Xenopus Connexin38 Forms Hemi-Gap-Junctional Channels in the Nonjunctional Plasma Membrane of *Xenopus* Oocytes

L. Ebihara
Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, North Chicago, Illinois 60064

ABSTRACT A nonselective cation current activated by depolarization (I_c) is present in the nonjunctional membrane of *Xenopus* oocytes. This current shares a number of properties with hemi-gap-junctional currents induced by exogenous gap-junctional proteins in oocytes and with a nonjunctional current seen in teleost retinal horizontal cells including nonselective permeability to small cations, block by external divalent cations, and slow activation kinetics. Here we study the effects of depleting or overexpressing Cx38 on I_c. Antisense depletion of Cx38 caused a marked reduction in I_c and blocked endogenous gap-junctional coupling in oocyte pairs. Conversely, expression of cloned Cx38 in oocytes increased the amplitude of I_c and enhanced gap-junctional coupling. Furthermore, there appeared to be a close correlation between the temperature sensitivity of I_c and the temperature sensitivity of assembly of endogenous gap-junctional channels in oocyte pairs. These results suggest that *Xenopus* connexin38 is involved in the generation of I_c.

INTRODUCTION

Gap junctions are composed of intercellular channels that allow the passage of ions and other small metabolites. Each channel is formed from two hemichannels or connexons in opposing cell membranes, which dock with each other via their extracellular domains to form a patent intercellular channel. The connexons are oligomers of 6 protein subunits called connexins. At least 12 different types of rodent connexins have been identified (Paul et al., 1991; Beyer et al., 1987; Zhang and Nicolson, 1989; White et al., 1992; Hennemann et al., 1992a,b,c; Willecke et al., 1991; Kanter et al., 1992).

In addition to forming gap-junctional channels, recent work suggests that some types of connexins can form poorly selective, voltage-gated channels blocked by extracellular calcium in the nonjunctional plasma membrane of single cells. DeVries and Schwartz (1992) reported that there was a nonjunctional membrane current in teleost retina horizontal cells that exhibits many of the properties expected of a hemi-gap-junctional current, including modulation by factors known to modulate the gap-junctional conductance between retinal horizontal cells such as dopamine, nitric oxide, and intracellular pH and permeability to the anionic dye, lucifer yellow. Additional evidence for the presence of hemi-gap-junctional channels comes from expression studies in oocytes that show that rat connexin46, chick connexin56, and bovine connexin44 can form channels in the nonjunctional plasma membrane that activate on depolarization (Paul et al., 1991; Ebihara and Steiner, 1993; Ebihara et al., 1995; Gupta et al., 1994).

Received for publication 15 November 1995 and in final form 8 May 1996.

Address reprint requests to Dr. Lisa Ebihara, Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064. Tel.: 708-578-3424; Fax: 708-578-3265.

0006-3495/96/08/742/07 $2.00

Amphibian oocytes are metabolically and electrically coupled to follicular cells through gap junctions. Gap junctions are also present between blastomeres of the amphibian embryo early in development before activation of the embryonic genome. Studies of the developmental regulation of gap-junctional proteins in the *Xenopus* embryo indicate that *Xenopus* connexin38 (Cx38) is the major gap-junctional protein in the *Xenopus* oocyte (Ebihara et al., 1989; Gimlich et al., 1990). Northern blot analysis and S1 nuclease protection assays show that Cx38 mRNA is present at the highest levels in the oocyte and egg. After fertilization, it gradually decreases through the blastula and gastrula stages and is negligible by the early neurula stage.

Besides gap-junctional channels, *Xenopus* oocytes exhibit a number of other endogenous channels, including calcium-activated chloride channels (Barish, 1983; Miledi, 1982; Miledi and Parker, 1984), calcium-independent chloride channels activated by hyperpolarization (Parker and Miledi, 1988), chloride channels activated by hypotonicity (Ackerman et al., 1994), and stretch-activated cation channels (Methfessel et al., 1986; Yang and Sachs, 1990). Recently, Arellano et al. (1995) investigated the properties of an endogenous, nonselective cation current (I_c or I_{creep}) that activates on depolarization and is blocked by divalent cations. Our present results indicate that I_c is due to the opening of endogenous hemi-gap-junctional channels in the nonjunctional plasma membrane of *Xenopus* oocytes. Furthermore, they suggest that caution must be taken to distinguish this current from hemi-gap-junctional currents induced by heterologously expressed gap-junctional proteins.

MATERIALS AND METHODS

In vitro transcription and translation

A DNA fragment containing the entire coding region of *Xenopus* Cx38 was digested with AlwNI and *Eco*RI, linked with *Eco*RI linkers, and subcloned into the *Eco*RI site of the RNA expression vector, SP64TII, between the 5′-

and the 3′-noncoding regions of *Xenopus* β-hemoglobin. This vector was derived from pSP64T (Krieg and Melton, 1984) as previously described (Ebihara et al., 1995).

Cx38 SP64TII was linearized with *Bam*HI and transcribed in vitro using SP6 RNA polymerase (Promega Corp., Madison, WI). The methylated RNA cap analogue, 5′m^{7}G(5′)ppp(5′)G (New England Biolabs, Beverly, MA), was added to the transcription buffer to produce capped RNAs. The transcripts were purified on a spun column (Boehringer Mannheim, Indianapolis, IN) to remove unincorporated rNTPs and precipitated with ethanol. The transcripts were then resuspended in diethyl pyrocarbonate-treated water, and aliquots were stored at −80°C. The quality and amount of cRNA was assessed visually using formaldehyde gel electrophoresis. To verify that the cRNA could be translated in vitro, a sample of the cRNA was translated in a rabbit reticulocyte lysate (Life Technologies, Gaithersburg, MD) in the presence of [^{35}S]methionine according to the manufacturer's instructions. The radioactively labeled translation product was separated on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel, fixed in 10% acetic acid, 40% methanol for 30 min at room temperature, and detected by fluorography.

Preparation of oocytes

Oocytes were prepared and injected with cRNA or oligonucleotides as previously described (Ebihara and Steiner, 1993). Phosphorothioate oligonucleotides (Oligos etc., OR) were used in these experiments, but identical results were obtained using unmodified deoxyoligonucleotides. The oligonucleotide antisense to mRNA for *Xenopus* Cx38 had the sequence 5′-GCT TTA GTA ATT CCC ATC CTG CCA TGT TTC-3′. This sequence corresponds to nucleotides −5 to +25 of the coding region of Cx38 (Ebihara et al., 1989). The scrambled antisense oligonucleotide had the sequence 5′-CTT TTG ACC GCT CAT CCC TAT AGT ATT TGC-3′. In the Cx38 depletion studies, oocytes were injected with 7.4 ng of antisense oligonucleotide or 4.6 ng of the permutated version of it 24–48 h before electrophysiological measurements. To study gap-junctional channel formation in paired oocytes, oocytes were stripped and paired as previously described (Ebihara and Steiner, 1993).

Metabolic labeling studies were performed by co-injecting oocytes with Cx38 cRNA and [^{35}S]methionine (Amersham, Arlington Heights, IL) and incubating them at 18°C for 6–8 h. The oocytes were then transferred to Eppendorf tubes and homogenized on ice in 5 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 1 mM EGTA containing 20 μM phenylmethylsulfonyl fluoride (lysis buffer) by passage through a 20-gauge needle. The homogenate was centrifuged at 6000 × *g* at 4°C for 5 min to pellet yolk granules. The supernatant was then centrifuged at 100,000 × *g* for 45 min at 4°C, and the membrane pellet was resuspended in fresh lysis buffer, separated by SDS-polyacrylamide gel electrophoresis, and detected by fluorography.

Electrophysiological measurements

Nonjunctional currents were measured in single oocytes using a two-microelectrode voltage-clamp technique. Junctional currents were measured in oocyte pairs using a dual two-microelectrode voltage-clamp technique (Spray et al., 1981). The experiments were controlled and data were collected using an analog-to-digital converter system (TL-1 DMA interface, 100 kHz; Axon Instruments, Foster City, CA) and PClamp6 software (Axon Instruments). Analysis and graphical presentation of the data were done with PClamp6 (Axon Instruments) and Fig P (Biosoft, Cambridge, UK) software. The external bath solution used for electrophysiological recordings was composed of (in mM): 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 15 HEPES, 0.3 $Ca(NO_3)_2$, 0.41 $CaCl_2$, and 0.82 $MgSO_4$, pH 7.4.

The base of the recording chamber was in contact with an inverted glass tempering beaker. Temperature of the bath solution was controlled by circulating heated or cooled water through the glass tempering beaker using a refrigerated circulating water bath (Hoefer Scientific, San Francisco, CA). Temperature was monitored using a thermister probe (YSI, Yellow Springs, OH) in the recording chamber during experiments. The temperature dependence of the experimental parameters was described by the temperature coefficient, Q_{10}, which was determined from the equation $Q_{10} = [X_1/X_2] \times [10/(T_2 - T_1)]$, where X_1 is the value of the experimental parameter at the lower temperature, T_1, and X_2 is the value of the parameter at the higher temperature, T_2.

RESULTS

Effect of injection of antisense oligonucleotide complementary to Cx38 mRNA

Oocytes were voltage-clamped to a holding potential of −40 mV, and a series of depolarizing steps were applied between −30 and +30 mV. At potentials more positive than −10 mV, a small, slowly activating endogenous current was observed with characteristics of the nonselective cation current (I_c) previously described by Arellano et al. (1995): slow activation kinetics, block by external divalent cations, and linear instantaneous *I-V* relationship. The amplitude of I_c showed considerable variability in different batches of oocytes but was similar in oocytes from the same frog.

Because the functional properties of I_c resembled those of currents attributed to hemi-gap-junctional channels, we decided to investigate the involvement of endogenous hemi-gap-junctional channels in the generation of I_c. First, we examined the effect of depletion of the major endogenous gap-junctional protein, *Xenopus* Cx38, by injecting oocytes with an antisense oligonucleotide complementary to the sequence of Cx38. This treatment has been previously been shown to dramatically reduce endogenous gap-junctional coupling in oocyte pairs (Barrio et al., 1991; Bruzzone et al., 1993; Hennemann et al., 1992c). The results of these experiments are summarized in Table 1. Fig. 1 shows representative current traces recorded from a cell injected with Cx38 antisense oligonucleotide. Injection of Cx38 antisense oligonucleotide produced a dramatic reduction in I_c at 24 h after injection. By 48 h after injection, I_c was completely abolished. In contrast, no reduction in I_c was observed in control, non-injected cells or in cells treated with nonsense oligonucleotides at either 24 or 48 h after injection.

TABLE 1 Effect of depletion of Cx38 on biophysical properties of I_c in oocytes

	Cx38 antisense	Control	Scramble
I_{max} (nA)	−9.35 ± 9.9 (n = 18, N = 6)	−106.2 ± 45.5 (n = 25, N = 7)	−104 ± 50.8 (n = 8, N = 3)
Resting potential (mV)	−49.25 ± 8.9 (n = 16, N = 6)	−49.3 ± 14.2 (n = 20, N = 7)	−35.4 ± 7.6 (n = 7, N = 3)
Slope conductance at −40 mV (μS)	1.44 ± 172 (n = 19, N = 6)	1.35 ± 418 (n = 27, N = 7)	1.36 ± 304 (n = 8, N = 3)

Oocytes were injected with Cx38 antisense oligonucleotide or a permuted version (scramble) of it 24–48 hours before recording. All experiments were performed at room temperature (22–23°C). I_{max} was determined from the initial amplitude of the tail current on repolarization to −40 mV after a 24-s step to 30 mV. Results are presented as means ± SEM of the indicated number of cells (n). N = number of toads.

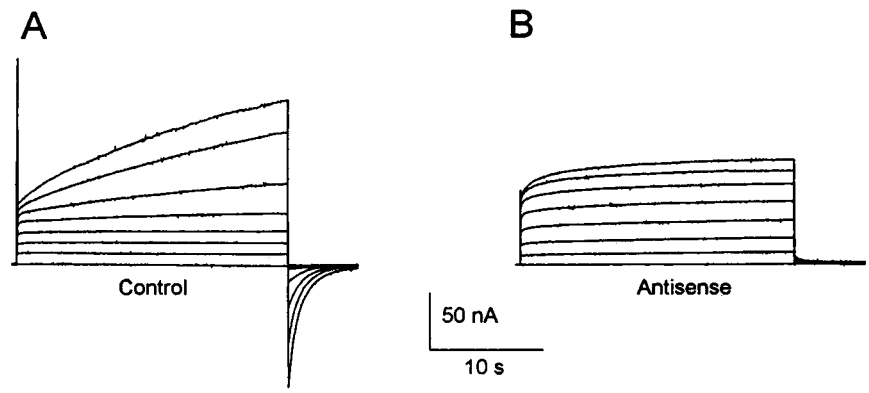

FIGURE 1 Effect of depletion of Cx38 on transmembrane currents in oocytes. Transmembrane currents recorded from control (*A*) and Cx38 antisense-injected (*B*) oocytes. Whole cell currents in oocytes were recorded in the two-microelectrode voltage-clamp configuration. Voltage clamp steps were applied in increments of 10 mV between −40 and +30 mV from a holding potential of −40 mV; the interpulse interval was 40 s, and the temperature was 22°C.

The effect of Cx38 antisense oligonucleotides appeared to be specific for I_c. No differences in resting potential or slope conductance at −40 mV were observed (Table 1). Other endogenous currents such as the voltage-dependent inward current and the calcium-activated chloride current were not affected by treatment with Cx38 antisense.

Injection of Cx38 cRNA enhances I_c

As another approach for examining the relationship between Cx38 and I_c, I_c was recorded in oocytes injected with Cx38 cRNA. The expression of Cx38 in Cx38 cRNA-injected oocytes was verified by co-injecting oocytes with [^{35}S]methionine. Fig. 2 shows an autoradiogram of an SDS-polyacrylamide gel loaded with plasma-membrane-enriched preparations from four oocytes/lane. Lane 1 was injected with water; lane 2 was injected with Cx38 cRNA. A major band with the same electrophoretic mobility as the in vitro translated Cx38 was observed in oocytes injected with Cx38 cRNA (lane 2). A band of similar size and intensity was not observed in water-injected controls.

Fig. 3B shows representative currents recorded from a Cx38 cRNA-injected oocyte 72 h after injection. Oocytes injected with Cx38 cRNA developed a large, slowly activating current, the properties of which resembled those of I_c. The amplitude of this current was significantly larger than that observed in control oocytes of the same age (Fig. 3 *A*). Fig. 3 *C* shows the amplitude of I_c as a function of time after injection. The amplitude of I_c (measured as the peak amplitude of the tail current at −40 mV) increased from

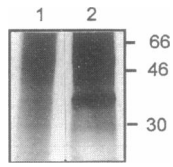

FIGURE 2 Cx38 cRNA is efficiently translated in *Xenopus* oocytes. Plasma-membrane-enriched preparations from oocytes injected with [^{35}S]methionine alone or Cx38 cRNA plus [^{35}S]methionine were run in lanes 1 and 2, respectively. The molecular mass (in kilodaltons) of protein standards are indicated on the right edge of the gel.

−95 ± 49.7 nA at day 0 (n = 16 oocytes) to −377.5 ± 114 nA at day 2 (n = 7 oocytes). The ability of Cx38 to induce gap-junctional coupling in oocyte pairs was also tested. Oocytes were injected with cRNA for Cx38, paired, and incubated overnight at 17°C. They were then incubated for 1–2 h at 24°C after which G_j was measured. Oocyte pairs injected with Cx38 cRNA had significantly higher levels of coupling than non-injected control pairs (Fig. 3 *D*). These results are similar to those previously obtained (Ebihara et al., 1989).

The I_c current expressed in oocytes injected with Cx38 cRNA activated with an exponential time course in response to a depolarizing voltage-clamp step (Fig. 4 *A*). The time course of activation became slower at more positive potentials. Unlike hemi-gap-junctional currents induced by the exogenous gap-junctional protein, rat connexin46 (Cx46), the activation kinetics of I_c did not depend on initial conditions. Changing the holding potential from −40 to −10 mV caused no significant change in the time course of activation in response to a depolarizing step to 30 mV. The voltage dependence of activation was determined from steady-state ionic currents (Fig. 4 *B*). The activation curve had a shallow voltage dependence and did not reach a maximal value over the voltage range between −10 and +30 mV. In several experiments, the reversal potential of the tail current was estimated to be ~−10 mV and the instantaneous *I-V* relation was linear. These findings are similar to the those previously reported by Arellano et al. (1995) and are consistent with the notion that I_c is nonselectively permeable to small cations such as sodium and potassium.

The Ca^{2+} dependence of I_c was measured by continuously superfusing cells with a series of solutions buffered to different calcium concentrations and determining the isochronal amplitude of the tail current at −40 mV after a 24-s pulse to 30 mV. An example of a typical experiment is shown in Fig. 4 *C*. Changing the external calcium concentration from 0.2 to 2 mM caused a marked reduction in the amplitude of I_c. I_c was maximal at calcium concentrations less than 0.5 mM and was mostly blocked at calcium concentrations greater than 2 mM. Another effect of increasing external calcium was to accelerate the time course

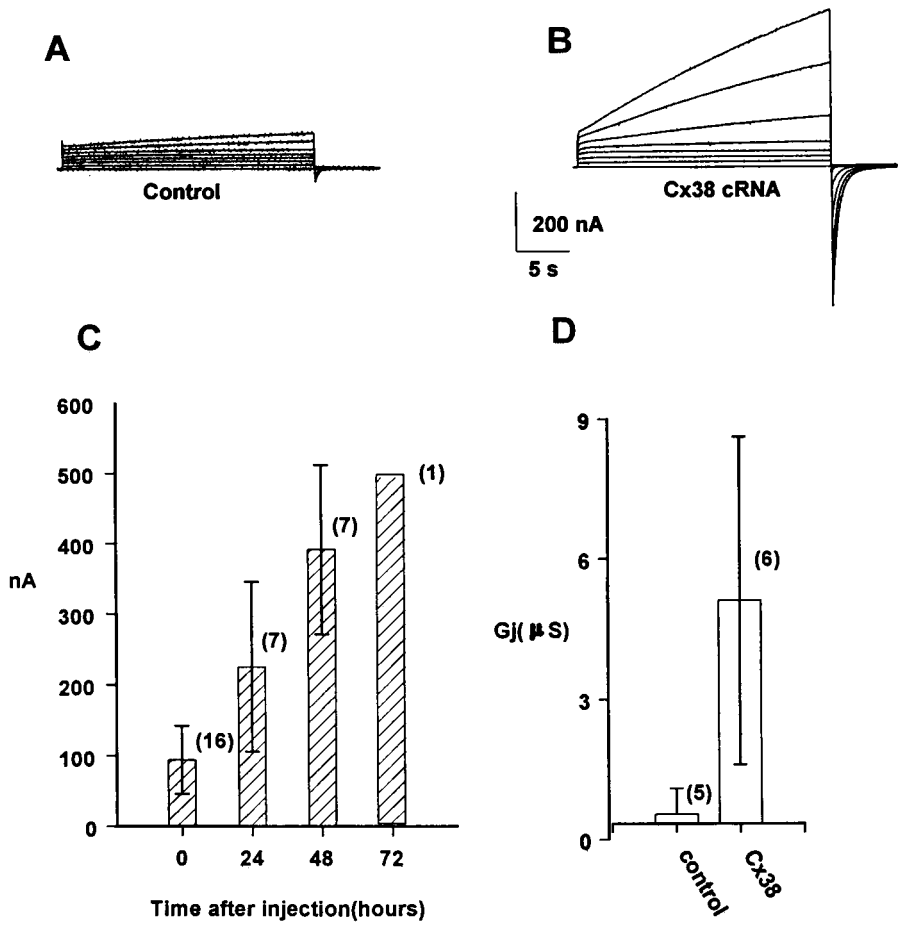

FIGURE 3 Overexpression of Cx38 increases the amplitude of I_c. (*A* and *B*) Currents in control (*A*) and Cx38 cRNA-injected (*B*) oocytes. Cx38 cRNA was injected 3 days before recording. Voltage clamp steps were applied in increments of 10 mV between −40 and +30 mV from a holding potential of −40 mV; the interpulse interval was 40 s. (*C*) Oocytes from three frogs were injected with Cx38 cRNA, and the initial amplitude of the I_c tail current (determined at −40 mV following a 24-s step to 30 mV) was recorded at different times after injection. Numbers in parentheses indicate number of oocytes tested. All experiments were performed at 23°C. (*D*) G_j was measured in non-injected oocyte pairs and in oocyte pairs injected with cRNA for Cx38. Numbers in parentheses indicate number of pairs tested.

of deactivation and reduce the steady-state holding current at −40 mV. The kinetics of activation were not altered by changing external calcium concentration.

Temperature dependence

One of the most striking characteristics of I_c was its sensitivity to changes in temperature. Fig. 5 *A* shows currents recorded from a Cx38 cRNA-injected oocyte at three different temperatures. The currents were elicited by applying a series of depolarizing voltage-clamp steps between −40 and +30 mV from a holding potential of −40 mV. Increasing the temperature by only a few degrees caused a marked increase in the amplitude of I_c and an acceleration of the activation and deactivation kinetics. These changes could be reversed by cooling the cell back to 21°C. To investigate whether the increase in I_c involved a temperature-dependent increase in the rate of transport of Cx38 from an intracellular compartment to the cell surface, we examined the effect of the fungal metabolite, brefeldin A, which had been previously shown to block export of proteins from distal Golgi compartments to the cell surface (Miller et al., 1992; Low et al., 1992). Preincubation of Cx38 cRNA-injected oocytes in 10 μg/ml brefeldin A for 1 h failed to inhibit the temperature-dependent increase in I_c (data not shown).

To study the effect of temperature on steady-state conductance, membrane currents were elicited by applying a train of hyperpolarizing voltage-clamp pulses from a holding potential of +10 mV to −50 mV while the temperature was slowly cycled between 20°C and 25°C (~0.5°C/min) (Fig. 5 *B*). Both the amplitude of the holding current at +10 mV and the amplitude of the initial tail current at −50 mV increased dramatically as the temperature was raised from 20°C to 25°C. Fig. 5 *C* shows the steady-state amplitude of I_c at +10 mV plotted as a function of temperature. Data obtained during heating and cooling are represented as open and filled symbols, respectively. Shifting the temperature from 20°C to 25°C caused a ~4.6-fold increase in amplitude corresponding to a Q_{10} of 21. Similar results were obtained in two other experiments.

We previously observed that oocyte pairs incubated at temperatures greater than 25°C exhibited much higher levels of endogenous coupling than pairs maintained at lower temperatures (Ebihara, unpublished observations). This suggests that there might be a correlation between the temperature sensitivity of I_c and the ability of Cx38 to assemble into gap-junctional channels. To further investigate this idea, the effect of temperature on the time course of gap-junctional channel assembly was measured (Fig. 6). In six pairs of non-injected oocytes from one frog, no endogenous

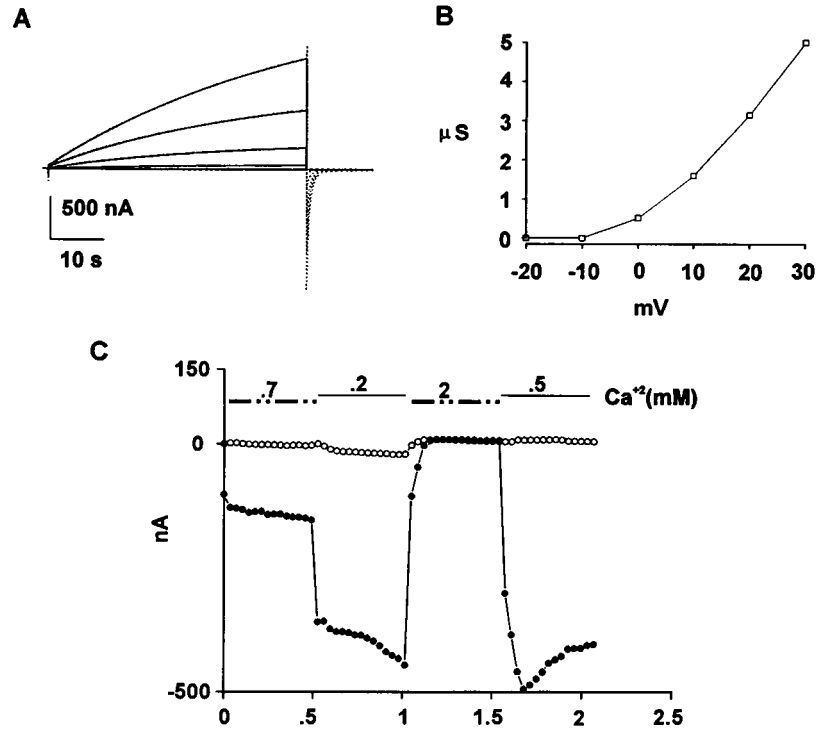

FIGURE 4 Properties of I_c in oocytes injected with Cx38 cRNA. (*A*) Time course of activation of I_c. I_c was recorded in response to a series of depolarizing voltage-clamp steps from a holding potential of −40 mV to potentials between −30 and +30 mV. The solid lines were drawn according to $I = A(1 - e(-t/\tau))$. τ increased from 19.3 s at 0 mV to 47.7 s at 30 mV. Data were corrected for a linear leakage component. Temperature was 23°C. (*B*) Steady-state activation curve. The steady-state conductance (G_∞) was determined by dividing I_c (∞) by V_{rev} where I_c (∞) was estimated by extrapolation of the currents recorded in *A* to $t = \infty$ and $V_{rev} \approx -10$ mV. (*C*) Dependence of I_c on extracellular Ca^{2+} concentration. The cells were continuously superfused with a series of solutions buffered to different calcium concentrations (*upper horizontal bars*). The isochronal tail current amplitude was measured after 24-s pulses to 30 mV and plotted as a function of time (●, Cx38 cRNA-injected oocyte; ○, Cx38 antisense-injected oocyte). The holding potential was −40 mV.

gap-junctional channel formation was observed when the pairs were maintained for 14–18 h at 19°C. Increasing the temperature to 22–23°C for 60 min failed to cause a significant increase in gap-junctional conductance, G_j. On the other hand, when the temperature was maintained at 26–28°C, there was a rapid increase in G_j in five out of five pairs tested. The increase in G_j could be only partially reversed by cooling the cell pairs back to 19°C, suggesting that, once formed, the connexins remain assembled as gap-junctional channels. Pretreatment of the oocyte pairs with

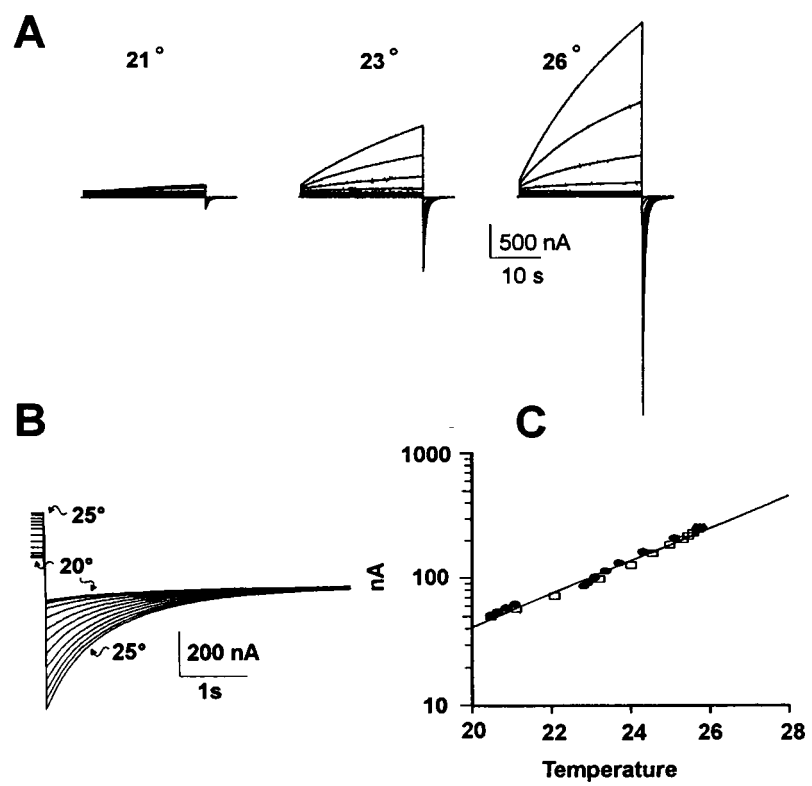

FIGURE 5 Effect of temperature on I_c. (*A*) Membrane currents recorded at three different temperatures. Currents were elicited by applying a series of depolarizing voltage-clamp steps from a holding potential of −40 mV to potentials between −30 and +30 mV. Increasing the temperature to 26°C dramatically increased the amplitude of I_c and accelerated the kinetics of activation. (*B*) Effect of temperature on steady-state amplitude of I_c. Superimposed currents were recorded during successive hyperpolarizing pulses to −50 mV from a holding potential of +10 mV as the bath temperature was cooled from 23°C to 20.4°C and then increased to 25.6°C. The interpulse interval was 2 min. Both the amplitude of the holding current at 10 mV and the initial amplitude of the tail current at −50 mV increased markedly as the temperature was increased from 20°C to 25°C. (*C*) The steady-state amplitude of I_c at +10 mV was plotted semilogarithmically versus temperature. Filled and open symbols represent data obtained during cooling and heating, respectively. The solid line is the best fit of a straight line to the data. Leakage current correction was made by subtracting the residue holding current after a hyperpolarizing prepulse to −50 mV determined at a temperature of 20°C.

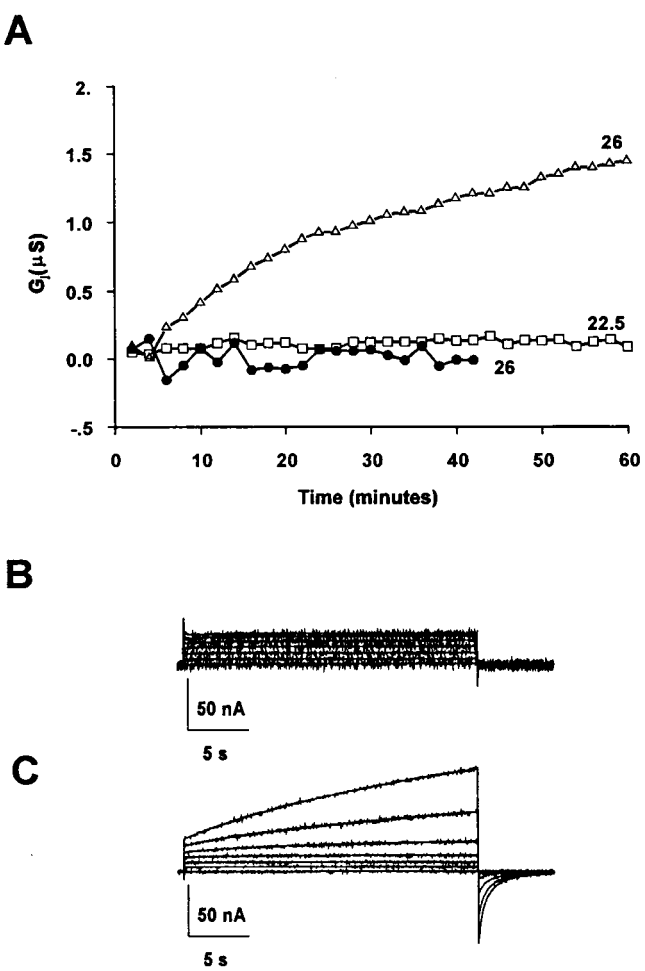

FIGURE 6 Effect of temperature on the rate of assembly of endogenous gap-junctional channels. (*A*) Gap-junctional conductance (G_j) is plotted as a function of time after changing the bath temperature to the indicated values. G_j was determined by applying a 20-mV test pulse to cell 1 at 180-s intervals and measuring the current response in cell 2. The holding potential was −40 mV. No endogenous gap-junctional channel formation was observed when pairs of control oocytes were incubated for 14–18 h at 19°C. Increasing the temperature to 22.6°C for 60 min failed to cause a significant increase in G_j (□). However, when the temperature was maintained at 26–27°C there was a rapid increase in G_j (△). In contrast, oocyte pairs pretreated with Cx38 antisense oligonucleotide (●) showed no increase in G_j in response to an increase in temperature to 26°C. (*B* and *C*) Nonjunctional currents recorded from Cx38 antisense-injected (*B*) and non-injected, control (*C*) pairs at 26–27°C. The nonjunctional current (I_m) was determined in dual voltage-clamp experiments as $I_m = I_1 - I_2$, where I_1 and I_2 are the currents recorded in cells 1 and 2, respectively, in response to a voltage-clamp step applied to cell 1. Data were recorded from the same pairs as in *A*.

Cx38 antisense oligonucleotide blocked both G_j and I_c at all temperatures (two pairs, one frog).

DISCUSSION

We have used the voltage-clamp technique to study the effect of depleting or overexpressing Cx38 on the endogenous, slowly activating outward current in *Xenopus* oocytes. Antisense depletion of Cx38 caused a marked reduction in I_c and blocked endogenous gap-junctional coupling in oocyte pairs. Conversely, expression of cloned Cx38 in oocytes increased the amplitude of I_c and enhanced gap-junctional coupling. Furthermore, there appeared to be a correlation between the temperature sensitivity of I_c and the temperature sensitivity of assembly of endogenous gap-junctional channels in oocyte pairs. These findings strongly suggest that Cx38 is involved in the generation of I_c.

The nonselective cation current described here shares a number of properties with nonjunctional currents observed in teleost retinal horizontal cells in solutions containing reduced calcium ions (DeVries and Schwartz, 1992) and with hemi-gap-junctional currents induced by exogenous gap-junctional proteins in *Xenopus* oocytes (Paul et al., 1991; Ebihara and Steiner, 1993; Ebihara et al., 1995; Gupta et al., 1994). All of the hemi-gap-junctional currents activated on depolarization were nonselectively permeable to small cations and were blocked by divalent cations. None of the currents showed significant shifts in reversal potential on replacement of all of the sodium chloride in the recording solution with various sodium salts, suggesting that the hemi-gap-junctional channels were either relatively impermeable to anions or that they discriminated very poorly between different anions. Another common feature observed for several of the channels was permeability to lucifer yellow (DeVries and Schwartz, 1992; Paul et al., 1991).

Despite these similarities, I_c could be readily distinguished from the hemi-gap-junctional current induced by the exogenous gap-junctional protein, rat Cx46, by differences in their activation kinetics. The kinetics of activation of the Cx46-induced nonjunctional current were complex and depended upon initial conditions (Ebihara and Steiner, 1993). In contrast, the kinetics of activation of I_c followed a monoexponential time course and were independent of initial conditions. The currents also exhibited differences in kinetics and steady-state voltage-dependent properties in Ca^{2+}-free test solutions (Ebihara et al., 1995; Ebihara and Steiner, 1993; Arellano et al., 1995).

Temperature dependence

The strong temperature-dependent enhancement of I_c could in principle reflect the activation of channels that had been previously inserted into the plasma membrane in an inactive form or the trafficking of Cx38 to the cell surface. However, the finding that this process was rapidly reversible on cooling and was not blocked by brefeldin A argues against the latter possibility. If the I_c channels are precursors in the formation of gap-junctional channels, then increasing the number of I_c channels in the membrane pool that are available for docking would be expected to increase the rate of gap-junctional channel formation although the rate of gap-junctional channel assembly would not necessarily parallel the rate of increase of I_c as the assembly of the gap-junctional chan-

nels is a complex process requiring multiple steps. Our experiments showing a correlation between the strong temperature dependence of the amplitude of I_c and the temperature dependence of assembly of endogenous connexins into gap-junctional channels are consistent with this notion.

In summary, the present results indicate that Cx38 is involved in the generation of an endogenous, nonselective cation current in *Xenopus* oocytes and suggest that the presence of functional gap-junctional hemichannels may be a more widespread phenomena than has been previously recognized. By studying the biophysical properties of hemi-gap-junctional channels in single oocytes using two-microelectrode voltage-clamp and single-channel recording techniques, it may be possible to obtain detailed information about the mechanisms underlying the permeability and modulation of a wide range of gap-junctional channels.

I thank E. Steiner for excellent technical assistance.
These studies were supported by National Institutes of Health grants EY10589 and HL45377.

REFERENCES

Ackerman, M. J., K. D. Wickman, and D. E. Clapham. 1994. Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J. Gen. Physiol.* 103:153–179.

Arellano, R. O., R. M. Woodward, and R. Miledi. 1995. A monovalent cationic conductance that is blocked by extracellular divalent cations in *Xenopus* oocytes. *J. Physiol.* 484.3:593–604.

Barish, M. E. 1983. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol.* 342:309–326.

Barrio, L. C., T. Suchyna, T. Bargiello, L. X. Xu, R. Roginsky, M. V. L. Bennett, and B. J. Nicholson. 1991. Voltage dependence of homo- and heterotypic cx26 and cx32 gap junctions expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA.* 88:8410–8414.

Beyer, E. C., D. L. Paul, and D. A. Goodenough. 1987. Connexin43: a protein from rat heart homologous to a gap junction protein from liver. *J. Cell Biol.* 105:2621–2629.

Bruzzone, R., J.-A. Haeffliger, R. L. Gimlich, and D. L. Paul. 1993. Connexin40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins. *Mol. Biol. Cell.* 4:7–20.

DeVries, S. H., and E. A. Schwartz. 1992. Hemi-gap-junction channels in solitary horizontal cells of the catfish retina. *J. Physiol.* 445:201–230.

Ebihara, L., V. M. Berthoud, and E. C. Beyer. 1995. Distinct behavior of connexin56 and connexin46 gap-junctional channels can be predicted from the behavior of their hemi-gap-junctional channels. *Biophys. J.* 68:1796–1803.

Ebihara, L., E. C. Beyer, K. I. Swenson, D. L. Paul, and D. A. Goodenough. 1989. Cloning and expression of a *Xenopus* embryonic gap junction protein. *Science.* 243:1194–1195.

Ebihara, L., and E. Steiner. 1993. Properties of a nonjunctional current expressed from a rat connexin46 cDNA in *Xenopus* oocytes. *J. Gen. Physiol.* 102:59–74.

Gimlich, R. L., N. M. Kumar, and N. B. Gilula. 1990. Differential regulation of the levels of three gap junction mRNAs in *Xenopus* embryos. *J. Cell Biol.* 110:597–605.

Gupta, V. K., V. M. Berthoud, N. Atal, J. A. Jarillo, L. C. Barrio, and E. C. Beyer. 1994. Bovine connexin44, a lens gap junction protein: molecular cloning, immunological characterization, and functional expression. *Invest. Ophthalmol. Vis. Sci.* 35:3747–3758.

Hennemann, H., E. Dahl, J. B. White, H.-J. Schwarz, P. A. Lalley, S. Chang, B. J. Nicholson, and K. Willecke. 1992a. Two gap junction genes, connexin 31.1 and 30.3 are closely linked on mouse chromosome 4 preferentially expressed in skin. *J. Biol. Chem.* 267:17225–17233.

Hennemann, H., H.-J. Schwarz, and K. Willecke. 1992b. Characterization of gap junction genes expressed in F9 embryonic carcinoma cells: molecular cloning of mouse connexin31 and -45 cDNAs. *Eur. J. Cell Biol.* 57:51–58.

Hennemann, H., T. Suchyna, H. Lichtenberg-Frate, S. Jungbluth, E. Dahl, H.-J. Schwarz, B. J. Nicholson, and K. Willecke. 1992c. Molecular cloning and functional expression of mouse connexin40, a second gap junction gene preferentially expressed in lung. *J. Cell Biol.* 117: 1299–1310.

Kanter, H. L., J. E. Saffitz, and E. C. Beyer. 1992. Cardiac myocytes express multiple gap junction proteins. *Circ. Res.* 70:438–444.

Krieg, P. A., and D. A. Melton. 1984. Functional messenger RNAs are produced by SPG in vitro transcription of cloned cDNAs. *Nucleic Acids Res.* 12:7057–7070.

Low, S. H., B. L. Tang, S. H. Wong, and W. Wong. 1992. Selective inhibition of protein targeting to the apical domain of MDCK cells by brefeldin A. *J. Cell. Biol.* 118:51–52.

Methfessel, C., V. Witzemann, T. Takahashi, M. Mishina, S. Numa, and B. Sakmann. 1986. Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor and sodium channels. *Pflugers Arch.* 407:577–588.

Miledi, R. 1982. A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proc. R. Soc. Lond. B Biol. Sci.* 215:491–497.

Miledi, R., and I. Parker. 1984. Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol.* 357:173–183.

Miller, S. G., L. Carnell, and H.-P. H. Moore. 1992. Post Golgi membrane traffic: brefeldin A inhibits export from distal Golgi compartments to the cell surface but not recycling. *J. Cell Biol.* 118:267–283.

Parker, I., and R. Miledi. 1988. A calcium-independent chloride current activated by hyperpolarization in *Xenopus* oocytes. *Proc. R. Soc. B.* 233:191–199.

Paul, D. L., L. Ebihara, L. J. Takemoto, K. I. Swenson, and D. A. Goodenough. 1991. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of *Xenopus* oocytes. *J. Cell Biol.* 115:1077–1089.

Spray, D. C., A. L. Harris, and M. V. L. Bennett. 1981. Equilibrium properties of voltage dependent junctional conductance. *J. Gen. Physiol.* 77:77–93.

White, T. W., R. Bruzzone, D. A. Goodenough, and D. L. Paul. 1992. Mouse cx50, a functional member of the connexin family of gap junction proteins, is the lens fiber protein MP70. *Mol. Biol. Cell.* 3:711–720.

Willecke, K., R. Heynkes, E. Dahl, H. Stutenkemper, S. Hennemann, T. Jungbluth, T. Suchyna, and B. J. Nicholson. 1991. Mouse connexin37: cloning and functional expression of a gap junction gene highly expressed in lung. *J. Cell Biol.* 114:1049–1057.

Yang, X., and F. Sachs. 1990. Characterization of stretch-activated ion channels in *Xenopus* oocytes. *J. Physiol.* 431:103–122.

Zhang, J.-T., and B. J. Nicolson. 1989. Sequence and tissue distribution of a second protein of hepatic gap junctions, Cx26, as deduced from its cDNA. *J. Cell Biol.* 109:3391–3401.