required to elucidate the effect of Cd\(^{2+}\) on peak current at positive potentials in Cx38 hemichannels.
Figure 55. Inactivation of CaCCs at hyperpolarizing potentials. A segment of a continuous current trace recorded in bath solution containing 1.8 mM CaCl$_2$ from a *Xenopus* oocyte injected with Cx32*43E1 Q56C RNA and preinjected with an anti-sense Cx38 oligonucleotide (Barrio et al., 1991). The oocyte was initially held at a voltage of −10 mV and then stepped to 50 mV. Subsequently, voltage pulses between −10 mV (15 s duration) and −70 mV (5 s duration) were repeatedly applied. The depolarizing step results in the activation of a large inward current (arrows) elicited by hyperpolarization to −70 mV that is never observed in bath solutions containing 1.8 mM MgCl$_2$. The inward current most likely represents activation of CaCC channels resulting from the influx of extracellular Ca$^{2+}$ through Q56C channels that are open at −10 mV. The inward current progressively inactivates with repeated membrane polarizations (arrows) until only Q56C currents remain. There is no transient current increase evident in the current attributable to Q56C. The result is consistent with the reported inactivation of CaCC channels by hyperpolarization (Eggermont, 2004; Hartzell et al., 2005).

REFERENCES


