Formation of Heterotypic Gap Junction Channels by Connexins 40 and 43

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Abstract—Gap junctions formed between transfected cells expressing connexin (Cx) 40 and Cx43 (Cx43-RIN, Cx40-HeLa, and Cx43-HeLa) revealed a relationship, $g_j=f(V_j)$, at steady state, that is typified by a nonsymmetrical behavior similar to that previously reported for other heterotypic channels (gap junction conductance $g_j$; transjunctional voltage $V_j$). The unitary conductance of the channels was sensitive to the polarity of $V_j$. A main state conductance of 61 pS was found when the Cx43 cell was stepped positively or the Cx40 cell negatively ($V_j=70 \text{ mV}$); the reverse polarities yielded a conductance of 100 pS. These heterotypic channels were permeable to carboxyfluorescein. In addition, two other heterotypic forms are illustrated to demonstrate that endogenous Cx45 expression cannot explain the results. The demonstration of heterotypic Cx40–Cx43 channels may have implications for the propagation of the electrical impulse in heart. For example, they may contribute to the slowing of the impulse propagation through the junctions between Purkinje fibers and ventricular muscle. The full text of this article is available at http://www.circresaha.org.

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Key Words: gap junction ■ ion channel ■ electrophysiology

Coexpression of connexins (Cx) has been demonstrated in a number of tissues including vascular smooth muscle and myocardium. For example, Cx43 and Cx40 are present in the sinoatrial (SA)-node of dogs and guinea pigs or Cx43, Cx40, and Cx45 in the atrioventricular (AV)-node of humans, rats, and guinea pigs. This finding immediately raises the question about the types of gap junction channels present. In principle, three types of channels can be constituted, ie, homotypic, heterotypic, and heteromeric channels.

Homotypic gap junction channels are defined to consist of two identical hemichannels made from one type of connexin. Such channels have been studied extensively under in vitro conditions (eg, see References 9–11). Heteromeric channels are defined to consist of two hemichannels composed of more than one type of connexin. However, the existence of such channels in situ is still controversial. Some authors concluded from in vitro and in vivo studies that heteromeric channels exist, exhibiting properties not profoundly different from those of their homotypic counterparts (but see also References 15). Other authors concluded from in vitro studies that there is no unambiguous functional evidence for the presence of heteromeric channels. Heterotypic channels consist of two different hemichannels, each of which is made of a different type of connexin. Such channels were demonstrated unequivocally in vitro, but not every homomeric hemichannel will form a functional gap junction channel with another homomeric hemichannel derived from a different connexin.

A number of investigators have reported that Cx40 and Cx43 are unable to form functional heterotypic channels between injected oocytes and transfected HeLa cells. This finding was explained as being the result of low levels of connexin expression and/or structural incompatibility between the extracellular loops E1 and E2 of Cx43 and Cx40. There are several reasons that heterotypic Cx40–Cx43 channels may exist. One argument relies on functional evidence. Examining isolated myocytes from adult rat hearts, two authors of the present study have recently obtained electrophysiological data indicative of heterotypic Cx40–Cx43 channels (L. Polonchuk, V.V., J.A. Haefliger, H. Eppenberger, R.W., unpublished data, 1998). Another argument is based on structural similarities. A comparison of the amino acid residues of Cx40 and Cx43 indicates that 80% of E1 and 53% of E2 are conserved. A similar degree of homology exists between Cx37 and Cx43, ie, 83% and 55% for E1 and E2, respectively, two candidates that have been shown to establish heterotypic channels between oocytes and transfected N2a cells.

The aim of the present study has been to investigate the possible formation of heterotypic gap junction channels between Cx40 and Cx43. For this purpose, we have used HeLa cells transfected with either Cx40 or Cx43 and RIN...
cells transfected with Cx43. The transfectants were cocultured (Cx43-RIN cells and Cx40-HeLa cells; Cx40-HeLa cells and Cx43-HeLa cells), and the putative formation of heterotypic channels was assessed using a dual-voltage clamp method. In contrast to previous studies, the analysis of multichannel currents as well as single-channel currents revealed properties consistent with the presence of heterotypic Cx40–Cx43 channels.

Materials and Methods

Cells and Culture Conditions

Experiments were carried out on human HeLa cells transfected with cDNA coding for mouse Cx40, Cx43, and Cx45 and rat islet tumor (RIN) cells transfected with cDNA coding for rat Cx43 and chicken Cx45 DNA. HeLa cells and RIN cells were grown in DMEM and RPMI 1640 medium, respectively, supplemented with 10% FCS, 100 µg/mL streptomycin, and 100 U/mL penicillin. The media also contained 0.4 mg/mL G418 (Geneticin, Life Technologies). The cells were passaged weekly, diluted 1:10, and kept at 37°C in a CO2 incubator (5% CO2, 95% ambient air). For later identification, some cultures were previously tagged with cell tracker green (5-chloromethyl-fluorescein diacetate; Molecular Probes).12 Tagged cultures were previously tagged with cell tracker green (5-

Electrophysiological measurements were carried out on cells cultured for 1 to 3 days.

Solutions

During experiments, the cells were superfused with bath solution containing (in mmol/L) CsCl 110, KCl 5, CaCl2 2, MgCl2 1, and HEPES 10 (pH 7.4). The patch pipettes were filled with saline containing (in mmol/L) CsCl 110, MgCl2 0.1, CaCl2 0.1, EGTA 3, and HEPES 10 (pH 7.2).

Electrical Measurements

Glass coverslips with adherent cells were transferred to an experimental chamber perfused with bath solution at room temperature (21°C to 23°C). The chamber was mounted on the stage of an inverted microscope (Olympus IMT2). Patch pipettes were pulled from glass capillaries (code 7052; A-M Systems) with a horizontal puller (Sutter Instruments). When filled, the resistance of the pipettes measured 1 to 3 MΩ. Experiments were carried out on mixed cell pairs. A dual-voltage clamp method and whole-cell recording were used to control the membrane potential of both cells and to measure currents.9,10 Each cell was attached to a patch pipette connected to a separate micromanipulator (WR-88; Narishige Scientific Instrument) and amplifier (Axopatch 200). Initially, the membrane potential of cell 1 and cell 2 was clamped to the same value, \( V_i = V_o \). \( V_i \) was then changed to establish a transjunctional voltage, \( V_j = V_o - V_i \). Currents recorded from cell 2 represent the sum of two components, the junctional current, \( I_j \), and the membrane current of cell 2, \( I_m \). The current obtained from cell 1 corresponds to \( I_j \). To measure \( I_j \), both cells were held at the same holding potential, ie, \( V_i = 0 \) mV. The voltage of one of the cells was then stepped to different levels.

Signal Recording and Analysis

Voltage and current signals were recorded on chart paper (Gould RS 2400; Gould Instruments) and videotape (Neurocorder DR-384; Neuro Data Instruments). For offline analysis, the current signals were filtered at 1 kHz (low-pass filter), digitized with a 12-bit A/D converter (DT21EZ, Data Translation), and stored with a personal computer. Data acquisition and analysis were performed with custom-made software.10,24 Curve-fitting and statistical analysis were done with SigmaPlot and SigmaStat, respectively (Jandel Scientific). The data are presented as mean values ±1 SEM.

Results

The formation of homotypic channels consisting of Cx40 or Cx43 has been demonstrated in several studies examining cells in primary culture11 and transfected cells.9,10,25,26 For illustration, Figures 1A and 1B show records from a pair of transfected Cx40-HeLa cells and a pair of transfected Cx43-RIN cells, respectively. Starting from a \( V_o \) of 0 mV, bipolar pulses of 800 ms in duration were administered to establish \( V_i \) gradients of identical amplitude and either polarity. \( V_o \) was then altered from ±10 to ±110 mV using increments of 20 mV. The associated currents \( I_j \) increased proportional with \( V_o \) and showed a voltage- and time-dependent inactivation. The amplitudes of \( I_j \) were determined at the beginning (\( I_{j, \text{inst}} \); inst: instantaneous) and end of each pulse (\( I_{j, ss} \); ss: steady state) to estimate the conductances \( g_{j, \text{inst}} \) and \( g_{j, ss} \), respec-
tively. The values of \( g_{j,\text{inst}} \) were normalized with respect to \( g_{j,\text{inst}} \) and plotted versus \( V_j \). As shown in Figure 1C, this resulted in a symmetrical relationship for both types of cell pairs (●: Cx40-HeLa cells; ○: Cx43-RIN cells). The analysis revealed the following Boltzmann parameters: Cx40 (continuous curve): \( V_{1/2}=56 \) mV, \( g_{j,\text{min}}=0.18, z=2.1 \); Cx43: \( V_{1/2}=66 \) mV, \( g_{j,\text{min}}=0.23, z=2.3 \) (dashed curve). These findings are consistent with previous reports on Cx40\(^{9,26} \) and Cx43 gap junctions.\(^{10,11,25} \)

In the case of pairs of Cx40-HeLa cells, the single-channel conductances of the main state and residual state, \( \gamma_{\text{max}} \) and \( \gamma_{\text{residual}} \), were strongly asymmetrical. The instantaneous plots \( j, \text{inst} \) peaked at \( 66 \) mV, \( g_{j,\text{inst}} \) peaked at \( 135 \) and \( 26 \) pS, respectively (data not shown). In the case of pairs of Cx43-RIN cells, these conductances averaged 76 and 18 pS (data not shown). These values are consistent with previous reports on Cx40\(^{9,26} \) and Cx43 channels.\(^{10,11,25} \)

To examine whether heterotypic Cx40–Cx43 channels are formed, Cx40-HeLa cells were cocultured with Cx43-RIN or Cx43-HeLa cells (see Materials and Methods). Dual whole-cell voltage-clamp experiments were performed on cell pairs with one cell stained with cell tracker and the other devoid of it. Of 23 cell pairs examined, 20 exhibited functional coupling at the macroscopic or microscopic current level, whereas 3 showed no coupling. In the case of Cx40-HeLa–Cx43-RIN preparations, \( g_{j} \) averaged \( 3.8 \pm 1.6 \) nS (\( n=12 \)); in the case of Cx40-HeLa–Cx43-HeLa preparations, \( g_{j} \) averaged \( 6.8 \pm 2.9 \) nS (\( n=8 \)).

To further explore the properties of \( I_j \) in mixed cell pairs, the bipolar pulse protocol (see Figures 1A and 1B) was used to alter \( V_j \) from \( \pm 10 \) to \( \pm 150 \) mV and generate a family of currents. Figure 2A illustrates an experiment with a Cx40-HeLa–Cx43-RIN cell pair. Inspection of the current signals indicated that the size and time course of \( I_j \) are not only sensitive to the amplitude of \( V_j \) but also to its polarity. This means \( I_j \) was asymmetrical at each \( V_j \). Specifically, stepping \( V_j \) to make the inside of the Cx43-RIN cell negative or the inside of the Cx40-HeLa cell positive led to a large \( I_{j,\text{inst}} \) with pronounced inactivation. Conversely, stepping \( V_j \) to make the inside of the Cx43-RIN cell positive or the inside of the Cx40-HeLa cell negative led to a small \( I_{j,\text{inst}} \) with marginal or no inactivation. Consistent with these findings, inversion of the \( V_j \) polarity during bipolar pulses revealed that \( I_j \) recovery is faster in the former case. Experiments performed on Cx40-HeLa–Cx43-HeLa preparations (data not shown) yielded \( I_j \) patterns comparable to those seen in Cx40-HeLa–Cx43-RIN cell pairs.

Figures 2C and 2D summarize the data gathered from Cx40-HeLa–Cx43-RIN (\( n=10 \)) and Cx40-HeLa–Cx43-HeLa preparations (\( n=6 \)). They show the normalized relationships \( g_{j,\text{inst}} \) versus \( f(V_j) \) (○) and \( g_{j,\text{inst}} \) versus \( f(V_j) \) (●). Both plots were strongly asymmetrical. The instantaneous \( g_{j} \) increased when the cell expressing Cx43 was made negative inside and decreased when it was made positive. The increase in \( g_{j,\text{inst}} \) peaked at \( \approx 1.15 \) between \( V_j=-100 \) and \( -150 \) mV. The decrease in \( g_{j,\text{inst}} \) reached a value of \( \approx 0.7 \) at \( V_j=150 \) mV. In contrast, the steady-state \( g_j \) declined when the Cx43 cell was made negative or positive. In the former case, it decreased in a sigmoidal fashion to a quasi-stable level at \( V_j \) of \( \approx 150 \) mV. In the latter case, it decreased gradually without reaching a plateau, ie, it followed nearly \( I_{j,\text{inst}} \). The continuous curves at negative values of \( V_j \) represent the best fit of data to the Boltzmann equation using the following parameters: Figure 2C: \( V_{1/2}=-82 \) mV, \( g_{j,\text{min}}=0.31, g_{j,\text{max}}=1.03, z=1.9 \), \( n=10 \); D: \( V_{1/2}=-89 \) mV, \( g_{j,\text{min}}=0.33, g_{j,\text{max}}=0.98, z=1.7 \), \( n=6 \). When compared with the respective homotypic channels (see Figure 1C), the heterotypic channels exhibit a more negative \( V_{1/2} \) and a broader voltage sensitivity. This is consistent with previous reports on heterotypic Cx26–Cx32\(^{16–18} \) and Cx37–Cx43 channels.\(^{12} \)

Diffusion experiments on Cx40-HeLa–Cx43-RIN cell pairs indicated that Cx40–Cx43 channels are permeable to 5-(and 6-) carboxyfluorescein (Molecular Probes), the fluorescent anionic dyes (Figure 2B).

Figure 3 illustrates experiments designed to study the \( V_j \) sensitivity of single heterotypic channels. Figure 3A shows records of a Cx40-HeLa–Cx43-RIN cell pair whose gap...
junction contained a single operational channel. Biphasic pulses were applied to cell 1 ($V_1$; Cx43-RIN cell) while gap junction currents were recorded from cell 2 ($I_2$; Cx40-HeLa cell). As illustrated for $V_f = \pm 70$, $\pm 90$, and $\pm 110$ mV, $I_2$ showed three discrete levels corresponding to the closed state (continuous lines), main state, and residual state (dashed lines). The analysis indicated that the unitary currents are smaller when cell 1 is depolarized (positive $V_f$) than when it is hyperpolarized (negative $V_f$). Moreover, residual currents were preferentially seen in the latter case, i.e., voltage gating was virtually absent at positive $V_f$. The observed single channel conductances were $\gamma_{f, \text{main}} = 60/100$ pS (positive/negative $V_f$); $\gamma_{f, \text{residual}} = 14$ to 20 pS (negative $V_f$).

Figure 3B shows an experiment carried out on a Cx40-HeLa–Cx43-RIN cell pair whose gap junction consisted of several operational channels. This time a bipolar voltage-ramp protocol was used to alter $V_f$ from $-50$ to $50$ mV and back to $-50$ mV again. The ramps evolved at a rate of 100 mV/200 ms (see $V_1$ and $V_2$). The associated junctional current changed linearly with time with a distinct break at $V_f = 0$ mV ($I_f$). The dashed lines were aligned to the shallower segments and correspond to a slope conductance of 0.64 nS, indicating the involvement of at least 10 channels. The steeper segment yielded a slope conductance of $\approx 1$ nS. Conceivably, the break in slope reflects the $V_f$ sensitivity of the channels. An involvement of channel inactivation seems unlikely because of the short duration of the ramp and the small amplitude of $V_f$.

Figure 3C summarizes the combined results from the Cx40-HeLa–Cx43-RIN cell pairs ($n = 7$). The plot $\gamma_{f, \text{main}} = f(V_f)$ (●) demonstrates that over the voltage range examined, i.e., $\pm 150$ mV, the channels exhibit a strong dependence on $V_f$ polarity and a weak dependence on $V_f$ amplitude. Because of limitations of resolution, it was not possible to gain reliable data for $V_f < 30$ mV. The plot $\gamma_{f, \text{residual}} = f(V_f)$ (▼) indicates that the channels also exhibit a weak dependence on $|V_f|$.

Figure 3D compares the I-V relationships of the single-channel data documented in Figure 3C and the multichannel data presented in Figure 2C. The currents $I_{f, \text{main}}$ (●) and $I_{f, \text{residual}}$ (▼) were normalized with respect to the values at $V_f = -150$ mV, averaged, and plotted versus $V_f$, respectively. The currents $I_{f, \text{residual}}$ (▼) were scaled to account for the normalization of the $I_{f, \text{main}}$ values, averaged, and plotted versus $V_f$. The analysis yielded the following slopes for the relationships $I_f$ normalized $= f(V_f)$: multichannel currents: $-3.93 \times 10^{-4}$ to $-6.85 \times 10^{-3}$ mV$^{-1}$ ($r^2 = 0.98/0.99$) for positive/negative $V_f$; single-channel currents, main state: $-3.90 \times 10^{-4}$ to $-6.74 \times 10^{-4}$ mV$^{-1}$ ($r^2 = 0.99/0.99$); residual state: $-8.99 \times 10^{-4}$ to $-7.23 \times 10^{-4}$ mV$^{-1}$ ($r^2 = 0.83/0.98$).

Involvement of Intrinsic Connexins. The possible contribution of endogenous coupling in the mixed-cell pairs was explored by studying a number of relevant combinations of HeLa and RIN cells. For this purpose, small test pulses (10 mV, 70 ms) were administered to one cell of a pair to determine $g_f$ without interference from voltage and time dependence. The Table summarizes the results gathered from the different types of cell pairs. A comparison of the $g_f$ data indicates that both HeLa cells and RIN cells express a marginal level of intrinsic connexins. Pairs consisting of identical parental cells (HeLa–HeLa and RIN–RIN) yielded a weak coupling (0.04 and 0.13 nS) or no coupling at all (90% and 83%). Similarly, mixed pairs containing one transfectant (Cx43-RIN–HeLa, Cx43-HeLa–HeLa, and Cx40-HeLa–HeLa) also showed weak coupling (not detectable, 0.04 nS, respectively) or no coupling (100%, 100%, and 87%, respectively). In contrast, mixed pairs consisting of two transfectants (Cx40HeLa–Cx43-RIN, Cx40-HeLa–Cx43-HeLa) yielded significant coupling (3.8 and 6.8 nS, respectively) and few coupling failures (0% and 20%, respectively). Hence, these pairs exhibit a 170- to 190-fold or larger $g_f$ than the respective control pairs (Cx40HeLa–Cx43-RIN versus Cx40-HeLa–RIN and Cx43-RIN–HeLa; Cx40-HeLa–Cx43-HeLa versus Cx40-HeLa–HeLa and Cx43-HeLa–HeLa). This suggests that coupling in pairs of transfected cells occurs by exogenous rather than
endogenous connexins. The $g_j$ data for homotypic pairs of Cx40-HeLa, Cx43-HeLa, and Cx43-RIN cells in the Table are comparable to those previously reported for these cell lines.\textsuperscript{9,23,27} Transfection increased the intercellular coupling at least 100-fold.\textsuperscript{27}

The Table also summarizes the unitary conductances determined from these experiments. Pairs of parental cells showed a value of 40 to 50 and 30 pS (HeLa–HeLa and RIN–RIN), respectively. This is in agreement with Cx45–Cx45 channels in HeLa cells.\textsuperscript{28,29} Pairs of Cx43-RIN cells and Cx43-HeLa cells yielded a value of 76 and 72 pS, respectively, indicative of Cx43–Cx43 channels,\textsuperscript{10,11,25} whereas pairs of Cx40-HeLa gave a value of 135 pS, comparable with Cx40–Cx40 channels.\textsuperscript{9,26} Pairs of Cx40-HeLa–HeLa or Cx40-HeLa–RIN might be expected to form Cx40–Cx45 channels. A few unitary conductance values yielded 30 to 40 pS, which are not consistent with the Cx40–Cx43 heterotypic channel in the present study. The unitary conductances quoted for the pairs Cx40-HeLa–Cx43-RIN and Cx40-HeLa–Cx43-HeLa refer to the values prevailing at $V_j = \pm 70$ mV. They were taken from the experiments already described in Figure 3. Finally, when examining Cx40 and Cx43 transfectants, we rarely noticed signs of endogenous channels (see also Reference 9). Conceivably, they may even be suppressed after transfection from a functional point of view.

The bipolar pulse protocol was then used to study multichannel currents from a Cx43-HeLa–Cx45-HeLa cell pair (Figure 4A) and a Cx40-HeLa–Cx45-HeLa cell pair (Figure 4B). In the former case, the stepping $V_f$ to make the inside of the Cx45-HeLa cell negative or the inside of the Cx43-HeLa cell positive resulted in an $I_{in}$ with pronounced inactivation. Conversely, stepping $V_f$ to make the inside of the Cx45-HeLa cell positive and the inside of the Cx43-HeLa cell negative led to an $I_{in}$ with distinct activation. In the latter case, the current signals revealed a similar picture, i.e., $I_{in}$ inactivated when the inside of the Cx45-HeLa cell was made negative or the inside of the Cx40-HeLa was made positive.

Figures 4C and 4D show the analysis of the current records depicted in Figures 4A and 4B. They represent the normalized relationships $g_j$ versus $V_j (\circ)$ and $g_s$ versus $V_j (\bullet)$. Both plots were strongly asymmetrical. In the case of Cx43-HeLa–Cx45-HeLa (Figure 4C), the instantaneous $g_j$ decrease was more pronounced when the cells expressing Cx45 were made negative. In the case of Cx40-HeLa–Cx45-HeLa (Figure 4D), the instantaneous $g_j$ decreased when Cx45 cells were made positive. In contrast, the steady-state $g_j$ increased when the cell expressing Cx45 was made positive inside and decreased when it was made negative. Note that $g_j$ was far from steady state at the end of the 400-ms pulse applied (see Figures 4A and 4B).

When compared with Cx40-HeLa–Cx43-RIN (see Figures 2A and 2C) and Cx40-HeLa–Cx43-HeLa cell pairs (see Figure 2D), the currents and conductances of Cx43-HeLa–Cx45-HeLa (Figures 4A and 4C) and Cx40-HeLa–Cx45-HeLa cell pairs (see Figures 4B and 4D) behave differently. They deviate with regard to $V_j$ dependence, gating polarity, current patterns, kinetics of inactivation, and the presence or absence of activation of $I_j$. This suggests that Cx43–Cx45 and Cx40–Cx45 channels are likely not to be involved in the Cx40-HeLa–Cx43-RIN and Cx40-HeLa–Cx43-HeLa cell pairs.

**Discussion**

Our experiments indicate that the incidence of electrical coupling in parental HeLa cells and RIN cells is low. After transfection with Cx40 or Cx43, coupling was enhanced ~200-fold in Cx40-HeLa–Cx43-HeLa and Cx40-HeLa–Cx43-RIN cell pairs. This suggests formation of heterotypic Cx40–Cx43 channels.

The multichannel and single-channel data presented indicate that Cx40–Cx43 channels are present in Cx40-HeLa–Cx43-HeLa and Cx40-HeLa–Cx43-RIN cell pairs. With regard to multichannel data, the relationships $g_s=f(V_j)$ and $g_{in}=f(V_j)$ were asymmetrical, a property seen in heterotypic gap junctions whose connexons exhibit widely different

### Electrophysiological Data Obtained From Different Types of Cell Pairs

<table>
<thead>
<tr>
<th>Investigated Cell Pairs</th>
<th>Coupled Cell Pairs</th>
<th>Total Conductance ($g_j$, nS)</th>
<th>Single-Channel Conductance ($g_{in}$, pS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIN–RIN</td>
<td>18</td>
<td>3</td>
<td>0.13</td>
</tr>
<tr>
<td>HeLa–HeLa</td>
<td>29</td>
<td>3</td>
<td>0.04</td>
</tr>
<tr>
<td>Cx43-RIN–Cx43-RIN</td>
<td>13</td>
<td>13</td>
<td>19.0 ± 3.9</td>
</tr>
<tr>
<td>Cx43-HeLa–Cx43-HeLa</td>
<td>11</td>
<td>11</td>
<td>21.4 ± 2.8</td>
</tr>
<tr>
<td>Cx40-HeLa–Cx40-HeLa</td>
<td>12</td>
<td>12</td>
<td>25.1 ± 5.9</td>
</tr>
<tr>
<td>Cx43-RIN–HeLa</td>
<td>10</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Cx43-HeLa–HeLa</td>
<td>11</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Cx40-HeLa–HeLa</td>
<td>15</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>Cx40-HeLa–RIN</td>
<td>9</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Cx40-HeLa–Cx43-RIN</td>
<td>15</td>
<td>12</td>
<td>3.80 ± 1.6</td>
</tr>
<tr>
<td>Cx40-HeLa–Cx43-HeLa</td>
<td>8</td>
<td>8</td>
<td>6.8 ± 2.0</td>
</tr>
</tbody>
</table>

*Cell pairs consisting of different types of cells were examined for total gap junction conductance, $g_j$, and unitary conductance, $g_{in}$. The numbers refer to mean values ±1 SEM. n.d. indicates not detectable.*
properties (eg., Cx26–Cx32,16–18 or Cx37–Cx43.12,21 In the case of Cx40–Cx43, rectification was prominent for both relationships, ie., \( g_{\text{ss}} = f(V_j) \) and \( g_{\text{inst}} = f(V_j) \) (see Figures 2C and 2D). Voltage gating was almost exclusively observed at negative \( V_j \). This is consistent with the notion that Cx40 is gating with positive polarity (V.V., R.W., P.R.B., unpublished observations, 1999) and Cx43 with negative polarity.30 With regard to single-channel data, the function \( \gamma_{\text{inst}} = f(V_j) \) was characterized by two phenomena, a strong dependence on \( V_j \) polarity and a weak dependence on \( V_j \) amplitude (see Figure 3C). Although the latter has been anticipated on theoretical grounds, the former is not.31 Because of limited resolution, values of \( g_{\text{inst}} \) (and \( g_{\text{residual}} \)) could not be determined accurately for \( V_j < 30 \text{ mV} \). Hence, the function \( g_{\text{inst}} = f(V_j) \) remains undefined over this voltage range. It may undergo an abrupt change at \( V_j = 0 \text{ mV} \) or a gradual change over a narrow range of \( V_j \) values, ie., from \(-30 \) to \( 30 \text{ mV} \). The function \( \gamma_{\text{residual}} = f(V_j) \) showed a distinct voltage dependence for either \( V_j \) polarity.

Significant interference from endogenous connexins is unlikely because of the low level of expression. This conclusion is based on several observations. For example, coupling between parental cells and between Cx40 or Cx43 transfectants and parental cells is rudimentary or absent (see Table). Moreover, the incidence of single-channel currents attributable to intrinsic connexins is extremely low in pairs of transfectants. Furthermore, pairs of Cx45-HeLa cells with Cx40-HeLa or Cx43-HeLa (Cx43-RIN) cells yielded asymmetrical relationships \( g_{\text{ss}} = f(V_j) \) distinctly different from those seen in Cx40-HeLa–Cx43-HeLa or Cx40-HeLa–Cx43-RIN cell pairs (compare Figures 2 and 4). Most striking is the change in \( V_j \) polarity responsible for channel gating. Such properties were not detectable in our data from Cx40-HeLa–Cx43-HeLa or Cx40-HeLa–Cx43-RIN cell pairs (compare Figures 2 and 4). This suggests that intrinsic connexins are expressed at a low level of expression. Finally, the properties of single channels seen between parental cells and between Cx40 or Cx43 transfectants and parental cells are different from those observed in Cx40-HeLa–Cx43-HeLa or Cx40-HeLa–Cx43-RIN cell pairs (see Table).

What are the mechanisms underlying rectification of \( g_{\text{inst}} \) and \( g_{\text{ss}} \) observed for Cx40–Cx43 gap junctions and gap junction channels? On the one hand, \( g_{\text{inst}} \) reflects the properties of voltage-sensitive gating of the channels. Hence, rectification of \( g_{\text{inst}} \) can be explained by the opposite gating polarity of Cx40 and Cx43 (see above). On the other hand, \( g_{\text{ss}} \) reflects the properties of \( \gamma_{\text{ss}} \). Hence, rectification of \( g_{\text{inst}} \) can be explained by the properties of hemichannels arranged in series. It has been shown that the symmetrical functions \( g_{\text{ss}} = f(V_j) \) of a homotypic gap junction require two hemichannels with an identical nonlinear \( I/V \) relationship.31 Compared with this, two hemichannels with different properties led to the asymmetrical functions characteristic of heterotypic gap junctions. This suggests that the hemichannel functions \( \gamma_{\text{ss}} = f(V_j) \) of Cx40 and Cx43 arranged in series determine the properties of \( \gamma_{\text{inst}} = f(V_j) \).

Rectification of \( \gamma_{\text{ss}} \) (and \( \gamma_{\text{residual}} \)) may be caused by interactions between ionic charge carriers and channel structures. Alternatively, it may reflect alterations of the effective channel geometry caused by the electric field. Another possibility is that the channels exhibit rapid gating beyond the time resolution of the data recording (1 to 2 ms). Yet another possibility is partial channel block. However, the data in Figure 3C argue against this because the block did not increase with increasing \( V_j \).

The heart is a prominent organ whose tissues coexpress Cx40, Cx43, and Cx45.32,33 During maturation, mouse hearts show overlapping (left ventricle) and complementary (atrium) expression of Cx40, Cx43, and Cx45.32–34 In adult hearts, Cx40 and Cx43 are distributed unequally: SA-node: Cx40>>Cx43; atrium: Cx40>>Cx43; AV-node: Cx43>>Cx40 (however, Cx45 is the major connexin); Purkinje fiber: Cx40>>Cx43; and ventricle: Cx43>>Cx40. Cx45 distribution is controversial. Coppen et al32 have demonstrated prevalent Cx45 expression in conduction tissues whereas Alcolea et al33 have presented data that indicate a downregulation of Cx45 throughout the pacemaker-conducting system such that the distribution appears to be slight in adult heart. Hence, prominent regions with coexpression of Cx40 and Cx43, and possibly Cx45, include junctional interphases in tissues such as Purkinje fibers and ventricular muscle or atrial
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References


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