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Functional Analysis of Selective Interactions among Rodent Connexins

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One consequence of the diversity in gap junction structural proteins is that cells expressing different connexins may come into contact and form intercellular channels that are mixed in connexin content. We have systematically examined the ability of adjacent cells expressing different connexins to communicate, and found that all connexins exhibit specificity in their interactions. Two extreme examples of selectivity were observed. Connexin40 (Cx40) was highly restricted in its ability to make heterotypic channels, functionally interacting with Cx37, but failing to do so when paired with Cx26, Cx32, Cx43, Cx46, and Cx50. In contrast, Cx46 interacted well with all connexins tested except Cx40. To explore the molecular basis of connexin compatibility and voltage gating, we utilized a chimera consisting of Cx32 from the N-terminus to the second transmembrane domain, fused to Cx43 from the middle cytoplasmic loop to the C-terminus. The chimeric connexin behaved like Cx43 with regard to selectivity and like Cx32 with regard to voltage dependence. Taken together, these results demonstrate that the second but not the first extracellular domain affects compatibility, whereas voltage gating is strongly influenced by sequences between the N-terminus and the second transmembrane domain.

INTRODUCTION

Cells communicate directly with their neighbors through specialized intercellular channels present in gap junctions. Genes encoding the structural components of these channels have been cloned and comprise a family of highly related proteins, the connexins (Cx). Connexins oligomerize into channels called connexons that span a single plasma membrane; complete intercellular channels spanning two plasma membranes are formed when connexons in adjacent cells align. Presently, thirteen different connexins have been identified and cloned in rodents (Dermietzel and Spray, 1993; White *et al.*, 1995). Individual connexins display unique spatial and temporal patterns of expression (Paul, 1985; Beyer *et al.*, 1989; Dermietzel *et al.*, 1989; Gimlich *et al.*, 1990; Nishi *et al.*, 1991; Risek and Gilula, 1991; Valdimarsson *et al.*, 1991; Goliger and Paul, 1994). In addition, channels composed of different connexins exhibit distinct functional properties with regard to unitary conductance (Fishman *et al.*, 1990; Moreno *et al.*, 1991; Veenstra *et al.*, 1994), gating by voltage (Bennett *et al.*, 1991; Chen and DeHaan, 1992; Veenstra *et al.*, 1992; Nicholson *et al.*, 1993) and by phosphorylation (Swenson *et al.*, 1990; Takens-Kwak and Jongsma, 1992; Kanemitsu and Lau, 1993; Moreno *et al.*, 1994), and permeability (Steinberg *et al.*, 1994; Veenstra *et al.*, 1994). An important issue to be resolved is how differences in connexin distribution or channel properties contribute to the function of organs constructing gap junctions from these proteins (Goodenough and Musil, 1993).

One consequence of connexin diversity is that cells expressing different connexins may come into contact

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and form intercellular channels that are mixed in connexin content. This idea is supported by Northern analyses of connexin mRNAs, which indicate that certain organs express multiple connexins (Willecke *et al.*, 1991; Haefliger *et al.*, 1992; Kanter *et al.*, 1992; Dermietzel and Spray, 1993; Paul *et al.*, 1993), and by the immunolocalization of multiple connexin proteins within single gap junctions (Nicholson *et al.*, 1987; Traub *et al.*, 1989; Paul *et al.*, 1991; Risek *et al.*, 1994). Because intercellular channels span two plasma membranes, they may be defined as homotypic, when both connexons are composed of the same connexin, or heterotypic, when each connexon contains a different connexin. Using in vitro functional expression systems, it has been shown that some connexins can form both homotypic and heterotypic channels (Dahl *et al.*, 1987; Swenson *et al.*, 1989; Werner *et al.*, 1989; Barrio *et al.*, 1991). These studies indicate that heterotypic intercellular channels display asymmetric properties with respect to voltage-dependent closure and support the suggestion that such channels could account for the asymmetric voltage sensitivity observed in vivo at rectifying electrotonic synapses (Giaume *et al.*, 1987).

Although it had been generally accepted that cellular coupling could be established between different cell types, we have previously used the paired oocyte system to demonstrate that some connexins are restricted in their ability to form heterotypic channels (Bruzzone *et al.*, 1993; White *et al.*, 1994b). The inability of certain connexin combinations to form heterotypic channels provides a simple mechanism to limit or segregate communication that could have profound biological consequences. Selective communication could explain the observation of "communication compartments," which are characterized by clusters of cells that are permeable to dyes within, but not between, adjacent groups (Lo and Gilula, 1979; de Laat *et al.*, 1980; Warner and Lawrence, 1982; Weir and Lo, 1982; Meda *et al.*, 1983; Blennerhasset and Caveney, 1984; Kam *et al.*, 1987; Chanson *et al.*, 1991; Lo Turco and Kriegstein, 1991; Yuste *et al.*, 1992).

The goal of this study was to examine whether selective formation of heterotypic channels is a common property shared by all members of the connexin family. We demonstrate that adjacent *Xenopus* oocytes that are programmed for expression of different connexins have a high probability of not establishing intercellular communication. Fourteen of twenty-seven heterotypic connexin combinations failed to become electrically coupled. Two extreme examples of selectivity were found. Cx40 was highly restricted in its ability to make heterotypic channels, functionally interacting with Cx37, but failing to do so when paired with Cx26, Cx32, Cx43, Cx46, or Cx50. In contrast, Cx46 was quite indiscriminate, forming intercellular channels with Cx26, Cx32, Cx43, and Cx50. Furthermore, we have used a chimeric connexin to define the domains involved in the regulation of compatibility between connexins and of voltage gating. Our results demonstrate that all connexins exhibit selectivity in heterotypic interactions and that the second but not the first extracellular domain affects compatibility, whereas voltage gating is strongly influenced by sequences between the N-terminus and the second transmembrane domain.

MATERIALS AND METHODS

In Vitro Transcription

Cx26, Cx32, Cx37, Cx40, Cx43, Cx46, and Cx50 were previously subcloned (Swenson *et al.*, 1989; Paul *et al.*, 1991; White *et al.*, 1992; Bruzzone *et al.*, 1993) into the transcription vector SP64T (Krieg and Melton, 1984). The strategy for the production of chimeric connexins and some properties of the chimera designated as 3243H4 have been previously described (Bruzzone *et al.*, 1991, 1994). Constructs were linearized with restriction endonucleases, and capped mRNAs were transcribed in vitro with SP6 RNA polymerase using the mMessage mMachine (Ambion Inc., Austin, TX) according to the manufacturer's instructions. Purity and yield of transcribed mRNA were assessed using agarose gel electrophoresis, and by comparing the intensity of ethidium bromide staining to a known standard (RNA ladder, Life Technologies, Grand Island, NY).

Preparation of Xenopus Oocytes

Oocytes were collected from *Xenopus laevis* females and processed for the paired oocyte expression assay as described (Swenson *et al.*, 1989), except that the final concentration of calcium in the modified Barth's medium was adjusted to 2.9 mM with $CaCl_2$ in experiments utilizing Cx46 (Ebihara and Steiner, 1993). To eliminate the possible contribution of endogenous intercellular channels to the measured conductance, defolliculated oocytes were injected with an antisense oligonucleotide (Barrio *et al.*, 1991) corresponding to a region within the coding sequence of *Xenopus* Cx38 (3 ng/oocyte, 5′-CTGACTGCTCGTCTGTCCACACAG-3′; Bruzzone *et al.*, 1993). The efficacy of the antisense treatment was tested by measuring the recruitment of *Xenopus* Cx38 into heterotypic channels with Cx43 or Cx37 in the presence of injected oligonucleotides. After incubation for 24 to 72 h at 19°C, antisense-treated oocytes were then injected with 40 nl of connexin mRNA (10–100 pg). It has been our experience that although all connexin mRNAs induce conductance in a dose-dependent manner, individual connexins vary in the specific concentration of mRNA required to achieve any given level of conductance. In the experiments described here, the amounts of connexin mRNAs were varied so that homotypic channels had similar levels of conductance regardless of the connexin type. For heterotypic channel formation, each oocyte was injected with the same concentration of mRNA used in the homotypic experiments, so that for each individual connexin the levels of homotypic vs. heterotypic conductance are comparable. It should be noted, however, that channels composed of a single connexin exhibit multiple conductance states (Chen and Dehaan 1992; Veenstra *et al.*, 1994), and that equal macroscopic conductances for different homotypic channels do not imply similar numbers of actual open channels. After RNA injection, oocytes were stripped of their vitelline membranes and paired in homotypic (same connexin mRNA in each oocyte) or heterotypic (different connexin mRNAs injected in each oocyte) configurations.

Electrophysiological Measurements

Intercellular communication was directly quantitated by double voltage clamp (Spray *et al.*, 1981) 24–48 h after oocyte pairing.

Voltage clamping of oocyte pairs was performed using two GeneClamp 500 amplifiers (Axon Instruments, Foster City, CA) controlled by an IBM-PC compatible computer through a Digidata 1200 interface (Axon Instruments). C-Lab II software (Indec System, Sunnyvale, CA) was used to program stimulus and data collection routines. Current outputs were filtered at 10 Hz for steady-state measurements, where the sampling interval was 150 ms and at 500 Hz for initial measurements, where the sampling interval was 1 ms. Electrodes had a resistance of 1–2 MΩ and were filled with 3 M KCl, 10 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, and 10 mM *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid, pH 7.4. Both cells of a pair were initially clamped at −40 mV to ensure zero transjunctional potential. To impose a transjunctional potential, one cell was depolarized 10 mV, while the other cell was held at −40 mV. Current delivered to the cell clamped at −40 mV during the voltage pulse was equal in magnitude to the junctional current, and was divided by the voltage to yield the conductance. To ensure adequate control of voltage across the transjunctional membrane and avoid the risk of overestimating the actual transjunctional potential at steady state (Wilders and Jongsma, 1992), oocyte pairs exhibiting conductance less than 5 μS were selected for analysis of voltage dependence. Transjunctional potentials of opposite polarities were generated by hyperpolarizing or depolarizing one cell in 10 mV steps, while clamping the second cell at −40 mV. After the imposition of voltage steps, initial currents were resolved at 5–10 ms, and steady state currents were measured at 30 s. Initial and steady-state conductance values were normalized to their value at ± 10 mV, and plotted against the transjunctional potential. Data were fit to a Boltzmann equation in the form: $G_j ss = (G_j max - G_j min)/\{1 + \exp(A[V - V_0])\} + G_j min$, where $G_j ss$ is the steady-state conductance, $G_j max$ is maximum conductance over the range of $V_j s$ tested, $G_j min$ is minimum conductance, A is proportional to the number of electron charges moving through the applied voltage, and V_0 is the voltage at which the decrease in $G_j ss$ is half maximal (Spray *et al.*, 1981).

RESULTS

The ability of different rodent connexins to form heterotypic channels was systematically tested using the paired *Xenopus* oocyte expression system (Swenson *et al.*, 1989; Dahl, 1992). Experiments were designed to examine the six possible interactions (three homotypic, three heterotypic) among subgroups containing three connexins each. The connexins in each subgroup were chosen so that one of the heterotypic combinations was previously known to be functional. Therefore, each set of experiments tested two novel heterotypic combinations. Five such combinations are illustrated in Figures 1 and 2.

As previously reported, connexins 26, 32, 37, 40, 43, 46, and 50 made homotypic channels in the oocyte system (Figure 1; Swenson *et al.*, 1989; Barrio *et al.*, 1991; White *et al.*, 1992; Bruzzone *et al.*, 1993; Ebihara and Steiner, 1993). To eliminate any possible contribution of endogenous *Xenopus* Cx38 (Swenson *et al.*, 1989; Werner *et al.*, 1989; Bruzzone *et al.*, 1993) to our measurements, we used antisense DNA oligonucleotide injection to deplete the endogenous connexin. The efficacy of this treatment was demonstrated by constructing Cx37/H_2O and Cx43/H_2O pairs, because Cx37 and Cx43 readily

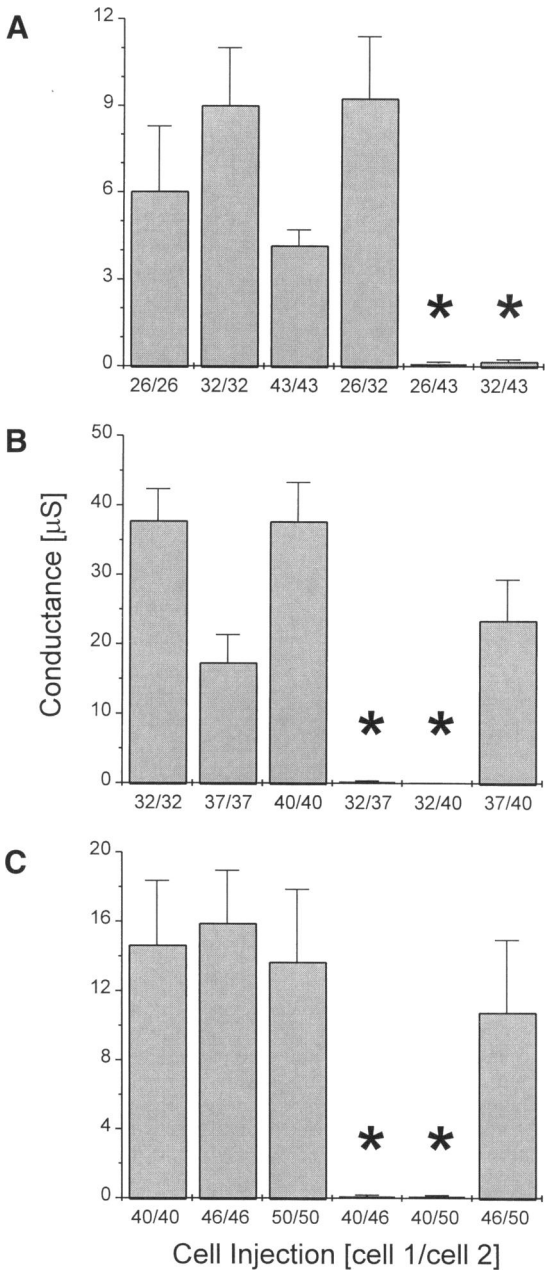

Figure 1. The formation of intercellular channels is a selective process dependent on connexin compatibility. Oocytes pre-treated with an oligonucleotide antisense to *Xenopus* Cx38 were injected with the specified connexin mRNAs and paired for 24–48 h before measuring junctional conductance by dual voltage clamp. Asterisks denote the novel combinations tested. (A) Heterotypic Cx26/Cx43 and Cx32/Cx43 pairs did not develop junctional conductance above background levels (cf. with Cx43/antisense in Table 1). Values are means ± SEM of 7–12 pairs from two to three independent experiments. (B) Cx32 failed to interact with either Cx37 or Cx40 (cf. with Cx37/antisense in Table 1). Values are means ± SEM of 6–20 pairs from two to three independent experiments. (C) Similarly, Cx40 was unable to form heterotypic channels with either Cx46 or Cx50 (cf. with antisense/antisense in Table 1). Values are means ± SEM of 6–16 pairs from two to three independent experiments.

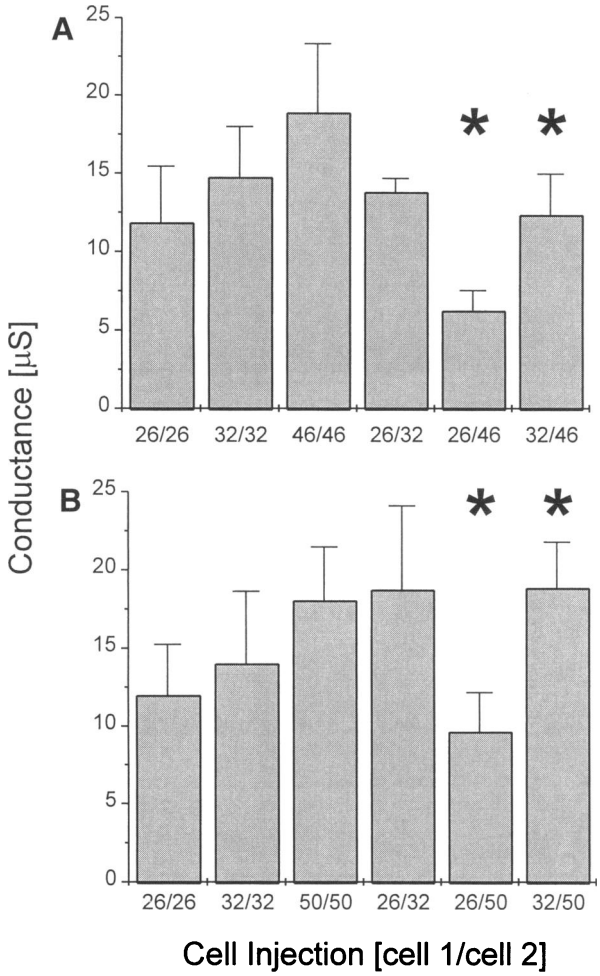

Figure 2. Compatible connexins form heterotypic intercellular channels. Oocytes pre-treated with an oligonucleotide antisense to *Xenopus* Cx38 were injected with the specified connexin mRNAs and paired for 24–48 h before measuring junctional conductance by dual voltage clamp. Asterisks denote the novel combinations tested. Cx46 (A) and Cx50 (B) efficiently interacted with both Cx26 and Cx32. Values are means ± SEM of 8–16 pairs from two to three independent experiments.

interact with *Xenopus* Cx38 (Bruzzone *et al.*, 1993). Antisense treatment resulted in a 95–98% reduction of conductance levels in these pairs (Table 1). Although the other connexins examined do not form heterotypic channels with *Xenopus* Cx38 (Swenson *et al.*, 1989; Werner *et al.*, 1989; Barrio *et al.*, 1991; Bruzzone *et al.*, 1993; White *et al.*, 1994b) antisense Cx38 treatment was always included.

Functional Connexins Do Not Always Form Heterotypic Channels

Heterotypic connexin combinations did not always result in detectable conductance. As previously reported, Cx26 formed channels with Cx32 (Barrio *et al.*, 1991), whereas Cx43 failed to form channels with both Cx26 and Cx32 (Figure 1A). The lack of interaction between Cx32 and Cx43 contradicts earlier reports of heterotypic channel formation by these two connexins (Swenson *et al.*, 1989; Werner *et al.*, 1989; see DISCUSSION). In addition, Cx37 interacted with Cx40 (Bruzzone *et al.*, 1993), whereas Cx32 was incompatible with both Cx37 and Cx40 (Figure 1B). Finally, Cx46 formed heterotypic channels with Cx50 (White *et al.*, 1994b), although Cx40 did not make channels with either Cx46 or Cx50 (Figure 1C). Cx40 also failed to make functional channels when paired with Cx26 (0.03 ± 0.01 μS, n = 7) and Cx43 (Bruzzone *et al.*, 1993). These data show that adjacent cells expressing different connexins regularly fail to become electrically coupled, and that all tested connexins show some limitation in their ability to form heterotypic channels. Cx40 failed to form channels when paired with Cx26, Cx32, Cx43, Cx46, or Cx50, making it the most highly restricted in its ability to interact with other connexins among the combinations examined.

Cx46 and Cx50 Were Relatively Unrestricted in Their Ability to Form Heterotypic Channels

Functional interaction was often observed in combinations that included Cx46 or Cx50. Cx46 formed heterotypic channels with both Cx26 and Cx32 (Figure 2A; White *et al.*, 1994a). In addition, Cx50 also interacted with Cx26 and Cx32 (Figure 2B). Cx46 also formed channels when paired with Cx43 and Cx50 (White *et al.*, 1994b), making it the least restricted in its ability to interact with other connexins among the combinations that we tested. A summary of these results is presented in Table 2, along with other homotypic and heterotypic combinations that have been

Table 1. An anti-*Xenopus* Cx38 oligonucleotide greatly reduces the endogenous contribution to junctional conductance

Oocyte injection cell 1/cell 2	Junctional conductance (μS)	No. of pairs
antisense/antisense	0.02 ± 0.01	12
Cx37/H_2O	4.63 ± 0.72	15
Cx37/antisense	0.22 ± 0.06	13
Cx43/H_2O	6.65 ± 2.40	14
Cx43/antisense	0.17 ± 0.07	14

Oocytes were injected with either an oligonucleotide antisense to a portion of mRNA coding for *Xenopus* Cx38 or mock-treated (H_2O) 24 h before the injection of the specified mRNAs. Cells pairs were analyzed for the development of junctional conductance as described in MATERIALS AND METHODS. Values are means ± SEM of the indicated number of pairs, pooled from three to four independent experiments.

previously published. Taken together, these data clearly illustrate that all connexins exhibit selectivity in their ability to form heterotypic channels.

Of the eleven novel heterotypic combinations tested, the four that yielded functional intercellular channels were analyzed for voltage gating properties. Plots of initial (5–10 ms) and steady-state (30 s) conductance (G_j) vs. transjunctional potential (V_j) revealed that heterotypic channels displayed unique voltage sensitivity (Figure 3). Cx26/Cx46 channels show reduced steady-state conductance for both positive and negative transjunctional potentials, and a slight reduction in initial conductance for positive values of V_j (Figure 3A). Cx26/Cx50 channels also showed steady-state closure for both polarities of V_j, as well as a greater initial reduction for positive transjunctional potentials (Figure 3B). In sharp contrast, both Cx32/Cx46 and Cx32/Cx50 channels were much more electrically asymmetric, showing fast rectification, i.e., a decrease in conductance for relative positivity and an increase in conductance for relative negativity of the Cx32-injected cell. Moreover, when the Cx32 cell was relatively positive, all voltage-dependent closure occurred during the initial 5–10 ms after the imposition of transjunctional potential (Figure 3, C and D).

The voltage sensitivity of the Cx26/Cx46, Cx26/Cx50, Cx32/Cx46, and Cx32/Cx50 heterotypic channels differed dramatically from homotypic channels composed of these connexins (Barrio *et al.*, 1991; White *et al.*, 1994a,b). Previously we had demonstrated that heterotypic Cx46/Cx50 and Cx46/Cx43 channels had different functional properties when compared with homotypic Cx46 channels (White *et al.*, 1994b). In all cases, the voltage sensitivity of the channel for relative positivity in the Cx46- or Cx50-containing cell varied depending on the identity of the connexin in the adjacent cell. These differences were quantitated by fitting the data to Boltzmann equations, the parameters for which are given in Table 3.

Molecular Domains Involved in the Formation and Gating of Heterotypic Channels

To elucidate the molecular determinants involved in the formation and gating of heterotypic channels we used a previously characterized chimeric connexin, composed of portions of Cx32 and Cx43, and examined its interactions with Cx46 and Cx50. The chimera, designated as 3243H4, consists of Cx32 from the amino-terminal through the second transmembrane domain, and Cx43 from the central cytoplasmic through the carboxy-terminal domain (Figure 4A; Bruzzone *et al.*, 1994). The rationale for this series of experiments relied on the finding that Cx46 and Cx50 showed different compatibilities with the parent molecules of the chimera. Cx46 formed channels with both Cx32 and Cx43, whereas Cx50 made channels with Cx32 but not Cx43 (Table 2). As previously reported, the chimera readily forms channels with H_20-injected oocytes, contributing the endogenous *Xenopus* Cx38. The assembly of these 3243H4/Cx38 channels is substantially inhibited by the injection of antisense Cx38 oligonucleotides (Figure 4B; Bruzzone *et al.*, 1994). Under the latter conditions, oocytes injected with 3243H4 mRNA developed high conductances when paired with Cx46-containing cells, whereas 3243H4/Cx50

Table 2. Rodent connexins exhibit selective compatibility in intercellular channel formation

	Partner connexins tested in paired *Xenopus* oocytes[a]									
Connexin	Develop conductance[b]					Do not develop conductance[c]				
26	26	32	46	50		31.1	40	43		
30.3	30.3									
31.1			?			26	31.1	32	43	
32	26	32	46	50		37	40	43		
33			?			33	37	43		
37	37	40	43			32	33			
40	37	40				26	32	43	46	50
43	37	43	46			26	32	33	40	50
46	26	32	43	46	50	40				
50	26	32	46	50		40	43			

[a] This table also includes previously reported combinations (Barrio *et al.*, 1991; Hennemann *et al.*, 1992a, b; Bruzzone *et al.*, 1993, 1994; White *et al.*, 1994b). New data from this study are shaded gray.

[b] These heterotypic channels developed similar conductance levels to homotypic control channels (Figures 1 and 2).

[c] These connexin combinations never developed conductance exceeding that of antisense-treated control oocyte pairs (Table 1).

? The question mark denotes that both Cx31.1 and Cx33 are unable to form homotypic channels between paired *Xenopus* oocytes.

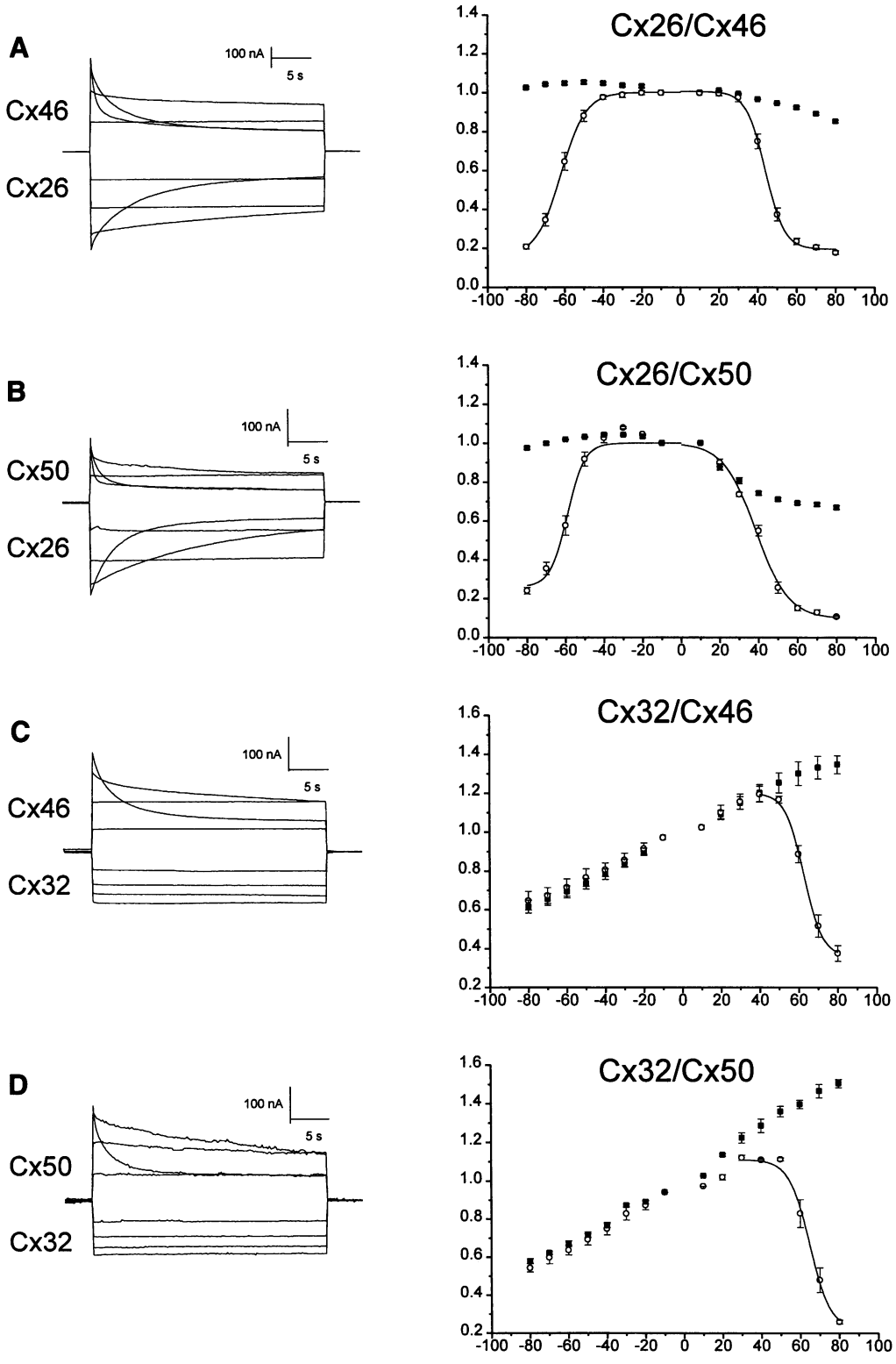

Figure 3. The physiological properties of heterotypic channels are modified by the identity of the connexin partners. Antisense-treated oocytes were injected with connexin mRNAs and incubated for 24–48 h after manual pairing. Voltage gating was analyzed by dual voltage clamp. The two paired oocytes were initially clamped at −40 mV to ensure zero transjunctional voltage (V_j). While one cell was held at a constant potential, V_j steps of opposite polarities were sequentially imposed on the other cell and the resulting junctional currents (I_j) and conductance (G_j) were recorded. Traces show the time-dependent decay of I_j induced by V_j steps of the duration of 30 s, applied in 20 mV increments. Plots describe the relationship of V_j to initial (filled squares) and steady-state (open circles) junctional conductances, normalized to the values obtained at ± 10 mV. V_j is defined as positive for depolarization of the right-hand cell (Cx46 or Cx50) relative to the left-hand cell (Cx26 or Cx32) and vice versa. All curves represent the best fits to Boltzmann equations whose parameters are given in Table 3. Values are means ± SEM of three to four pairs. (A and B) Both heterotypic channels containing Cx26 exhibited some degree of asymmetry in the steady-state currents with a marginal effect on the fast component of channel closure. (C and D) Both combinations with Cx32 were characterized by a fast rectification and a marked asymmetry of the slow voltage-dependent component.

pairs were not coupled above background levels (compare to 3243H4/antisense). Thus, with regard to selectivity, the chimera behaved more like Cx43 than Cx32, suggesting that sequences comprised between

Table 3. Boltzmann parameters of novel heterotypic channels

Channel	V_j	A	V_0	$G_j$max	$G_j$min
Cx26/Cx46	+	0.20	44	1.00	0.20
Cx26/Cx46	−	0.15	62	1.00	0.16
*Cx32/Cx46	+	0.21	63	1.15	0.34
Cx26/Cx50	+	0.12	39	1.00	0.10
Cx26/Cx50	−	0.22	59	1.00	0.26
*Cx32/Cx50	+	0.19	65	1.11	0.22
*3243H4/Cx46	+	0.12	28	1.13	0.07

Steady-state junctional conductance was measured and fit to a Boltzmann equation of the form given in the text. The plus and minus signs for V_j indicate the polarity of the transjunctional potential. Thus, positive potentials indicate relative positivity of the right-hand (e.g. Cx46 in row 1) relative to the left-hand connexin (e.g. Cx26 in row 1).

*For these combinations, negative V_js were not well fit by a Boltzmann relation.

the central cytoplasmic and carboxy-terminal domains regulate connexin compatibility. These results support our hypothesis that the second extracellular domain is a major determinant of selectivity, as previously suggested by domain swapping experiments between Cx46 and Cx50 in which compatibility and discrimination segregated with the identity of the second extracellular region contributed by each connexon (White *et al.*, 1994b).

Analysis of voltage gating in 3243H4/Cx46 heterotypic channels revealed a pronounced asymmetry, where all voltage-dependent closure occurred during the initial 5–10 ms when the 3243H4 cell was relatively positive (Figure 4C). These properties were very similar to those displayed by Cx32/Cx46 channels (Figure 3C) and differed significantly from those exhibited by Cx43/Cx46 where no fast rectification was observed (White *et al.*, 1994b). Therefore, with regard to voltage dependence, the chimera behaved more like Cx32 than Cx43, suggesting that connexin sequences between the N-terminus and the end of the second transmembrane domain regulate voltage gating.

DISCUSSION

Adjacent cells are able to contribute different connexins to the intercellular channel, which spans two plasma membranes. In the paired *Xenopus* oocyte assay, the two cells can be injected with mRNA encoding different connexins to test whether heterotypic channels are formed. Early tissue culture experiments suggested that intercellular communication was a promiscuous phenomenon, likely to occur between all contacting cell types (Michalke and Loewenstein, 1971; Epstein and Gilula, 1977), although some examples of selective coupling have been reported (Kettenmann *et al.*, 1983; Kam *et al.*, 1987; Mesnil *et al.*, 1987). In this study, we have demonstrated that most of the rodent connexins are restricted in their heterotypic interactions. Specifically, we show that adjacent *Xenopus* oocytes expressing different connexins have a high probability of not establishing intercellular communication. These observations are likely to apply to other cell types as well, as it has been demonstrated that mammary epithelial cells transfected with Cx43 do not communicate with transfected cells expressing Cx26 (Tomasetto *et al.*, 1993). Thus, the establishment of intercellular communication is not a uniformly permissive process, but one which is controlled by the expression of alternative connexins. Our results also demonstrate that the second extracellular domain plays a critical role in determining compatibility. Furthermore, the physiological properties of a connexon containing a given connexin are specified in part by sequences from the amino terminus through the second transmembrane domain.

In the present study no coupling was detected between heterotypic pairs expressing Cx32 and Cx43; however, it had been suggested that these two connexins were able to form heterotypic channels (Swenson *et al.*, 1989; Werner *et al.*, 1989). A major difference between this and those two studies was our elimination of endogenous *Xenopus* Cx38, using antisense oligonucleotides. Cx43-injected oocytes are known to become highly coupled to uninjected cells in the absence of antisense treatment (Swenson *et al.*, 1989; Werner *et al.*, 1989; White *et al.*, 1992, 1994b; Bruzzone *et al.*, 1993, 1994), complicating the interpretation of the earlier reports. Although no voltage gating data for Cx32/Cx43 channels were shown in these earlier reports, it was suggested that these pairs could be distinguished from Cx43/Cx38 pairs by a symmetrical lack of voltage sensitivity (Swenson *et al.*, 1989). This is also difficult to reconcile with the dramatic asymmetric changes in voltage gating normally exhibited by Cx32 in heterotypic channels (i.e., with Cx26, Cx46, and Cx50, see below). When *Xenopus* Cx38 is inhibited by injecting oocytes with antisense oligonucleotides, we have found that cells expressing Cx43 failed to become electrically coupled to cells expressing Cx32. A lack of interaction between Cx32 and Cx43 in antisense-treated oocytes has also been observed independently in another laboratory (B.J. Nicholson, personal communication).

What are the molecular mechanisms by which connexins select compatible partners? On the basis of primary sequence relationships, connexins are divided into two groups: α and β (Risek *et al.*, 1990; Kumar and Gilula, 1992). Although this subdivision could have provided a general means to discriminate between family members, our data indicate that connexin selectivity is not simply based on group identity. Although Cx40, Cx46, and Cx50 are all group α con-

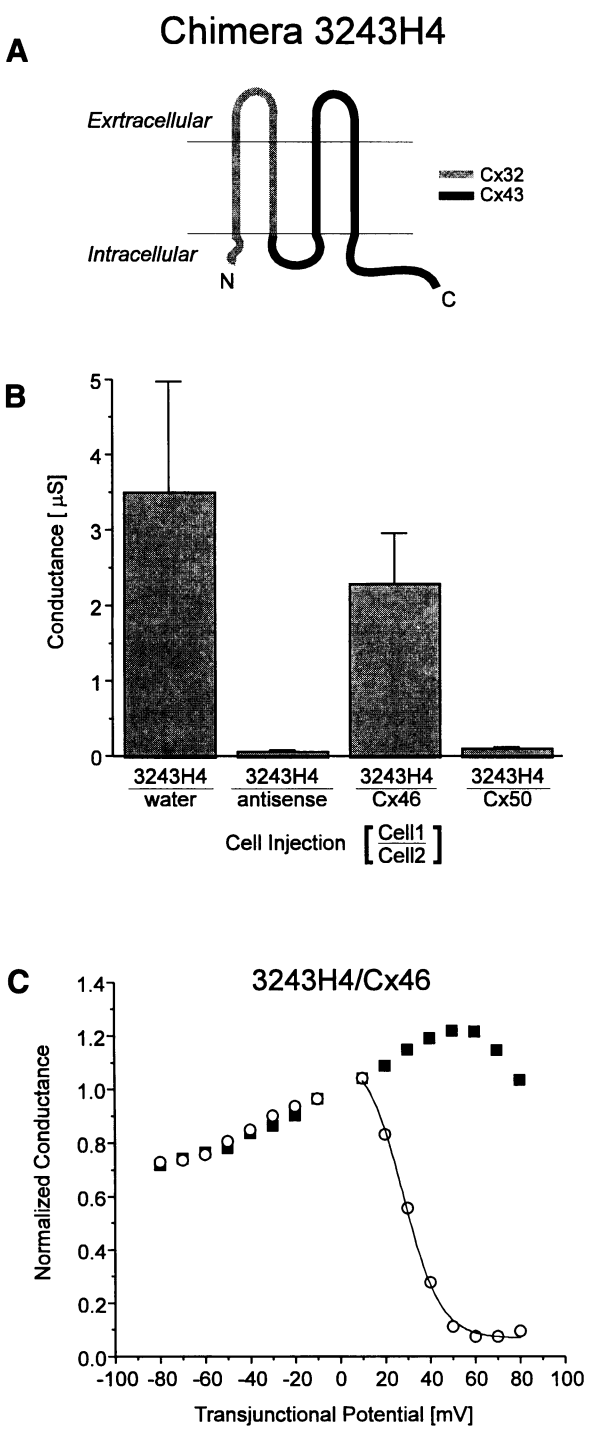

Figure 4. Different domains specify compatibility and voltage gating behavior. (A) Schematic representation and membrane topology of the chimeric construct 3243H4. Portions corresponding to Cx32 (amino acid residues 1–96) are depicted in gray, whereas portions corresponding to Cx43 (amino acid residues 98–382) are depicted in black. (B) Antisense-treated oocytes were used to study the interactions of 3243H4 with Cx46 and Cx50. The chimera readily formed heterotypic channels with Cx46 whereas it discriminated against Cx50. Values are means ± SEM of 5–10 pairs from three independent experiments. (C) Voltage dependence of 3243H4/Cx46 channels. Plots describe the relationship of V_j to initial (filled squares) and steady-state (open circles) junctional conductances, normalized to the values obtained at ± 10 mV. V_j is defined as positive for depolarization of the Cx46 side relative to the 3243H4 side. The solid line represents the best fit to a Boltzmann equation, the parameters for which are given in Table 3. Values are representative of three independent experiments.

nexins, Cx40 does not interact with either Cx46 or Cx50. Furthermore, Cx32 and Cx26, which are group β, both readily interact with group α Cx46 and Cx50.

The selectivity behavior of the chimeric connexin 3243H4 is consistent with a model in which compatibility is controlled by sequences comprising the second extracellular domain. Connexin topological models, which are based on hydropathy plots, proteolysis studies, and experimental analysis with site-specific antibodies (Goodenough *et al.*, 1988; Hertzberg *et al.*, 1988; Milks *et al.*, 1988; Yancey *et al.*, 1989), indicate that there are two extracellular domains. It is reasonable to speculate that these regions, parts of which must physically interact, participate in the process of recognition. Indeed, mutational analysis had emphasized the role of conserved cysteine residues in both domains for the development of functional homotypic channels (Dahl *et al.*, 1992). To determine the relative importance of each extracellular domain in the process of discrimination, we constructed a chimera whose extracellular sequences are derived from two connexins with different patterns of selectivity. The ability to form intercellular channels segregated with the second extracellular domain, consistent with previous studies using other chimeric connexins (White *et al.*, 1994b). The general applicability of this model requires further testing.

The electrical behavior of the chimera 3243H4 is most consistent with a model in which sequences from the N-terminus to the end of the second transmembrane domain contribute to voltage gating. The characteristic rectification exhibited by Cx32 in heterotypic configurations (Barrio *et al.*, 1991; Rubin *et al.*, 1992; Suchyna *et al.*, 1993; Figure 3, C and D) was maintained in this chimera where those domains were derived from Cx32 (Figure 4C). Although our studies do not permit us to define the specific residues more precisely, they are in agreement with previous reports that have separately implicated the N-terminus (Verselis *et al.*, 1994), the first extracellular domain (Rubin *et al.*, 1992; Verselis *et al.*, 1994) and the second transmembrane domain (Suchyna *et al.*, 1993) as components of the voltage gating mechanism.

Cx32 is unique in that it imposes dominant electrical properties when heterotypic channels are formed (Cx32/Cx26, Barrio *et al.*, 1991; Cx32/Cx46, Cx32/Cx50, present work), whereas other connexins exhibit unpredictable changes in electrical behavior (Henne-

mann *et al.*, 1992b; Bruzzone *et al.*, 1994; White *et al.*, 1994b). The rectification observed in heterotypic Cx32/Cx26 intercellular channels occurs because connexons composed of Cx26 and Cx32 close in response to voltages of opposite polarities (Verselis *et al.*, 1994). Cx26, like most other connexins, closes in response to relative positivity at the cytoplasmic end of the channel. In contrast, Cx32 closes when relatively negative, and this results in rectification when Cx32 is heterotypically paired with other connexins. It would appear that Cx32 retains this polarity of closure when paired with Cx46 or Cx50 (Figure 3, C and D), although it has been recently demonstrated that polarity of voltage gating is not an intrinsic property of the connexon. For example, the polarity of voltage gating of Cx46 connexons reverses upon incorporation into an intercellular channel (White *et al.*, 1994a). In addition, mutation of a proline residue in the second transmembrane domain of Cx26 resulted in connexons that also had a dominant rectifying phenotype when paired with either wild-type Cx26 or Cx32, which should exhibit opposite polarities of voltage gating (Suchyna *et al.*, 1993). Together, these studies do not identify a single domain within connexins that dictates voltage properties of gap junction channels, but suggest that complex interactions between different domains, and possibly between opposing connexons, are involved in the voltage gating of intercellular channels.

The ability of some connexins to form heterotypic channels could lead to greater functional diversity. Allosteric interactions between opposing connexons can, as we have shown, generate novel electrical properties. This model has been invoked to explain the behavior of rectifying electrical synapses where only orthodromic impulse propagation is allowed (Giaume *et al.*, 1987). Although our analysis is limited to voltage gating, other properties, including permeability to ions, second messengers, and metabolites may be modified by heterotypic interaction. For example, passage of microinjected dyes was readily detected from astrocytes to oligodendrocytes but rarely in the opposite direction (Robinson *et al.*, 1993; see also Finkelstein, 1994 for an alternative view). Although not extensively documented, it has been suggested that different connexins are expressed in these two cell types (Dermietzel *et al.*, 1989). Similarly, unidirectional movement of Ca^{2+} from astrocytes to neurons has been reported after triggered calcium transients (Nedergaard, 1994).

What are the possible biological consequences of selective communication? Connexin selectivity could explain the observation of communication compartments. In many animal tissues, communication occurs within but not between physically adjacent groups of cells, even in the absence of an obvious anatomical boundary (Lo and Gilula, 1979; de Laat *et al.*, 1980; Warner and Lawrence, 1982; Weir and Lo, 1982; Meda *et al.*, 1983; Blennerhasset and Caveney, 1984; Kam *et al.*, 1987; Chanson *et al.*, 1991; Lo Turco and Kriegstein, 1991; Yuste *et al.*, 1992). This behavior could result from the expression of incompatible connexins by neighboring groups of cells. For example, Cx40, which we have shown to be extremely limited in its ability to interact, is prominently expressed in the Purkinje fibers of the cardiac conduction system (Bastide *et al.*, 1993; Gourdie *et al.*, 1993; Gros *et al.*, 1994). It would likely be deleterious to the coordinated propagation of excitation in the myocardium if Purkinje fibers could indiscriminately form electrical contacts with surrounding myocardial cells. We hypothesize that Purkinje fibers may form electrical connections only between themselves and with a subset of myocardial cells that express Cx40. Now that the patterns of connexin interaction have been established, it should be possible to better correlate connexin distribution with the presence of communication compartments. Other factors in addition to the expression of compatible connexins may also influence the ability of adjacent cells to communicate. For example, dye transfer mediated by Cx43 has been shown to depend on the proper expression and interaction of cell adhesion molecules (Mège *et al.*, 1988; Meyer *et al.*, 1992). Consequently, the expression of compatible connexins is a necessary, but not sufficient, step for adjacent cells to establish intercellular communication.

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REFERENCES

Bastide, B., Neyses, L., Ganten, D., Paul, M., Willecke, K., and Traub, O. (1993). Gap junction protein connexin40 is preferentially expressed in vascular endothelium and conductive bundles of rat myocardium and is increased under hypertensive conditions. Circ. Res. *73*, 1138–1149.

Barrio, L.C., Suchyna, T., Bargiello, T., Xu, L.X., Roginski, R.S., Bennett, M.V.L., and Nicholson, B.J. (1991). Gap junctions formed by connexins 26 and 32 alone and in combination are differently affected by voltage. Proc. Natl. Acad. Sci. USA *88*, 8410–8414.

Bennett, M.V.L., Barrio, L.C., Bargiello, T.A., Spray, D.C., Hertzberg, E., and Sáez, J.C. (1991). Gap junctions: new tools, new answers, new questions. Neuron *6*, 305–320.

Beyer, E.C., Kistler, J., Paul, D.L., and Goodenough, D.A. (1989). Antisera directed against connexin43 peptides react with a 43-kD protein localized to gap junctions in myocardium and other tissues. J. Cell Biol. *108*, 595–605.

Blennerhasset, M.G., and Caveney, S. (1984). Separation of developmental compartments by a cell type with reduced junctional permeability. Nature *309*, 361–364.

Bruzzone, R., Chanson, M., Swenson, K.I., Bosco, D., Meda, P., and Paul, D.L. (1991). Gap junctions and cell-to-cell signalling. In: Cell

Signalling: Experimental Strategies, ed. E. Reid, G.M.W. Cook, and J.P. Luzio, Cambridge, UK: The Royal Society of Chemistry, 341–358.

Bruzzone, R., Haefliger, J.-A., Gimlich, R.L., and Paul, D.L. (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.

Bruzzone, R., White, T.W., and Paul, D.L. (1994). Expression of chimeric connexins reveals new properties of the formation and gating behavior of gap junction channels. J. Cell Sci. *107*, 955–967.

Chanson, M., Orci, L., and Meda, P. (1991). Extent and modulation of junctional communication between pancreatic acinar cells in vivo. Am. J. Physiol. *261*, G28-G36.

Chen, Y-H., and DeHaan, R.L. (1992). Multiple-channel conductance states and voltage regulation of embryonic chick cardiac gap junctions. J. Membr. Biol. *127*, 95–111.

Dahl, G. (1992). The *Xenopus* oocyte cell-cell channel assay for functional analysis of gap junction proteins. In: Cell-Cell Interactions: A Practical Approach, ed. B.R. Stevenson, W.J. Gallin, and D.L. Paul, New York: Oxford University Press, 143–165.

Dahl, G., Miller, T., Paul, D., Voellmy, R., and Werner, R. (1987). Expression of functional cell-cell channels from cloned rat liver gap junction complementary DNA. Science *236*, 1290–1293.

Dahl, G., Werner, R., Levine, E., and Rabadan-Diehl, C. (1992). Mutational analysis of gap junction formation. Biophys J. *62*, 172–182.

de Laat, S.W., Tertoolen, L.G.J., Dorresteijn, A.W.C., and van den Biggelaar, J.A.M. (1980). Intercellular communication patterns are involved in early molluscan determination. Nature *287*, 546–548.

Dermietzel, R., and Spray, D.C. (1993). Gap junctions in the brain: where, what type, how many and why? Trends Neurosci. *16*, 186–192.

Dermietzel, R., Traub, O., Hwang, T.K., Beyer, E., Bennett, M.V.L., Spray, D.C., and Willecke, K. (1989). Differential expression of three gap junction proteins in developing and mature brain tissues. Proc. Natl. Acad. Sci. USA *86*, 10148–10152.

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

Epstein, M.L., and Gilula, N.B. (1977). A study of communication specificity between cells in culture. J. Cell Biol. *75*, 769–787.

Finkelstein, A. (1994). Gap junctions and intercellular communications. Science *265*, 1017–1018.

Fishman, G.I., Spray, D.C., and Leinwand, L.A. (1990). Molecular characterization and functional expression of the human cardiac gap junction channel. J. Cell Biol. *111*, 589–598.

Giaume, C., Kado, R.T., and Korn, H. (1987). Voltage clamp analysis of a crayfish rectifying synapse. J. Physiol. *386*, 91–112.

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

Goliger, J.A., and Paul, D.L. (1994). Expression of gap junction proteins Cx26, Cx31.1, Cx37, and Cx43 in developing and mature rat epidermis. Dev. Dyn. *200*, 1–13.

Goodenough, D.A., and Musil, L.S. (1993). Gap junctions and tissue business: problems and strategies for developing specific functional reagents. J. Cell Sci. Suppl. *17*, 133–138.

Goodenough, D.A., Paul, D.L, and Jesaitis, L. (1988). Topological distribution of two connexin32 antigenic sites in intact and split rodent hepatocyte gap junctions. J. Cell Biol. *107*, 1817–1824.

Gourdie, R.G., Severs, N.J., Green, C.R., Rothery, S., Germroth, P., and Thompson, R.P. (1993). The spatial distribution and relative abundance of gap-junctional connexin40 and connexin43 correlate to functional properties of components of the cardiac atrioventricular conduction system. J. Cell Sci. *105*, 985–991.

Gros, D., Jarry-Guichard, T., Ten Velde, I., de Maziere, A., van Kempen, M.J.A., Davoust, J., Briand, J.P., Moorman, A.F.M., and Jongsma, H.J. (1994). Restricted distribution of connexin40, a gap junctional protein, in mammalian heart. Circ. Res. *74*, 839–851.

Haefliger, J.-A., Bruzzone, R., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., and Paul, D.L. (1992). Four novel members of the connexin family of gap junction proteins: molecular cloning, expression, and chromosome mapping. J. Biol. Chem. *267*, 2057–2064.

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

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

Hertzberg, E.L., Disher, R.M., Tiller, A.A., Zhou, Y., and Cook, R.G. (1988). Topology of the M_r 27,000 liver gap junction protein. J. Biol. Chem. *263*, 19105–19111.

Kam, E., Watt, F.M., and Pitts, J.D. (1987). Patterns of junctional communication in skin: studies on cultured keratinocytes. Exp. Cell Res. *173*, 431–438.

Kanemitsu, M.Y., and Lau, A.F. (1993). Epidermal growth factor stimulates the disruption of gap junctional communication and connexin43 phosphorylation independent of 12-*O*-tetradecanoylphorbol 13-acetate sensitive protein kinase C: the possible involvement of mitogen-activated protein kinase. Mol. Biol. Cell *4*, 837–848.

Kanter, H.L., Saffitz, J.E., and Beyer, E.C. (1993). Canine cardiac myocytes express multiple gap junction proteins. Circ. Res. *70*, 438–444.

Kettenmann, H., Orkand, R.K., and Schachner, M. (1983). Coupling among identified cells in mammalian nervous system cultures. J. Neurosci. *3*, 506–516.

Krieg, P.A., and Melton, D.A. (1984). Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. Nucleic Acids Res. *12*, 7057–7070.

Kumar, N.M., and Gilula, N.B. (1992). Molecular biology and genetics of gap junction channels. Semin. Cell Biol. *3*, 3–16.

Lo, C.W., and Gilula, N.B. (1979). Gap junctional communication in the pre-implantation mouse embryo. Cell *18*, 411–422.

Lo Turco, J.J., and Kriegstein, A.R. (1991). Clusters of coupled neuroblasts in embryonic neocortex. Science *252*, 563–566.

Meda, P., Michaels, R.L., Halban, P.A., Orci, L., and Sheridan, J.D. (1983). In vivo modulation of gap junctions and dye coupling between B-cells of the intact pancreatic islets. Diabetes *32*, 858–868.

Mège, R-N., Matsuzaki, F., Gallin, W.J., Goldberg, J.I., Cunningham, B.A., and Edelman, G.M. (1988). Construction of epithelioid sheets by transfection of mouse sarcoma cells with cDNAs for chicken cell adhesion molecules. Proc. Natl. Acad. Sci. USA *85*, 7274–7278.

Mesnil, M., Fraslin, J.M., Piccoli, C., Yamasaki, H., and Guguen-Guillouzo, C. (1987). Cell contact but not junctional communication (dye coupling) with biliary epithelial cells is required for hepatocytes to maintain differentiated functions. Exp. Cell Res. *173*, 524–533.

Meyer, R.A., Laird, D.W., Revel, J.P., and Johnson, R.G. (1992). Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies. J. Cell Biol. *119*, 179–189.

Michalke, W., and Loewenstein, W.R. (1971). Communication between cells of different type. Nature *232*, 121–122.

Milks, L.C., Kumar, N.M., Houghten, R., Unwin, N., and Gilula, N.B. (1988). Topology of the 32-kd liver gap junction protein determined by site-directed antibody localizations. EMBO J. *7*, 2967–2975.

Moreno, A.P., Eghbali, B., and Spray, D.C. (1991). Connexin32 gap junction channels in stably transfected cells: unitary conductance. Biophys. J. *60*, 1254–1256.

Moreno, A.P., Sáez, J.C., Fishman, G.I., and Spray, D.C. (1994). Human connexin43 gap junction channels: regulation of unitary conductances by phosphorylation. Circ. Res. *74*, 1050–1057.

Nedergaard, M. (1994). Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. Science *263*, 1768–1771.

Nicholson, B., Dermietzel, R., Teplow, D., Traub, O., Willecke, K., and Revel, J.-P. (1987). Two homologous protein components of hepatic gap junctions. Nature *329*, 732–734.

Nicholson, B.J., Suchyna, T., Xu, L.X., Hammernick, P., Cao, F.L., Fourtner, C., Barrio, L., and Bennett, M.V.L. (1993). Divergent properties of different connexins expressed in *Xenopus* oocytes. Prog. Cell Res. *3*, 3–13.

Nishi, M., Kumar, N.M., and Gilula, N.B. (1991). Developmental regulation of gap junction gene expression during mouse embryonic development. Dev. Biol. *146*, 117–130.

Paul, D.L. (1985). Antibody against liver gap junction 27-kD protein is tissue specific and cross-reacts with a 54-kD protein. In: Gap Junctions, ed. M.V.L. Bennett and D.C. Spray, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 107–122.

Paul, D.L., Bruzzone, R., and Haefliger, J.-A. (1993). Identification of novel connexins by reduced stringency hybridization and PCR amplification using degenerate primers. Prog. Cell Res. *3*, 15–20.

Paul, D.L., Ebihara, L., Takemoto, L.J., Swenson, K.I., and Goodenough, D.A. (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.

Risek, B., and Gilula, N.B. (1991). Spatiotemporal expression of three gap junction gene products involved in fetomaternal communication during rat pregnancy. Development *113*, 165–181.

Risek, B., Guthrie, S., Kumar, N., and Gilula, N.B. (1990). Modulation of gap junction transcript and protein expression during pregnancy in the rat. J. Cell Biol. *110*, 269–282.

Risek, B., Klier, G., and Gilula, N.B. (1994). Developmental regulation and structural organization of connexins in epidermal gap junctions. Dev. Biol. *164*, 183–196.

Robinson, S.R., Hampson, E.C.G.M., Munro, M.N., and Vaney, D.I. (1993). Unidirectional coupling of gap junctions between neuroglia. Science *262*, 1072–1074.

Rubin, J.B., Verselis, V.K., Bennett, M.V.L., and Bargiello, T.A. (1992). Molecular analysis of voltage dependence of heterotypic gap junctions formed by connexins 26 and 32. Biophys. J. *62*, 183–195.

Spray, D.C., Harris, A.L., and Bennett, M.V.L. (1981). Equilibrium properties of a voltage-dependent junctional conductance. J. Gen. Physiol. *77*, 75–93.

Steinberg, T.H., Civitelli, R., Geist, S.T., Robertson, A.J., Hick, E., Veenstra, R.D., Wang, H.-Z., Warlow, P.M., Westphale, E.M., Laing, J., and Beyer, E.C. (1994). Connexin43 and connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells. EMBO J. *13*, 744–750.

Suchyna, T., Xu, L.X., Gao, F., Fourtner, C.R., and Nicholson, B.J. (1993). Identification of a proline residue as a transduction element involved in the voltage gating of gap junctions. Nature *365*, 847–849.

Swenson, K.I., Jordan, J.R., Beyer, E.C., and Paul, D.L. (1989). Formation of gap junctions by expression of connexins in *Xenopus* oocyte pairs. Cell *57*, 145–155.

Swenson, K.I., Piwnica-Worms, H., McNamee, H., and Paul, D.L. (1990). Tyrosine phosphorylation of the gap junctional protein connexin43 is required for the Pp $60^{v\text{-}src}$ induced inhibition of communication. Mol. Biol. Cell *1*, 989-1002.

Takens-Kwak, B.R., and Jongsma, H.J. (1992). Cardiac gap junctions: three distinct single channel conductances and their modulation by phosphorylating treatments. Pflügers Arch. *422*, 198–200.

Tomasetto, C., Neveu, M.J., Daley, J., Horan, P.K., and Sager, R. (1993). Specificity of gap junction communication among human mammary cells and connexin transfectants in culture. J. Cell Biol. *122*, 157–167.

Traub, O., Look, J., Dermietzel, R., Brummer, D., Husler, D., and Willecke, K. (1989). Comparative characterization of the 21-kD and 26-kD gap junction proteins in murine liver and cultured hepatocytes. J. Cell Biol. *108*, 1039–1051.

Valdimarsson, G., De Sousa, P.A., Beyer, E.C., Paul, D.L., and Kidder, G.M. (1991). Zygotic expression of the connexin43 gene supplies subunits for gap junction assembly during mouse preimplantation development. Mol. Reprod. Dev. *30*, 18–26.

Veenstra, R.D., Wang, H.-Z., Beyer, E.C., Ramanan, S.V., and Brink, P.R. (1994). Connexin37 forms high conductance gap junction channels with subconductance state activity and selective dye and ionic permeabilities. Biophys. J. *66*, 1915–1928.

Veenstra, R.D., Wang, H.-Z., Westphale, E.M., and Beyer, E.C. (1992). Multiple connexins confer distinct regulatory and conductance properties of gap junctions in developing heart. Circ. Res. *71*, 1277–1283.

Verselis, V.K., Ginter, C.S., and Bargiello, T.A. (1994). Opposite voltage gating polarities of two closely related connexins. Nature *368*, 348–351.

Warner, A.E., and Lawrence, P.A. (1982). Permeability of gap junctions at the segmental border in insect epidermis. Cell *28*, 243–252.

Weir, M.P., and Lo, C.W. (1982). Gap junctional communication compartments in the *Drosophila* wing disk. Proc. Natl. Acad. Sci. USA *79*, 3232–3235.

Werner, R., Levine, E., Rabadan-Diehl, C., and Dahl, G. (1989). Formation of hybrid cell-cell channels. Proc. Natl. Acad. Sci. USA *86*, 5380–5384.

White, T.W., Bruzzone, R., Goodenough, D.A., and Paul, D.L. (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.

White, T.W., Bruzzone, R., Goodenough, D.A., and Paul, D.L. (1994a). Voltage gating of connexins. Nature *371*, 208–209.

White, T.W., Bruzzone, R., and Paul, D.L. (1995). The connexin family of intercellular channel forming proteins. Kidney Int. (*in press*).

White, T.W., Bruzzone, R., Wolfram, S., Paul, D.L., and Goodenough, D.A. (1994b). Selective interactions among the multiple connexin proteins expressed in the vertebrate lens: the second extracellular domain is a determinant of compatibility between connexins. J. Cell Biol. *125*, 879–892.

Wilders, R., and Jongsma, H.J. (1992). Limitations of the dual voltage clamp method in assaying conductance and kinetics of gap junction channels. Biophys. J. *63*, 942–953.

Willecke, K., Hennemann, H., Dahl, E., Jungbluth, R., and Heynkes, R. (1991). The diversity of connexin genes encoding gap junctional proteins. Eur. J. Cell Biol. *56*, 1–7.

Yancey, S.B., John, S.A., Ratneshwar, L., Austin, B.J., and Revel, J.-P. (1989). The 43-kD polypeptide of heart gap junctions: immunolocalization, topology, and functional domains. J. Cell Biol. *108*, 2241–2254.

Yuste, R., Peinado, A., and Katz, L.C. (1992). A system of neuronal domains in developing neocortex. Science *257*, 665–668.