A new gap junction gene isolated from the mouse genome codes for a connexin protein of 261 amino acids. Because of its theoretical molecular mass of 30.366 kDa, it is named connexin-30. Within the connexin gene family, this protein is most closely related to connexin-26 (77% amino acid sequence identity). The coding region of mouse connexin-30 is uninterrupted by introns and is detected in the mouse genome as a single copy gene that is assigned to mouse chromosome 14 by analysis of mouse × hamster somatic cell hybrids. Abundant amounts of connexin-30 mRNA (two transcripts of 2.0 and 2.3 kilobase pairs) were found after 4 weeks of postnatal development in mouse brain and skin. Microinjection of connexin-30 cRNA into Xenopus oocytes induced formation of functional gap junction channels that gated somewhat asymmetrically in response to transjunctional voltage and at significantly lower voltage ($V_o = +38$ and $-46$ mV) than the closely homologous connexin-26 channels ($V_o = 89$ mV). Heterotypic pairings of connexin-30 with connexin-26 and connexin-32 produced channels with highly asymmetric and rectifying voltage gating, respectively. This suggests that the polarity of voltage gating and the cationic selectivity of connexin-30 are similar to those of its closest homologue, connexin-26.

The connexin gene family codes for the protein subunits of gap junction channels that mediate direct diffusion of ions and metabolites between the cytoplasm of adjacent cells (reviewed by Bennett et al. (1991), Beyer (1993), Paul (1995), and White et al. (1995)). Functional gap junctions have been suggested to be involved in metabolic cooperation between cells, synchronization of cellular physiological activities, growth control, and regulation of development.

To date, 12 different connexin genes have been characterized in the mouse or rat genome (Willecke et al., 1991a; Haefliger et al., 1992; White et al., 1992) and assigned to different chromosomal localizations (Haefliger et al., 1992; Schwarz et al., 1992, 1994). Connexin genes are expressed in a cell type-specific manner with overlapping specificity. Based on analyses of amino acid sequences and labeling of membrane-embedded connexins with peptide-specific antibodies (Milks et al., 1988; Yancey et al., 1989; Zhang and Nicholson, 1994), a general topology of connexin proteins was proposed. It was deduced that the polypeptide chain of connexins spans the plasma membrane four times, with amino- and carboxyl-terminal regions facing the cytoplasm. By comparison within the gene family, connexins show very high sequence identities in the transmembrane regions and in the two extracellular loops, which are presumably responsible for the docking of two hemichannels. Major differences have been found in the central cytoplasmic loop and carboxyl-terminal tail in terms of length as well as sequence. Cloned connexin (Cx)$^+$ genes have been functionally expressed in Xenopus oocytes and cultured mammalian cells (reviewed by Paul (1995) and White et al. (1995)). These reconstitution experiments have shown that gap junction channels have unique properties depending on the type of connexin(s) constituting the channel.

Reconstitution of connexin channels in cultured mammalian cells has revealed that channel conductance can depend on phosphorylation of the Cx43 protein (Moreno et al., 1994) and that connexin channels exhibit different permeabilities to tracer molecules (Elfag et al., 1995). Furthermore, connexins show specificity in terms of the functional docking of their hemichannels. Some combinations are compatible (e.g. Cx26 and Cx32 (Barrio et al., 1991) as well as Cx40 and Cx37 (Hennemann et al., 1992a)), while others (Cx40 and Cx43) do not form functional gap junctions in Xenopus oocytes (Bruzzone et al., 1993) or cultured mammalian cells (Elfag et al., 1995).

To understand all aspects of gap junctional communication between different mammalian cells, it is necessary to characterize all functional connexin channels and their protein constituents. In this paper, we describe the characterization of Cx30, a new member of the murine connexin gene family, which is highly expressed in adult skin and brain, but is not detected in embryonic and fetal brain. This expression pattern is clearly different from that of the closely related Cx26 gene. When expressed in Xenopus oocytes, Cx30 and Cx26 channels show the same voltage gating polarity. However, the specific parameters, describing the voltage sensitivity of these channels, vary between Cx30 and Cx26. This system of two highly sequence-related connexin proteins can be used to search for the molecular basis of the differences in channel gating and other parameters.

MATERIALS AND METHODS
Isolation of Genomic Mouse Cx30 DNA—Previously, we isolated 25 connexin homologous recombinant EMBL3 phage clones by screening of

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1 The abbreviations used are: Cx, connexin; kb, kilobase pair(s).
a genomic C57/B16 mouse library with a rat Cx26 probe (Willecke et al., 1991b). The procedure used to determine which connexin genes were represented among the recombinant phages is described by Henne mann et al. (1992a). Several phage clones were identified that did not hybridize to any of the known connexin genes under stringent conditions (50% formamide, 5 × SSC at 42 °C). DNA of one of these latter phage clones was sequenced, and the RNA polymerase (T7 RNA polymerase) was isolated according to the protocols and reagents in the RNAid kit (BIO 101, Inc. Vista, CA). Production of predominantly full-length transcripts was confirmed by electrophoresis in 1% agarose. The final concentration of Cx30 rRNA for injection was ~0.2 μg/μl.

Xenopus oocytes were stripped of the follicular membrane after a 1-h treatment with collagenase (2 mg/ml). Each oocyte (1.5–2.0 ng a 5′-GTG TAG TAA TTC CCA TCC TGC CAT GTT TC-3′) which is complementary to Xenopus Cx38 (commencing at nucleotide 5) alone or in combination with 8 ng (40 nl) of Cx30 rRNA. Similar amounts of rRNAs of Cx26 or Cx32, prepared as described above, from templates described by Barrio et al. (1991), were injected for analysis of the heterotypic channels. Oocytes were subse quently incubated in L-15 medium at room temperature for 1 day, after which the vitelline membranes were manually removed, and the oocytes were paired in various combinations (i.e. Cx30/O, Cx30/Cx32, Cx30/Cx30, Cx32/Cx32, and Cx32/Cx26). Functional expression was examined by a dual-cell voltage clamp.

For electrophysiological measurements, the two-electrode voltage-clamp procedure was used to clamp each oocyte (Harris et al., 1981). The membrane potentials of the cells were measured, and the more negative one was used as the holding potential for both, typically ~40 to ~60 mV. Transjunctional voltage (Vj) was applied by clamping one cell at the holding potential and inducing different voltage steps of 1–30 s duration in the other cell. Records of both voltage and current in each cell provide direct measures of transjunctional voltage and current. Net conductance between oocytes was determined from the slope of the i-V relation.

The conductance at the initial moment of the voltage step (G0) was obtained by fitting the decaying current to an exponential and extrapolating the current traces to t = 0. The steady-state conductance (Gw; i.e. the conductance after the decaying current reached a steady-state level) was obtained as the offset term of the exponential fitting.

RESULTS

Cloning of the Mouse Cx30 Gene—Previously, we had screened a mouse genomic library of EMBL3 phages with rat Cx26 cDNA using low stringency hybridization. The phages (Willecke et al., 1991b). Here, we describe isolation of a recombinant phage clone that did not hybridize under stringent conditions to any of the known murine connexin genes. Restriction mapping and Southern blot hybridization revealed, within a 10.5-kb insert, two KpnI fragments that cross-hybridized to Cx26 rat liver cDNA (Fig. 1). These fragments were subcloned and sequenced. They contained a complete connexin homologous open reading frame, as revealed by comparison with previously characterized connexin genes and cDNAs (Fig. 2). The ATG start codon was located in a consensus translation initiation context (Kozak, 1991). The open reading frame coded for a protein of 261 amino acids with a predicted molecular mass of 30,336 Da. We designated this new connexin as mouse Cx30 according to the nomenclature suggested by Beyer et al. (1987).

Analysis of Mouse Cx30 Amino Acid Sequence—The deduced amino acid sequence of mouse Cx30 shows the typical features of a connexin protein: it contains four potential transmembrane regions (the third of which exhibits a marked amphipathic character) and two putative extracellular loops with conserved cysteine residues, compared with other connexins (see Bennett et al. [1991]). The transmembrane domains (underlined in Fig. 2) were predicted by the algorithm of Rao and Argos (1986) and alignment to topological domains of rat Cx32 that were previously deduced from site-specific antibody studies (Milks et al., 1988). Each of the two putative extracellular loops of mouse Cx30 contains three cysteine residues in the sequences Cx4,Cx4,C and Cx4,Cx4,C. Cx30 shows the highest overall amino acid identity to mouse Cx26 (77%), mouse Cx32 (57%), and
Based on these comparisons, mouse Cx30 can be classified in the β-group of connexins (Gimlich et al., 1990).

Table I shows the predicted pattern of amino acid identities among mouse Cx30, Cx26, and Cx43 (Hennemann et al., 1992a, 1992d) and Xenopus Cx30 (Gimlich et al., 1988) according to topological domains, as previously established for Cx32, Cx43, and Cx26 (Milks et al., 1988; Yancey et al., 1989; Zhang and Nicholson, 1994). It is evident that mouse Cx30 shows greater similarity to mouse Cx26 than to any other connexin protein sequence. Mouse Cx30 is probably not the rodent analogue of Xenopus Cx30, with which it shares only 56% amino acid identity. In particular, the cytoplasmic region C, which exhibits high divergence among connexins, shows 72% amino acid identity between mouse Cx30 and Cx26. For comparison, this domain shows only 11 and 29% sequence identities to mouse Cx43 and Xenopus Cx30, respectively. Fig. 3 illustrates the phylogenetic tree of all known murine connexin genes based on comparison of their amino acid sequences. It shows the close relationship and the relatively late divergence in evolution of Cx30 and Cx26 genes.

Genomic Organization and Chromosomal Localization—Comparison of connexin cDNAs with their respective genes has revealed a common feature of mammalian connexin genes: the complete coding region is located within a single exon. This coding exon is separated from the promoter by a large intron that can be between 3.8 kb (Cx26; Hennemann et al., 1992d) and 8.5 kb long (Cx43; Yu et al., 1994). The genomic organization of mouse Cx30 seems to comply with these criteria: the open reading frame is uninterrupted by introns. However, a possible splice acceptor site is located at positions 225 to 238 upstream of the start codon (Fig. 2), similar to other characterized connexin genomic sequences (Fishman et al., 1991; Willecke et al., 1991a; Hennemann et al., 1992a). Potential transmembrane regions according to the algorithm of Rao and Argos (1986) are underlined. Connexin-specific conserved cysteine residues in the extracellular domain are shown in boldface letters. The nucleotide sequence data are available from GenBank™/EMBL/DDBJ under accession no. 270023.
mouse Cx30 gene, in extension of the nomenclature used for designation of known mouse connexin genes (cf. Schwarz et al. (1992, 1994)).

Expression of Cx30 mRNA—Total RNA from several mouse tissues was prepared (Chomczynski and Sacchi, 1987), electrophoretically separated, and hybridized under stringent conditions (50% formamide, 5 x SSC at 42°C) to the mouse Cx30 gene probe described under "Materials and Methods." Preliminary experiments had shown that probe specificity and stringency of hybridization were sufficient to prevent cross-hybridization to Cx26 mRNA. Fig. 5A illustrates that expression of Cx30 mRNA is most abundant in adult brain and skin. Less abundant expression was detected in the uterus, lung, and eye tissue. Very low expression was seen in the testis and sciatic nerve. Cx30 mRNA was not detected in the liver, which exhibits abundant expression of the highly related Cx26 gene (Zhang and Nicholson, 1989). In all tissues that express Cx30 transcripts, two mRNAs species of 2.3 and 2.0 kb were detected, the latter one being much more abundant. Interestingly, the ratio of these two transcripts differed between mouse tissues. In adult brain and testis, the 2.3-kb Cx30 transcript contributed 35 and 50%, respectively, to the total amount of Cx30 mRNA as determined by quantitative densitometric evaluation of the double bands. In adult skin, uterus, and lung, the 2.0-kb mRNA was 10-fold more abundant than the 2.3-kb transcript. We have normalized the relative amounts of Cx30 transcripts (signals of 2.0- and 2.3-kb mRNAs added) to the amount of poly(A)⁺ mRNA in these tissues (see "Materials and Methods"). Normalized expression levels are indicated in Fig. 5B.

Furthermore, we have studied in more detail whether expression of Cx30 mRNA in the brain is developmentally regulated. Cx30 transcripts were not detected before birth. Weak expression was seen after 2 weeks of postnatal brain develop-

### Table I

Amino acid identities of the putative topological domains of mouse Cx30 compared with those of mouse Cx26 and Cx43 and Xenopus Cx30. Number in parentheses represent percent amino acid similarity (i.e., residues of similar chemical properties).

| Putative topology of mouse Cx30 | Residues in mouse Cx30 | Amino acid identities |
|---------------------------------|------------------------|----------------------|
|                                 |                        | To mouse Cx26 | To mouse Cx43 | To Xenopus Cx30 |
|                                 |                        | %                  | %                  | %                  |
| Cytoplasmic region A            | 1–21                   | 91 (95)           | 38 (62)           | 57 (76)            |
| Transmembrane region 1          | 22–41                  | 75 (95)           | 50 (80)           | 55 (90)            |
| Extracellular region B          | 42–74                  | 91 (94)           | 66 (72)           | 73 (76)            |
| Transmembrane region 2          | 75–94                  | 95 (95)           | 60 (70)           | 95 (95)            |
| Cytoplasmic region C            | 95–130                 | 72 (86)           | 11 (17)           | 29 (49)            |
| Transmembrane region 3          | 131–150                | 85 (95)           | 50 (60)           | 85 (95)            |
| Extracellular region D          | 151–188                | 66 (79)           | 51 (70)           | 61 (76)            |
| Transmembrane region 4          | 189–208                | 75 (90)           | 25 (70)           | 65 (90)            |
| Cytoplasmic region E            | 209–261                | 56 (75)           | 8 (11)            | 32 (45)            |

* For residues 209–224 (16 amino acids only). The Cx26 protein sequence is 35 residues shorter than the Cx30 sequence.
When the amounts of Cx30 mRNA in mouse brain were standardized by hybridization to a cytochrome c DNA probe (Hennemann et al., 1992b), it became obvious that the brain at 4 and 6 weeks and in adult mice contained similar amounts of Cx30 transcripts (Fig. 6).

Functional Expression of Mouse Cx30 in Xenopus Oocytes—Capped Cx30 RNA was synthesized in vitro using T7 RNA polymerase and injected into stage VI Xenopus oocytes. Following incubation overnight at 19 °C, oocytes were stripped of their vitelline envelopes and paired for a further 16–20 h before recording intercellular conductance with two-electrode voltage clamps. For this experimental series, these conditions yielded coupled oocytes from injections of control cRNA for Cx32 at 50% of the time. This frequency was lower than usual as the toads were entering their "nonproductive" season. The background of endogenous coupling was eliminated as described previously by injection of an antisense oligonucleotide to bases 25 to 25 of Xenopus Cx38 (numbering from the initiation codon for translation) (Ebihara et al., 1989).

Cx30 formed functional homotypic channels in Xenopus oocytes (13 out of 25 pairs) with conductances ranging from 0.5 to 3 microsiemens. Quantitative analysis of junctional currents induced by Cx30 showed that initial junctional conductance \((G_i)\) decreased with increasing positive or negative \(V_j\) (Fig. 7).

\[ G_i \text{ showed an asymmetry, with a slight decline in response to hyperpolarizing voltages and a larger decline with depolarizing voltages. Part or all of this decline in } G_i \text{ at higher } V_j \text{ may result from systematic errors introduced from extrapolation to } t = 0 \text{ of the exponential fits to the current decays as the time constants become markedly shorter with increasing } V_j \text{ (Fig. 7A). In fact, the dependence of the time constant of the current decay on } V_j \text{ was the steepest we have yet recorded of any connexin. The voltage gating responses of } G_{mn} \text{ of Cx30 channels, obtained from depolarizing and hyperpolarizing pulses, are described by slightly different Boltzmann relationships, with parameters of } A = 0.13, V_o = +37.7 \text{ mV, and } G_{mn} = 0.25 \text{ for depolarizing} \]

| TABLE II | Segregation of mouse Cx30 genes and mouse chromosomes in mouse × Chinese hamster somatic cell hybrids |
|----------------|--------------------------------------------------|
| **Cell hybrid** | **Mouse chromosome** |
| **Mouse Cx30 gene** |
| EBS 1 | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X |
| EBS 2 | + + + + (+)* |
| EBS 4 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 5 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 9 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 11 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 13 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 15 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 17 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 3A | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 3A | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 15A | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 51 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 58 | + + + + + + + + + + + + + + + + + + + + + + |
| EBS 13A | + + + + + + + + + + + + + + + + + + + + + + |

**Concordant hybrids** | 9 6 7 10 11 7 5 7 8 11 6 5 14 4 7 5 8 8 4 |
| **Discordant hybrids** | 6 9 8 5 4 8 10 8 8 7 4 9 10 1 11 8 10 7 7 11 |
| **Discordancy (%)** | 40 60 53 33 26 53 53 26 66 66 73 53 66 46 46 73 |

* (+) indicates that a fragment of the chromosome was present.

**FIG. 5.** Tissue-specific expression of mouse Cx30 mRNA as detected by Northern blot hybridization and autoradiography. A, total RNA (20 μg/lane) from adult mouse tissues was electrophoresed, blotted, and probed with a genomic mouse Cx30 probe (as described under "Materials and Methods"). Two transcripts of 2.0 and 2.3 kb were detected, being most abundant in the brain, skin, and uterus. B, expression of Cx30 mRNAs was standardized against abundance of poly(A)⁺ RNA in each tissue sample using [³H]oligo(dT) (see "Materials and Methods"). The amount of Cx30 transcripts in the brain was set equal to 100%. Note that autoradiographic intensities of both Cx30 mRNA transcripts were added for this comparison.

**FIG. 6.** Developmental expression of Cx30 mRNA in mouse brain. Samples of total RNA (20 μg/lane) were isolated from mouse brain at the developmental stages indicated and subjected to Northern blot hybridization as described in the legend to Fig. 5. Significant amounts of Cx30 mRNA were detected 2 weeks after birth and became most abundant in adult brain.

Concordant hybrids | 9 6 7 10 11 7 5 7 8 11 6 5 14 4 7 5 8 8 4 |
| Discordant hybrids | 6 9 8 5 4 8 10 8 8 7 4 9 10 1 11 8 10 7 7 11 |
| Discordancy (%) | 40 60 53 33 26 53 53 26 66 66 73 53 66 46 46 73

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Fig. 7. Electrical coupling of Cx30. A, recordings of transjunctional current through Cx30 gap junctions expressed in oocytes. Voltage pulses of 1-s duration, ranging from 5 to 105 mV and from −5 to −105 mV, in 10-mV increments were applied to one cell, while the other was clamped at its resting potential of −60 mV. Note the marked increase in the rate of current decay with increasing voltage. Values of Gss and Gmin were obtained by extrapolations of exponential fits of the current decay to t = 0 and t = infinity, respectively. B, plots of Gss (normalized to the values obtained at + or −5 mV) or steady-state conductance (Gss/Gi, Gmin normalized to Gi at the same Vj) versus Vj showed asymmetric declines at higher voltages that are likely to be due, at least in part, to the limited time resolution of the system as channel kinetics became faster with increasing Vj. Normalized Gss values from depolarizing (positive Vj) or hyperpolarizing (negative Vj) voltage pulses were fit by distinct Boltzmann relations with the following parameters: A = 0.13, V0 = 37.7 mV, and Gmin = 0.25 for positive Vj, and A = 0.10, V0 = 45.7 mV, and Gmin = 0.26 for negative Vj. C, plots of Gss and Gmin versus Vj for Cx32/Cx30 heterotypic pairings, with the Cx30 cell defined as the positive pole. Both Gss and Gmin were normalized to conductance at 0 mV (interpolated between + and −5 mV). Rectification of Gss and the unilateral gating response of Gmin were similar to those observed in Cx32/Cx30 heterotypic combinations, with steeper instantaneous rectification (slope of Gss versus Vj = 0.010 mV−1). The drop in Gss at larger positive Vj was presumably analogous to that described for the homotypic channels in B. The unilateral gating response of Gss can be obtained by fitting Gss/Gi versus Vj to a Boltzmann relation with the parameters A = 0.136, V0 = 69.8 mV, and Gmin = 0.28 (inset). D, plots of Gss and Gss/Gi (normalized as described for B) versus Vj for Cx26/Cx30 heterotypic pairings. Positive Vj was defined as depolarizing pulses in the Cx30-expressing oocyte or as hyperpolarizing pulses in the Cx26-expressing oocyte. Gating characteristics at positive Vj were similar to those of Cx30 homotypic channels, although a Boltzmann fit to the data yielded slightly different parameters (A = 0.18, V0 = 59.2 mV, and Gmin = 0.28). A similar increase in the V0 of the Cx26 hemichannel would move its voltage response beyond the recorded range.

Rectification values and A = 0.10, V0 = −45.7 mV, and Gmin = 0.26 for hyperpolarizing pulses. Paired t tests of the Gss values for equivalent hyper- and depolarizing Vj pulses demonstrated this asymmetry to be significant at the 0.05 to 0.02 probability level. The asymmetry in Gss is independent of that seen in Gi, as we plot the Gss/Gi ratio in Fig. 7. The asymmetric responses of both Gi and Gss versus Vj are consistent with a sensitivity to transmembrane or inside-outside voltage (Vicr). However, preliminary studies utilizing different holding potentials for both oocytes ranging from Vm = 120 to 0 mV revealed no obvious sensitivity of conductance to Vicr, although it remains possible that the relatively minor asymmetries seen in Fig. 7, if arising to Vicr may require more extensive statistical analysis to detect.

Cx30 also formed functional heterotypic channels with Cx32 (7 out of 13 pairs) and Cx26 (7 out of 12 pairs) in Xenopus oocytes, with junctional conductances ranging from 1 to 5 microsiemens and from 1 to 8 microsiemens, respectively. The Cx32/Cx30 pairing produced highly rectifying channels that showed only a slow voltage gating response when the Cx30 side was made relatively positive (Fig. 7C). Boltzmann parameters describing this response were obtained from replotted of Gss/Gi versus Vj (see Fig. 7C, inset) and fitting, with values of A = 0.136, V0 = 69.8 mV, and Gmin = 0.28. This behavior was analogous to that reported for Cx32/Cx26 pairs. Rapid rectification, also characteristic of Cx32/Cx26 heterotypic junctions, was evident in the Gi plot of the Cx32/Cx30 response to Vj. The increase in conductance with positive Vj (defined with respect to the Cx26- or Cx30-expressing oocyte) was even steeper with Cx30 (0.009 mV−1) than with Cx26 (0.0055 mV−1) (Barrio et al., 1991).

Cx30/Cx26 pairings did not show significant rectification of Gi other than that likely to be attributable to an extrapolation of the rapidly decaying currents at large positive Vj. The Gss plot showed an asymmetry that was somewhat predictable from the respective homotypic behaviors of Cx30 and Cx26.
The heterotypic interaction appeared to cause an increase in the $V_o$ of each of the hemichannel responses compared with the homotypic case, such that no significant gating of the Cx26 hemichannel was evident within the range tested.

**DISCUSSION**

The new mouse Cx30 gene is closely related to the mouse Cx26 gene, with which it shares 77% sequence identity, more than with any other connexin gene described so far. We have assigned the Cx30 gene to mouse chromosome 14, which also contains the genes for Cx26 and Cx46 (Schwarz et al., 1992). Thus, presumably the Cx30 and Cx26 genes arose by gene duplication. The dendrogram of all murine connexin genes (Fig. 3) supports the notion of a common origin of these genes, late in evolution relative to other connexin genes. Whereas the cytoplasmic loop of the mouse $\beta$-connexin proteins Cx26 and Cx32 shows only 37% sequence identity (cf. Fig. 4B of Hennemann et al. (1992c), for example), the Cx26 and Cx30 proteins exhibit 72% identity in this region.

Several findings support our conclusion that mouse Cx30 is a functional connexin gene. First, the genomic Cx30 sequence has all the features characteristic of other functional rodent connexin genes, i.e. the reading frame is uninterrupted by introns, but there is a possible intron upstream of the initiation site for translation. Second, the Cx30 amino acid sequence deduced from the nucleotide sequence contains four potential transmembrane regions characteristic of connexins. The extracellular cysteine residues found in all connexin proteins and potentially involved in recognition and docking of hemichannels (John and Revel, 1991; Dahl et al., 1992) are conserved as well. Third, the coding region of Cx30 hybridizes to two specific transcripts of 2.3 and 2.0 kb in several mouse tissues. Fourth, when Cx30 cRNA is expressed in Xenopus oocytes, it forms gap junction channels exhibiting unique voltage dependence.

The different sizes of the mouse Cx30 mRNA could be due to different start points of transcription, alternative splicing, or different lengths of the 3′-untranslated region. Alternative splicing has recently been detected in the rodent Cx32 gene. Schwann cells express Cx32 mRNA using an alternative promoter located in the large intron upstream of the coding exon (Neuhaus et al., 1995; Sohl et al., 1996). In addition, a third Cx32 transcript is expressed in embryonic stem cells. Two additional mouse connexin genes, Cx30.3 and Cx31, exhibit transcripts of two different sizes (reviewed by Willecke et al. (1991a)). Since a large intron is conserved in the 5′-untranslated region of all connexin genes, one can speculate that an alternative promoter usage may be a common feature for regulation of connexin gene expression.

Upon expression of the cDNA of Cx30 in the Xenopus oocyte system, robust currents between cell pairs were recorded. Surprisingly, the voltage sensitivity of these currents was slightly asymmetric, showing greater decrements in both $G_0$ and $G_{as}$ with depolarizing voltage pulses (see Fig. 7, A and B). The kinetics of the current decay showed a strong dependence on $V_j$ for both polarities. Given the poor temporal resolution of the oocyte system (clamping times are typically 10 ms), this posed a problem for obtaining accurate estimates of $G_j$ at high voltages. Despite efforts to extrapolate the exponential decays to zero, some apparent decreases in $G_j$ were seen. While this is likely to be due to extrapolation errors, we cannot discount the possibility that this reflects a real $V_j$-activated gate with kinetics that are not resolved by our clamps.

The steady-state conductance of these channels showed asymmetrical gating in response to $V_j$, even when $G_{as}$ values were normalized to $G_0$ for each value of $V_j$. This was most evident in Boltzmann fits of the data that yielded different parameters for hyperpolarizing and depolarizing voltage pulses, most notably with respect to $V_o$, ($-45$ and $37$ mV, respectively). This asymmetry could arise from sensitivity of the channels to transmembrane voltage differences, although this would have to be of the opposite polarity to that seen for the closest homologue studied to date, Cx36. However, no overt dependence of transjunctional conductance on the original holding potential of the cell (from $-120$ to $0$ mV) was observed. An alternative possibility could be an asymmetric contribution of heterotypic channels between Cx30 and Xenopus Cx38, although this seems unlikely as no conductance was ever seen to develop between Cx30 and endogenous Xenopus Cx38. Finally, it is formally possible that the docking process between connexins could differentially affect the voltage gates in the two hemichannels. Such a phenomenon has not been seen to date and seems unlikely given the reported mirror image symmetry of the two halves of a gap junction.

Another surprising result is the lack of similarity in the gating profiles in response to $V_o$ for Cx30 and its close homologue, Cx26. Cx30 channels responded at significantly lower voltages ($V_o = 37\text{–}45$ mV) than Cx26 ($V_o = 89$ mV) and also showed faster kinetics of closure by at least an order of magnitude (see Fig. 7A). Noticeably, the time constant of decay is more strongly dependent on $V_o$ than that of any other connexin we have studied in oocytes.

Other general characteristics of Cx30, revealed in heterotypic pairings with Cx26 or Cx32, were more similar to the properties of Cx26. Heterotypic pairings with Cx26 showed asymmetrical voltage gating (Fig. 7D), consistent with each hemichannel gating with positive voltage at its cytoplasmic face, but with $V_o$ increased over that seen in homotypic channels (cf. $V_o = 59$ mV for Cx30 homotypic channels and $V_o > 105$ mV with $V_o = 89$ mV for Cx26 homotypic channels). Such increases in $V_o$ may reflect increases in the activation energy required for channel closure, a property that may be influenced by the nature of the docking interaction between hemichannels.

Cx32/Cx30 heterotypic channels showed even greater rectification characteristics than reported for Cx32/Cx26 (Fig. 7C) (cf. Barrio et al. (1991)). Based on previous analyses of Cx32/Cx26 channels (Verselis et al., 1994), the asymmetric $G_{as}$ response of Cx32/Cx30 channels to $V_j$ further supports the conclusion that Cx30 channels gate when their cytoplasmic ends are relatively positive. The rapid rectification seen in the $G_j$ versus $V_j$ plot of Cx32/Cx26 has recently been demonstrated not to represent gating, but to reflect the properties of each channel that changes conductance with voltage (Bukauskas et al., 1995). It is largely explained by significant differences in the relative selectivity of the two channels for cations and anions (Cx32, slightly anionically selective; Cx26, cationically selective). By this criterion, Cx30 and Cx26 would be expected to share similar ionic selectivity, although the steeper rectification of $G_j$ seen in Cx32/Cx30 channels (0.010 mV$^{-1}$) compared with Cx32/Cx26 channels (0.005 mV$^{-1}$) might suggest that Cx30 channels are more strongly cationically selective than Cx26 channels.

The expression pattern of mouse Cx30 transcripts was different from that of Cx26 mRNA. For example, no expression of Cx30 was found in the liver and pancreas, which expressed relatively high levels of Cx26 (Zhang and Nicholson, 1989). Both genes were expressed in the brain, skin, and uterus. The
quantitative comparison of Cx30 mRNA expressed in different tissues (Fig. 5) showed that expression was most abundant after 4 weeks of postnatal development in the brain. This was clearly different from Cx26, which is more highly expressed in prenatal brain and decreases after birth (Dermietzel et al., 1989). Cx30 gap junction channels appear to be characteristic of adult mouse brain.

The expression of both Cx30 and Cx32 (the latter associated with oligodendrocytes and some neurons; see Dermietzel and Spray (1993)) raises the interesting possibility of formation of rectifying channels such as those reported above in the oocyte system. In a tissue showing the rapid local fluctuations in voltage seen in the central nervous system, such channels could play a significant role in preferentially directing impulse propagation. A critical future goal will be to use in situ hybridization and peptide-specific antibodies to resolve the expression pattern of mouse Cx30 at the cellular level. This will reveal whether Cx30 is expressed in neurons or glial cells. Electrophysiological experiments, reviewed by Dermietzel and Spray (1993), have indicated that the CA3 region of rat hippocampus contains gap junctions that do not react with antibodies to Cx43, Cx32, or Cx26 proteins. Cx30 is an interesting candidate for analysis of unidentified gap junction proteins and channels in the brain. Furthermore, since there are relatively few amino acid differences between Cx26 and Cx30 proteins, it is possible that some of the described antibodies, directed to Cx26-derived peptides or the total Cx26 protein, may cross-react with the Cx30 protein. Thus, the specificity of Cx26 antibodies has to be reanalyzed and carefully compared with that of Cx30 antibodies. These experiments are currently underway in our laboratory. Both the similarities and differences in the channel properties of Cx30 and Cx26, given their high level of sequence identity, raise exciting prospects for identification of specific domains, or even residues, that influence characteristics such as voltage, gating kinetics, and permeability.

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