J. Membrane Biol. 116, 187–194 (1990)

The Journal of
Membrane Biology

Topical Review

Connexin Family of Gap Junction Proteins

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Introduction

Gap junctions are composed of aggregations of membrane channels, called *connexons*, joined with similar connexons in adjacent cells to form intercellular pathways for the diffusion of ions and small molecules (Caspar et al., 1977; Makowski et al., 1977). The resulting intercellular communication is unique in that adjacent cells exchange cytoplasmic molecules directly, with no secretion into the extracellular space (Bennett, 1966; Loewenstein, 1966). Due to the large size of the intercellular channels formed by connexon pairs, the exchange of molecules between cells is nonspecific, and includes the entire pool of ions and small metabolites in each cell (Gilula, Reeves & Steinbach, 1972; Pitts & Simms, 1977; Simpson, Rose & Loewenstein, 1977; Goodenough, Dick & Lyons, 1980). This form of intercellular communication is ideally suited for the role of intercellular buffering of cytoplasmic ions (Corsaro & Migeon, 1977; Ledbetter & Lubin, 1979), synchronization of cellular behavior, such as the coordinated contraction of myocardial cells (Barr, Dewey & Berger, 1965) and the cell-to-cell coordination of metachronal waves (Moss & Tamm, 1987; Sanderson, Chow & Dirksen, 1988). Involvement of gap junction-mediated intercellular communication has also been suggested for growth control and embryonic differentiation (Loewenstein, 1966; Furshpan & Potter, 1968; Warner, Guthrie & Gilula, 1984; Loewenstein & Azarnia, 1988). Due to the sharing of low molecular weight substrate pools, gap junctions will also function to suppress the deleterious effects of somatic cell mutation in a variety of enzymes (Subak-Sharpe, Burk & Pitts, 1969; Cox et al., 1970).

Recent studies have shown that the gap junctions in many tissues are formed by members of a family of related proteins for which the generic name *connexin* has been suggested (Beyer, Paul & Goodenough, 1987). In this paper, we will briefly highlight the structural and biochemical studies that suggest the presence of related, but differing gap junction proteins. We will describe the cDNA cloning studies that have predicted the structures of several connexins and the biochemical and immunohistochemical studies used to test the models derived from the sequences. And, finally, we will describe the ongoing studies using the cloned gap junction cDNAs to investigate the relation between the structural similarities and differences among connexins and gap junction physiology.

Gap Junction Structure

The gap junction was originally characterized by its appearance in thin-section electron micrographs as a pair of membranes of variable area separated by a 2-nm "gap" (Robertson, 1963; Revel & Karnovsky, 1967). Freeze-fracture replicas have shown that the structure is characterized by a plaque-shaped, differentiated region of the plasma membrane containing a dense array of intramembrane particles (connexons) on the P-fracture face and a complementary array of depressions or pits on the E-fracture face (Kreutziger, 1968; Goodenough & Revel, 1970).

However, gap junctions do not all appear structurally and functionally identical. Myocardial gap junctions are thicker in profile than those in liver, implying that in the heart either additional proteins are associated with the cytoplasmic surfaces or that the heart junctional protein is larger (Goodenough, Paul & Culbert, 1978; Kensler & Goodenough, 1980; Manjunath, Goings & Page, 1984). Gap junctions from liver and lens epithelium may develop a

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Key Words gap junctions · connexin · intercellular communication · molecular cloning

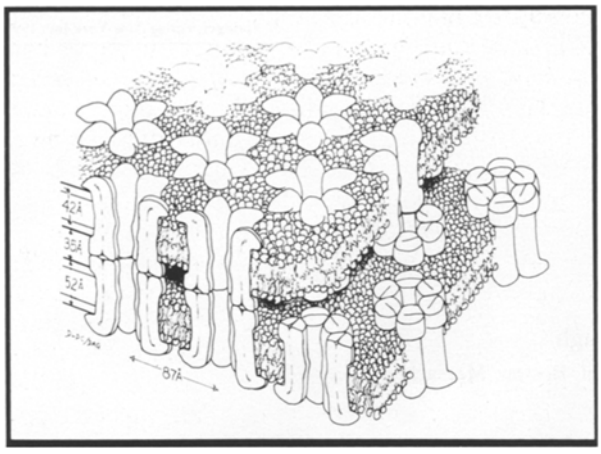

Fig. 1. A low-resolution diagram of one of the forms of isolated gap junctions from mouse liver, based on data from x-ray diffraction and electron microscopy. The protein subunits are arrayed in hexamers (connexons) in each of the paired junctional membranes, here shown separated by a 35 Å extracellular gap. Two apposed connexons join in mirror symmetry to form an axial channel connecting the cytoplasms of the participating cells. Modeling studies of high-angle x-ray data are consistent with predominantly alpha-helical secondary structure in the transmembrane portions of the protein subunits (Tibbitts et al., 1989). Three-dimensional structural analyses of x-ray and frozen hydrated specimens (Makowski, 1988; Caspar et al., 1988) reveal connexon-connexon interactions at the threefold axes on the cytoplasmic surfaces (not shown here). This figure is reprinted with permission from *The Journal of Cell Biology*, 1977, Vol. 74, p. 643, by copyright permission of the Rockefeller University Press

near-crystalline structure in response to isolation or chemical fixation, but the gap junctions between lens fiber cells do not, suggesting other biochemical differences (Goodenough & Stoeckenius, 1972; Schuetze & Goodenough, 1982; Miller & Goodenough, 1985, 1986). Many studies have suggested physiologic differences between gap junctions in different cell types (Flagg-Newton & Loewenstein, 1980; Schuetze & Goodenough, 1982; Spray & Bennett, 1985; White et al., 1985); certainly such physiologic differences might be due to differences in the constituent gap junction proteins.

Procedures have been developed for the isolation of gap junctions from liver (Goodenough & Stoeckenius, 1972; Hertzberg & Gilula, 1979). These isolated gap junctions retain their in vivo appearance in thin sections, but negative stain and freeze-fracture images reveal that, as a consequence of the isolation procedure, the apparent disordered arrays of particles seen in the whole tissue become tightly crystallized. The crystalline nature of the connexons in isolated liver junctions has facilitated study of some of their molecular structure using x-ray diffraction and low-dose Fourier microscopy (Makowski et al., 1977; Baker et al., 1982; Unwin & Ennis, 1984; Caspar et al., 1988; Sosinsky et al., 1988). These studies have visualized the connexon as a hexameric assembly of integral membrane proteins (connexins) delineating an axial transmembrane channel (*see* Fig. 1). The volume of the electron density of a single protein can be used to predict a molecular weight of about 24 kD (Makowski et al., 1984). The discrepancy between this estimate and the values of 27 and 32 kD for the liver gap junction protein gained from protein biochemistry and molecular biology comes from uncertainties in the analysis and proteolysis of the specimens (discussed in Makowski, 1988). In addition, certain features of the gap junction structure are easily disordered or damaged during specimen preparation, making them invisible in Fourier analysis (Caspar et al., 1988).

Based on primary sequence data, models of alpha helical folding of connexin32 traversing the junctional membrane have been published (Hertzberg et al., 1988; Milks et al., 1988). Secondary structural data available in x-ray diffraction patterns from isolated gap junction plaques have been compared with simulated powder and fiber diffraction from alpha helices, beta sheets, and whole proteins with known different structural motifs. Gap junction diffraction correlates most closely with up-and-down alpha helix bundle proteins, in which the helices are tilted between 25° and 55° from the membrane plane (Tibbitts et al., 1989), suggesting the presence of alpha helical structure in the transmembrane portions of the connexin32 protein (Milks et al., 1988).

Biochemical Studies

In addition to the liver junctions, methods have been developed for the isolation of myocardial gap junctions (Kensler & Goodenough, 1980; Manjunath et al., 1984) and of lens fiber plasma membranes which contain 5–10% gap junction profiles (Bloemendahl et al., 1972; Alcala, Lieska & Maisel, 1975; Broekhyse, Kulhman & Stols, 1976; Goodenough, 1979; Paul & Goodenough, 1983). SDS-PAGE of these isolated preparations has shown that the isolated liver gap junctions are composed primarily of a 27-kD polypeptide, accompanied by proteolysis fragments, aggregates, and a 21-kD polypeptide (Henderson, Eibl & Weber, 1979; Hertzberg & Gilula, 1979; Finbow et al., 1980). In the mouse, the 21-kD polypeptide is present at 50% the abundance of the 27-kD one, while in the rat its relative abundance is only 10% (Nicholson et al., 1987). Isolated myocardial gap junctions contain a 43–47 kD polypeptide, cleaved by endogenous pro-

teases to 34, 32, and 29 kD bands (Kensler & Goodenough, 1980; Manjunath et al., 1987). Isolated bovine and ovine lens fiber plasma membranes contain a predominant polypeptide of 26 kD, called MP26 or MIP26, and numerous other polypeptides, notably one of 70 kD (MP70) (Kistler, Kirkland & Bullivant, 1985). N-terminal sequencing of these proteins by Edman degradation has shown that the liver 27 and 21 kD (Nicholson et al., 1987), the heart 43–47 kD and its degradation products (Gros, Nicholson & Revel, 1983; Nicholson et al., 1985), and the lens 70 kD (Kistler, Christie & Bullivant, 1988) are homologous proteins, while the lens 26 kD appears unrelated (Nicholson et al., 1983).

Polyclonal and monoclonal antisera have been raised to the liver 27 kD, the lens MP26, and the lens MP70. Immunocytochemical studies have shown that anti-27 kD and anti-MP70 antisera bind directly to the cytoplasmic surfaces of gap junctions from liver and lens, respectively (Janssen-Timmen et al., 1983; Paul, 1985, 1986; Stevenson et al., 1986; Gruijters et al., 1987; Milks et al., 1988; Goodenough, Paul & Jesaitis, 1988). In some laboratories, anti-MP26 has been localized to both the junctional and nonjunctional membranes of lens fibers (Bok, Dockstader & Horwitz, 1982; Fitzgerald, Bok & Horwitz, 1983; Sas et al., 1985), while in others, the antisera can only be localized to the nonjunctional membranes (Paul & Goodenough, 1983; Zampighi et al., 1989). In a novel study, Gruijters (1989) reports that MP26 is associated only briefly with lens fiber junctions during their assembly. The antisera have also been used to show that while MP26 and MP70 appear to be lens-specific proteins, the 27-kD molecule is not unique to the liver, localizing to junctions in other tissues, as well, e.g., stomach, exocrine pancreas, renal tubules, and brain (Dermietzel et al., 1984; Paul, 1985). On Western blots, some antisera have proven to be specific for the 27-kD molecule, while others will cross-react with junctional proteins in other tissues (Hertzberg & Skibbens, 1984; Paul, 1985). Taken together, these antibody data corroborate the results from the Edman degradation studies: gap junctions between different cell types may be made from different members of a protein family that share some structure, but also contain unique domains.

Molecular Studies

cDNAs coding for the liver 27-kD molecule have been cloned from both rat and human sources (Paul, 1986; Kumar & Gilula, 1986). They both code for a protein of 32 kD. Since the mobility of this protein on SDS-PAGE varies with different electrophoresis conditions making assignment of an M_r inaccurate, and since the 27-kD protein is not unique to liver, we have proposed a nomenclature using the generic term *connexin* for the protein family, with an indication of species and a numeric suffix designating the predicted molecular mass in kilodaltons. Thus, the 27-kD protein from rat liver is termed *rat connexin32*. An alternative nomenclature system has recently been suggested which assigns greek letters to different classes of gap junction proteins according to their order of appearance in embryonic development (Risek et al., 1990). The identity of rat connexin32 as a gap junction protein has been confirmed by demonstrating that an antiserum raised against a bacterial fusion protein reacts with the gap junction structure by immunocytochemistry and by demonstrating the production of functional channels after expression of connexin32 in paired *Xenopus* oocytes (Paul, 1986; Dahl et al., 1987; and *see below*).

The connexin32 cDNA was used to isolate cDNAs coding for other connexins from a number of mammalian sources. By screening a rat myocardial library with the connexin32 cDNA at reduced stringency, a cDNA has been cloned which codes for a homologous polypeptide of 43 kD, termed *connexin43* (Beyer et al., 1987). Connexin43 shows about 43% overall homology with connexin32, but contains certain regions with many more identical amino acids (Fig. 2 and *see* discussion below). A second cDNA from rat liver has been cloned which codes for the 21-kD protein (Zhang & Nicholson, 1989); this cDNA codes for a 26-kD protein and would thus be termed *connexin26*. Connexin26 shares structural features over the length of the molecule with connexins32 and -43 (*see* Fig. 2). The 70-kD lens protein MP70 has not been cloned, but a cDNA which predicts a 46-kD polypeptide (*connexin46*) has been isolated from a lens cDNA library which shares exact amino acid sequence with the N-terminus of MP70 (Beyer, Goodenough & Paul, 1988; Kistler et al., 1988). The relationship of MP70 to connexin46 has not yet been clarified; the anti-MP70 monoclonal antibody does not see a 46-kD species in lens Western blots. Northern analysis of lens RNA probed with connexin46 reveals a 3.0-kb message. The cDNA coding for MP26 has been cloned; it has no sequence similarity to any of the known connexins (Gorin et al., 1984).

The low-stringency cloning strategies have also identified connexins in other vertebrates. *Xenopus connexin30* has been cloned from a liver cDNA library (Gimlich, Kumar & Gilula, 1988); it shows 71% identical amino acids to the rat or human connexin32 (Fig. 2). An additional *Xenopus* connexin, expressed only in oocytes and embryos, *Xenopus*

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                                                                  *
Rat Cx26  M-DWGTLQSILGGVNKHSTSIGKIWLTVLFIFRIMILVVAAKEVWGDEQADFVCNTLQPG   59
Rat Cx32  M-NWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAAESVWGDEKSSFICNTLQPG   59
Rat Cx43  MGDWSALGKLLDKVQAYSTAGGKVWLSVLFIFRILLLGTAVESAWGDEQSAFRCNTQQPG   60
Xen Cx30  M-NWAGLYAILSGVNRHSTSIGRIWLSVVFIFRIMVLVAAAESVWGDEKSAFTCNTQQPG   59
Xen Cx38  MAGWELLKLLLDDVQEHSTLIGKVWLTVLFIFRIFILSVAGESVWTDEQSDFICNTQQPG   60

          *   *
Rat Cx26  CKNVCYDHYFPISHIRLWALQLIMVSTPALLVAMHVAYRRHEKKRKFMKGEIKNEFKD--   117
Rat Cx32  CNSVCYDHFFPISHVRLWSLQLILVSTPALLVAMHVAHQQHIEKKMLRLEGHGDPLHL--   117
Rat Cx43  CENVCYDKSFPISHVRFWVLQIIFVSVPTLLYLAHVFYVMRKEEKLNKKEEELKVAQTDG   120
Xen Cx30  CNSVCYDHFFPISHIRLWALQLIIVSTPALLVAMHVAHLQHQEKKELRLSGHVKDQEL--   117
Xen Cx38  CTNVCYDQAFPISHVRYWVLQFLFVSTPTLTYLGHMVYLSKKEEKERQKENESRILVA--   117

Rat Cx26  ----------------IEEIKTQKVRIEGSLWWTYTTSIFFRVIFEAVFMYVFYIMYNGF   161
Rat Cx32  ----------------EEVKRHKVHISGTLWWTYVISVVFRLLFEAVFMYVFYLLYPGY   160
Rat Cx43  VNVEMHLKQIEIKKFKYGIEEHGKVKMRGGLLRTYIISILFKSVFEVAFLLIQWYIY-GF   179
Xen Cx30  -----------------AEVKKHKVKISGTLWWTYISSVFFRIIFEAAFMYIFYLIYPGY   160
Xen Cx38  ----------NEAQTEVYSSATKKIRIQGPLMCTYTTSVVFKSIFEAGFLLGQWYIY-GF   165

                  *    *      *
Rat Cx26  FMQRLVKCNAWPCPNTVDCFISRPTEKTVFTVFMISVSGICILLNITELCYLFIRYCSGK   221
Rat Cx32  AMVRLVKCEAFPCPNTVDCFVSRPTEKTVFTVFMLAASGICIILNVAEVVYLIIRACARR   220
Rat Cx43  SLSAVYTCKRDPCPHQVDCFLSRPTEKTIFIIFMLVVSLVSLALNIIELFYVFFKGVKDR   239
Xen Cx30  SMIRLVKCDAYPCPNTVDCFVSRPTEKTIFTVFMLVASGVCIVLNVAEVFFLIAQACTRR   220
Xen Cx38  VMSPIFVCERIPCKHKVECFVSRPMEKTIFIIFMLVVSLISLLLNLMELIHLSFKCFQHG   225

Rat Cx26  SKRPV                                                         226
Rat Cx32  AQRRSNPPSRKGSGFGHRLSPEYKQNEINKLLSEQDGSLKDILRRSPGTGAGLAEKSDRC   280
Rat Cx43  VKGRSDPYHATTGPLSPSKDCGSPKYAYFNGCSSPTAPLSPMSPPGYKLVTGDRNNSSCR   299
Xen Cx30  A-RR-----HRDSG-S--ISKEHQQNEMN-LL-ITGGS---IIKRSP---AGQ-EKGDHC   262
Xen Cx38  IKEGATCPPTGIPFNGAGNRMPPQEYTNPPSSNQDIDLPAYNKMSGGHNWSSIQMEQQVN   285

Rat Cx32  SAC                                                           283
Rat Cx43  NYNKQASEQNWANYSAEQNRMGQAGSTISNSHAQPFDFPDDNQNAKKVAAGHELQPLAIV   359
Xen Cx30  STS                                                           265
Xen Cx38  GLVKPKCQCDCWSQSAISVVVSGAPGIISNMDAVKRNHQTSSKQQYV                334

Rat Cx43  DQRPSSRASSRASSRPRPDDLEI                                       382
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Fig. 2. Comparison of the amino acid sequences for five connexins as derived from the cloned cDNAs. The alignments of rat connexin32 and -43 are shown as in Beyer et al. (1987) and those of rat connexin32 and *Xenopus* connexin30 are shown as in Gimlich et al. (1988). The sequences of rat connexin26 (Zhang & Nicholson, 1989) and *Xenopus* connexin38 were optimally aligned manually. Residues which are identical at a given position in half or more of the connexins are shown in boldface type. Dashed lines represent spaces added to optimize alignment. This comparison shows that all of the connexins contain two regions with many identical residues. Between the two homologous regions and at the C-terminal ends of the connexins the sequences show little similarity

connexin38, shows 32–41% homology to rat connexin32 and -43 (Ebihara et al., 1989; *see* Fig. 2). Other unique connexins are also expressed in the developing chick embryo (E.C. Beyer, *in preparation*).

The amino acid sequences derived from the cloned cDNAs have been used to predict the structures of the connexins. Hydropathy plots of connexin32 predict four hydrophobic domains, with a large carboxy-terminal hydrophilic tail. Three smaller hydrophilic domains separate the hydrophobic regions (Paul, 1986). These data, together with that from proteolysis studies of isolated junctions, have been used to construct topology models for the relation of this polypeptide to the junctional plasma membrane, assuming that each hydrophobic domain represents a transmembrane segment of the molecule (Beyer et al., 1987; Zimmer et al., 1987). This model has been tested by examining the protease sensitivity of isolated liver gap junctions and by the mapping of site-specific antisera by immunocytochemistry. The controlled proteolytic cleavage has demonstrated that both the N- and C-termini of connexin32 face the cytoplasm, and that an additional cytoplasmically-accessible proteolytic site is located between the second and third transmembrane segments (Zimmer et al., 1987; Hertzberg et al., 1988). Antisera have been raised against syn-

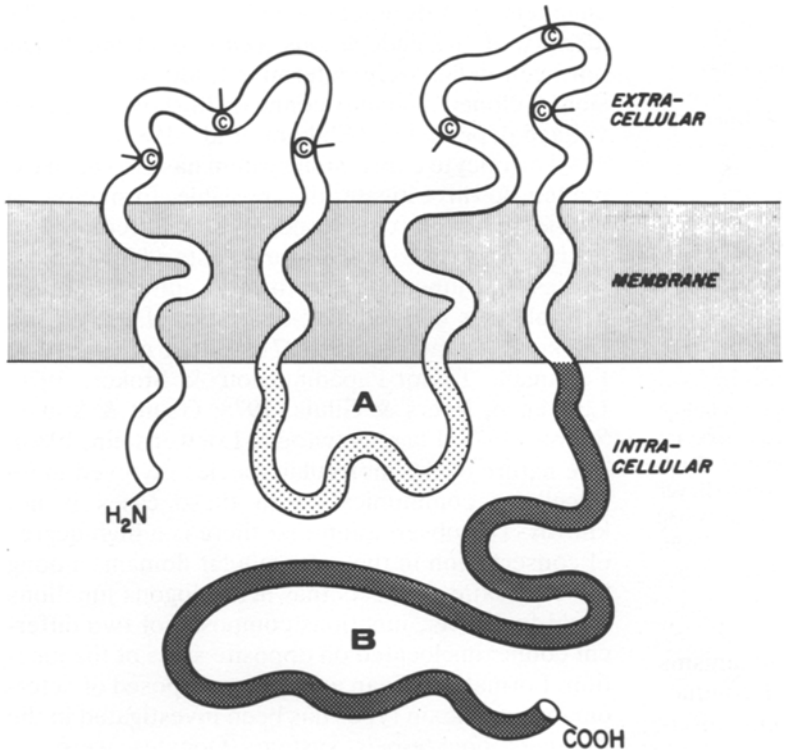

Fig. 3. Structure and topology of the connexins relative to the junctional plasma membrane. A model of the topology of the subunit gap junction proteins has been developed based on hydropathy plots and tested by proteolysis and immunocytochemical studies (*see* text). That model, which is depicted here, predicts that the connexins have four transmembrane spans and have both their amino- and carboxy-termini located on the intracellular face of the junctional membrane. Unshaded portions represent the regions of the connexins which are relatively more conserved among all members of the family: the four transmembrane and two extracellular domains. The two extracellular domains each contain three invariant cysteines (represented by circled *C*). In contrast, the cytoplasmic loop in the middle of the connexins (*A*) and the cytoplasmic carboxy-terminal tail (*B*) are entirely different among the connexins, both in sequence and in length. The sizes of these regions in different connexin molecules are shown in the Table

thetic oligopeptides representing various segments of connexin32 and have been used to map the topology in the electron microscope (Goodenough et al., 1988; Milks et al., 1988). These studies have confirmed the proteolysis studies. They have shown that the amino-terminus, the carboxy terminus and a loop in the middle of the protein are all located on the cytoplasmic face of the junctional membrane. And, to the degree that the harsh experimental conditions do not alter the protein topology, they also demonstrate that both extracellular domains of connexin32 can be detected on the extracellular surfaces of the junctional membranes (*see* Fig. 3).

Studies have suggested that connexin43 has a similar structure. Hydropathy analysis reveals a similar set of four hydrophobic domains to those in connexin32. Modelling of connexin43 in a comparable manner to connexin32 suggests that those hydrophobic/transmembrane domains and the extracellular regions correspond to the homologous regions between connexin43 and -32 (Beyer et al., 1987). Proteolysis studies of isolated cardiac gap junctions combined with the localization of antisynthetic peptide antisera have confirmed the three separate cytoplasmic domains and an overall membrane topology similar to that of connexin32 (Beyer et al., 1989; Yancey et al., 1989).

Such studies mapping sites by proteolysis and immunocytochemistry have not been conducted for the other connexins, but sequence comparison and analogy to the connexin32/43 studies allow some prediction of their structure. All of the sequenced connexins share the four hydrophobic domains corresponding to the transmembrane regions in connexin32. Comparison of the primary sequences of all the known connexins reveals that the two predicted extracellular domains are the most conserved regions, each containing three invariant cysteines. The transmembrane domains are somewhat less well conserved. The cytoplasmic domains, with the exception of the short N-terminal region, differ markedly between connexins both in sequence and in length. This is summarized in Fig. 3, in which the two cytoplasmic domains, labeled *A* and *B* are shown, together with their sizes in numbers of predicted amino acids in the accompanying table. *Xenopus* connexin38 has a novel feature: it contains a unique predicted hydrophobic domain in the C-terminal tail (Ebihara et al., 1989). It is not known if this fifth hydrophobic segment represents an additional transmembrane excursion placing the C-terminus of connexin38 extracellularly.

On the basis of these comparisons, one would predict that the connexins could interact heterologously with each other; that is, a connexon composed of connexin32 could join with a connexon composed of connexin43 to form an intercellular channel. The divergences in the cytoplasmic re-

Table.

	Length in amino acids	
	Domain A	Domain B
Rat connexin26	37	16
Rat connexin32	36	74
Rat connexin43	55	154
Rat connexin46	55	187
Xenopus connexin30	36	56
Xenopus connexin38	43	118
Chick connexin42	58	139
Chick connexin45	78	143

The lengths of the variable cytoplasmic domains depicted in Fig. 3 were determined from the connexin amino acid sequences: rat connexin26 (Zhang & Nicholson, 1989), rat connexin32 (Paul, 1986), rat connexin43 (Beyer et al., 1987), rat connexin46 (Beyer et al., 1988; D.L. Paul, *unpublished data*), *Xenopus* connexin30 (Gimlich et al., 1988), *Xenopus* connexin38 (Ebihara et al., 1989), and chick connexin-42 and -45 (E.C. Beyer, *unpublished data*).

gions might be indicative of alternative mechanisms of channel gating, or of different channel conductances. It is known that gap junctions joining different cell types have variable sensitivities to modulators of intercellular communication and that unique unitary channel conductances have been measured in different experimental systems (Burt & Spray, 1988; DeHaan, 1988). In a novel approach, Brink and Fan (1989) have been able to patch-clamp gap junction membranes from the cytoplasmic surface in giant axons of the earthworm; in this case, only one class of gap junctional channel conductance was observed.

Physiological Studies Using the Cloned Connexin cDNAs

The availability of these cloned connexin cDNAs and specific antibody probes is facilitating more detailed investigation of their physiological functions. A powerful system has been developed (Werner et al., 1985; Dahl et al., 1987) involving the expression of injected gap junction mRNAs in paired *Xenopus* oocytes and the subsequent study of the channels induced between the two cells by voltage-clamp techniques. In this system, homologous injection of two oocytes with mRNA for rat connexin32 or connexin43, for example, induces the development of high junctional conductance between the paired cells up to three orders of magnitude above background levels (Dahl et al., 1987; Swenson et al., 1989; Werner et al., 1989). Junctional conductance induced by the exogenous mRNA can be further distinguished from the endogenous background channels by differences in voltage sensitivity. The identity of the endogenous *Xenopus* channels has not been definitively determined, although expression of cloned *Xenopus* connexin38 shows a similar voltage dependence (Ebihara et al., 1989).

The oocyte expression system has also been exploited to investigate the possible formation of hybrid junctions composed of two different connexins. An extensive literature exists which demonstrates that junctions can form in culture between heterologous cells (Subak-Sharpe et al., 1969; Michalke & Loewenstein, 1971; Gilula et al., 1972; Fentiman, Taylor-Papadimitriou & Stoker, 1976; Lawrence, Beers & Gilula, 1978; Gaunt & Subak-Sharpe, 1979; Flagg-Newton & Loewenstein, 1980). The nature of the molecular species involved in intercellular communication in these cases is not known. The observation that there is a high degree of conservation in the extracellular domains among the connexins suggests that heterologous junctions could form, i.e., junctions composed of two different connexins located on opposite sides of the junction. Formation of gap junctions composed of heterologous connexin types has been investigated in the *Xenopus* oocyte-pair system. Oocytes were injected with either connexin32 or connexin43 and then paired as connexin 32/43. These pairs of oocytes expressing both connexin32 and connexin43 displayed voltage-insensitive high junctional conductances similar to oocyte pairs injected with the same connexin, indicating that heterologous channels have formed (Swenson et al., 1989; Werner et al., 1989). Proof that heterologous channels can form is provided by the fortuitous finding that connexin43, but not connexin32, can form high conductances with the oocyte's endogenous channels in heterologous pairs. In this case, the resultant hybrid channels are asymmetrically voltage sensitive, showing physiological properties of both the endogenous and connexin43 channels.

Regulation of Expression of Connexins

Immunofluorescence studies of the distribution of connexins in various tissues and organs reveals complicated overlapping patterns. Connexin43 and connexin32 are both found in proximal convoluted tubule cells of the mouse kidney, connexin43 and MP70 are both found in the mouse lens (Beyer et al., 1989). In the liver, connexin32 and connexin26 are found together in the same junctional plaques, but show different distributions throughout the hepatic lobule, connexin26 being more concentrated in the periportal zones (Nicholson et al., 1987). There are not yet any clear functional or develop-

mental explanations to the patterns which have been seen thus far.

In the *Xenopus* embryo, there is a temporal pattern of expression of two connexins. *Xenopus* connexin38 mRNA is plentiful in the oocyte, then decreases to an undetectable level by the neurula stage (Ebihara et al., 1989). In an overlapping pattern, *Xenopus* connexin30 (Gimlich et al., 1988) is first detected at the midgastrula stage, then increases with further development. In the mouse, initial activation of the zygotic genome results in transcription of connexin43 but not connexin32 (Barron et al., 1989), yet preimplantation mouse embryos develop junctions with connexin32 (Lee, Gilula & Warner, 1987) indicating that these early gap junctions are derived from maternal stores.

We are indebted to our colleagues Katherine I. Swenson, Linda S. Musil, Lisa Ebihara, Joerg Kistler, Stanley Bullivant, John Jordan and Analise Horah. Studies reported in this review were supported by grants GM18974, GM37751, and EY02430 from the National Institutes of Health.

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Received 11 January 1990; revised 20 February 1990