Journal of Bioenergetics and Biomembranes, Vol. 28, No. 4, 1996

Connexin Expression Systems: To What Extent Do They Reflect the Situation in the Animal?

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Received February 6, 1996; accepted March 8, 1996

Intercellular communication is mediated by specialized cell–cell contact areas known as gap junctions. Connexins are the constitutive proteins of gap junction intercellular channels. Various cell expression systems are used to express connexins and, in turn, these expression systems can then be tested for their ability to form functional cell–cell channels. In this review, expression of murine endogenous connexins in primary cells and established cell lines is compared with results obtained by expression of exogenous connexins in *Xenopus* oocytes and cultured mammalian cells. In addition, first reports on characterization of connexin-deficient mice are discussed.

KEY WORDS: Gap junctions; lipid bilayers; *Xenopus* oocytes; established cell lines; primary cells; connexin-deficient mice.

INTRODUCTION

Connexins (Cx) are the subunit proteins of hexameric hemichannels termed connexons. Two hemichannels in apposed plasma membranes of neighboring eukaryotic cells can dock to each other and form a channel through which ions and metabolites can diffuse from cell to cell. Aggregates of very few to many thousands of channels can be visualized by electron microscopy and are called gap junction plaques. It is assumed that gap junction plaques are the main sites of direct intercellular communication. At present, it is not clear to what extent dispersed single channels contribute to total gap junctional communication. Since 1986 it has been shown that the murine genome contains at least 13 different connexin genes that are expressed in cell type specific, but overlapping patterns (Table I). In many, if not all, cell types investigated, at least two different connexin genes are expressed. We do not know the functional reasons for this expression pattern. It could mean that cellular physiology in animals requires gap junctional channels which are comprised of more than one type of connexin protein subunit. Alternatively, two or more different, noninteracting channels may be required for intercellular communication, if their permeabilities were different or differently regulated. We shall see below that examples for each of these alternatives seem to exist.

Since more than one type of connexin proteins is expressed in the same cell, it is necessary to study the different types of connexin channels independently of each other in appropriate experimental systems. Only when the properties of channels comprised of a single type of connexin protein are known will it be possible to unravel the function of different connexin proteins contributing to the same (heteromeric) hemichannels within one cell or to different (heterotypic) hemichannels.

Another reason for characterizing connexins in experimental expression systems is to evaluate the contribution of different connexin domains or amino acid residues toward the function as channel subunits. Using peptide-specific connexin antibodies and proteolytic degradation of the membrane embedded proteins, it has been shown that connexin32, −43, and −26 exhibit very similar topology: The polypeptide chain transverses the membrane four times and the

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0145-479X/96/0800-0319$09.50/0

Table I. Connexin Genes in Mouse and Rat Genome

Cx gene	Group	M_r	Tissue or cell type
Cx26	β_2	26.5	Hepatocytes, pinealocytes, leptomeningealocytes, pancreas, endometrium
Cx30[a]	β_6	30.4	Brain, skin
Cx30.3	β_5	30.3	Skin
Cx31	β_3	31	Keratinocytes, placenta
Cx31.1	β_4	31.1	Skin
Cx32	β_1	32	Hepatocytes, neurons, kidney, oligodendrocytes, pancreas, Schwann cells
Cx33	α_7	32.9	Testes
Cx37	α_4	37.6	Lung, endothelium
Cx40	α_5	40.4	Lung, endothelium, smooth muscle cells; conductive myocardium,
Cx43	α_1	43.1	working myocardium, myometrium, endothelium, astrocytes, fibroblasts
Cx45	α_6	45	Kidney, skin
Cx46	α_3	46	Lens fiber cells
Cx50	α_8	50	Lens fiber cells

[a] The mouse connexin gene 30 was isolated in our laboratory and will be described in Dahl *et al.* (1996), submitted for publication. M_r, relative molecular weight. The nomenclature using Greek letters is described in Kumar and Gilula (1992). Note that the list of tissue or cell type specific expression pattern is incomplete.

amino- and carboxy termini are located on the cytoplasmic side. Each of the two extracellular loops contains three cysteine residues, presumably interacting with each other and involved in the docking of two hemichannels when they form the complete channel. Since the central cytoplasmic loop and C-terminal domain differ largely between various connexin proteins, it has been suggested that they contribute to the individual function of each member of this protein family. For general reviews on the molecular biology of gap junctions, see Bennett *et al.* (1991), Beyer (1993), Kumar and Gilula (1992), Paul (1995), and White *et al.* (1995a).

In this review we want to compare the results obtained with different expression systems for connexins. It is not our intention to list all papers which were published using the different systems. We rather want to point out the advantages and disadvantages of each expression system by quoting only a few selected publications. Furthermore, since our own group is studying expression of mouse and rat connexins, most examples will be taken from analyses of murine connexin genes. Each expression system of connexin genes allows the study of only certain aspects of the complex situation in the living animal. It can be expected that the conclusions deduced from expression systems will be checked in mouse mutants, using the various techniques of gene targeting.

Relatively few reports have been published describing the *in vitro* reconstitution of gap junction channels from rat liver (Young *et al.*, 1987; Mazet *et al.*, 1992) and sheep lens (Donaldson and Kistler, 1992) in lipid bilayers. Harris *et al.* (1992) and Buehler *et al.* (1995) reported the reconstitution of Cx32 and Cx26 channels, respectively. Functional reconstitution in lipid bilayers requires relatively large amounts of purified connexin proteins which can be obtained in the baculo virus expression system. Expression of rat Cx26 connexons in lipid bilayers yielded channels with different conductances (Buehler *et al.*, 1995).

Below we shall first discuss expression of endogenous connexin genes in cultured primary cells. Then we shall compare expression of exogenous connexin genes in *Xenopus* oocytes and established cultured mammalian cells. Finally, recent results obtained with transgenic, connexin-deficient mice will be reviewed.

EXPRESSION OF ENDOGENOUS CONNEXIN EXPRESSION IN CULTURED PRIMARY CELLS

Primary mammalian cells in culture have a limited life span and usually require a higher concentration of growth factors than their transformed derivatives. Several reports describe the expression of connexin genes in cultured primary cells. For example, Cx43 is expressed in murine fibroblasts (Crow *et al.*, 1990), cardiac myocytes (Laird *et al.*, 1991), and astrocytes (Giaume *et al.*, 1991; Dermietzel *et al.*, 1991). More recently, Cx45 protein has been found in cultured neonatal ventricular myocytes (Darrow *et al.*, 1995). Cx43 and Cx26 have been detected in primary leptomeningeal cells (Spray *et al.*, 1991) and keratinocytes (Brissette *et al.*, 1994) that also express Cx31. Primary pinealocytes express Cx26 (Saez *et al.*, 1991), whereas Cx32 and Cx26 are co-expressed in primary mouse hepatocytes (Stutenkemper *et al.*, 1992). Cx37 mRNA has been detected in primary vascular endothelial cells (Reed *et al.*, 1991).

Several investigations described expression of connexins in established cell lines. For example, Cx31, Cx31.1, and Cx30.3 were detected in the mouse keratinocyte derived cells HEL30 and HEL37 (Hennemann

et al., 1992a,b). Expression of Cx40 (Beyer *et al.*, 1992) and Cx45 (Laing *et al.*, 1994) have been studied in the A7r5 cell line from rat aorta smooth muscle.

Primary cells in continued culture tend to lose some of their characteristic traits of differentiation. This can include expression of certain connexins, for example Cx32 and Cx26 in hepatocytes or Cx40 in endothelial cells and conductive myocardiocytes. Immortalization of hepatocytes, for example, using SV40 large T antigen, accelerates dedifferentiation, although newly immortalized cells can maintain a high degree of differentiation for several generations (Stutenkemper *et al.*, 1992). Several hepatocyte derived cell lines, after prolonged culture, show decrease or loss of expression of Cx32 and Cx26, but express Cx43 instead which is very low or absent in primary hepatocytes. It seems that the expression of Cx43 is turned on when mouse hepatocytes or endothelial cells are kept in culture for several generations, whereas expression of cell type specific connexins is often turned off. This raises the question why the maintenance of some highly differentiated cell types is associated with expression of specific connexin proteins. It is tempting to speculate that this may be caused by distinct properties (gating or permeability) of channels formed by these connexins. The observations that several tumorigenic cell lines show largely decreased or absent expression of connexins has led to the suggestion that loss of connexin gene expression or intercellular communication via gap junctions may contribute to loss of growth control in tumorigenesis (cf. Holder *et al.*, 1993).

EXPRESSION OF EXOGENOUS CONNEXINS IN *XENOPUS* OOCYTES

The expression of rat Cx32 in *Xenopus* oocytes and measurements of electrical conductance in paired oocytes were first reported by Dahl *et al.* (1987). Subsequently the oocyte expression system has been used in several laboratories as standard assay for the function of wild type and mutated connexins. The advantages of this expression system are obvious: Usually high levels of expression can be obtained by injection of connexin cRNA into oocytes. The oocyte assay is faster than expression of exogenous connexins in mammalian cell lines. Furthermore, electrical measurements of total conductance can be carried out more easily with pairs of big oocytes than with small mammalian cells. Unfortunately, paired oocytes stripped of the vitelline membrane which surrounds the oocyte exhibit residual electrical coupling under conditions when no exogenous connexin RNA has been injected. This endogenous coupling is due to expression of *Xenopus* Cx38. Barrio *et al.* (1991) have shown that the expression in oocytes of endogenous Cx38 channels can be largely suppressed by pre-injection of antisense Cx38 oligonucleotides. Another endogenous connexin, XeCx43, does not appear to contribute to coupling between oocytes, since no functional XeCx43 protein has been detected in these cells (Gimlich *et al.*, 1990). Many rat or mouse connexins have been expressed in the *Xenopus* oocyte system: Cx43 (Swenson *et al.*, 1989; Werner *et al.*, 1989), Cx32 (Dahl *et al.*, 1987), Cx37 (Willecke *et al.*, 1991), Cx40 (Hennemann *et al.*, 1992b; Bruzzone *et al.*, 1993), and Cx50 (White *et al.*, 1992).

In all these cases functional homotypic connexin channels between paired oocytes were detected. It was found that the conductance of different intercellular channels was dependent on transjunctional voltage to various extents (cf. White *et al.*, 1995a). Interestingly, rat Cx46 expressed in *Xenopus* oocytes formed hemichannels which were leaky and led to lysis of the oocytes, but could be osmotically stabilized by addition of ficoll to the bath medium (Paul *et al.*, 1991). Furthermore, Cx31.1 and Cx33 did not form homotypic intercellular channels in paired *Xenopus* oocytes. (White *et al.*, 1995a). It has been reported in an abstract that Cx33 is inhibitory to Cx37 but not to Cx43 in the oocyte assay (Chang *et al.*, 1994). The biological role of such inhibitory connexins is not yet clear.

Heterotypic channels formed from hemichannels, each comprised of a different type of connexin, have been intensively studied in paired oocytes (cf. White *et al.*, 1995a). In this system, it has been first shown that Cx40 and Cx43 hemichannels, both expressed in cardiomyocytes, do not form functional channels with each other (Bruzzone *et al.*, 1993). Furthermore, Cx50 and Cx43, which are expressed in lens fiber cells, are incompatible with each other (White *et al.*, 1994). Recently, selectivity has also been reported for other connexins tested in the oocyte assay (White *et al.*, 1995b). Incompatible connexins may fulfill a biological role by defining the borders of communication compartments described in early embryogenesis.

Heterotypic channels can have novel properties due to allosteric interaction of the two hemichannels (Barrio *et al.*, 1991). The voltage-dependent gating of one hemichannel is dependent on the connexin type in the other hemichannel. The biological relevance of

voltage dependence in heterotypic connexin channels is not clear at present. Voltage differences between cells that are large enough to induce closure of gap junctions can be expected to occur between injured and noninjured cells in tissues. Furthermore, relatively large voltage differences have been measured between different compartments of the developing *Xenopus* embryo (Blackshaw and Warner, 1976). Voltage-dependent heterotypic gap junctions have been suggested to cause rectification of electrotonic synapses (Giaume *et al.*, 1987).

Chimeric connexin molecules, where one connexin domain has been exchanged for an analogous one from a different connexin protein, have been expressed in the oocyte system, in order to search for the voltage sensitive region(s) or to study the interaction between channel subunits. Using Cx32/Cx26 chimeras, it was concluded that the first extracellular loop may contribute to voltage sensitivity (Rubin *et al.*, 1992). However, other amino acid residues, for example a proline residue in the second transmembrane domain, also influence rectification of conductance (Suchyna *et al.*, 1993). It seems that voltage dependence is caused by interaction of several connexin domains. The same general conclusion holds true for determinants of compatibility. However, experiments with Cx43/Cx32 chimeras in the oocyte expression system suggest that the second extracellular domain E2 (cf. Fig. 1) is a determinant of compatibility (Bruzzone *et al.*, 1994a). This has also been reported for Cx46/Cx50 chimeras expressed in the oocyte system (White *et al.*, 1994).

In another study with mutated Cx32 it was shown that the extracellular cysteine residues are essential for the function of Cx32 channels (Dahl *et al.*, 1991). Furthermore, the interaction of Cx32 hemichannels could be inhibited by Cx32 sequence specific oligopeptides added to the bath medium of oocytes before pairing (Dahl *et al.*, 1994).

Finally, it should be pointed out that rat Cx43, when expressed in *Xenopus* oocytes, is found in the lowest M_r form, presumed to be nonphosphorylated, although it forms conductive channels (Swenson *et al.*, 1989). Thus, a higher order of Cx43 phosphorylation is not required for electrically conductive channels, consistent with the observation that Cx43 is detected in certain rat tissues, for example in astrocytes, predominantly in the lowest M_r form (Kadle *et al.*, 1991). The functional role of phosphorylation in Cx43 and other connexin proteins needs to be clarified.

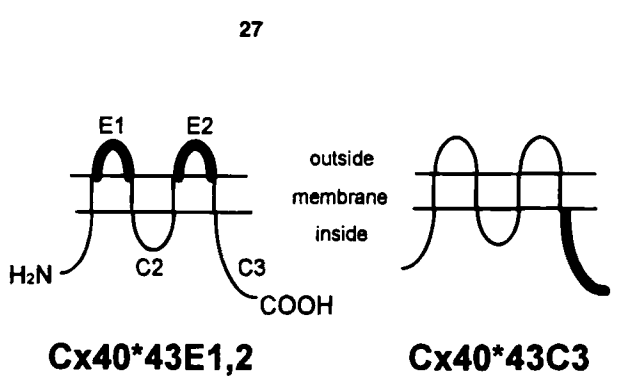

Fig. 1. Schematic representation of two chimeric molecules constructed by exchanging domains between Cx40 and Cx43. Thin lines represent Cx40 sequences and fat lines Cx43 sequences. The presumed general topology of membrane-embedded connexin proteins is schematically indicated.

EXPRESSION OF EXOGENOUS CONNEXINS IN CULTURED CELL LINES

Next to *Xenopus* oocytes, cultured mammalian cell lines provide the second major expression system for exogenous connexin genes. However, as mentioned above, the more differentiated traits are expressed in established mammalian cells, the better these cells are usually coupled via endogenous connexin channels. All recipient cells used for expression of exogenous connexins exhibit low endogenous coupling but are largely dedifferentiated and highly tumorigenic. Thus, it is difficult to draw from this expression system meaningful conclusions regarding the role of connexins in cell differentiation and growth control. A number of reports have shown that expression of rat Cx32, Cx43, or Cx26 in tumorigenic cell lines, such as human SK-Hep-1 adenocarcinoma (Eghbali *et al.*, 1990), rat C6 glioma (Zhu *et al.*, 1991), or human HeLa cervix carcinoma cells (Mesnil *et al.*, 1995) led to prolonged times of appearance of tumors (Eghbali *et al.*, 1991), decreased growth rate of transfected cells (Zhu *et al.*, 1991), or suppression of tumorigenesis in nude mice (Rose *et al.*, 1993; Mesnil *et al.*, 1995). In all these cases, the exogenous connexin gene had been transfected into mammalian cell lines under control of a highly active (viral) promoter. It is not clear how the partial reversion of the transformed phenotype occurs at the molecular level. Since functional exogenous connexin channels can be expressed in recipient cells at least 10-fold higher (50- to 100-fold higher in HeLa cells) than endogenous connexins, characterization of exogenous connexin channels can be accom-

plished in this system. Elfgang *et al.* (1995) have expressed seven murine connexin DNAs (Cx26, −31, −32, −37, −40, −43, and 45) in human HeLa cells and compared them with regard to channel permeability and selective interaction of hemichannels. It turned out that several connexin channels differed in their permeability toward the positively charged tracers propidium iodide and ethidium bromide. In addition, other permeability differences were noticed, for example, regarding Cx40 and Cx43 channels. Since the measurements of permeability were taken 5 min after microinjection of the fluorescent dye, they probably reflect steady-state conditions. Kinetic studies indicate that Cx26, Cx32, and Cx45 channels show progressively decreasing permeabilities to Lucifer yellow in the HeLa cell system and in oocytes (Cx32 > Cx26) (Cao *et al.*, 1994). It has been suggested that different permeabilities of connexin channels to nonphysiological tracers may reflect different permeabilities to second messengers, ions, and metabolites (Steinberg *et al.*, 1994; Elfgang *et al.*, 1995). This could be of biological relevance since second-messenger molecules such as cAMP, cGMP, and inositol 1,4,5-triphosphate are considered to be long-range messengers, although their average half-life is only between 1 and 60 sec. Thus, subtle permeability differences between connexin channels could be biologically important for quick intercellular equilibration of second-messenger molecules triggered, for example, by hormonal stimuli. Elfgang *et al.* (1995) investigated the selectivity of heterotypic channel formation in the HeLa expression system, using the dye Lucifer yellow to test cell–cell transfer. They confirmed previous findings in the *Xenopus* expression system (Bruzzone *et al.*, 1993) that Cx40 and Cx43 are incompatible, although each of these connexins can form functional channels with Cx37 or Cx45. Furthermore, Cx31 HeLa transfectants expressed functional homotypic channels but did not couple to any of the other CxHeLa transfectants. The incompatibility of Cx31 and Cx43 could be biologically relevant, since they are expressed in extraembryonic tissue and the gastrulating embryo, respectively (Dahl *et al.*, 1996), which form apposed but independent communication compartments.

Different permeabilities to fluorescent dyes have also been found in rat osteoblastic cell lines that expressed Cx43 or, predominantly, Cx45. Whereas Cx43-expressing cells showed efficient transfer of Lucifer yellow, Cx45-expressing cells did not (Steinberg *et al.*, 1994). More recently, Koval *et al.* (1995) reported that transfected chick Cx45 DNA altered the permeability between cells expressing Cx43, consistent with the notion that the permeability of heterotypic or heteromeric connexin channels may differ from that of corresponding homotypic channels. Veenstra *et al.* (1994a,b) have found that exogenous connexin channels expressed in mouse N2A neuroblastoma cells exhibit different ion selectivity and permeability to 6-carboxyfluorescein. In elegant experiments, they were able to measure the conductance and permeability of distinct connexin channels, simultaneously.

The expression system of exogenous connexins in cultured cell lines has been extensively used to measure the unitary conductance of each channel. This has not been possible so far in the oocyte expression system. Moreno *et al.* (1994) found that phosphorylation modified unitary conductance and voltage-dependent kinetics of Cx43 gap junction channels expressed in SK-Hep cells. However, mutants of Cx43 in which phosphorylated serines were replaced by alanine residues could form functional channels, suggesting that these sites of phosphorylation are not necessary for appropriate assembly and functional activity of this connexin channel (Spray *et al.*, 1995).

Bukauskas *et al.* (1995a) have used a new method for measuring conductance of connexin channels in HeLa Cx40 transfectants. Single cells, impaled by electrodes, were pushed together, and the first transjunctional currents due to newly formed channels were recorded. With this technique, heterotypic connexin channels between different connexin-transfected HeLa cells have been investigated (Bukauskas *et al.*, 1995b). It was shown that heterotypic Cx26/Cx32 channels have novel properties compared to the corresponding homotypic connexin channels, confirming and extending conclusions from previous work with the oocyte system (Barrio *et al.*, 1991; Verselis *et al.*, 1994).

Using the HeLa expression system, we have recently investigated why Cx40 and Cx43 channels are incompatible. Several domain exchange mutants of Cx40 were constructed in which extracellular or intracellular domains were exchanged for the corresponding domains of Cx43. After expression in HeLa cells, the permeability of these mutants to neurobiotin (*N*-2[aminoethyl]biotinamide hydrochloride) was compared with the electrical conductance in homotypic and heterotypic combinations with Cx43 and Cx40. We found that the exchange of the two extracellular domains E1 and E2 (Fig. 1) allowed formation of functional channels with Cx43 and Cx40. The

exchange of only one extracellular domain did not yield functional homotypic or heterotypic channels. The voltage dependence of conductance and the permeability of the mutant Cx40*43E1,2 (Fig. 1) were different from Cx40 or Cx43 homotypic channels. Exchange of the C-terminal region of Cx40 for that of Cx43 in the mutant Cx40*43C3 (Fig. 1) did not yield functional homotypic channels when expressed in HeLa cells (Haubrich *et al.*, manuscript in preparation, 1996). We conclude that the extracellular domains of Cx43 can be directed to dock to the extracellular domains of Cx40 and form functional channels when the rest of both hemichannels is identical to Cx40. Thus, it seems that docking and gating of heterotypic channels are dependent on extracellular domains that are influenced by intracellular domains.

COMPARISON OF EXOGENOUS CONNEXIN EXPRESSION IN *XENOPUS* OOCYTES AND CULTURED CELLS

Functional expression of wild type and mutated exogenous connexin genes has been accomplished in both *Xenopus* oocytes and mammalian cells. Differences between these two experimental systems were found with expression of rat Cx46 which needs osmotic stabilization in oocytes (Paul *et al.*, 1991) but forms functional homotypic channels after transfection in HeLa cells (Elfgang *et al.*, unpublished results). Furthermore, murine Cx31 has not been functionally expressed in oocytes but forms channels in transfected HeLa cells (Elfgang *et al.*, 1995). It is not clear whether these results reflect general differences between the two systems. However, post-translational processing and modification (i.e., phosphorylation) are different between the two experimental systems. Future investigations will have to show which aspects of biosynthesis, function, or degradation of gap junctions are influenced by post-translational processing and phosphorylation. Each of the expression systems is artificial. An advantage of the oocyte system is the ease of manipulation which allows relatively fast experiments. On the other hand, unitary conductances can, so far, be measured only with connexin channels expressed in mammalian cells. In both expression systems, interaction of exogenous connexins with a low level of endogenous connexins can take place. This can be eliminated to some extent in the oocyte system by preincubation with antisense oligonucleotides directed to endogenous Cx38. The functional consequences of correct intracellular location of exogenous connexin channels can hardly be studied in paired oocytes. Even highly tumorigenic cell lines, such as HeLa, N2A, or SKHep1 cells, do not represent the morphology of differentiated mammalian cells. Thus, the specific location of gap junctional channels between two established cells may differ from that of highly differentiated mammalian cells in living organs. Gap junction mediated intercellular communication through Cx43 channels requires expression of E-cadherin on the surface of transformed mouse epidermal cells (Jongen *et al.*, 1991). It is not yet known whether other cellular adhesion proteins contribute specifically to gap junctional function. If this turns out to be the case, it could probably be better studied with mutated mammalian cell lines than with oocytes.

Currently, one can conclude that each of the two expression systems for connexins has advantages and disadvantages. It depends on the specific question being asked and the expertise in the laboratory, which system one prefers. As usual, in cell biological experiments a complex process (in this case physiologically relevant gap junctional intercellular communication) has to be taken apart and studied in an artificial expression system before one can interpret the complex situation in the living organism. Recently, this last step has been eased by gene targeting in mice. Thus, we shall briefly review the first results of connexin gene manipulation in this animal.

CONNEXIN-DEFICIENT TRANSGENIC MICE

Using homologous recombination in cultured embryonic stem cells, it is now possible to inactivate or "knock out" any known gene of the mouse genome, mostly by replacing part of the coding sequence carrying the selected gene with an expression cassette (for review, see Melton, 1994). The corrected recombinant embryonic stem cells are transferred into blastocysts which, after introduction into the uterus of a pseudo-pregnant mouse, can develop into chimeric mice. If germ cells of these animals were derived from recombinant embryonic stem cells, further breeding of these mice will lead to mice which are heterozygous and, finally, possibly homozygous for the targeted, deficient gene. This technique opens the possibility to study the consequences of connexin gene defects in the mouse, if these "knock-out" animals are viable. Reaume *et al.* (1995) have inactivated the mouse Cx43 gene and have found that

the homozygous deficient mice die shortly after birth due to malformations of the heart. The blood flow from the right ventricle to the pulmonary arteries was impaired and oxygenation was prevented postnatally. It is not known how the missing expression of Cx43 inhibited the normal development of the heart. Since Cx43 is expressed very early in development, it is surprising that Cx43 deficient mice survived until term. Possibly, prenatal expression of Cx40 (Delorme *et al.*, 1996) allowed the function of the fetal heart until birth.

More information with regard to the molecular mechanism of connexin function can be obtained from mice deficient in Cx32, recently characterized in our laboratory (Nelles *et al.*, 1996). These mice are fertile and phenotypically normal, although they weigh, on average, less than the corresponding wild type mice. The total area of gap junctional plaques in the liver of Cx32 (−/−) mice is much smaller than in Cx32 (+/+) mice, although cultured embryonic hepatocytes from Cx32 (−/−) mice still exhibit some transfer of micro-injected Lucifer yellow to nearest-neighbor cells. Furthermore, Cx32 (−/−) liver contains less Cx26 protein but the same amount of Cx26 mRNA as wild type liver, suggesting that Cx32 protein can stabilize the Cx26 protein in heterotypic or heteromeric channels. When sympathetic nerves of isolated Cx32 (−/−) liver were electrically stimulated, much less glucose was mobilized than in wild type liver. These results show that noradrenaline, released from the endings of sympathetic nerves, can trigger in Cx32-deficient liver glucose mobilization only in periportal hepatocytes that are close to the nerve endings. In wild type liver, the noradrenaline-triggered intracellular second messenger, presumably inositol 1,4,5-triphosphate, can penetrate through Cx32 gap junctions into neighboring hepatocytes and provide maximal glucose mobilization. This could be a general mechanism for the function of gap junctions in tissues innervated by sympathetic nerves. Gap junctions mediate maximal response of the innervated tissue to the nerve stimulus. Since sympathetic nerves trigger "fight and flight" responses, gap junctions in the innervated tissues have the function to maximize this response, for example, glucose mobilization in liver. Interestingly, human liver is more densely innervated than mouse liver. Thus, human Charcot–Marie–Tooth (X type) patients who are deficient in the Cx32 gene may show less effect on hepatic glucose mobilization than the corresponding Cx32 "knock-out" mice. Human CMTX patients begin to suffer in their second decade of life from demyelination, due to defective expression of Cx32 in Schwann cells, which leads to lower conduction in peripheral nerves. Cx32 (−/−) mice show normal conduction in sciatic nerves at four months of age. It is possible, however, that they develop peripheral neuropathological symptoms when they get older.

These results show that much information can be obtained from a null mutation in the mouse Cx32 gene, when no functional protein is synthesized in the animal. In several cases investigated, human CMTX patients express defective Cx32 protein due to point mutations in the Cx32 gene (Bruzzone *et al.*, 1994b). This protein could lead to transdominant negative effects, if it inhibits the function of other connexin proteins. This could also explain the differences in phenotypes between CMTX patients and Cx32 (−/−) mice which do not contain any Cx32 protein.

Much of the knowledge needed to interpret the phenotype of connexin-deficient mice will be derived from the expression systems such as *Xenopus* oocytes or cultured mammalian cell lines. Thus, it is obvious that further studies of connexin expression in "artificial" expression systems will be necessary to interpret the physiological consequences of connexin mutations in the experimental animal and in human patients. Only by comparison with the results obtained in expression systems will it be possible to understand the biological function of gap junctions in the living organism.

ACKNOWLEDGMENT

Work in the authors' laboratory on the molecular biology of gap junctions has been supported by the Deutsche Forschungsgemeinschaft (SFB 284), the Dr.-Mildred-Scheel-Stiftung, and the Fonds der Chemischen Industrie.

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