Plasma Membrane Channels Formed by Connexins: Their Regulation and Functions

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other proteins). The physiological role of gap junctions in several tissues has been elucidated by the discovery of mutant connexins associated with genetic diseases and by the generation of mice with targeted ablation of specific connexin genes. The observed phenotypes range from specific tissue dysfunction to embryonic lethality.

I. GENERAL COMMENTS

In this review, we provide a brief historical background regarding gap junction structure and physiology and then concentrate on reviewing several areas of active research in the field of gap junctions including studies of the molecular composition and biochemical regulation of these channels, recent studies of hemichannels, and investigations of the role of gap junction channels and hemichannels in various normal physiological processes and diseases in vertebrates.

Due to limitations of space, some of the earlier investigations and some specific areas of gap junction biology that have shown significant advance in recent years (e.g., nervous system) have not been considered in detail in the current review. The reader is referred to previous reviews for more detailed information on earlier studies (35, 48, 66, 67, 190, 539, 551). Several reviews have been published recently on gap junction structure (534, 548, 665), trafficking of protein subunits (154, 314, 317, 666), channel gating (36, 59, 108), pharmacological regulation (45, 499, 550), and functional features of the channels (353, 443, 540, 614). Other reviews have covered the roles and regulation of gap junctions in development (338, 352) and in various mature tissues and organs, including vascular tissue (60, 91), urogenital smooth muscle (269), the heart (50) and cardiovascular tissues and organs, including vascular tissue (60, 91), urogenital smooth muscle (269), the heart (50) and cardiovascular system (128), pancreas (377), endocrine glands (395), the nervous system (65, 122, 123, 168, 546, 552), and the immune system (11, 502, 503). Other reports describe the regulation and role of gap junctions in tissue injury (120) and the involvement of gap junctional communication in the phenotypes observed in connexin knock-out animals and/or different diseases (134, 540, 647, 662).

II. BACKGROUND

A. Intercellular Communication

The first indications that a pathway for direct cell-to-cell communication might exist substantially preceded discovery of the gap junction structure. Weidmann (637), working with strips of myocardium, found that the space constant for the spread of current extends beyond the expected value for a single Purkinje fiber. He suggested that this phenomenon might be explained by the existence of a low-resistance intercellular pathway. Stronger evidence supporting the existence of such a pathway was provided by the discovery of electrical transmission at the giant crayfish motor synapses (170). Later, this type of pathway was demonstrated in other excitable tissues (22, 34, 162) and in nonexcitable cell types (184, 356, 432, 468, 482). Today, it is accepted that cells of most invertebrate and vertebrate tissues can communicate with their neighbors via a low-resistance intercellular pathway. In vertebrates, only a few cell types do not form gap junctions in their fully differentiated state, including red blood cells, spermatozoa, and skeletal muscle. Nevertheless, the progenitors of these cells do express gap junctions (98, 386, 469, 495).

B. Gap Junction Structure

Electron microscopy studies provided evidence for a structure responsible for intercellular electrical transmission. Robertson (491) demonstrated the structural units of the neuronal junction. In studies of excitable tissues, a close apposition between the plasma membranes of adjacent cells was observed when current transfer occurred, but it was absent when electrical transmission was not detectable (22, 440, 482). This intercellular junction has been given several names, including nexus, macula communicans, and gap junction. The designation “gap junction” from the work of Revel and Karnovsky (481) has prevailed despite the contradiction between the function and the morphological characteristics that it denotes.

The structure of gap junctions and their constituent channels have been significantly elucidated. In transmission electron micrographs of ultrathin tissue sections, gap junctions appear as regions where the plasma membranes of adjacent cells closely approach each other, but appear to be separated by a small gap of 2–3 nm (33, 481, 491) (Fig. 1A). Electron micrographs of freeze-fracture replicas of vertebrate junctions show 8.5- to 9.5-nm particles on the P face either free or in plaque-like arrays with complementary pits on the E face (Fig. 1C). In many cases, these particles (termed connexons) are regularly ordered in a hexagonal pattern that has allowed detailed structural studies by other techniques. Studies of gap junctions using atomic force microscopy (AFM) (226, 318) show a dense packing of particles with a center-to-center distance of 9–10 nm and a similar long-range hexagonal packing. The particles contain a porelike structure with a diameter of 2 nm, a depth of 1 nm, and a width of 3.8 nm based on freeze-fracture and AFM analyses (226, 318, 481). X-ray diffraction examination of isolated gap junction membranes (78, 362) and Fourier analysis of low-dose electron micrographs (395) show that each particle or connexon within a gap junction forms a cylinder with an apparent aqueous pore in the center. The wall of the connexon is formed of six protein subunits (362). Other studies suggest that these subunits may move with respect to each other and control channel opening (594) (Fig. 3, inset 1).
The structure of the gap junction channel has been refined in recent years (Fig. 1D). Using gap junctions isolated from cells expressing a recombinant truncated gap junction protein, Unger et al. (592) demonstrated the dodecameric structure of the channel at a resolution of 7 Å in the membrane plane and 21 Å in the vertical direction; each hemichannel contains 24 α-helices corresponding to the four transmembrane domains of the six protein subunits (592, 593). These structures were also suggested by circular dichroism (77) and X-ray patterns obtained from oriented gap junctions (577). The outer diameter of the hemichannel is ~70 Å at the intracellular region and ~50 Å at the extracellular regions giving the complete channel an hour glass-like appearance. The channel pore narrows from ~40 Å diameter at the cytoplasmic side to ~15 Å at the extracellular side of the bilayer and then widens to ~25 Å in the extracellular region (451, 592, 593). The connexon interfaces within a channel must be tightly bound to form a tight seal. Thus the docking domain provides a high resistance to the leakage of current carrying ions between the channel lumen and the extracellular space. Recently, conformational changes of the cytoplasmic and extracellular surfaces in gap junction plaques containing channels made of native connexin26, a protein subunit, have been imaged using AFM (394). The carboxy terminus reversibly collapses when increased forces are applied to the AFM stylus. The presence of Ca$^{2+}$ in the buffer solution reduces the diameter of the extracellular channel entrance from 1.5 to 0.6 nm, a conformational change fully reversible and specific among the divalent cations. Calcium induces the formation of microdomains, and the plaque height increases in 0.6 nm (from 17.4 to 18 nm), indicating that Ca$^{2+}$ induces conformational changes affecting the structure of connexin26 membrane channels (394).

III. MOLECULAR COMPONENTS OF GAP JUNCTIONS

A. Gap Junction Proteins

Gap junction-enriched preparations, similar to those utilized for structural studies, were used for biochemical
different protein subunits (52, 53). In some cases, a prepolypeptide connexin (in kilodaltons) to distinguish different members of this family, a standard nomenclature indicating the predicted molecular mass of the protein (often abbreviated Cx) followed by a suffix was needed. The most widely used system uses the molecular mass has led to the use of a decimal point to distinguish different, polypeptide of 43 kDa corresponding to a rat liver gap junctional protein showed that it differed from both the liver and heart proteins (284).

B. The Family of Chordate Gap Junction Protein Subunits

The production of antibodies to the main protein in isolated liver gap junction preparations, and the synthesis of oligonucleotide probes based on the amino-terminal sequence information, allowed the isolation of rat and human cDNA clones encoding a liver gap junction protein of 32 kDa (300, 442). Proof that this protein produces gap junction channels came from its expression in Xenopus oocytes (138, 566, 639). It became evident that there was a family of subunit gap junction proteins when Beyer et al. (52) isolated cDNA clones encoding a related, but clearly different, polypeptide of 43 kDa corresponding to a rat heart gap junction protein, which they named connexin43 (Cx43). While connexins were originally denoted according to the tissue of origin or the apparent size of a polypeptide as determined by SDS-PAGE, it soon became clear that such designations were inappropriate, because many of these proteins are not uniquely expressed in a single tissue (52), and their mobilities on SDS-PAGE may vary with electrophoresis conditions (196). Therefore, to distinguish members of this family, a standard nomenclature was needed. The most widely used system uses the word connexin (often abbreviated Cx) followed by a suffix indicating the predicted molecular mass of the polypeptide connexin (in kilodaltons) to distinguish different protein subunits (52, 53). In some cases, a prefix is added to indicate the species of origin. The finding that two (or more) connexins may have similar molecular mass has led to the use of a decimal point to distinguish them (e.g., mCx30.3 or mCx31.1). Some confusion has also been created when orthologous and functionally homologous connexins have different molecular masses (e.g., rat Cx46 vs. bovine Cx44 vs. chicken Cx56). An alternative (but also problematic and less widely used) nomenclature in which connexins are classified in subclasses according to amino acid sequence homology has also been proposed (423, 486).

Subsequently, a number of cloning strategies including hybridization screening of genomic and cDNA libraries at reduced stringency and use of the polymerase chain reaction with degenerate/consensus primers have been successfully applied to identify additional members of the connexin family (for recent review, see Ref. 653).

C. Connexin Genes

Recently, the screening of the mouse and human genomic databases has revealed 19 and 20 connexin genes in the mouse and human genome, respectively (55, 359, 417, 653). A dendrogram for all identified human connexins is shown in Fig. 2A. The genes for various connexins have been localized to human (32, 92, 161, 214, 233, 271, 417, 654) and mouse (9, 93, 207, 215, 233, 521) chromosomes. Connexin genes are found in many different chromosomes, but there is a cluster of connexins in a region of mouse chromosome 4 (214) which is syntenic to a region in human chromosome 1. The genes for many connexins have been cloned, and the gene structure of other connexins has been partially assessed by DNA blotting and by the polymerase chain reaction (for a review, see Ref. 653). The available information indicates that many connexin genes have a similar organization, and virtually all have only single copies in the haploid genome (653). The sequence similarities between connexins and their similar gene structures suggest that they have arisen by gene duplication. In humans, a Cx43 pseudogene has been identified (163).

Most connexin genes have a first exon containing only 5'-untranslated (UTR) sequences and a large second exon containing the complete coding region as well as all remaining untranslated sequences (Fig. 2B). Exceptions to this gene structure are the Cx32, Cx36, and Cx45 genes. The human (and rodent) Cx32 gene contains alternative first exons (containing only 5'-UTR) whose use is tissue specific (411, 545, 547); some bovine Cx32 transcripts have three exons, two of which contain only 5'-UTR (135). The Cx36 (and the Morone americana Cx34.7) gene contains two exons, both of which contain untranslated and translated sequences. The coding region is interrupted by an intron (32, 97, 422, 423, 544). The Cx45 gene contains three exons; exons 1 and 2 contain only 5'-UTR and exon 3 contains the full coding region and 3'-UTR (19, 242); all Cx45 transcripts examined contain exons 2 and 3, while some also contain exon 1 (242).
D. Transcriptional Regulation and Gene Promoters

In various cell types, different treatments are known to increase the levels of connexin mRNA due to enhanced transcription. For example, cAMP induces elevation of the Cx43 transcription rate in Morris hepatoma cells (380). Moreover, the transcription rates of Cx32 and Cx26, but not Cx43, are upregulated by glucocorticoids in liver-derived cells (479), and the phorbol ester [12-O-tetradecanoylphorbol-13-acetate (TPA)] induces transcriptional up-regulation of Cx26 in human immortalized MCF-10 mammary epithelial cells (340). Also, the large increase of Cx43 in myometrium before parturition is at least in part due to increased transcription (433, 486). Thus knowledge of connexin gene structure and identification of regulatory elements will allow us to understand the transcriptional regulation of connexin expression.

The basal promoter of the rat Cx32 gene was first localized to a region −179 to 0 or −134 to 0, immediately upstream of the first exon (17). The mouse Cx32 gene contains two putative binding sites for the transcription factor HNF-1 and consensus motifs for NF-1 as well as NFκB within the 680 bp upstream of the main transcription start site (215). Cx32 cDNA clones from rat or mouse liver, sciatic nerve, and embryonic stem cells differ (545). They correspond to different splicing variants with a different 5′-UTR or exon 1, each with its own promoter region (545).

The human Cx32 gene can be transcribed from two tissue-specific promoters, P1 and P2, leading to the production of two mRNAs with different 5′-UTRs through the use of alternative exons (411). The P1 promoter is functional in liver and pancreas and is located more than 8 kb upstream of the translation start codon, while the P2 promoter is functional in nerve cells and is located 497 bp upstream of the translation start codon (411). Cx32 expression in vitro is strongly activated by direct binding of SOX10, a common transcription factor for other myelin genes (56). Methylation of the Cx32 (and Cx43) promoter at Msp I/Hpa II restriction sites correlates well with cell type-dependent decreased transcriptional activity (459).

Sequence analysis of mouse Cx37 gene has revealed that the regions flanking exon 1 contain a consensus “TATA box” 43 bp 5′ from the transcription start site preceded by several putative transcription factor binding sites and a 282-bp truncated L1Md transposon (529). Determination of the structure and sequence of the Cx43 gene and analysis of trans-activation have elucidated some of the hormonal and pharmacological regulation of Cx43 expression. Sullivan et al. (565) and Yu et al. (674) cloned the mouse and rat Cx43 genes, identified the transcriptional start site, and showed that there were a number of putative transcription factor binding sites within the 5′-flanking region including a TATA box, AP-1, AP-2, Sp1, CRE sites, and half-palindromic estrogen response elements. The basal promoter is contained within −110 bp 5′ to the start site (88). DNase I footprinting assays have confirmed the use of AP-1 (−44 to −36) and Sp1 (−77 to −69 and −59 to −48) consensus sequences; moreover, mutation of this AP-1 site abolishes phorbol...
ester-induced transactivation of Cx43 gene transcription in uterine smooth muscle cells (178). Transfection studies using Cx43 constructs linked to luciferase have shown the responsiveness of Cx43 transcription to estrogen, parathyroid hormone, and prostaglandin (94, 674). cAMP, which can also increase Cx43 expression (380), may serve as the mediator of the parathyroid hormone and prostaglandin effects (519) through the Cx43 promoter cAMP response elements (18). The mechanism of the estrogen-induced response is not so clear; Piersanti and Lye (460) have suggested that it occurs indirectly through production of c-fos, which interacts with the AP-1 sites. Electrophoretic mobility shift assays have demonstrated c-jun and Sp1 binding at the AP1 and Sp1 sites, respectively (141). In positions −480 and −464 of the rat gene, there is a thyroid hormone binding sequence that is able to activate the promoter (561).

Rat Cx43 and Cx30 transcription may be transactivated by the Wnt-1 signaling pathway, probably through TCF/LEF binding elements (376, 600). These are HMG box transcription factors that bind to β-catenin in response to activation of the Wnt-1 signaling pathway, and not through a cAMP-induced pathway (600). Functional analysis demonstrated that mouse Cx43 promoter constructs could be activated in zebrafish embryonic neural crest cells (87), suggesting that the sequence motifs and transcriptional regulation involved in targeting Cx43 expression to neural crest cells are evolutionarily conserved in zebrafish and mouse embryos.

The Cx40 mRNA sequence is identical in all tissues studied (198). Thus transcriptional regulation may account for the differences in levels of Cx40 transcripts detected during development and among different tissues. Transient transfection studies have demonstrated that the basal promoter activity of the Cx40 gene lies within 300 bp upstream of the transcription start site and that a strong negative regulatory element is present in the intron in the region from +100 to +297 bp (530). Moreover, potential transcription binding sites include AP-1, AP-2, Sp1, TRE, p53, and a TATA box located near exon 1 (550). Transcription of Cx40 is transactivated by Tbx5 (T-box transcription factor 5) and the homeodomain transcription factor Nkx2–5 (62). Heterozygous and homozygous Tbx5-deficient mice have similar features to the human Holt-Oram syndrome (62), and it has been suggested that decreased Cx40 transcription may contribute to the cardiac conduction defects observed in this syndrome.

The mouse Cx26 promoter contains at least two transcription start sites and is located in a very GC-rich region without a TATA box, reminiscent of promoters of housekeeping genes (215). Consensus sequences for a metal response element, NFκB, and six GC boxes were found within 600 bp upstream of the Cx26 transcription start sites in mouse and human DNA (215, 276). Moreover, the human sequence contains a YY1-like binding site and a consensus mammary gland factor binding site (276). The region −140 to −113 of the Cx26 promoter includes an Sp binding site overlapping with an AP-2 binding site which are responsible for the upregulation of Cx26 in the myometrium during pregnancy and in the mammary gland during lactation (590).

The Cx31 gene promoter region contains five putative binding sites for the GATA transcription factor, a NFκB element, a CAAT box, and E-box/E-box related sequences (172). Interestingly, if part of the intron sequence is deleted, the promoter activity is lost (172).

E. Posttranscriptional Regulation of Connexin mRNA Levels

The abundance of mRNA can be affected by conditions that increase or reduce its stability. The 5′-UTR of the Cx43 mRNA contains a strong internal ribosome entry site that confers increased transcription levels (518; for a review, see Ref. 638).

Elevated cAMP concentrations increase the levels of Cx32 mRNA in primary cultures of rat hepatocytes without detectable changes in the transcriptional rate, suggesting an enhanced Cx32 mRNA stability (505). Moreover, during liver regeneration, the reduction in Cx26 and Cx32 mRNA occurs without changes in transcriptional rate, suggesting a posttranscriptional mechanism (293). In this system, the administration of cycloheximide, a protein synthesis inhibitor, completely inhibits the disappearance of Cx26 mRNA, but not of Cx32 mRNA, suggesting the participation of protein factors in destabilization of the Cx26 mRNA (293). The reduction in liver Cx32 steady-state mRNA levels induced by bacterial lipopolysaccharide appears to occur at the posttranscriptional level, since the rate of degradation of this message is higher than the rate of transcription of the gene (185).

F. Connexin Topology and Secondary Structure

The amino acid sequences derived from each cloned cDNA have been used to predict the structure of connexins. Hydropathy plots of several connexins predict the presence of four hydrophobic domains, with a carboxy-terminal hydrophilic tail. In addition, three hydrophilic domains separate the hydrophobic regions (52, 220, 442) (Fig. 3, inset 2). The possible connexin membrane topologies derived from these data were tested by examining the protease sensitivity of isolated liver and heart gap junctions. Antibodies raised against synthetic oligopeptides representing various segments of Cx26, Cx32, and Cx43 were used to map the connexin topology. The amino terminus, the carboxy terminus, and a loop in the middle of the protein are all located on the cytoplasmic side of the junctional membrane, and the predicted extracellular
connexin domains can be detected on the extracellular surfaces of the junctional membranes (218, 382, 471, 663, 679, 682) (Fig. 3, inset 2). The four transmembrane spanning regions and the extracellular loops contain many identical residues or conservative substitutions among the different connexins. In contrast, the cytoplasmic domains are unique to each connexin; the cytoplasmic loop and the carboxy terminus vary extensively in length and amino acid composition.

Sequence analysis and expression of wild-type or mutant connexins are approaches that are actively being used to determine residues within these sequences that may be responsible for various channel properties. The transmembrane regions must be responsible for spanning the plasma membrane and forming the aqueous pore. Three transmembrane regions M1, M2, and M4 contain mainly hydrophobic amino acid residues, while M3 also contains a number of charged (positive and negative) amino acid residues. Therefore, M3 has been modeled as an amphipathic α-helix, and it has been suggested that its hydrophilic face might line the pore of the gap junction channel (35). Structural studies (593) indicate that regions of two α-helices line the inner wall of the channel. Scanning cysteine mutagenesis of Cx46 hemichannels in which amino acid residues from M1 and M3 were replaced by cysteines indicated that both transmembrane regions contribute to the lining of the pore (681). Scanning cysteine mutagenesis of Cx32 channels suggests that M2 also contributes to the lining of the pore (543).

The extracellular regions must be responsible for the “docking” of connexons which occurs by collision of two connexons, each provided by each of two adjacent cells.
This process apparently occurs through noncovalent forces (180). White and co-workers (643, 648) demonstrated the importance of the second extracellular loop (E2) in determining which connexins can interact to form heterotypic channels. Synthetic peptides homologous to the extracellular loop 2 of Cx40 or Cx43 selectively block the function of the corresponding connexin (304). Werner et al. (640) showed that site-directed mutagenesis of amino acid residues within the extracellular loops that differ among connexins alter but do not prevent gap junctional communication. Biochemical studies have shown that cysteines in the extracellular loops participate in forming intramolecular but not intermolecular disulfide bonds (252, 475). Mutation of any of these cysteines to serines prevents gap junction channel formation (109, 110), emphasizing the importance of the secondary and tertiary structures imposed on the connexins by the disulfide bonds. Alteration of the position of the cysteines in Cx32 can seriously impair the ability to form functional channels (165); these data have been used to model this region as adopting a β-sheet conformation. The cysteine residues involved in formation of the intramolecular disulfide bonds in Cx43 have been studied using site-directed mutagenesis (580); however, these differ from those proposed for Cx32 (165). In expression systems, different connexins form channels with differing properties including unitary conductance, selectivity/permeability, and gating/regulation (23, 73, 142, 144, 162, 292, 367, 389, 391, 617–620). Some of the unique properties must be conferred by the unique portions of the connexin sequences (many of which are located within or near the cytoplasm). The main differences in the primary sequence between two closely related connexins, Cx26 and Cx30, are located in the cytoplasmic loop and carboxy-terminal domains. Exchange of one or both of these domains has demonstrated that the cytoplasmic domains interfere directly or indirectly with the permeability, conductance, and voltage gating of the channels in HeLa cells transfected with Cx26/30 chimeras (367).

IV. BIOCHEMISTRY

A. Phosphorylation of Connexins

All studied connexins with the exception of Cx26 (508, 585) are phosphoproteins (for previous reviews, see Refs. 100, 322, 506). Connexin phosphorylation has been implicated in the regulation of intercellular communication through a number of mechanisms, including connexin biosynthesis, trafficking, assembly, membrane insertion, channel gating, internalization, and degradation (16, 42, 44, 146, 217, 229, 320, 330, 390, 401, 426, 470, 567, 586, 670, 669). It is likely that some connexin phosphorylation occurs before insertion into the plasma membrane, but some must occur at the plasma membrane to affect gating. Nevertheless, the specific phosphorylation sites and functional role for most of them have not been elucidated.

The most extensively studied connexin in terms of modification by protein kinases and phosphatases is Cx43. This protein contains several consensus sites for phosphorylation in the carboxy terminus, several of which have been identified as target sites for specific protein kinases. Fusion proteins containing the cytoplasmic tail of Cx43 or synthetic peptides corresponding to the deduced sequences in this region are phosphorylated by protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and cdc2 kinase (323, 507, 575, 634). The identified phosphorylation sites are Ser-368 and Ser-372 phosphorylated by PKC (507) and Ser-255, Ser-279, and Ser-282 phosphorylated by MAPK (633, 634). Although phosphorylated Cx43 can be found at the plasma membrane (400), phosphorylation of Cx43 could also happen in intracellular compartments (103, 315).

Epidermal growth factor (EGF)-, vascular endothelial growth factor (VEGF)-, fibroblast growth factor 2 (FGF-2)-, and platelet-derived growth factor (PDGF)-induced Cx43 phosphorylation can be mediated by intracellular kinase pathways that activate MAPK also termed ERK1/2 (132, 229, 230, 263, 564, 664) (Fig. 4). In some systems, this effect appears to occur in a PKC-independent manner (449), but in other systems it can be prevented by PKC inhibition, but not by inhibition of the ERK1/2 pathway (133). In T51B cells, this effect requires the activation of both PKC and ERK1/2 (229). MAPK activation may also mediate the induction of Cx43 phosphorylation by lysophosphatidic acid (LPA) (221). The observations are consistent with the variations in cross-talk between different protein kinase-dependent pathways in different cell types (e.g., differences in the relative activity of each pathway and different subcellular compartmentalization of different protein kinases). It has been confirmed that Cx43 is a MAPK substrate in vivo and that phosphorylation on Ser-255, Ser-279, and/or Ser-282 is sufficient to disrupt gap junctional intercellular communication (634). Accordingly, T33 A31 fibroblasts transfected with a carboxy-terminal truncated Cx43 remain highly coupled after treatment with PDGF (388). Nevertheless, other studies suggest that other unknown factors besides phosphorylation are required for disruption of Cx43-mediated cell-to-cell communication (231, 232). The consensus sites for MAPK and cdc-2 kinase present in Cx43 are apparently shared, since the two-dimensional tryptic maps of the carboxy terminus phosphorylated by MAPK or cdc-2 kinase appear identical (507). Yet, comparison of two-dimensional phosphotryptic peptide analysis of the p34(cdc2)/cyclin B kinase-phosphorylated carboxy terminus and of the P3 phosphoform of Cx43 immunoprecipitated from mitotic cells reveals several
differences. This suggests that in vivo Cx43 phosphorylation would involve the participation of other protein kinase(s), which may be activated by the p34cdc2/cyclin B kinase (321).

Numerous reports have described changes in the state of phosphorylation of Cx43 through PKC-dependent pathways. The pattern of Cx43 phosphorylation and the functional consequences are cell type dependent (for reviews, see Refs. 322, 506). Ser-368 in Cx43 appears to be an important site that may underlie the cellular uncoupling that follows TPA treatment, since mutation of Ser-368 partially prevents this effect (323, 349).

The products of various oncogenes are tyrosine kinases that disrupt gap junctional communication by phosphorylation of tyrosine residues in Cx43. Oncogene products with tyrosine kinase activity phosphorylate Cx43 in vivo (102, 160, 302) and in vitro (357). Similarly, c-Src seems to be involved in loss of gap junctional communication induced when ligands bind to c-Src-associated receptors (466). The interaction with v-Src is mediated by a proline-rich motif of Cx43, Pro-274-Pro-284 (264). Moreover, in vitro studies indicate that Cx43 is a substrate for v-Src tyrosine kinase and that phosphorylation of Cx43 occurs in Tyr-247 and Tyr-265 (345). Expression in Cx43-null cells of Cx43 constructs in which Tyr-247 and/or Tyr-265 were replaced with phenylalanines suggests that phosphorylation of both tyrosine residues is required for the complete v-Src-induced disruption of gap junction channel communication (345).

In rat (but not human) Cx43, Ser-257 is flanked by a proline and a lysine, making it a possible site for phosphorylation by cGMP-dependent protein kinase. Accordingly, the extent of phosphorylation of rat Cx43, but not of human Cx43, expressed in SkHep1 cells is increased by 8-bromo-cGMP (306).

Some studies have shown that activation of cAMP-dependent protein kinase (cAMP-dPK) leads to a rapid augmentation in Cx43 phosphorylation as detected by immunoblotting (193) and increased intercellular communication (69, 186). However, direct phosphorylation of Cx43 by cAMP-dPK has not been documented. The polypeptides corresponding to the carboxy terminus of Cx43 are not phosphorylated by cAMP-dPK (509, 575). Moreover, activation of cAMP-dPK does not change the incorporation into Cx43 transfected into fibroblasts derived from Cx43-null mice (575).

Cx32 is phosphorylated by cAMP-dPK, PKC, Ca\textsuperscript{2+}/calmodulin-dependent kinase II, and EGF receptor or Ca\textsuperscript{2+}/calmodulin-dependent protein kinase have not been reported.

The stoichiometry of Cx32 phosphorylation in vitro by PKC or cAMP-dPK is between 0.1 and 1 mol phosphate/mol Cx32 (508, 511, 570), suggesting that not every channel or not every subunit within a channel is phosphorylated. The stoichiometry of in vivo phosphorylation and identification of the cell compartment(s) in which Cx32 phosphorylation occurs have not been reported. Cx32 is phosphorylated in Ser-233 by both cAMP-dPK and PKC (508). It is likely that PKC phosphorylates other amino acid residues that are not phosphorylated by cAMP-dPK (570). Increased incorporation of \textsuperscript{32}P into Cx32 is observed when isolated liver gap junctions (previously phosphorylated by cAMP-dPK) are incubated with PKC (570); moreover, two-dimensional tryptic phosphopeptide maps of Cx32 immunoprecipitated from hepatocytes treated with PKC activators show a higher number of \textsuperscript{32}P-labeled peptides than the maps of Cx32 obtained from cells treated with cAMP-dPK activators (508). Phosphorylation of Ser-233 is not required for formation of functional channels (640), but its possible role in other functions has not been studied. The tyrosine residues phosphorylated after activation of EGF receptor (130) have not been identified.

Lens connexins are phosphoproteins, and changes in their state of phosphorylation correlate with changes in cell-cell communication (39, 248, 514, 670). In ovine lens, Cx49 is phosphorylated by casein kinase I (89), and inhibition of casein kinase I increases intercellular communication between cultured lens cells (90). Moreover, Cx45.6 is phosphorylated at Ser-363 by casein kinase II; this phosphorylation inhibits the cleavage of the connexin carboxy terminal mediated by caspase-3-like protease (669). Cx46 is phosphorylated in serine and threonine by PKC in the lens cortex (514). Two phosphorylation sites have been identified in Cx56, Ser-118 and Ser-493. These sites are phosphorylated under basal conditions in lentioid-containing cultures. Upon activation of PKC, phosphorylation is enhanced in Ser-118 (39). Phosphorylation of Ser-118 correlates with a reduction in intercellular communication (39). The Cx56 phosphorylation and reduced intercellular communication induced by a tumor promoter phorbol ester appear to be mediated by PKC-γ (44).

Phosphorylation of other connexins (e.g., Cx31, Cx37, Cx40, and Cx45) in transfected cells has been demonstrated directly by incorporation of \textsuperscript{32}P into the protein or shift in their electrophoretic mobilities after treatments that increase protein kinase activity (313, 328, 583, 584, 610, 613). Mouse and human Cx37 expressed in cell lines are phosphorylated mainly in serine residues (328, 584). Mouse Cx45 is detected as a doublet that is reduced to a single 46-kDa band after treatment with alkaline phosphatase (613). After mutation or deletion of nine serine resi-
dues in the carboxy terminus of Cx45, phosphorylation is decreased by 90%, indicating that those serine residues represent main phosphorylation sites in the protein (217). Activation of cAMP-dPK leads to an electrophoretic mobility shift of human Cx40 that correlates with increased cell-to-cell communication (610). However, the identification of amino acid residues with functional significance and dissection of protein kinases responsible for their phosphorylation await further studies.

Finally, treatment of some cells with phosphatase inhibitors leads to increased Cx43 phosphorylation, suggesting the involvement of phosphoprotein phosphatases 2A and 2B (42, 104, 342). The phosphatases that mediate dephosphorylation of other connexins have not been reported.

B. Other Protein Modifications

The amino termini of several connexins contain potential consensus N-glycosylation sites, but they are not utilized (474, 630), presumably because they are located in the cytoplasm and therefore are not accessible to the glycosylating enzymes. Fatty acylation of connexins has been suggested by metabolic labeling studies (585), but in-depth studies have not been published.

Modification of Cx43 by ubiquitination has been demonstrated (309, 312, 501) (Fig. 3). The residue to which ubiquitin molecules are added has not been identified, but it is likely to be a lysine.

Cleavage of the carboxy terminus of lens fiber-specific connexins has been proposed to occur naturally during maturation of lens fiber cells (283). Studies of the ovine Cx50 have identified calpain as the enzyme that removes a 32-kDa portion of the carboxy tail of Cx50 (344, 669, 670). However, a similar phenomenon may not occur in chicken lenses, since cleavage of Cx56 was not observed when lens cortex and lens nucleus homogenates were prepared using EDTA (40).

C. Interaction and Colocalization of Connexins With Other Proteins

Connexins may interact with other proteins within the cell. Because gap junction isolation methods involve the use of relatively harsh detergents, interacting proteins do not copurify with connexins. However, other strategies have suggested some possible associated proteins. Blotting experiments have suggested Cx32 could bind calmodulin (604, 682). In addition, Cx43 contains SH2 domains that may be important for its interaction with tyrosine kinases such as pp60src (264, 358). Recent studies have suggested an association of Cx43, Cx45, Cx46, and Cx31.9 with the peripheral membrane protein ZO-1 (181, 310, 416, 417, 581) (Fig. 3), which was originally identified as a component of tight junctions. Binding of Cx32, but not Cx26, to another tight junction protein, occludin, has also been demonstrated by coimmunoprecipitation experiments (288, 289). The expression of Cx32, but not Cx32 truncated at position 220, wild-type Cx26, or Cx43, in Cx32-null hepatocytes enhances the expression of the tight junction proteins claudin, occludin, and ZO-1, which colocalize with Cx32, suggesting that the Cx32-mediated intercellular communication participates in the formation of tight junctions (290). With the use of a yeast two-hybrid protein interaction screen, the interaction of Cx43 through its carboxy terminus with the second PDZ domain of the ZO-1 protein has been demonstrated (181). The v-Src-induced disruption of gap junction plaques may occur through interaction of v-Src with ZO-1 (579).

Connexins appear to interact with caveolin-1, a structural protein of lipid raft domains (112, 520); this interaction seems to be cell type specific, and its functional significance is unknown. Colocalization of connexins with other membrane proteins, including FGF receptors (268), aquaporins (476), or cytoplasmic proteins such as calmodulin (549) and cytoskeletal proteins (182) has been demonstrated.

V. FORMATION AND DEGRADATION OF GAP JUNCTIONS

A. Connexin Biosynthesis and Gap Junction Assembly

The development of specific anti-connexin antibodies has made possible in vitro studies of connexin biosynthesis by metabolic labeling and immunoprecipitation (reviewed in Refs. 314, 317). The biosynthetic studies have been most extensive for Cx43. Pulse-chase studies show that Cx43 is initially synthesized as a 40- to 42-kDa polypeptide that is subsequently posttranslationally modified to forms with slightly slower mobilities on SDS-PAGE due to phosphorylation of serine residues (102, 160, 399, 400). Multiple phosphates are added to Cx43, and at least some of this phosphorylation occurs in a serine-rich sequence near the carboxy terminus. Phosphorylation of Cx43 to yield a phosphoform (P1) occurs soon after translation. Inhibition of protein trafficking with monensin or brefeldin A reveals that partial phosphorylation of Cx43 occurs before its exit from the Golgi apparatus (315, 470).

The location at which connexins oligomerize into connexons is connexin-type dependent (116, 129, 370, 402, 474); as examples, it appears that Cx32 assembles in the endoplasmic reticulum (ER) (or ER/Golgi intermediate compartment) while Cx43 assembles in the trans-Golgi network (116, 129, 370, 402, 474). With the use of an in vitro cell-free transcription/translation system, it has
been demonstrated that Cx26 hemichannels are integrated directly into plasma membranes in a posttranslational manner (5). In cells expressing two connexins, it is possible to find hemichannels formed by both connexins (heteromeric) (43, 246, 355, 372, 556) or hemichannels constituted by one or the other protein (homomeric). In studies performed in liver, oligomeric intermediates of Cx26 are detected in an ER/Golgi intermediate compartment while oligomers containing both Cx26 and Cx32 are preferentially detected in a Golgi membrane fraction (129). This might have implications for the formation of heteromeric connexons (see below). Newly synthesized connexons appear to be transported to the plasma membrane through two different pathways: one sensitive to brefeldin A (a drug that disrupts the Golgi compartment) and one sensitive to nocodazole (a drug that disassembles microtubules) (179, 370).

Connexons are inserted into the plasma membrane in a closed configuration (Fig. 3, inset 1) perhaps at regions of cadherin/catenin-mediated cell adhesion or near tight junctions (169). Formation of gap junctions requires appropriate cell adhesion, especially that mediated by Ca\(^{2+}\)-dependent molecules (cadherins) (258, 400). Musil et al. (400) have demonstrated that Cx43 localizes intracellularly and not at gap junctional plaques in the noncommunicating cell lines L929 and S180. The Cx43 in these cells is incompletely phosphorylated. However, transfection of the S180 cells with the cell adhesion molecule LCAM (E-cadherin) restores gap junctional communication, phosphorylation of Cx43 (to a “P2” form), and presence of gap junction plaques. These findings suggest a relation between the ability of cells to more extensively phosphorylate Cx43 and the ability to form communicating junctions. They also suggest a hierarchy of events in the formation of intercellular junctions: a cell adhesion event is required before formation of gap junction plaques. Similar observations regarding the requirement of cadherin-mediated cell adhesion for development of gap junctions have been made in epidermal cells (258). Cell-to-cell contact sites mediated by the cadherin-catenin complex also seem to act as foci for gap junction formation as shown by colocalization of connexins with E-cadherin or \(\beta\)-catenin during gap junction formation in regenerating liver (169).

Moreover, antibodies to A-CAM (N-cadherin) or peptides representing extracellular domains of Cx43 inhibit gap junction formation in Novikoff hepatoma cells (381). However, the effects of increased cell adhesion seem to be cell type specific, since expression of exogenous cadherin decreased gap junctional communication between mouse L cells but increased it in Morris hepatoma cells (632). A role for Ca\(^{2+}\)-independent cell adhesion molecules (e.g., N-CAM) has also been suggested (270).

Incorporation of connexons made of Cx43 into gap junction plaques correlates with resistance to solubilization in Triton X-100 and with additional phosphorylation (401). However, some studies suggest that phosphorylation may not be an absolute requirement for assembly or insertion of connexons into the plasma membrane. In exogenous expression systems, Cx26, a nonphosphorylated protein, is able to induce junctional currents (23). Moreover, truncated Cx43 or Cx32 constructs devoid of some phosphorylatable serine residues from their carboxy termini can still induce intercellular coupling (162, 351, 640). In addition, phosphorylation of Cx43 can occur in Madin-Darby canine kidney cells in the absence of Ca\(^{2+}\)-dependent cell adhesion molecule activity and does not correlate with existence of intercellular coupling (42).

Formation of gap junction plaques requires clustering of gap junction channels. Increased levels of cAMP induce clustering of Cx43 gap junction channels and enhanced gap junction plaque growth through a mechanism that seems to be cell type dependent. In some cell types, this process depends on polymerized actin while in others it depends on intact microtubules and trafficking through intracellular membrane compartments (158, 256, 445, 631). It has recently been suggested that the carboxy terminus of Cx43 (including Ser-364) plays a role in the cAMP-induced clustering of gap junction channels (575). The differences observed between cell types might reflect differences in the source from which additional connexons come (i.e., a pool at or near the plasma membrane or a pool at earlier locations within the biosynthetic/exocytic pathway).

Connexins that are coexpressed in the same cell can localize to the same gap junction plaque (404, 413, 428, 585, 115, 228, 600, 668) or to distinct membrane domains (203, 553), suggesting the presence of a sequence motif in connexins that determines its topographic fate. Although differences in primary sequence between connexins might explain the delivery of hemichannels to specific plasma membrane domains, these sequences have not been identified for most connexins. It has been reported that the transmembrane domains, but none of the cytoplasmic domains, are required for the delivery of Cx32 to its specific destination in cultured epithelial cells (337). How gap junction channels aggregate and form small junctional plaques is not yet resolved. In ultrastructural studies, small particle aggregates have been found within particle poor, flattened regions of the plasma membrane as junctions are beginning to form between reaggregated cells (255). Growth of gap junctions occurs by incorporation of additional gap junction channels to the plasma membrane followed by their incorporation to the periphery of existing gap junction plaques (174, 331). Imaging of living cells transfected with fluorescent protein-tagged connexins suggests that connexins are delivered to the gap junction plaque in cytoplasmic vesicles (260) that move along microtubules (331). Large junctional plaques can form by coalescence of small junctional plaques (227, 282).
Some incompletely resolved issues are as follows: how connexins are brought together to assemble into connexons, how these connexons are incorporated into the plasma membrane, how gap junctional channels become part of a plaque, and which other proteins/factors participate in these processes. It is possible that no other factors are necessary for channel or plaque formation because purified connexons tend to aggregate in filaments and sheets (557).

B. Gap Junction and Connexin Degradation

Proteins in gap junctions are apparently rather short-lived. Based on in vivo labeling, Fallon and Goodenough (157) estimated that the half-life of a major polypeptide in liver gap junctions was in the range of 5 h. Similarly, pulse-chase experiments have shown that connexins have half-lives of 1–3 h in cultured cells (102, 115, 316, 586). A similar half-life has been measured for Cx43 in intact lens and heart (29, 399). However, at least some of the lens fiber connexins are more stable, with half-lives of 2–3 days or more (38, 247).

Morphological and biochemical approaches have recently been applied to begin to elucidate the mechanisms of gap junction degradation (reviewed in Ref. 49). Electron microscopy studies have implied endosomal internalization of entire junctions forming “annular gap junctions” (Figs. 1B and 3) followed by degradation in lysosomal, multivesicular body, or autophagosomal compartments (260, 325, 326, 375, 397, 408, 447, 489, 533, 616). In vivo, these structures have been most frequently found following pathological insults such as ischemia or during tissue remodeling. Cell fractionation and immunoelectron microscopy studies have shown an association between connexins and lysosomes in some cultured cells (515, 616). Murray et al. (398) found that in SW-13 adrenal cortical tumor cells, many of the cytoplasmic annular Cx43 gap junction profiles are associated with myosin II, but not with tubulin, or vimentin-containing fibers. Disruption of microfilaments resulted in a decrease in the number and an increase in the size of these annular gap junctions, suggesting a role for myosin-containing cytoskeletal elements in annular gap junction turnover (327, 398). A recombinant Cx43 containing tetracysteine tags was recently used to demonstrate that older Cx43 is removed from the center of plaques into pleiomorphic vesicles of widely varying sizes (174).

Recently, investigators have used biochemical or immunochromical techniques to study proteolysis of the subunit gap junction proteins. Studies of Cx43 proteolysis in E36 Chinese hamster ovary cells have shown the major involvement of the ubiquitin-proteasome pathway in Cx43 degradation. In these cells, treatment with the protease inhibitor N-acetyl-leucyl-leucyl-norleucinal (ALLN) induces accumulation of Cx43 and a significant prolongation of its half-life. Also, Cx43 degradation is abolished when ts20 Chinese hamster ovary cells, which contain a thermolabile ubiquitin activating enzyme, E1, are incubated at the restrictive temperature (309). Immunofluorescence studies in both primary cultures of cardiac myocytes and in established cell lines have shown that Cx43 gap junction plaques as well as the Cx43 polypeptide exhibit rapid turnover rates. The rapid disappearance of Cx43 staining can be blocked by inhibitors of either the proteasome or the lysosome, implicating both proteolytic systems in gap junction degradation (290, 311, 312). These studies have also shown that connexin degradation could be stimulated by cellular stress such as heat shock (311). It is possible that the proteasome is mainly involved in degradation of misfolded connexins (as occurs for many other proteins); this process is called ER-associated degradation and serves as a quality control check point. Misfolded connexins likely are dislocated from the ER and then degraded by the proteasome. The involvement of this pathway during connexin biosynthesis has been recently demonstrated for wild-type Cx43 (612). The participation of both proteasomal and lysosomal pathways in the degradation of Cx43 in the heart has also been demonstrated (29).

The published studies suggest that the preference of one degradative pathway over the other is cell type dependent and that within the same cell type the degradative pathway chosen might depend on the metabolic/pathological state of the cell. The experiments do not allow differentiation between the possibilities of a sequential (e.g., the carboxy tail of Cx43 is degraded in the proteasome while the core of the protein embedded in the membrane is degraded by the lysosome) or parallel (Cx43 can be degraded by the proteasome or the lysosome) pathway for degradation of connexins.

VI. HEMICHANNELS

Although for several decades hemichannels found at nonjunctional membranes were thought to remain permanently closed to avoid cell death, data reported during the last decade have documented the existence of regulatable hemichannel opening in cultured cells. Nevertheless, it is still not completely known whether cell surface hemichannels have different permeability properties than gap junction channels formed by the same connexin type. Moreover, it is unknown whether physiological stimuli may induce brief hemichannel openings that allow transfer of small molecules between the intracellular and extracellular compartments.

A. Opening and Closing Hemichannels

The opening of connexin hemichannels was first described based on the swelling and death of Xenopus oo-
cytes injected with the RNA of Cx46 (444). Shortly thereafter, DeVries and Schwartz (125) reported that cultured solitary horizontal cells of the catfish retina express an endogenous current mediated by hemichannels that open upon reduction of the extracellular \([\text{Ca}^{2+}]\). The current is reduced by external \([\text{Ca}^{2+}]\) higher than 1 mM, treatment with dopamine, or a weak acid and coincides with blockade of Lucifer yellow uptake. Subsequently, the electrophysiological characterization of macroscopic and single hemichannel currents have been reported for various connexins exogenously expressed in Xenopus oocytes or mammalian cell lines (137, 139, 205, 291, 341, 462, 471, 562, 589, 597, 599).

Opening of hemichannels formed by different connexin types is rather infrequent or absent under resting conditions but can be regulated by several factors. Low extracellular \([\text{Ca}^{2+}]\) enhances the opening of hemichannels in the Xenopus oocyte expression system (137, 139), in Novikoff cells (341), in some cardiac myocytes (291), in astrocytes (225, 562), and in transfected human osteoblast-like cells (492). In addition, opening of Cx43 hemichannels has been induced by mechanical stimulation in cultured astrocytes (562). Agonists of hemichannel opening have also been described. The hemichannel-mediated currents expressed by retinal horizontal cells bathed in low extracellular \([\text{Ca}^{2+}]\) are enhanced by the antimalarial drugs quinidine or quinine (363). Quinidine-induced dye uptake revealing opening of Cx43 hemichannels has also been observed in astrocytes (562), but not in Cx43 RNA-injected Xenopus oocytes (644). The quinine-induced hemichannel opening cannot be explained solely by the quinine-induced alkalinization because it is still observed using 80 mM buffered HEPES in the recording patch pipette (131). Alendronate, a drug used widely in the treatment of bone diseases, induces opening of patch pipette (131). Alendronate, a drug used widely in the treatment of bone diseases, induces opening of patch pipette (131).

Connexins, including rat Cx46, bovine Cx44, and chicken Cx56, form functional hemichannels in Xenopus oocytes bathed in low or even normal extracellular \([\text{Ca}^{2+}]\) (137, 139, 205, 444). However, lens fibers have high input resistance, suggesting the absence of open hemichannels at the plasma membrane under physiological conditions. This apparent controversy might be explained by differences in the posttranscriptional modification of these connexins in oocytes with respect to that in lens fibers. These connexins are phosphoproteins and are detected as multiple bands in immunoblots of lens homogenates (40, 205, 444), but when expressed in Xenopus oocytes, they show the same electrophoretic mobilities as in vitro translated proteins (137, 205, 444). Sheep lens fibers containing Cx49 show an increase in dye coupling when treated with a casein kinase I inhibitor, suggesting that phosphorylation of the lens fiber connexin by the endogenous casein kinase I activity leads to closure of gap junction channels (89, 90). A similar mechanism might help in keeping lens fiber hemichannels closed to maintain the high input resistance of lens cells.

Opening of Cx43 hemichannels induced by low extracellular \([\text{Ca}^{2+}]\) can be blocked by activation of a PKC-dependent pathway (341, 350), suggesting the involvement of protein phosphorylation as a gating mechanism that maintains the hemichannels closed. Likewise, the membrane current mediated by Cx46 hemichannels expressed in Xenopus oocytes is greatly reduced by activation of a PKC-dependent mechanism (245, 412). Moreover, Cx43 is a substrate for MAPK (633). MAPK can be activated by tyrosine kinase receptors, such as the EGF receptor, or through transduction pathways that activate PKC leading to direct or indirect phosphorylation of Cx43 via PKC or MAPK as proposed for Cx43 forming gap junction channels (Fig. 4). A similar mechanism might operate on Cx43 hemichannels. In support of this hypothesis, it is known that hemichannels of MAPK-phosphorylated Cx43 reconstituted in lipid vesicles remain preferentially closed, but dephosphorylated Cx43 forms opening hemichannels (280). Nevertheless, it is difficult to conceive that under resting conditions MAPK would keep the hemichannels closed without affecting gap junctional communication. Alternatively, Cx43 forming hemichannels could be phosphorylated by a protein kinase activity with several isoenzymes that show cellular compartmentalization (e.g., PKC). Fibroblasts from Cx43-null mice transfected with Cx43 mutated at Ser-368, a change that confers resistance to PKC-induced closure of gap junction channels (323), show a decrease in hemichannel opening after treatment with a phorbol ester to activate PKC (349).

Closure of hemichannels has been observed after extracellular application of lanthanide cations, \(\text{La}^{3+}\) (99, 251, 280, 291), or \(\text{Gd}^{3+}\) (562), or treatment with gap junction channel blockers, such as octanol, heptanol, carbonoxolone, oleamide, halothane, and 18-\(\alpha\) and 18-\(\beta\)-glycyr rhretinic acid. Moreover, closure of hemichannels formed by Cx30, Cx44, Cx46, Cx50, or Cx56 can be induced by hyperpolarization of the plasma membrane (137, 139, 205, 587, 599). In horizontal cells bathed in \(\text{Ca}^{2+}\)-free saline and exposed to positive holding potentials, open hemichannels are closed by retinoic acid (678). With the use of cysteine replacement mutagenesis, the voltage gate in Cx46 hemichannels has been localized extracellularly to the amino acid residue at position 35 (453). Extracellular acidification also closes hemichannels formed by various connexins (28, 588, 676). The membrane current mediated by hemichannels is greatly reduced by 1–2 mM extracellular \(\text{Ca}^{2+}\) (137, 139, 454). It has been proposed that \(\text{Ca}^{2+}\) induces a regional closure of the pore (454). \(\text{Mg}^{2+}\) can partially substitute for the \(\text{Ca}^{2+}\) blocking effect on Cx46 hemichannels (139).

The conductance of homomeric hemichannels formed by Cx30, Cx32, Cx43, Cx45, Cx46, and Cx50 has been measured using voltage clamp in the cell-attached or
the excised-patch configuration (140, 149, 587, 597, 599). The unitary conductance determined for homomeric connexin hemichannels ranges from 31 to 352 pS (140, 149, 587, 599).

In support of the existence of conduits permeable to small molecules, it has been demonstrated that dextran polymers of high molecular weight conjugated to different fluorophores are not taken up by cells expressing hemichannels (99, 562). Moreover, it has been demonstrated that Cx43 hemichannels are permeable to Lucifer yellow, ethidium bromide, carboxyfluorescein, 7-hydroxycoumarin-3-carboxylic acid, and fura 2 (99, 291, 341, 350, 462, 562, 607). Direct or indirect measurements have demonstrated the permeability of Cx43 hemichannels to other small molecules [e.g., NAD$^+$, ATP and inositol trisphosphate (IP$_3$)] (15, 63, 64, 492, 562). Similarly, cells expressing Cx32 release ATP, suggesting that Cx32 hemichannels are permeable to this nucleotide (15).

Alterations in hemichannel features by genetic mutation have also been documented. Two mutations linked to congenital cataracts, Cx46Asn63Ser and Cx46 frame-shift 380, were impaired in their ability to form functional hemichannels (438). Studies on Cx50 have revealed that the His176Gln mutant is oocyte lethal and that the His161Asn mutant does not form detectable hemichannels (28). Moreover, the double mutant Cx50His161Asn/His176Gln neither forms hemichannels nor kills the oocytes (28). Hemichannels formed by a CMTX-associated Cx32 mutant, Cx32Ser85Cys, show increased currents compared with those made of wild-type Cx32 (2). A chimeric connexin consisting of Cx32 where the first extracellular loop sequence is replaced by the corresponding Cx43 sequence induces a membrane conductance in single Xenopus oocytes (455). Finally, some carboxy-terminal mutations of Cx32, including some of those identified in CMTX, prevent the formation of functional connexons (79).

**B. Hemichannel Functions**

Recent reports indicate that hemichannel opening could play relevant functions under physiological and pathological conditions. Quist et al. (471) proposed that...
Cx43 hemichannels regulate the cell volume in response to changes in extracellular physiological \([\text{Ca}^{2+}]\) in an isosmotic situation. In addition, Cx43 hemichannels mediate the release of \(\text{NAD}^+\) to the extracellular medium in fibroblasts (63, 64). This provides an answer to the old paradox of having intracellular \(\text{NAD}^+\) and NAD +- by immuno-fluorescence (262, 243). It has been proposed that opening of Cx26 hemichannels would lead to depolarization of cell dendrites mediating a negative feedback that opening of Cx43 hemichannels provides a paracrine signal mechanism that results in a delayed calcium transient in neuronal cells in coculture, which is strongly inhibited by glutamate receptor blockers (621). Moreover, mechanical stimulation elicits release of ATP through Cx43 hemichannels in astrocytes and could mediate the propagation of \(\text{Ca}^{2+}\) signals for intercellular communication in astrocytes and other nonexcitable cells (562).

The detection of Cx26 hemichannels on the dendrite surface of retinal horizontal cells has been demonstrated by immunofluorescence (262, 243). It has been proposed that opening of Cx26 hemichannels would lead to depolarization of cell dendrites mediating a negative feedback mechanism that modulates the activity of \(\text{Ca}^{2+}\) channels and subsequent glutamate release from cones (262). Massive opening of hemichannels made of Cx43, the most ubiquitous connexin, has been demonstrated in astrocytes and cardiomyocytes subjected to metabolic inhibition to mimic ischemia (99, 251, 291, 339). The metabolic inhibition-induced opening of Cx43 hemichannels would speed up mechanisms leading to cell death (99). Moreover, the increased opening of hemichannels made of a CMTX-associated Cx32 mutant (Cx32Ser85Cys) could be deleterious for glial cells and normal neural function (2).

VII. GAP JUNCTIONS IN CARDIOVASCULAR, DIGESTIVE, REPRODUCTIVE, AND IMMUNE SYSTEMS

A. Cardiovascular System

Gap junctions between cardiac myocytes are found in specialized plasma membrane regions known as the intercalated disks adjacent to adherens junctions (which facilitate mechanical coupling between cells). Gap junction channels in the heart appear to play a critical role by allowing the intercellular passage of current-carrying ions, which facilitates action potential propagation. Differences in the abundance, size, and location of gap junctions in different cardiac regions (117, 118) may contribute to differences in their properties of electrical conduction. As examples, Saffitz and colleagues (360, 512) have shown that cardiac tissues that differ in their anisotropy of conduction (infarct border zones vs. normal myocardium or crista terminalis vs. ventricular myocardium) show marked differences in their relative abundance of “end-to-end” versus “side-to-side” gap junctions. Moreover, because gap junction channels made of different connexins have different conductance and gating properties, differences in expression patterns of connexins may also contribute to differences in cardiac conduction.

Several connexins including Cx43, Cx40 (or its ortholog, chicken Cx42), and Cx45 are expressed in cardiac tissues (266, 267), but they have different distributions in specialized cardiac tissues. Cx43 appears to be the major connexin in the working myocardium of the ventricle (117, 118, 605). In atrial myocytes, Cx43 is coexpressed with Cx40 (117, 118, 191, 512, 625). Studies of cardiac tissues from several different species (human, dog, cow, rabbit) have shown that Cx43 is rare or absent in cells of the specialized conducting regions of the sinus and atrioventricular nodes (14, 117, 118, 307, 430, 431). However, some studies have found Cx43-expressing cells that might form specialized pathways for electrical conduction out of nodal zones (307, 622, 623). The expression of Cx40 in the heart is more restricted than that of Cx43. Cx40 is abundant in atrial myocytes and cells of the His-Purkinje system and is also found in cells of the sinoatrial and atrioventricular nodes (24, 117, 191, 199, 266). Although Cx40 is not present in adult mammalian ventricular myocytes, its ortholog (Cx42) is abundant in the ventricular myocardium of the developing chicken heart (384). Cx45 expression is detectable in cells of the atra, ventricle, sinus and atrioventricular nodes, and His bundle (117, 266). The absolute amounts of different connexins in cardiac myocytes have not been determined; rather, different abundances have been considered based on the intensity of immunostaining. This has led to some debate regarding the significance of levels of Cx45 in ventricular myocardium (101, 253).

Gap junctions are also present in distributions that link all of the different cell types in the walls of blood vessels. Gap junctions may play multiple roles in facilitating interactions between these cells (as recently reviewed in Refs. 420, 532); most prominently, endothelial cell gap junctions may be critical for the propagation of vasomotor signals (523). In general, all types of vessel wall cells express Cx43, in vivo and in vitro. In vivo, Cx40 mRNA or protein has been demonstrated in large and small vessel endothelium and in smooth muscle from several species but not others. Cx40 is a major gap junction protein of endothelial cells of the adult vasculature in most organs.
(346, 606), but it shows heterogeneous expression along the vasculature (528). Other than expression in the ovary and perhaps in some lung cells, Cx37 expression appears to be virtually exclusively limited to endothelial cells (478), but there may be substantial variability in expression in vivo and in culture, depending on vascular bed type and species. The differential distribution of Cx37 and Cx43 suggests that they are involved in more dynamic processes than Cx40. However, interpretations of their patterns of expression along vessel walls are controversial. Cx43 is highly localized to sites of disturbed flow in rat aortic endothelium, but Cx37 and Cx40 are more uniformly distributed (173). While arterial smooth muscle cells abundantly express Cx43, they may also express Cx40 (346) and Cx45 (222, 287, 298). The patterns of expression of connexins among endothelial and smooth muscle cells are modulated under pathological conditions including early human coronary atherosclerosis or arterial wall injury (54, 208, 209, 305, 464, 465, 667).

Myocardial gap junctions and connexins may be critical for the coordinated electrical activation of the heart. A variety of alterations in their number and distribution have been observed in diseased hearts (265, 532). Disorganization of gap junctions and/or reduced expression of Cx43 have been observed and may explain increased arrhythmogenesis in human and animal ventricular myocardium associated with ischemia or infarction (452, 531). Decreased Cx43 at plasma membrane appositions has been found between cardiac myocytes infected with Trypanosoma cruzi, the Chagas disease agent, suggesting a potential cause for cardiac conduction disturbances observed in affected individuals (71). Levels of Cx40 increase 3.1-fold, and those of Cx43 decrease 3.3-fold in the myocardium of hypertensive rats (24). In contrast, Cx40 levels were reduced, and its distribution was altered without any changes in Cx43 in association with models of atrial fibrillation (603).

Recently, molecular biology approaches have been used to alter the expression of cardiovascular connexins to elucidate their functions. Reaume et al. (477) used homologous recombination to produce Cx43-null mice. These animals survive until birth and look essentially normal. At birth, they have beating hearts, but they appear cyanotic and die shortly thereafter, apparently due to a lack of blood flow to their lungs due to obstruction of the right ventricular outflow tract. Thus it appears that the presence of Cx43 is not an absolute requirement for the heart to beat or for the anatomic development of the heart. Perhaps other coexpressed connexins (such as Cx45) can explain the coupling of Cx43-null ventricular myocytes, but the amount of Cx45 is also diminished in Cx43-deficient mice (253).

The importance of Cx43 for normal cardiac conduction and velocity has been elucidated by studies of mice that are heterozygous for the knock-out genotype (Cx43+/−). These mice appear phenotypically normal and reproduce normally. However, epicardial conduction is slowed in the ventricle (but not the atrium) (202, 576). In addition, the QRS complex of the electrocardiogram is widened, implying ventricular conduction delay (202, 576). Finally, tissue-specific knock-out strategies have been used to produce mice with cardiac-specific loss of Cx43; these animals show normal heart structure and contractile function, but they develop sudden cardiac death due to spontaneous ventricular arrhythmia by ~2 months of age (206). It has been recently suggested that mutations in Cx43 associated with oculodentodigital dysplasia might be responsible for the cardiac conduction abnormalities observed in some of the families (446).

An endothelial cell-specific Cx43 knock-out mouse shows hypotension and bradycardia (343). The hypotensive and bradycardic phenotypes correlate with elevated plasma levels of nitric oxide and angiotensins I and II, suggesting the importance of Cx43 gap junctions for maintenance of vascular tone. An extensive series of transgenic experiments (using wild-type or mutant Cx43) by Lo et al. (354) have emphasized the role of Cx43 expressed by cardiac neural crest cells for the proper development of the heart. For example, Huang et al. (235) have shown that Cx43 gene dosage is critical for the function of neural crest cells during cardiac development.

The importance of Cx40 in conduction through the specialized cardiac conduction system is emphasized by the analysis of Cx40-null mice. These animals develop to adulthood and reproduce normally, suggesting that Cx40 is not necessary for cardiac development, since the cardiac structure and chambers appear normal. However, electrocardiograms show wide and bizarre QRS complexes (47, 281, 542, 611) consistent with a right bundle branch block pattern (likely due to the absence of this connexin from the atrioventricular conduction pathways). These animals may also have defects in propagation of endothelium-dependent vasodilator responses (127). Cx40-deficient mice retain gap junctional communication between aortic endothelial cells, although with altered characteristics with respect to wild-type mice; moreover, Cx37 and Cx43 are upregulated and redistributed (297), suggesting a compensatory mechanism. Cx40-(and Cx37-)null animals exhibit no obvious vascular abnormalities.

Cx45-deficient mice die during embryogenesis due to abnormalities of vascular development and cardiogenesis (298, 299). Endothelial cell development and positioning are normal, but further formation of vascular trees is impaired and the smooth muscle layer of major arteries fails to develop. The hearts of these animals are dilated, and apoptosis is observed in almost all tissues by embryonic day 9.5.
B. Digestive System

1. Gastrointestinal tract

Gap junctions, connexins, and functional intercellular coupling have been identified in multiple portions of the gastrointestinal tract. The distribution of different connexins in the various organs and tissues of the digestive system is shown in Table 1 based on a compilation of diverse published studies. Along the gastrointestinal tract, Cx26 and Cx32 are found in many of the epithelial cells, whereas Cx43 is found in most of the smooth muscle cells.

Within the intestine, gap junctions are present in the circular muscle layer (reviewed in Ref. 114) where they allow the formation of a muscular syncytium that can synchronize contractile activity. Intestinal smooth muscle cells are electrically coupled (1, 21, 143, 177, 212, 572), and they show intercellular passage of microinjected dyes (675). In contrast, gap junctions appear to be absent in the longitudinal muscle layer (212), which may explain why the contraction of canine duodenal circular muscle correlates much better with spike potentials than does contraction of longitudinal muscle.

Cells in different layers of the intestine may show electrical (143, 572) but not dye coupling (675) with each other. This coupling might be mediated indirectly through interstitial cells and fibrocytes (572). Gap junctions between interstitial cells of Cajal of the guinea pig ileum have recently been functionally demonstrated (31). Functional gap junctions between cells of Cajal and smooth muscle cells have also been demonstrated (348), but their role in the transmission of contraction signals is still a matter of debate (113). Recently, reduced Cx43 expression has been observed in tissues from patients with Hirschprung’s disease (410), suggesting that impaired intercellular communication between interstitial cells of Cajal and smooth muscle cells may be partly responsible for the motility dysfunction in this disease.

Several studies suggest a role for gap junctions in gastric ulcers or cancers. The amount of Cx32 and Cx43 is reduced in the mucosa surrounding a chronic gastric ulcer, but it reverts to normal after antiulcer treatment (383, 427). Blockade of rat gastric gland gap junctions with 18α-glycyrrhetinic acid prevents dye coupling, calcium wave propagation, and acid secretion (472). Moreover, gap junction blockade (induced with octanol) significantly inhibits the recovery of gastric mucosa from acid-induced injury (568). It has also been proposed that inhibition of gap junctions weakens the barrier function of gastric mucosa and subsequently contributes to the damaged barrier function following ischemia-reperfusion (241). Moreover, analysis of tissue specimens from gastric cancers has shown loss of Cx43 expression (427, 591).

2. Accessory glands

Different cell types in the salivary glands contain different connexins (Table 1). During the ontogeny of the rat submandibular gland, Cx43 appears in myoepithelial cells on gestational day 17, and Cx32 appears in acinar cells on day 18 (237). The differential distribution of connexins suggests that Cx26- and Cx32-containing junctions participate in the secretory function of acinar cells and Cx43 participates in the contractile function of myoepithelial cells.

In the pancreas, connexin expression and gap junction function are very different in the exocrine and endocrine cells. Gap junctions are abundant, and Cx26 and Cx32 are found in acinar cells (Table 1). As in other secretory organs, it is believed that intercellular communication may enhance secretion by coordinating the response of coupled cells within an acini to a secretagogue (378, 558). Functional gap junctions do not appear to be

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**TABLE 1. Distribution of connexins in the digestive system**

<table>
<thead>
<tr>
<th>Segment/Organ</th>
<th>Connexins</th>
<th>Cell Type</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>26, 43</td>
<td>Keratinocytes</td>
<td>513</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>Developing tooth</td>
<td>461</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>26, 32</td>
<td>Acinar cells</td>
<td>224, 303, 396, 538</td>
</tr>
<tr>
<td>(Parotid, sublingual, and</td>
<td>43</td>
<td>Myoepithelial cells</td>
<td>237, 396, 537, 591</td>
</tr>
<tr>
<td>submandibular glands)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>26, 36, 43, 45</td>
<td>Endocrine β-cells</td>
<td>86, 379, 526</td>
</tr>
<tr>
<td></td>
<td>26, 32</td>
<td>Exocrine cells</td>
<td>379</td>
</tr>
<tr>
<td>Liver</td>
<td>26, 32</td>
<td>Hepatocytes</td>
<td>413, 585</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>Stellate cells, oval cells, Kupffer cells,</td>
<td>41, 413, 585, 680</td>
</tr>
<tr>
<td></td>
<td></td>
<td>endothelial cells, cholangiocytes, and cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of the Glisson capsule</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>26, 43</td>
<td>Epithelial cells</td>
<td>159, 652</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Sphincter muscle cells</td>
<td>629</td>
</tr>
<tr>
<td>Stomach</td>
<td>26, 32, 43</td>
<td>Epithelial cells</td>
<td>159, 308, 472, 568</td>
</tr>
<tr>
<td>Gastroduodenal junction</td>
<td>43</td>
<td>Muscle cells</td>
<td>238</td>
</tr>
<tr>
<td>Small intestine</td>
<td>43, 45</td>
<td>Muscle cells</td>
<td>159, 177, 405</td>
</tr>
<tr>
<td></td>
<td>40, 43, 45</td>
<td>Cells of Cajal</td>
<td>405, 410, 524, 525, 629</td>
</tr>
<tr>
<td>Colon</td>
<td>40, 43</td>
<td>Muscle cells</td>
<td>629</td>
</tr>
</tbody>
</table>
necessary for zymogen secretion, since dissociated pancreatic acinar cells show the same rate of exocytosis as intact acini (72). However, connexin expression may be necessary for proper control of secretory function, since pancreatic amylase secretion is increased in Cx32-deficient mice (81). Gap junction function in pancreatic duct cells may also contribute to the pancreatic dysfunction observed in cystic fibrosis patients. Chloride currents evoked by cAMP analogs increase intercellular cell coupling, and intercellular communication is impaired when mutant cystic fibrosis transmembrane conductance regulator (CFTR) is expressed (83), but this effect is reversed by transfection of wild-type CFTR (82).

In the endocrine islets of the pancreas, several connexins including Cx26, Cx43, and Cx45 have been found (Table 1), but Cx36 now appears to be most involved in insulin secretion. In Cx36-null mice, the development and differentiation of β-cells are normal, but insulin secretion in response to a glucose challenge is reduced (70). While in vitro studies suggest that Cx43 expression is necessary for insulin production (627), Cx43-null mouse embryos have normal endocrine pancreatic ultrastructure, insulin content, and secretion (86). The connexin type or abundance may also be crucial for the normal islet function, since insulin secretion in response to glucose is reduced in transgenic mice with targeted expression of Cx32 in pancreatic β-cells despite increased levels of insulin, improved electrical synchronization, and increased intracellular calcium levels (85). It has been suggested that gap junctions between islet cells are important for synchronizing glucose-induced intracellular calcium oscillations (259). However, published evidence contradicts this hypothesis, since these calcium waves are not affected by gap junction blockers and may be observed in the absence of cell contacts (46).

FIG. 5. Transfer of three different intercellular signals through gap junctions. A and B: a phase-contrast micrograph of a pair of superior cervical ganglion neurons is shown in A. Each neuron is impaled with a microelectrode; one electrode is used to inject current into one of the cells and the voltage deflections generated in each cell are recorded. An example of one of those recordings is shown in B. This cell-cell signaling corresponds to an electrotonic potential that propagates from one cell to another in milliseconds or less. C and D: phase-contrast (C) and fluorescent (D) images of a cluster of MDCK cells in culture showing intercellular transfer of Lucifer yellow. This process occurs by simple diffusion through gap junctions and takes from several seconds to a couple of minutes. E–L: levels of intracellular free \([\text{Ca}^{2+}]\) measured by ratio imaging after loading the cells with fura 2. E: Nomarski image of a hepatocyte triplet showing the microelectrode used for microinjections into the top cell. F–H: intercellular propagation of a Ca\(^{2+}\) wave generated by the intracellular microinjection of IP\(_3\). The increase in intracellular free \([\text{Ca}^{2+}]\) in adjacent cells occurred sequentially and was not due to \([\text{Ca}^{2+}]\) diffusion because the increase in free \([\text{Ca}^{2+}]\) occurred in discrete cellular regions. I–L: intracellular free \([\text{Ca}^{2+}]\) measured in cells bathed in \([\text{Ca}^{2+}]\)-free extracellular medium after \([\text{Ca}^{2+}]\) was injected into the cell with an electrode positioned as drawn. A localized increase in intracellular free \([\text{Ca}^{2+}]\) was evident in the right side of the lower cell. This occurred 2 s after \([\text{Ca}^{2+}]\) microinjection and before visualization of \([\text{Ca}^{2+}]\) diffusion into the neighboring cell, suggesting that the microinjected \([\text{Ca}^{2+}]\) induced the generation of a diffusible \([\text{Ca}^{2+}]\)-releasing agent that permeated through gap junctions to the adjacent cell. This process is regenerative and takes a few seconds. Magnification bar: 20 μm in A, 45 μm in E–L, 60 μm for C and D. [E–L modified from Sáez et al. (504).]
Rodent hepatocytes are morphologically and metabolically heterogeneous, yet electrical coupling between hepatocytes occurs over a relatively long distance within the liver (0.5 mm, ~25 hepatocytes), which accounts for the similarity in membrane potentials among hepatocytes (334). Consistent with the notion that gap junctions provide a pathway for synchronization of electrical responses, the glucagon-induced hyperpolarization is higher in periportal (Z1 zone) than in pericentral (Z3 zone) hepatocytes in isolated liver perfused with octanol, a gap junction blocker (334). Moreover, dye coupling (119, 407) and ligand-induced Ca\(^{2+}\) waves (393, 406, 490) have been demonstrated in intact rat liver. Therefore, gap junctions between hepatocytes allow propagation of electrotonic potentials and Ca\(^{2+}\) waves as well as the intercellular exchange of small molecules by simple diffusion (Fig. 5).

In rodent liver, the propagation of vasopressin-induced Ca\(^{2+}\) waves occurs in the absence of extracellular Ca\(^{2+}\) (490), suggesting the participation of IP\(_3\)-induced Ca\(^{2+}\) release from intracellular stores instead of Ca\(^{2+}\) influx. These Ca\(^{2+}\) waves generated by a subthreshold concentration of vasopressin propagate from terminal hepatic venules (THV) to terminal portal venules (TPV), a direction opposite to the blood flow (Fig. 6), ruling out the involvement of an ATP-mediated paracrine intercellular communication mechanism at the apical region of the cells. Moreover, perfusion of liver with ATP causes rapid transient elevations in intracellular free [Ca\(^{2+}\)] in single hepatocytes or groups of hepatocytes throughout the lobule without a specific anatomic location for ATP-susceptible cells (393). In contrast, studies in rat hepatocyte triplets or quadruplets (578) or in perfused liver (527) have demonstrated that Ca\(^{2+}\) waves induced by vasopressin start at cells bearing high vasopressin receptor density, as proposed previously (41).

The propagation of Ca\(^{2+}\) waves would require as mentioned above a regenerative system for increases in intracellular [Ca\(^{2+}\)] such as Ca\(^{2+}\)-activated phospholipase C and IP\(_3\) receptors (Fig. 6). It has been demonstrated that IP\(_3\) also permeates hepatocyte gap junctions (504) and that coordination of Ca\(^{2+}\) oscillations is fully dependent on the intercellular diffusion of IP\(_3\) and not Ca\(^{2+}\) between neighboring hepatocytes (95, 136). Immunofluorescence studies have demonstrated the presence of protein G q, phospholipase C, and IP\(_3\) receptors in hepatocytes (223, 582). The importance of gap junctional communication and IP\(_3\)-sensitive Ca\(^{2+}\) stores in coordinating increases in intracellular [Ca\(^{2+}\)] between epithelial cells from a community with an heterogeneous density of vasopressin receptors has been demonstrated recently (336).

While nonparenchymal cells of the liver express Cx43, hepatocytes express Cx26 and Cx32 (41, 188, 413, 585, 680). In hepatocytes, these connexins form homotypic and heterotypic gap junction channels as demonstrated by single-channel analysis in cells of Cx32-null and wild-type mice (598). Cx26 and Cx32 gap junction channels show differences in permeability and charge selectivity when expressed in HeLa cells (73, 144). Hepatocytes from Cx32-null mice show a strong reduction in IP\(_3\) permeability compared with wild-type hepatocytes (419). Likewise, the intercellular propagation of IP\(_3\)-induced
Ca$^{2+}$ waves is three- to fourfold more efficient in Cx32 than in Cx26 transfected HeLa cells (418).

In support of the notion that hepatocytes require gap junctional communication for an efficient metabolic response, it has been reported that Cx32-null mice show close to a 70% reduction in nerve stimulation-induced glucose release compared with wild-type mice (409). Similarly, Cx32-null mice show a drastic reduction in glucagon- and norepinephrine-induced glucose release (563). Moreover, with the use of dissociated hepatocytes as well as reaggregated hepatocytes and gap junction blockers, a reduction of ~70% in vasopressin-induced glycogenolysis has been observed (153). In addition to their involvement in liver metabolic responses, gap junctions participate in bile flow because 18α-glycyrrhetinic acid exacerbates the decrease induced by vasopressin (407). Cx32-null mice also show a dilated bile canaliculi, and the nerve-dependent decrease in hepatic bile flow is attenuated drastically (574).

A reduced number of gap junctions between hepatocytes occurs in liver cirrhosis and in chronic viral hepatitis (661), two pathological conditions likely to be associated with an inflammatory response. During liver inflammation, Cx26 and Cx32 are downregulated (119, 188), a process that can be mimicked by soluble factors released by activated Kupffer cells (188). The inflammatory response is a factor common to diverse pathologies; thus reduced gap junctional communication might contribute to the metabolic malfunctioning of the liver observed in various diseases. In addition, several studies have demonstrated the role of gap junction in cell growth and tumor promotion. In this respect, the incidence of liver tumors in Cx32-null mice is much higher than in wild-type animals (156, 573). The Cx32 deficiency enhances the growth of liver tumors irrespective of the genetic background of the mouse strain used (385).

C. Reproductive System

1. Gap junctions in the female reproductive system

Gap junctions interconnect cells in several different tissues of the female reproductive system. In the ovarian follicle, they are important for coordinating functions of granulosa or stromal cells and for permitting interactions of the developing oocyte with the surrounding follicular cells. In the myometrium, they allow the electrical synchronization of muscle cells to facilitate contractions in labor.

Ultrastructural studies have established the presence of gap junctions between rat follicular cells (6, 183). Since this observation, several connexins have been identified in ovarian cells from different mammalian species, including Cx26, Cx32, Cx30.3, Cx37, Cx40, Cx41, Cx43, Cx45, Cx57, and Cx60 (51, 192, 195, 240, 254, 368, 374, 421, 428, 541, 596, 672). Cx43 is very abundant and appears to be the primary connexin connecting the granulosa cells (51). Expression of Cx43 is evident in oocytes from sexually mature animals, but not from fetuses (374). A cycle of Cx43 expression has been detected during follicular growth; Cx43 abundance is elevated before ovulation and decreases when levels of luteinizing hormone are high and during follicular atresia (194, 650, 651). However, other investigators have detected gap junctions and Cx43 in the corpus luteum (275, 374). Thus ovarian Cx43 is developmentally and hormonally regulated. Two mechanisms may be involved in the inhibition of ovarian Cx43 gap junction function during the maturation of the rat oocyte: gating of gap junction channels due to increased Cx43 phosphorylation (192) and inhibition of Cx43 expression (193). Lawrence et al. (333) established the functional ability of gap junctions between granulosa cells to pass cAMP-dependent signals. Analyses of oocytes from Cx43 mutant or null mice support a critical role for Cx43 gap junctions between granulosa cells in the regulation of granulosa cell development. When these oocytes are studied in vitro or after grafting beneath the kidney capsule of adult females, folliculogenesis can reach only the initial steps (i.e., multilaminar follicles are absent in oocytes from Cx43-null mice but present in oocytes from wild-type mice) (4, 261). Similarly, maturation of bovine oocytes is partially impaired by adenoviral transfection of cumulus-oocyte complexes with an antisense Cx43 cDNA (626).

Gap junctions also link the growing female gamete with its surrounding somatic cells in developing follicles (13). This heterologous intercellular communication allows the cumulus cells to maintain the meiotic arrest of the oocyte, since meiotic maturation resumes if intercellular communication is disrupted (183). There may be a reciprocal transfer of signals through gap junctions between somatic cells and the oocyte in which follicular cells provide signals for oocyte growth and the oocyte provides regulatory signals for folliculogenesis (61, 148, 601, 602). It is likely that cAMP is the maturation inhibitory signal that is passed through gap junctions from the granulosa cells to the oocyte (333, 636). Calcium signals are also generated in cumulus cells and transported toward the oocyte in response to ATP (635), but they may not be related to oocyte maturation. Cx37 is a critical component of these heterocellular interactions, since this protein is found between the developing oocyte and surrounding cumulus cells. Female Cx37-null mice are infertile and produce numerous inappropriate corpus lutea (541). Follicle development arrests at the type 4 preantral stage, and oocyte growth ceases at a diameter that is only 74% of control size; the oocytes arrest in a G2 state and cannot enter M phase (initiate meiotic maturation) unless treated with a phosphatase inhibitor (74).

In the uterus, gap junction structures are present...
connecting the smooth muscle cells of the myometrium and connecting the epithelial cells of the endometrium. Dramatic changes in the abundance of gap junctions and intercellular communication precede the onset of labor and likely facilitate the organization of uterine contraction during parturition (176). Cx43 is abundantly found between the myometrial cells (51) and may be the most important connexin for the regulation of uterine activity associated with labor. However, multiple connexins have been identified in these cells including Cx26, Cx40, and Cx45 (7, 433). Cx43 is colocalized with Cx40 and with Cx45 in myometrial smooth muscle (7, 278, 279). Cx26 has been identified in the epithelium of the implantation chamber of the pregnant, but not in nonpregnant or pseudopregnant rabbits (655). Cx26, Cx43, and Cx45 show different temporal and cell type-specific patterns of expression during pregnancy and postpartum (51, 441, 485, 656).

The expression of myometrial Cx43 and Cx26 is hormonally regulated during the onset of labor. Rather than the individual hormone levels, it appears that the increased ratio of estrogen to progesterone is the most important factor. At term, Cx43 mRNA increases (by transcriptional regulation as discussed above) and Cx43 protein trafficking is altered (201, 213, 487). However, the abundance of Cx43 in other organs is minimally affected (486).

In the human, rat, mouse, and hamster oviduct, Cx43 and Cx26 have been identified between epithelial cells, and Cx43 has been found between smooth muscle cells (216). The abundance of connexins correlated with sexual maturity, and the levels of Cx43 correlated with high levels of estrogens (216).

2. Gap junctions in the male reproductive system

Several connexin mRNAs have been found in seminiferous tubules by RT-PCR analysis (488). Immunohistochemical studies have demonstrated that Cx43 is the most prominent connexin in testes. Cx43 expression begins at early stages of mouse embryonic gonads and increases with time in Leydig and Sertoli cells (448, 450). A continuous increase in Cx43 expression is observed postnatally until adulthood, when Cx43 expression in Sertoli cells depends on the stage of the seminiferous epithelium (26, 58, 489). In rat testes, Cx33 colocalizes with Cx43 in Sertoli-Sertoli gap junction plaques (571). The expression of Cx33 is delayed with respect to that of Cx43 appearing at postnatal day 15; Cx33 accumulates at Sertoli cell interfaces until stage VII, the stage at which its expression decreases together with that of Cx43 (571). Consistent with these observations, in situ dye coupling has been detected between Leydig cells, between peritubular cells, between Sertoli cells, and between Sertoli and basal germ cells in rodent species (26, 489). Electrical coupling has been demonstrated between Leydig cells (615). Cx26 and Cx32 have not been found in Leydig cells (615) but are present in the apical regions of the seminiferous epithelium of mature rats (58, 489). Analysis of testes from neonatal Cx43-null mice demonstrate that Cx43 is required for germ line expansion, but not for steroidogenesis (261, 463, 494). In human testis, Cx43 is first expressed during puberty (560).

Connexin expression in the rat testis and epididymis is hormonally regulated. Human chorionic gonadotropin administration in vivo and in vitro diminishes Cx43 mRNA and protein in rat Leydig cells (673). Cx43 is differentially expressed in an androgen-dependent manner by different cells of the epididymis (107). Also, retinoid X receptor β (RXRβ)-deficient mice exhibit abnormal spermatogenesis due to altered Sertoli cell function and present decreased levels of Cx43 transcripts (26). This suggests that retinoids, through the RXRβ receptors, could be involved in the control of Cx43 gene expression in Sertoli cells. As described above, the expression of Cx43 can occur under the control of c-jun transcriptional factors (460). It has also been observed that jun-d-null mice are viable but infertile; they present impaired spermatogenesis that correlates with a drastic reduction or abolishment of Cx43 expression (25). In agreement with these findings, immunohistochemical studies have shown a direct correlation between severe spermatogenic impairment in men and loss of Cx43 immunoreactivity in seminiferous tubules (560). Thus gap junctions in testicular cells may play a role in gonad development and spermatogenesis, and their contribution may be critical to male fertility.

D. Immune System

Morphological and functional demonstration of gap junctions and connexin identification in cells of the immune system (published between 1972 and 1999) has been reviewed previously (11, 502, 503). This section summarizes the latest reports with particular emphasis on conditions that control the expression of connexins in different cells and their possible functional roles in particular steps of the immune response.

With the use of in vivo and in vitro approaches, Cx43 and gap junctional communication have been identified in the bone marrow between stromal cells and between stromal and hematopoietic cells (295). It has been hypothesized that gap junctions participate in hematopoiesis, and therefore, they may also play a role in leukemia (387, 497). However, Cx43-mediated communication between stromal and hematopoietic stem cells may not be an absolute requirement (496).

Gap junctions may provide a direct signaling pathway for transmigration, angiogenic, and metastatic processes. Recently, it has been demonstrated that HTLV-1-
transformed lymphocytes (which are related to T-cell leukemia-lymphoma) form functional gap junctions with endothelial cells in vitro (145). Nevertheless, inhibition of gap junctions formed between human lymphocytes and endothelial cells does not abolish lymphocyte transmigration through a human umbilical vein endothelial cell monolayer (434). But, these cells do not form a tight paracellular seal that would offer resistance for cell transmigration. Yet, gap junction blockers (octanol or 18alpha-glycyrrhetinic acid) reduce by ~60% the number of human monocytes that transmigrate across a blood-brain barrier model (151).

In primary lymph nodes, functional coupling between thymocytes as well as between thymocytes and thymic epithelial cells has been demonstrated (10, 75). These cell junctions are at least in part composed of Cx43 (10). In secondary lymph organs, Cx43 has also been detected in human follicular dendritic cells (294), and Cx40 and Cx43 are expressed in human tonsil-derived T and B lymphocytes (436). Moreover, dye coupling between human cultured dendritic cells and B lymphocytes (296) and Cx43 immunoreactivity between halogeneic mouse Langerhans cells and T lymphocytes in culture (510) have been observed, suggesting the possible role of gap junctions in antigen presentation and/or lymphocyte proliferation. Moreover, synthetic peptides with sequence corresponding to a region of the extracellular loop 1 of connexins, but not unrelated peptides, reduce the proliferation response of concanavalin A-stimulated lymphocytes (510). In addition, the inhibition of B-lymphocyte gap junctions with connexin-mimetic peptides or 18alpha-glycyrrhetinic acid drastically reduces the secretion of immunoglobulins and the expression of interleukin-10 mRNA, suggesting that intercellular communication mediated through gap junctions favors these functions (435).

Epithelial and endothelial cell barriers, members of the immune system, express connexins and form homocellular gap junctions (329, 346, 347, 532, 608) and could form heterocellular gap junctions with migratory cells of the immune system (204, 244, 369). Nevertheless, circulating nonactivated leukocytes, including monocytes, polymorphonuclear cells, and lymphocytes, do not express, at least, Cx32, Cx40, or Cx43 (11, 57, 244, 465). Consistent with the lack of connexin expression, gap junctional communication has not been detected between freshly isolated monocytes (12, 465), lymphocytes (510), or polymorphonuclear cells (57). However, dye coupling and Cx40 and Cx43 have been detected in T and/or B lymphocytes freshly isolated from human blood (436), raising controversy about connexin expression in these cells. Species differences or isolation procedures, some of which are known to induce cell activation, might explain these differences.

Mediators of inflammation induce opposite effects on gap junction expression in leukocytes and endothelial and parenchymal cells. In vitro treatment with tumor necrosis factor (TNF)-alpha plus interferon (IFN)-gamma induces expression of Cx43 in human monocytes (151) and rodent microglia (152). TNF-alpha plus other yet unidentified factor(s) released from activated endothelial cells induce Cx40 and Cx43 in human polymorphonuclear cells (57). Moreover, cerebral and hepatic fixed macrophages, microglia, and Kupffer cells express at least Cx43 at inflammatory foci or after treatment with proinflammatory agents (152, 188, 305, 465). Moreover, expression of Cx43 and Cx32 by murine bone marrow cultured mast cells and the growth factor-independent murine mast cell line C57 has been documented (624). Nevertheless, the possible functional role of these connexins remains unknown.

In agreement with the requirement for an increase in intracellular calcium concentration for activation of cells of the immune system, it has been found that a calcium ionophore induces the expression of Cx43 in cultured rat microglia (371). The increased levels of at least Cx43 by monocytes/macrophages and microglia occur with an increase in the levels of Cx43 mRNA (371, 465), suggesting activation of Cx43 mRNA transcription. In contrast to the findings in myeloid and lymphoid cells, the expression of connexins in endothelial (234, 609) and epithelial cells such as hepatocytes (188) is reduced by cytokines.

Further studies may help elucidate the mechanisms by which soluble factors control the expression of connexins and thus the formation of homocellular and heterocellular gap junctions between cells of the immune system. The possible functional role of hemichannels in the immune response has not been reported but deserves to be studied.

VIII. GAP JUNCTION CHANNELS AND DISEASE

Gap junctions have been implicated in many cellular processes, but the lack of specific blockers has limited the study of their physiological role in vivo. New information on the physiological roles of vertebrate connexins has emerged from genetic studies. Mutations in connexin genes correlate with a variety of human diseases, including demyelinating neuropathies, deafness, epidermal diseases, and lens cataracts. Genetic modification of connexins in mice has provided valuable information on connexin function and significance of their diversity. In addition, targeted ablation of mouse connexin genes has revealed basic insights into the functional diversity of the connexin gene family.

A. Connexin Mutations Related to Human Peripheral Neuropathy

The first disease demonstrated to rely on a connexin mutation was the X-linked form of Charcot-Marie-Tooth...
disease (CMTX), a demyelinating syndrome associated with Schwann cell defects that produce progressive degeneration of peripheral nerves. Cx32 protein is located in the paranodal loops and Schmidt-Lantermann incisures in myelinating Schwann cells (516) (Fig. 7). Cx32 channels form intracellular connections between adjacent loops of noncompacted myelin in one cell (reflexive gap junctions). These autocellular junctions provide a radial diffusion pathway between the Schwann cell nucleus and adaxonal membranes that is ~300 times shorter than the corresponding distance along the circumferential membrane (20). Communication through these junctions likely provides metabolic and nutritional support for these bits of cytoplasm. More than 160 CMTX-associated mutations in the Cx32 gene have been identified since 1993 (3, 37). Cx32 mutants identified in patients with CMTX include missense, frameshift, deletion, and nonsense mutations. Some of these mutations lead to complete loss of function with no expression of functional channels (68, 79, 124, 425, 429, 480, 671). Mutations within the noncoding region of the Cx32 gene have also been reported; some lead to a reduced level or lack of Cx32 mRNA (239, 403), while another affects mRNA translation (236). It has been described that SOX-10, a transcriptional factor responsible for the expression of Cx32 and other myelin proteins, fails to activate the mutated Cx32 promoter (56). Other mutant forms of Cx32 fail to leave the Golgi apparatus, leading to an abnormal accumulation in the cytoplasm (124). Other Cx32 mutants form functional channels, but their voltage-gating properties are abnormal, thus impeding normal intercellular communication (79, 124, 425, 429, 480). Yet, one mutation restricts the channel pore diameter, possibly affecting the type of signaling molecules that cross between adjacent loops of myelin (425). A mutant Cx32 (Ser85Cys) that forms functional gap junction channels shows increased hemichannel currents with respect to wild-type Cx32, a phenomenon that has been ascribed to increased open probability (2). In this case, the loss of ionic gradients and small metabolites and the increased influx of Ca²⁺ through putative Cx32 hemichannels might damage Schwann cells leading to the diseased state (2). Rat Schwann cells also express Cx29, but its pattern of distribution differs from that of Cx32 (8). In addition, low levels of Cx43 have been detected in peripheral myelin (364). Despite the expression of other connexin types in myelinating cells, dysfunctions due to Cx32 mutations predominate. Neuropathies due to mutations in other connexins may remain unrecognized if they are lethal due to abnormalities caused in the other tissues where they are expressed.

One intriguing aspect of the observed phenotype in individuals carrying Cx32 mutants is that functional abnormalities are restricted to the peripheral nervous system. It is well documented that Cx32 is a major component of gap junctions in the liver and many other cell types in different organs such as neurons, follicular cells, pancreatic acinar cells, lacrimal acinar cells, and oligodendrocytes (67). Deletion of Cx32 in mice leads not only...
to neuropathy but also to reduced nerve stimulation- and hormone-induced hepatic glucose release (409, 563) and reduced bile flow (574) following sympathetic stimulation, reduced fluid secretion by lacrimal glands (628), and an increased amylase secretion from the exocrine pancreas (81). The absence of Cx32 results in a distinct pattern of gene dysregulation in Schwann cells (415). However, Cx32-null mice show a normal organization of central nervous system myelin (517). This might result in part from the distribution of Cx32 in the central nervous system (i.e., mainly in oligodendrocyte cell bodies and proximal processes, but not in paranodes) and from myelin differences between the parasympathetic nervous system and the CNS (i.e., oligodendrocytes in central nervous system wrap around axons less times, so the width of the myelin sheath is smaller). Accordingly, the functional role of Cx32 might be employed by other connexins (e.g., Cx45 in the rat) that are coexpressed with Cx32 in oligodendrocytes (301). In fact, some human Cx32 mutations produce subclinical evidence of CNS dysfunction. These mutations, at least in vitro, do not produce a trans-dominant negative effect over Cx45 communication-competent HeLa cells (280), which would be in agreement with the possibility of Cx45 compensating for the lack of Cx32.

B. Connexin Mutations Related to Deafness and Skin Disorders

In mammals, Cx26 and Cx30 are expressed by non-sensory epithelial cells among which hair cells are dispersed, and by connective tissue cells at more distal locations from hair cells (271, 277, 332). Connective tissue cells, fibrocytes, and spiral ligaments and spiral limbs, express at least Cx31 (658).

Congenital deafness is a very frequent disorder occurring in ~1 in 1,000 live births. Autosomal recessive (DFNB1) and autosomal dominant (DFNA3) forms of genetic deafness have been associated with more than 50 mutations within the coding region of Cx26 [reviewed by Kelsell et al. (273), White (641), and Lefebvre and van de Water (335)]. (For an extensive list, visit the Connexins and Deafness Homepage: http://www.iro.es/cx26deaf.html.) Recessive mutations frequently result in a severely truncated Cx26 that is unlikely to retain any channel activity (76, 121, 150, 272, 677). In contrast, dominant mutations encode full-length products containing nonconservative amino acid substitutions (80, 274, 392, 484, 646). The Cx26Met34Thr mutant (274) was previously described as a dominant negative, and in vitro data demonstrated that the wild-type Cx26 could not form functional channels when coexpressed with the mutant Cx26 (646). However, some controversy about the interpretation of these results has been raised by the frequent finding that individuals heterozygous for the Cx26Met34Thr mutation have normal hearing (121, 272, 522). Although the precise role of Cx26 in the etiology of nonsyndromic deafness remains unknown, it has been proposed that junctional communication influences the ionic environment of the inner ear sensory epithelia. Thus gap junctions would be involved in recirculation of K\(^+\) via the interstitial space through the syncytium formed by cochlear supporting cells (166, 257, 424), similar to the spatial buffering of K\(^+\) by astrocytes in the CNS. The use of Cx26-null mice to investigate the role of Cx26 in the inner ear was not possible because of its embryonic lethality (171). The role of Cx26 in cochlear function and cell survival has been demonstrated recently after tissue-specific ablation of Cx26 (96). In ear-specific homozygous Cx26(OtogCre)-null mice, the inner ear develops normally, but after the onset of hearing, cell death is evident extending to the cochlear epithelial network and sensory hair cells. These mice have hearing impairment, but not vestibular dysfunction. Because cell death initially affects the supporting cells of the inner hair cell, it has been suggested that the inner hair cell response to sound stimulation could be triggered by the observed cell death.

Some Cx26 mutations are also linked to skin disorders, such as palmoplantar keratoderma (164, 210, 273, 484). Mutations of Cx31 and Cx30 are also associated with both deafness and epidermal disorders (197, 319, 483, 657). Recently, it has been shown that a new Cx26 mutation (Cx26AE42) associated with auditory and skin disorders inhibited the intercellular conductance in paired Xenopus oocytes when coexpressed with wild-type Cx43 (498). These results suggest that dominant negative Cx26 mutations can produce a disease phenotype in the skin if they interfere with the function of wild-type Cx43 also found in the same cells.

C. Connexin Mutations Related to Human Cataracts

The eye lens is an avascular organ formed by an epithelial cell layer covering the anterior surface of the organ and a large mass of fiber cells which form the bulk of the organ. The fiber cells contain few organelles, yet they must survive for the life span of the organism. Because there is no blood supply to the organ, most cells receive their nutrients by diffusion from the aqueous humor in which the organ is suspended. Thus it has been hypothesized that these cells are maintained by fluid, ion, nutrient, and metabolite movement through an extensive network of gap junctions (189). Mathias et al. (373) have shown the presence of circulating currents in the lens that would serve to drive water and ions between lens cells. Several connexins have been identified in the lens. While epithelial cells express Cx43 (51) and Cx50 (also known...
as MP70) (111), fiber cells express Cx46 and Cx50 in mammals (285, 444, 642) or Cx56 and Cx45.6 in chickens (40, 250, 500) (Fig. 8). The existence of gap junctions connecting epithelial and fiber cells has been demonstrated with the dye coupling technique in the normal adult rat lens (473). Accordingly, Cx43/Cx50 double knock-out mice do not show dye coupling between epithelial and fiber lens cells (649).

Point mutations of the Cx50 and Cx46 genes have been identified in patients with inherited zonular pulverulent cataract (361, 536). When expressed in Xenopus oocytes, Cx46 and Cx50 mutations do not form functional channels (437, 438). Similarly, the No2 mouse, which develops congenital cataracts, carries a mutation within the Cx50 sequence (559). Both Cx50-null and No2 mice develop cataracts and exhibit microphtalmia (559, 646), indicating involvement of Cx50 in lens development. In Xenopus oocytes, this mouse Cx50 mutant does not form functional gap junctions (659). Recently published data indicate that Cx43 and Cx50 are not required for prenatal lens development but both are required for organ homeostasis (649). Cx46 knock-out mice exhibit normal lens growth and development, but progressive cataractogenesis is evident 3 wk after birth (187). Because microphthalmia is present in Cx50-, but not in Cx46-null mice, it has been proposed that an early postnatal growth signal may propagate through Cx50 but not Cx46 gap junction channels (647). Because Cx46- and Cx50-null mice develop lens opacities, it is likely that channels made of a single connexin type cannot compensate for the functional role of gap junctions when both connexin types are expressed. It remains to be determined whether heteromeric gap junction channels made of Cx46 and Cx50 have different permeability than homomeric Cx46 or Cx50 channels.

IX. PHYLOGENY

While functionally equivalent intercellular channels and gap junction structures are found in many lower multicellular organisms, no connexin genes have been identified in any invertebrate organism (456). Invertebrate gap junctions are made of protein subunits termed innexins which lack sequence homology with connexins, but do exhibit similar membrane topology (175, 554). A phylogenetic study on connexins in working myocardium of chordates has demonstrated positive immunoreactivity in the myocardium of Ascidian, a primitive chordate (30). In turn, innexin family members have been detected in a variety of animal phyla, including Platyhelminthes, Nemotoda, Arthropoda, Mollusca, and Chordata. The finding of two human innexin homologs would imply that intercellular communication might not only depend on the distribution and activity of connexin-containing gap junctions, but also on that of innexins (439).

With the use of the Xenopus oocyte expression system, some of these proteins have been shown to form functional gap junctions (324, 458) with pH and voltage gating somewhat similar to those of vertebrate gap junctions (324). In the Caenorhabditis elegans genome, 25 innexins have been identified. Similar to vertebrates, C. elegans tissues and single cells express more than one innexin; some innexins are expressed in many tissues while others show a more restricted pattern of expression (555). In the fruitfly Drosophila, specific innexins play a relevant role in the development of the gastrointestinal tract (27) and the nervous system (105). Innexins are not interchangeable in the development of neural function in the Drosophila visual system (106). Cloning of an innexin expressed in annelida has been recently described (467). Reviews on gap junction in invertebrates have been reported (457, 555). The possibility of generating specific mutants both in Drosophila melanogaster and C. elegans will be an important approach to learn about the functional significance of gap junctions in different tissues of invertebrates.

X. PERSPECTIVES

The introduction of new techniques and approaches has increased our knowledge of gap junctions at several levels. It is anticipated that in the near future they will lead to an understanding of the multiplicity of different connexins and which of their functions are unique or common to other members of the family. Connexin mutants will allow identification of the molecular determi-
nant proteins of gating (e.g., voltage, pH, Ca$^{2+}$, other chemicals), permeability (to ions and molecules of different size), oligomerization with other connexins, docking of hemichannels, and interactions with other proteins. Structural studies of gap junctions at even higher resolution may elucidate the molecular events leading to closing and opening of gap junction channels or hemichannels. The use of cell type-specific promoters to knock out connexins in different cell types or at different times during development will clarify their roles. Conceivably, molecules that modify channel/hemichannel behavior (including channel blockers) in a connexin type-specific manner will be identified, allowing their use for pharmacological (and maybe therapeutic) purposes. Genetic studies may uncover additional diseases associated with other connexins. Finally, the possible physiological role(s) of hemichannels may be determined.

NOTE ADDED IN PROOF

Söhl et al. (544a) have suggested that the human ortholog of mouse Cx29 corresponds to Cx30.2, while Altevogt et al. (8) have suggested it corresponds to Cx31.3 due to the presence of an intron within the carboxy terminus.

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