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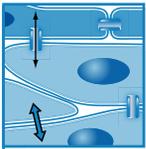
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CONNEXINS: KEY MEDIATORS OF ENDOCRINE FUNCTION

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Bosco D, Haefliger J-A, Meda P. Connexins: Key Mediators of Endocrine Function. *Physiol Rev* 91: 1393–1445, 2011; doi:10.1152/physrev.00027.2010.—The appearance of multicellular organisms imposed the development of several mechanisms for cell-to-cell communication, whereby different types of cells coordinate their function.

Some of these mechanisms depend on the intercellular diffusion of signal molecules in the extracellular spaces, whereas others require cell-to-cell contact. Among the latter mechanisms, those provided by the proteins of the connexin family are widespread in most tissues. Connexin signaling is achieved via direct exchanges of cytosolic molecules between adjacent cells at gap junctions, for cell-to-cell coupling, and possibly also involves the formation of membrane “hemichannels,” for the extracellular release of cytosolic signals, direct interactions between connexins and other cell proteins, and coordinated influence on the expression of multiple genes. Connexin signaling appears to be an obligatory attribute of all multicellular exocrine and endocrine glands. Specifically, the experimental evidence we review here points to a direct participation of the Cx36 isoform in the function of the insulin-producing β -cells of the endocrine pancreas, and of the Cx40 isoform in the function of the renin-producing juxtaglomerular epithelioid cells of the kidney cortex.

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I. INTRODUCTION

The appearance of multicellular systems, some 800 million years ago, has implicated the development of mechanisms for electrical and chemical cell-to-cell communication (42, 51, 60, 122, 332, 430, 431) whereby individual cells sense the activity of both adjacent and distant partners, and coordinate accordingly their own activity. With the progressive growth and diverging differentiation of multicellular systems, the chemical signaling has rapidly imposed the development of signaling units sizably larger than the unicellular exocrine systems, which are considered their primordial precursors (20, 34, 35, 302, 303, 433, 435, 436, 521). Thus, in mammals, most of the vital endocrine functions are now dependent on the proper functioning of multicellular glands.

With evolution, the differentiation, maintenance, and proper moment-to-moment functioning of these glands has come under the control of a complex regulatory network in which signals diffusing in the intercellular spaces interplay with signaling cascades dependent on the surface

proteins that concentrate at cell contacts (126, 547). In vertebrates, a consistent feature of this network is the proteins of the connexin family (335, 339, 344, 356, 480). Recent experimental evidence has revealed that connexin signaling is key for the proper functioning of multiple endocrine glands, and, at least in some of them, may exert a hitherto unsuspected influence, that predominates that of other cell-to-cell signaling systems.

Here, we review the bare essentials of the conditions imposed by multicellularity and endocrine function, with specific regard to the evolution of multiple cell-to-cell signaling mechanisms. We then focus on what are connexins, and summarize the data showing the key role of these proteins in the normal and pathophysiological functioning of the insulin-producing β -cells of the endocrine pancreatic islets, and of the renin-producing juxtaglomerular epithelioid cells of the renal cortex.

II. MULTICELLULARITY

A. When, Why, How

Once, during evolution, a primitive organism divided and the two daughter partners did not separate (case of aquatic organism), and/or two primitive unicellulars adhered to each other (case of terrestrial organisms) for the first time, probably as a result of a random event in the then essentially liquid earth environment, to form the primordial multicellular organism (39, 41, 42, 162, 261). From microfossil

records we know that such an event took place between cyanobacteria already 3.5 billions years ago, i.e., relatively soon after the earth crust solidified (466). This event occurred a number of times (39, 41, 42, 162, 163, 164, 430, 431, 573), suggesting that there was significant selection pressure for it in the ancient unicellular world (42). Presumably as a result of several independent and random mutations of the genome (42, 430, 431), one of such events, about 800 millions year ago in the Precambrian period, led to the advent of the increasingly larger and complex multicellular algae, fungi, plants, and animals we can still observe (40, 41, 162–164). Indeed, within the next 100 million years, most of the main structures and organizations (cell and tissue characteristics, body plans, and *pyla*) appeared, which closely resemble the ones existing today (62, 162).

Genomes are strikingly comparable in uni- and multicellular organisms (2, 430, 431, 474), but the latter show a much higher diversity, presumably as a result of the recruitment by multicellular organisms of genes from several distinct unicellular ancestors (430, 431). As a result of this recruitment, spontaneous genetic mutations (blocking cell separation after division, favoring adherence of two unicellulars) and/or environmental changes (increased oxygen concentration, competition for limited supply of phosphorus, emergence of predation) resulted in increased size of the new organism (39, 164). In turn, this change led to cell diversity, since size imposed new metabolic and structural changes (e.g., the acquisition of cells capable of transporting oxygen to the innermost cells, of separating different compartments) (40). These changes also led to the divergent differentiation of multiple cell types, due to the loss of the reproductive capacity of some cells, the spatial segregation of other cells from their partners, and/or their temporal specialization, inducing different genes to be expressed at different times and in different cells/regions of the organism (40, 163). In this case, the new organism progressively transformed from a mere assembly of independent cells (as seen in prokaryotes), which appears to have been selectively neutral (163), into a coordinated society of interacting partners. The latter appear to have acquired a selective advantage with regard to feeding, defense and/or escape behaviors (40), inasmuch as phylogeny shows a trend towards increased organism complexity (40, 42, 430, 431). In turn, multicellularity provided a new, major platform for evolution (162, 163). Compared with unicellulars, which have little control on their environment and mostly respond to it, multicellulars could largely produce their intercellular conditions and evolve in response to this milieu interieur that somewhat protected them from external changes (163). Thus multicellularity greatly expanded the range of contexts within which the eukaryotic cells could live.

B. Cell-to-Cell Communication

Proper functioning of multicellular systems obligatorily depends on a communication network for cross-talk be-

tween the component cells. Via such a network, adjacent and distant cells coordinate, and at times synchronize their function with that of other cells, to optimize their adaptation to the environment (60, 355). This signaling system, which was presumably initiated by the diffusion across the cell membrane of a small signaling molecule (39, 302–304, 434–436), has been largely diversified by natural selection, resulting in a complex array of cross-talking and to some extent overlapping cell communication mechanisms (547), which use different structures and signal molecules, and that we now know in animals (FIG. 1).

A widespread mechanism for intercellular communication is via the diffusion in the extracellular spaces of hormones and neurotransmitters that are simultaneously sensed by those cells bearing cognate receptors (302–304, 434–436). This system ensures a highly specific signaling between both distant (hormonal and neural communication) and nearby cells (paracrine communication), sometimes even affects the very same cell that generated the signal (autocrine feedback loop). Another form of cell-to-cell communication is provided by the diffusion in the extracellular spaces of ions and molecules that enter the cells by free diffusion through the lipid membrane bilayers or by way of specific transporters/channels (10, 50). Cell-to-cell coordination is then achieved by the simultaneous up- or downmodulation of specific metabolic and effectors pathways in several cells. In spite of the diversity of the structures and signals used to establish cellular cross-talk, all these communication mechanisms are referred to as “indirect,” inasmuch as they necessitate the mediation of a signal diffusing in the intercellular space, to mediate the transfer of information from one cell to another. A further variation of an “indirect” cell-to-cell communication is the mediation of the extracellular cell-to-cell signal by components of the extracellular matrix, to which closeby and distant cells may simultaneously, and dynamically attach by way of specific integrin proteins, that simultaneously signal multiple cells in both in-out and out-in directions (126, 547).

Most cell types also communicate in a “direct” way, i.e., by mechanisms that do not require an extracellular mediator and, thus, obligatorily require the contact of the communicating cells (126, 547). Why these mechanisms were pressure selected is still a matter of debate. An attractive hypothesis is that the extracellular signals diffusing from one cell to another may have been excessively diluted in the first multicellular organisms that essentially lived in an aqueous environment. Pressure selection could then have favored the emergence of alternatives in which the signals would directly flow from cell to cell, either by a structural wiring or by way of the protected bidirectional diffusion of signals enclosed by the cell membranes of the communicating cells. At any rate, a widespread mechanism for such direct cell-to-cell communications is via a large variety of cell adhesion glycoproteins (CAMs). These molecules play a prominent

role in establishing and maintaining, in a highly dynamic equilibrium, cell-to-cell contacts (126, 547). CAMs are well suited to transmit information, since they closely approximate the membranes of adjacent cells by homo- and hetero-

philic interactions within the extracellular space with similar or different CAM isoforms, and by associating in the cytosol with a variety of adaptor proteins that functionally link CAMs to specific cytoskeletal elements. The latter then act as information transducers at both cytoplasm and nucleus levels. Direct cell-to-cell communication is also mediated by connexins (200, 211, 212, 213), the nonglycosylated proteins that oligomerize to form hydrophilic units, referred to as connexons. These structures are usually inserted into regions of the cell membrane that face an adjacent cell. At these sites, referred to as gap junctions, the connexon of one cell pair with a similar structure made by another cell, bridging the intervening extracellular space to establish a hydrophilic cell-to-cell channel, which functionally joins two cytoplasms. Through such channels, adjacent cells may bidirectionally exchange a variety of cytosolic molecules, including current-carrying ions and many metabolites (200, 201, 211–213).

Uni- and multicellular organisms share many mechanisms of chemical communication (39, 521), indicating that cell-to-cell signaling predates the origin of metazoan (2, 259, 474). Still, metazoan cells communicate by many more different ways that are not used by prokaryotes (162, 164). Most likely, the primary chemical messengers used for cell-to-cell information were nutrients and toxins, and primary receptors were membrane enzymes binding these environmental molecules (434, 521). Subsequent structural modifications then allowed for a divergent evolution of binding activities (434, 521). Signaling transduction and regulatory pathways selected by unicellular organisms for versatility, robustness, and redundancy operate in different cell types, compartments, and times, within multicellular systems, in which they selectively affect but some cells. These variations, which progressively imposed an intricate, cross-talking array of many signals, acting rapidly or slowly, locally or at distance, complicate the identification of the primitive signal system adopted at the onset of multicellularity (16, 39, 163). By selectively affecting different types of differentiated cells, multiple signals presumably facilitated the non-lethal phenotypic variations that were instrumental for selection (162, 163, 164), allowing for the emergence of proteins for spatial differentiation, cell adhesion, as well as receptor and transduction systems. The conservation of these signaling mechanisms in metazoans indicates a non-dispensable role.

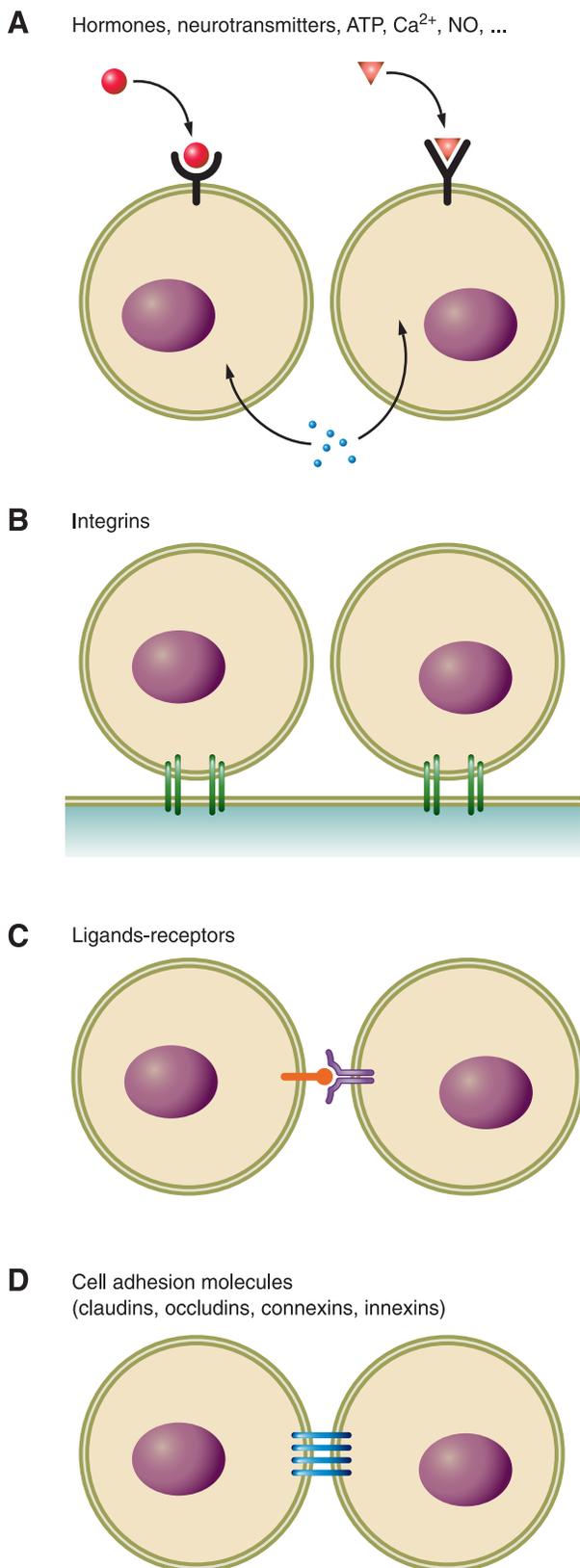


FIGURE 1. Different mechanisms allow for cell-to-cell interactions in multicellular systems. Indirect mechanisms of communication between cells involve multiple signal molecules diffusing in the extracellular spaces (*top panel*), and the integrin-dependent interactions of cells with molecules of the extracellular matrix (*second panel from top*). Direct mechanisms of communication between cells in contact involve heterophilic ligand-receptor interactions (*third panel from top*) and homophilic interactions between cell adhesion and junctional molecules (*bottom panel*).

C. Connexins, Innexins, and Pannexins

Among the proteins that became conserved in most phyla of invertebrates and vertebrates metazoans, several are transmembrane, tetraspan molecules that form channels for the flux of large cytosolic and extracellular molecules (10, 50, 200, 201, 211–213, 316, 367, 404, 405, 456, 485, 508). These proteins differ in vertebrate and invertebrate species (134, 167, 291) and are expressed in most cell types, with few exceptions (291, 343, 347, 460). There is no longer any question that vertebrate channels that mediate the cytosolic exchanges of cytosolic molecules at gap junctions are made by various combinations of different connexin (Cx) proteins (200, 201, 211–213, 316). In invertebrates, comparable but not identical structures are made by proteins (Ogre, Passover, Uncoordinated, and Shaking B) that form the OPUS family (404, 405, 456). More than 25 other junctional proteins revealing significant similarities, and now collectively referred to as innexins, have been identified, and it is clear that many other forms are expressed in various invertebrate species (81, 221, 404, 405, 586). Innexins share with connexins a similar structure and membrane topography, but no homology in primary amino acid sequence (289, 404, 405, 586). Notably, connexins display three conserved cysteine residues within each of the two extracellular loops, whereas innexins only carry two such residues. Sequencing of mammalian genomes has further revealed a third family comprising only three proteins that feature 20% similarity with innexins, and were termed pannexins (390, 456, 508). Like connexins and innexins, all three pannexins display NH₂- and COOH-terminal domains within the cytoplasm, large extracellular and cytoplasmic loop domains, and four membrane-spanning segments. Like innexins but in contrast to connexins, pannexins contain two Cys residues in each extracellular loop (390, 456, 508). However, and in marked contrast to both innexins and connexins, pannexins display consensus sequences for glycosylation (37, 103, 221, 456).

Expression and deletion studies in a variety of systems have established that connexin hexamers, termed connexons, concentrate at gap junction domains of the cell membrane, where the intercellular space is reduced to a gap 2–3 nm wide. At these sites, the connexons of one cell align with, and strongly bind to, the connexons of an adjacent cell, establishing a continuous intercellular hydrophilic pathway for the cell-to-cell exchange of multiple types of cytosolic molecules (200, 201, 211–213, 494, 496). The functional importance of this electrical and metabolic cell-to-cell coupling is shown by a variety of striking and tissue-specific phenotypes that can be experimentally induced after overexpression or knockout of individual connexin isoforms, as well as after the knock-in replacement of one isoform by another (200, 201, 211–213, 278, 411, 495, 569). It is further stressed by the identification of a number of diseases that are undoubtedly linked to connexin mutations (120, 121, 165, 200, 211–213, 267, 287, 347, 400, 426, 569).

Similarly, innexins oligomerize to form intercellular channels that cluster at gap junctions of invertebrate cells (17, 82, 88, 102, 289). In contrast, pannexons usually do not form cell-to-cell channels (103, 456, 457, 508), probably due to the glycosylation of the extracellular loops of pannexins (37, 38). Rather, these proteins usually form channels in nonjunctional domains of the cell membrane (103, 456, 457, 508). The temporary opening of such channels, which may also be made by several connexins and innexins (53, 55, 97, 116, 384, 385, 394, 456, 508), allows for the leakage of cytosolic molecules, notably ATP and glutamate, into the extracellular medium, and for the reverse uptake into cells of large extracellular and membrane-impermeant tracers (174, 445, 457, 508). Thus the three analogous families of connexin, innexin, and pannexin proteins, which oligomerize to form hydrophilic membrane channels permeable to both current-carrying ions and larger signal molecules, contribute to both direct (connexins and innexins) and indirect forms of cell-to-cell communication (mostly pannexins). The expression of these proteins in essentially all cell types of every existing multicellular systems, with a large overlap of pannexins and connexins in vertebrates, plaids for an obligatory role in the acquisition and maintenance of multicellularity. In view of their consistent variety of expression in different tissues, it is conceivable that the intercellular channels and nonjunctional channels these proteins make have evolutionarily adapted to fulfill specific roles in different environments. This contention is supported by a variety of protein and cell specific phenotypes that result from the experimental deletion and overexpression of selected isoforms of these proteins (200, 201, 211–214, 494, 496), and, at least for connexins, by the increasing finding of clinical diseases linked to specific mutations or single nucleotide polymorphisms (121, 122, 144–146, 165, 200, 205, 211–213, 267, 278, 287, 327, 346, 400, 426, 495, 569, 590). The association of another reported Cx43 mutation to some cardiac malformations (105) has not been confirmed. Eventually, quantitative changes in the levels and/or function of other connexins have been putatively attributed to a variety of acquired diseases (256, 300, 345).

III. CONNEXINS

A. The Mammalian Genes and Proteins

Twenty and twenty-one genes coding for connexins, each coding for a distinct protein, and distributed over many chromosomes, are found in the mouse and the human genome, respectively (23, 200, 201, 211–213, 496). Nineteen can be grouped as orthologous pairs (for the nomenclature of the connexin genes, see <http://www.genenames.org/genefamily/gj.php> for humans; <http://www.informatics.jax.org/> for rodents) (FIG. 2 AND TABLE 1). Nomenclature distinguishes connexins on the basis of the species of origin (m = mouse, h = human) and appends to the family name (Cx)

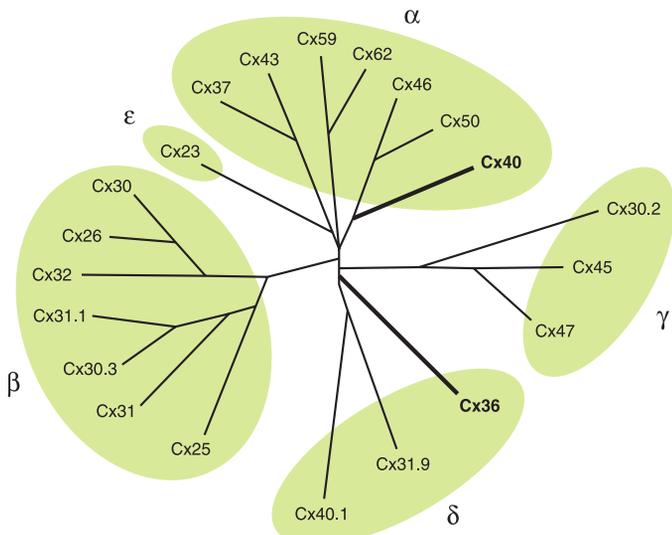


FIGURE 2. The human connexin family comprise 21 members. Each connexin is coded by a different gene. The phylogenetic tree of the connexin genes reveals that they are part of five distinct subgroups, referred to as α , β , γ , δ , and ϵ , respectively. Cx36 and Cx40, which are the main focus of this review, are parts of the δ and α groups, respectively.

the molecular mass predicted by the cloned cDNA sequence. For example, the 36- and 40-kDa proteins, which are the main focus of this review, are termed Cx36 and Cx40, respectively (**FIG. 2 AND TABLE 1**). Connexin genes share a common general structure (**FIG. 3**). Most connexin genes comprise in sequence a 5'-untranslated region (5'-UTR) on exon 1, which is separated from the complete coding region, usually as a single, uninterrupted reading frame, and the subsequent 3'-untranslated region (3'-UTR), on exon 2. In some connexin genes (e.g., the *Gjd2* gene which codes for Cx36), the coding region is interrupted by an intron, featuring a small part of the reading frame on exon 1 and the rest, in an uninterrupted region, on exon 2 (200, 201, 211–213, 496). There are different splice isoforms of several connexin genes (e.g., the *Gjb1* gene which codes for Cx32), indicating that different 5'-UTRs can be spliced in a consecutive and/or alternate manner, possibly due to the use of alternative promoters (200, 201, 211–213, 496). The expression of the connexin genes is selectively regulated during prenatal, neonatal, and adult life. However, the molecular control of this regulation is still poorly investigated. The basal expression of several connexin genes has been attributed to the ubiquitous transcription factor SP1, that recognizes GC boxes. Thus Sp-1 binding sites have been identified in the genes coding for Cx32, Cx36, Cx40, and Cx43 (12, 311, 536). Additional elements may be more specific of certain connexin isoforms. Thus the promoter region of the Cx36 gene comprises a neuron-restrictive silencer element (NRSE) that binds the RE-1 silencing transcription factor REST (324), which contributes to the basal promoter activity of the *Gjd2* gene. The pattern of connexin expression varies widely in different cell types (200, 201, 211–213, 291, 342, 356, 496). The consistence of these differences in a variety of cell species

shows that the spatial expression of connexins is also tightly controlled. Several factors, including Nkx2–5, GATA4, and GATA5, have been implicated in this control (52, 88, 217, 311, 520). In the case of Cx36, NRSE plays a central role, inasmuch as the overexpression of REST, a protein that negatively regulates the expression of numerous genes through the recruitment of histone deacetylase to their promoter, and which is expressed by most cell types but pancreatic beta cells and neurons (83, 84, 463, 464), inhibits the native expression of Cx36 in primary beta cells (324).

Biosynthesis of connexin polypeptides appears to proceed like that of most other membrane proteins (134, 286), first by docking of the nascent polypeptide, which lacks a signal peptide, to a protein channel of the endoplasmic reticulum (ER) membrane. It is during the integration into this membrane that the topographic arrangement of most connexins is established (134, 286). Connexin proteins have been highly conserved, in both sequence and topography, throughout evolution (23). They all consist of four membrane-spanning domains, linked by two extracellular and one intracellular loops (**FIG. 4**), that result in the cytoplasmic localization of both NH₂- and COOH-terminal regions (23, 200, 201, 211–213, 496). The two extracellular loops, which are among the most conserved regions of the proteins, contain three cysteine residues in all but one connexin isoform (23, 200, 201, 211–213, 496). The four transmembrane domains are also well conserved and form α -helical sheets that contribute to the wall of the connexon and line its central hydrophilic space (23, 200, 201, 211–213, 496). The intracellular loop, on the other hand, is highly variable. The NH₂-terminal region is of similar length in all Cxs. In contrast, the COOH-terminal region differs in length and sequence, facilitating the generation of isoform-specific antibodies (23, 200, 201, 211–213, 496). Some connexins are expressed in many cell types, whereas others are restricted to one or a few cell types (132, 197, 200, 201, 211–213, 329, 458, 459, 496).

B. The Posttranslational Modifications

Out of the three families of tetraspan proteins that form cell-to-cell and/or nonjunctional channels, only pannexins are extensively N-glycosylated on the second extracellular loop (37, 38, 390, 456, 508). This posttranslational modification is thought to be important for the proper intracellular trafficking of these proteins, and their insertion as pannexons at the cell membrane. It is likely that this glycosylated expansion is also the major impediment for pannexins to form cell-to-cell channels under most conditions, by preventing the close approximation of the pannexons made by adjacent cells, but possibly under rare conditions of large overexpression (53, 456, 508). Indeed, the experimental deletion of the glycosylation permitted the ready formation of pannexin cell-to-cell channels permeable to current-carrying ions and larger molecules (37, 38). Connexins and

Table 1. *The family of connexins*

Human				Mouse			
Cx	Gene	ID no.	Chromosome	Chromosome	ID no.	Gene	Cx
hCx23			6	10			mCx23
hCx25	GJB7	(375519)	6q15				
hCx26	GJB2	(2706)	13q11-q12	14d1-e1	(14619)	Gjb2	mCx26
hCx29	GJE1	(349149)	7q22.1	5g2	(118446)	Gje1	mCx29
hCx30	GJB6	(10804)	1 13q11-12.1	14c2	(14623)	Gjb6	mCx30
hCx31.9	GJA11	(125111)	17q21.2	11d	(353155)	Gja11	mCx30.2
hCx30.3	GJB4	(127534)	1p34.3	4d1-d3	(14621)	Gjb4	mCx30.3
hCx31	GJB3	(2707)	1p34	4d2.2	(14620)	Gjb3	mCx31
hCx31.1	GJB5	(2709)	1p35.1	4d2.2	(14622)	Gjb5	mCx31.1
hCx32	GJB1	(2705)	Xq13.1	Xd-f4	(14618)	Gjb1	mCx32
				Xf4	(414089)	Gja6	mCx33
hCx36	GJA9	(57369)	15q14	2f3	(14617)	Gja9	mCx36
hCx37	GJA4	(2701)	1p35.1	4d2.2	(14612)	Gja4	mCx37
hCx40.1		(219770)	10p11.21	18a1	(225152)		mCx39
hCx40	GJA5	(2702)	1q21.1	3f2.1	(14613)	Gja5	mCx40
hCx43	GJA1	(2697)	6q21-q23.2	10b4	(14609)	Gja1	mCx43
hCx45	GJA7	(10052)	17q21.31	11e1	(14615)	Gja7	mCx45
hCx46	GJA3	(2700)	13q11-q12	14c2	(14611)	Gja3	mCx46
hCx47	GJA12	(57165)	1q41-q42	11b1.3	(118454)	Gja12	mCx47
hCx50	GJA8	(2703)	1q21.1	3f2.1	(14616)	Gja8	mCx50
hCx59	GJA10	(81025)	1p34	4a5	(14610)	Gja10	mCx57
hCx62		(84694)	6q15-q16				

The lines in bold outline the genes coding for mouse connexin Cx36 and Cx40, which are the main topic of this review.

innexins, which readily form cell-to-cell channels with comparable permeabilities in most tissues, are not glycosylated (FIG. 4).

Connexins may undergo phosphorylation on either serine/threonine or tyrosine residues (200, 201, 211–213, 496). At least eight kinases, notably protein kinase A (PKA) and protein kinase C (PKC), and three phosphatases physiologically control the phosphorylation of various connexins (361, 444, 497, 498, 566). Still, some connexin isoforms (e.g., Cx26) may not be phosphorylatable, and others (e.g., Cx36) may only be posttranslationally modified only under certain conditions (200, 201, 211–213, 361, 444, 494, 497, 498, 550, 566). Connexin phosphorylation is often associated with changes in the conductance, permeability, and/or gating of the junctional channels that may vary with the connexin isoform (11, 481, 527) and, for a given connexin species, with the type of cell expressing it (73). In turn, these changes may affect the assembly of connexins into gap junction plaques (9, 288, 372), their proteolytic degradation (131), and their biosynthesis (379). Thus phosphorylation may contribute to regulate the half-life of connexins, which is unusually short compared with that of many other integral membrane proteins, and typically in the range of 3–5 h (200, 211–213, 494–496). It also affects the functional properties of established cell-to-cell channels, which has

best been documented for Cx43, whose channel conductance and permeability decrease with phosphorylation (497, 498).

Connexins also interact with ubiquitin, at least one ubiquitin ligase and other functionally related proteins (207, 264, 297–299). Recent experimental evidence indicates that connexin ubiquitination takes place at the plasma membrane and is rapidly regulated by both epidermal growth and some phorbol esters (207, 264, 297–299). Mono- and poly-ubiquitination differentially regulate the endocytosis of gap junction plaques made of Cx43, the intracellular trafficking of this connexin isoform, and its sequential targeting to early endosomes, the proteasome complex and, ultimately, to lysosomal compartments (207, 264, 297–299). However, ubiquitination also controls the degradation of connexins at the level of the ER, i.e., at an early quality control step (264). It remains to be shown whether the ubiquitination control also applies to other, if not all, connexin isoforms (207, 264). Still, the regulation it underlies could rapidly affect the levels of functional connexin nonjunctional and cell-to-cell channels, since the half-life of most connexins is significantly shorter than that of many other integral membrane proteins. The ubiquitination control could also provide an explanation for the altered intracellular trafficking of mutated connexins, which has been ob-

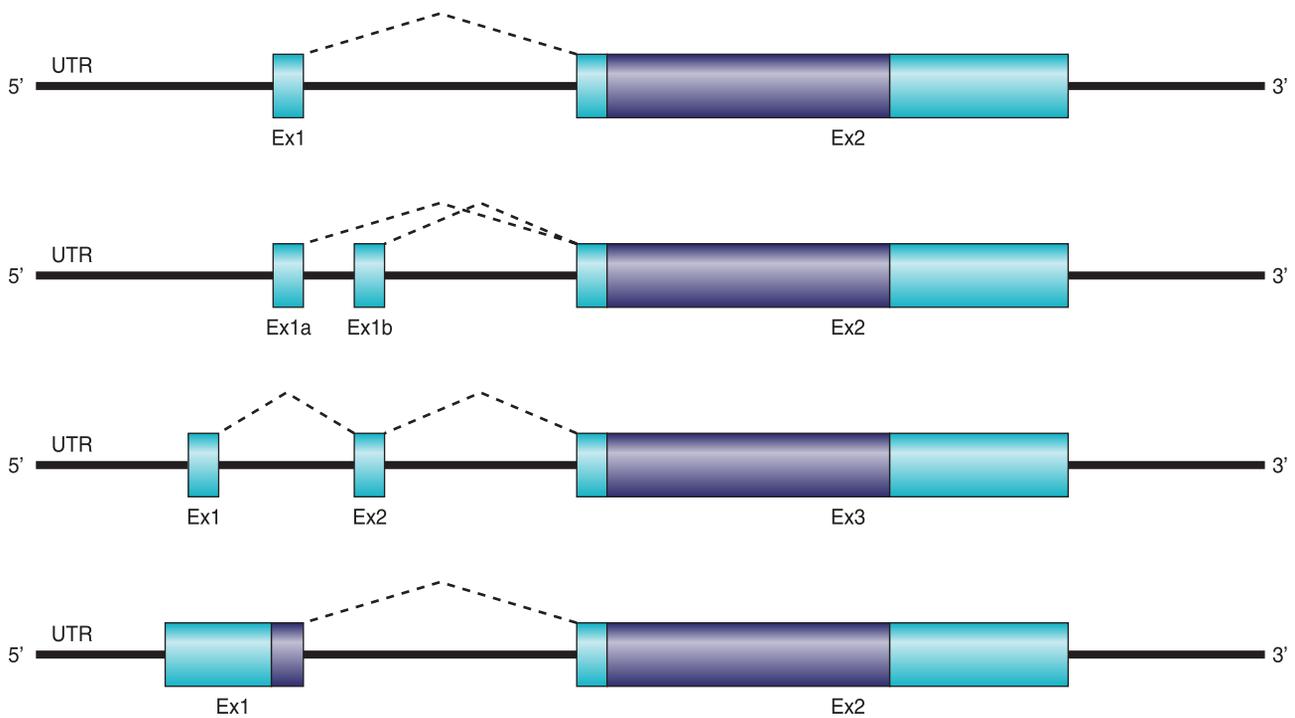


FIGURE 3. Connexin genes show a similar structure. Most connexin genes comprise 1–3 exons, and usually the entire coding sequence (dark blue) is uninterrupted within exon 2 or 3. However, some connexins, like Cx36, have their coding sequence in 2 separate exons (*bottom panel*). Some connexin genes have exons that are alternatively transcribed by different promoters in different tissues (*second panel from top*). Others have different exons that are alternatively spliced, resulting in different transcripts (*third panel from top*).

served in several connexin-linked genetic diseases (286, 287). Some connexins may also become phrenylated during their trafficking to the cell membrane (224).

C. The Assembly Into Junctional Channels

During the trafficking across the ER, the Golgi apparatus and the *trans*-Golgi network, six connexin molecules oligomerize around a central hydrophilic space to form a tubular structure called a connexon (134, 200, 201, 211–213, 275, 286), in a way that appears to be Cx-isoform specific (134, 275, 325) (FIG. 5). Depending on whether a single or more connexin species oligomerize, connexons are called homo- or heteromeric, respectively (275). After interaction with chaperone proteins (112), connexons are transported to the membrane mostly by way of Golgi-derived vesicles (112, 134, 286), although direct delivery of the proteins by fusion of the ER and plasma membrane has also been proposed (112, 134, 286). Vesicles are transported by a mechanism that may vary for different connexins in terms of their dependence on microtubules (138, 240, 286, 539–541). In many cases, microtubules appear to be dispensable for connexin transport to the cell surface, but may improve the efficiency of this transport (138, 240, 286, 532, 540, 541).

Connexons appear to be first inserted in cholesterol-rich and lipid raft domains of the nonjunctional cell membrane

(321, 353, 468). From these sites, and when cells express calcium-dependent cell adhesion molecules that allow their contact, connexons concentrate in regions of close, straight membrane apposition, where the intercellular space is reduced to a gap 2–3 nm wide (200, 201, 211–213, 496). Because of these characteristics, these regions are referred to as gap junctions (200, 201, 211–213, 496) (FIGS. 6 and 7). At these sites, the close apposition of the membranes favors the alignment of the connexons made by adjacent cells, resulting in a tubular structure, made by two connexons, which extends from one cytoplasm, across a first cell membrane, the intercellular gap and a second membrane, up to the cytoplasm of another cell. This two-cell structure forms the wall of a hydrophilic channel which is funnel-shaped and has a central, hydrophilic functional bore of 2.5–3 nm in diameter (200, 201, 211–213, 496), that functionally connects adjacent cytoplasm (FIGS. 5–7). Depending on whether the two interacting connexons are made of the same or different connexins, gap junction plaques are made by homo- and heterotypic channels (or a combination of the two), which feature distinct biophysical characteristics (200, 201, 211–213, 496). In culture, most cell types nonspecifically couple via gap junction channels (132), as in vitro conditions usually favor the default expression of Cx43. However, not all connexins are indiscriminatively compatible for formation of cell-to-cell channels, particularly if they are from different subgroups of the connexin family (200,

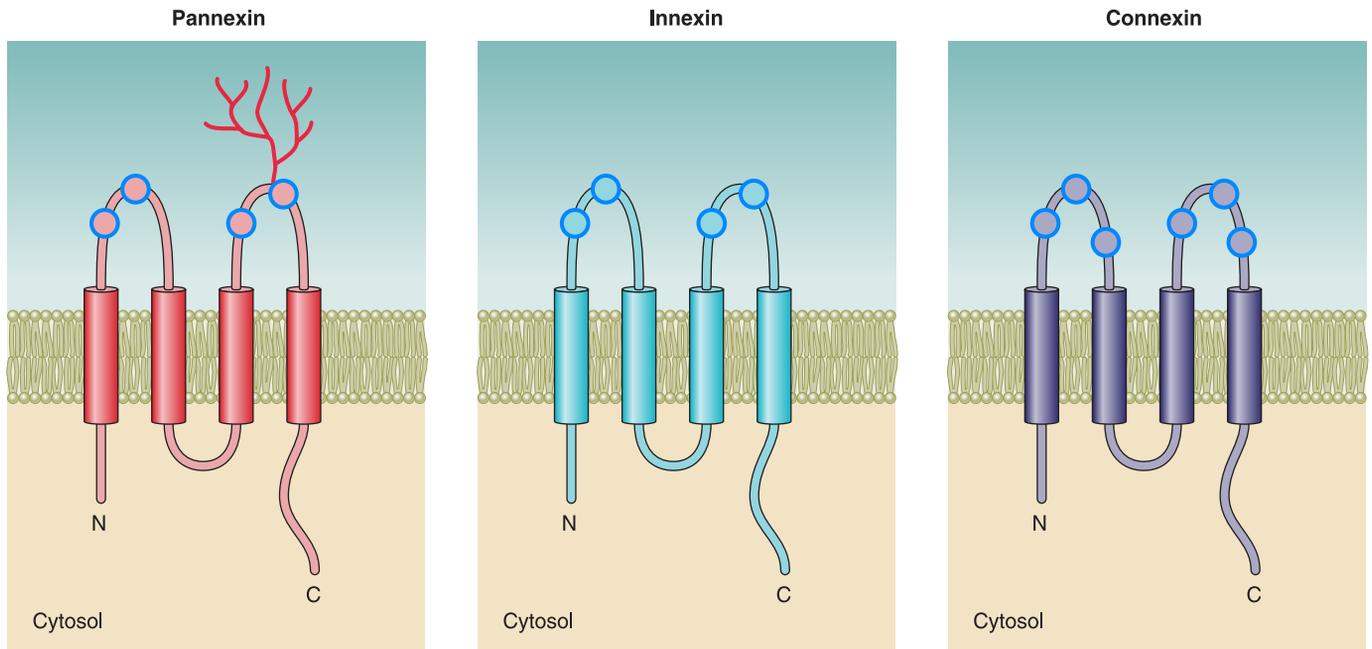


FIGURE 4. Pannexins, innexins, and connexins share a common membrane topography. The 3 families of proteins that form channels permeable to second messengers and metabolites are integral, tetraspan membrane proteins. All members of the 3 families have their NH₂ and COOH termini within the cytoplasm, and 2 extracellular loops that extend in the extracellular space. These loops contain 2 (pannexins and innexins) or 3 (connexins) highly conserved Cys residues (*circles*). Pannexins, but not innexins and connexins, feature a N-glycosylated branching on the second extracellular loop.

201, 211–213, 554, 555, 566). Gap junction plaques are highly dynamic membrane regions, which increase in size by the apposition of new connexons at their periphery (14, 135, 153, 201, 211–213, 290, 291, 502) and are rapidly turned over. Connexins have an unusually short half-life (1–5 h) so that, while newly synthesized connexons are added at the periphery of gap junction plaques, old connexons are removed from their center (13, 135, 153, 201, 211–213, 290, 291). This turnover is thought to involve the invagination of a vesicle, limited by a gap junction membrane, into one of the two interacting cells, the release in the cytosol of so-called “annular gap junctions” (112, 290, 291), and the degradation of its connexons via both the lysosomal and proteasomal pathways (135, 264, 286, 290, 291, 476).

The intercellular, gap junction channels are unique in two respects. First, they are two-cell structures. Second, they not only conduct current-carrying ions but are also permeable to larger molecules, including metabolites, nucleotides, morphogens, vitamin cofactors, small peptides, and fragments of nucleic acids (30, 169, 170, 200, 201, 211–213) (**FIG. 7**). The cell-to-cell exchanges of these molecules via junctional channels are referred to as ionic and metabolic (or cell-to-cell) coupling, respectively. For most ions and molecules, this coupling is bidirectional between the two coupled cells and is driven by diffusion along an electrochemical gradient (**FIG. 8**). However, both the conductance and permeability of junctional channels show a high degree of selectivity (200, 201, 211–213, 345). This selectivity

(**FIG. 9**) is determined both by the connexin composition of the channel and by the funnel-shaped geometry of the hydrophilic channel space, which is longer than that of other channels as it spans two membranes, the intercellular gap and part of the cytoplasm. The channel has a functional diameter of 2.5–3 nm (200, 201, 211–213). Channels made by different connexins can discriminate between closely related molecules such as cGMP and cAMP, and some channels favor the passage of nucleotides rather than the cognate base, presumably due to the negative electrical charge that results from phosphorylation of the former molecules (30, 169, 170). The observation that some channels favor the passage of anions whereas others are almost uniquely selective for cations indicates that the regulation of junctional channels is determined by a combinatorial effect of multiple parameters, including size, charge, and hydrophilicity (80, 201, 554, 555). Like other membrane channels, the opening of junctional channels is regulated, such that the average open probability of individual junctional channels is ~10%. How changes in the connexon structure account for the opening and gating of the cell-to-cell channels remains to be fully elucidated. Experimental gating can be achieved by imposing a voltage difference across the channel, by changing the resting membrane potential, by decreasing the cytosolic pH, or by increasing the concentration of free cytosolic Ca²⁺ (200, 201, 211–213, 345). Whether any of these factors is of physiological relevance remains to be determined, as, individually, each factor is rarely effective in all cell types, and usually needs to be

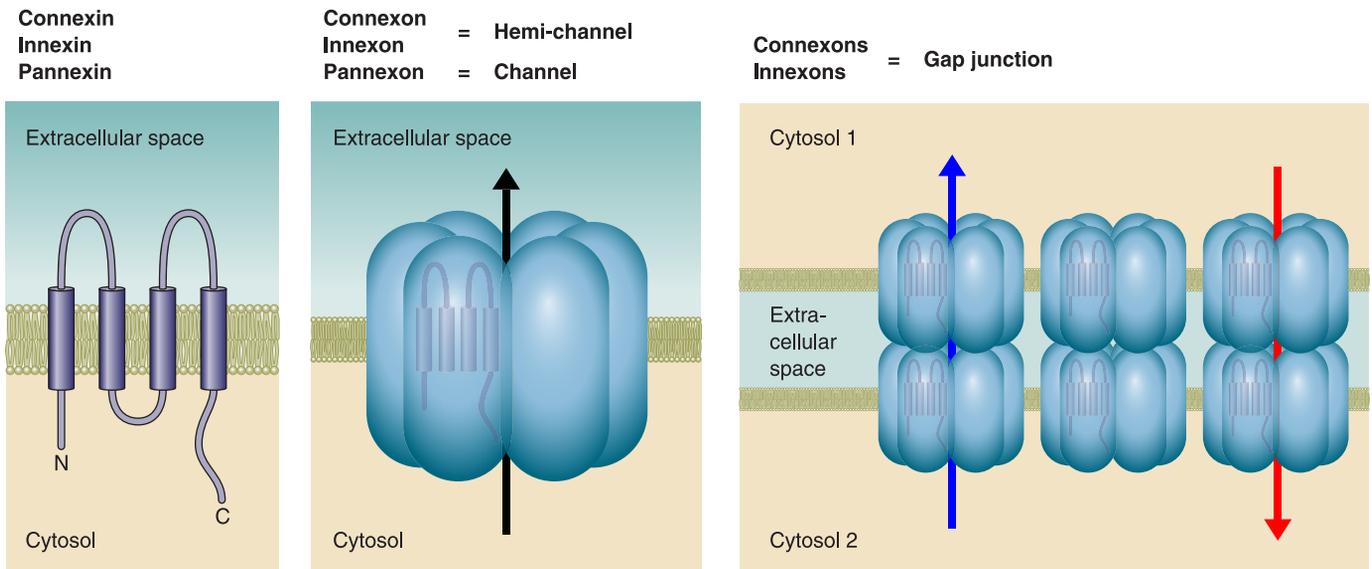


FIGURE 5. Connexins form “hemi-channels” and gap junctions between most cell types. *Top:* all connexins are nonglycosylated, integral membrane proteins that cross four times the phospholipid bilayer of the cell membrane and feature both the NH_2 and COOH terminus in the cytosol. *Middle:* during the intracellular trafficking, six connexins (as well as innexins and pannexins) oligomerize around a central hydrophilic space to form a “hemi-channel,” referred to as a connexon (or innexon or pannexon, respectively) that becomes inserted into the plasma membrane. *Bottom:* several connexons (or innexons, but not pannexons) concentrate within the plasma membrane to form domains referred to as gap junctions. The intercellular channels which are established by the pairing of 2 connexons, across the extracellular space, allow for the bidirectional, diffusion-driven exchange of cytosolic molecules from one cell to another, along electrochemical gradients (arrows).

drastically altered (except pH), to levels presumably not encountered in vivo. Gap junction channels may also be closed by a variety of drugs, most of which incorporate into the lipid bilayer of the membrane and act as chemical uncouplers (118, 200, 102, 211–213, 513). A few molecules have also been reported to enhance gap junction function, either by increasing the number of junctional channels (171, 200, 201, 211–213, 339, 340, 343) or by improving their gating and/or open probability (118, 200, 201, 209–213, 510, 513).

D. The Assembly Into “Hemi-channels”

Some connexons may also become inserted in nonjunctional domains of the cell membrane, i.e., at sites in which the membrane faces the extracellular medium and is not closely apposed to the membrane of another cell (FIG. 5). In this case, each connexon forms a so-called “hemi-channel” (53, 116, 394, 508), i.e., does not pair with a connexon from an adjacent cell. These structures were found to allow for the leakage of cytosolic molecules, notably ATP and glutamate into the extracellular medium, and to permit the reverse uptake into cells of large extracellular and membrane-impermeant tracers (96, 103, 174, 445, 456, 457, 508, 523). (FIG. 9). These fluxes were abolished by drugs known to block gap junction channels (216, 271, 306, 416). While proteins of the pannexin and innexin families can also form nonjunctional channels (15, 55, 89, 225, 315, 397, 456, 457, 542), and may do so more frequently than

connexins, there is now undisputed evidence that Cx46 and Cx50 form such structures when expressed in *Xenopus* oocytes (511), and probably also in the lens (588). It is also widely believed that Cx43 can also form such structures in a variety of cell types (201, 445, 458, 508). Other reports have proposed that this property is shared by several other connexin isoforms, including Cx23, Cx26, Cx30, Cx30.2, Cx31.9, Cx32, Cx35, Cx36, Cx37, Cx41.8, Cx45, Cx45.6, Cx55.5, and Cx56 (59, 130, 173, 174, 234, 378, 384, 385, 445, 462, 543, 544, 567). However, it has proven difficult to unambiguously demonstrate the presence of functionally open and unpaired connexons made by these proteins (458, 508).

The biophysical properties of connexin “hemi-channels” differ from those of the gap junction channels formed by the same connexin. Thus their unitary conductance (200–500 pS) is about twice the main state conductance of the same connexons in gap junctions (103, 200, 424, 457, 508, 562), although the unitary conductance of certain connexin hemichannels is much lower (e.g., those attributed to either Cx31.9 or Cx36 are <20 pS) (59). Hemi-channels feature little selectivity for the major current carrying ions, and a sizable open probability at positive resting potentials (103, 200, 457, 508). Most hemi-channels show an in-out permeability to ATP, and an out-in permeability to propidium iodide, Lucifer yellow, and 6 carboxyfluorescein (103, 200, 457, 508, 518, 526). Opening of all types of hemi-channels is induced mostly by supraphysiological membrane depo-

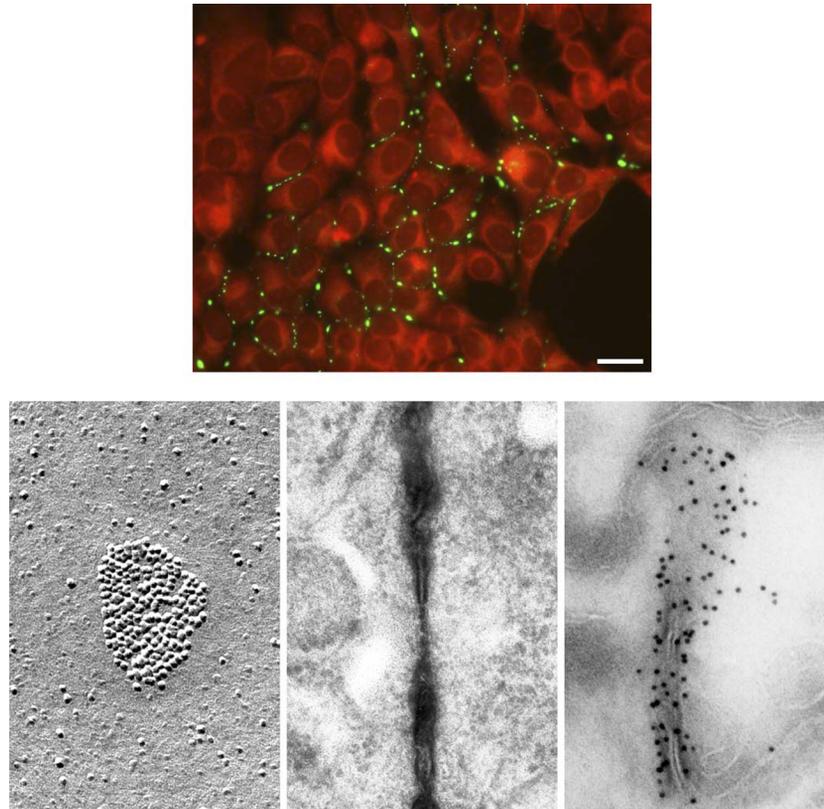


FIGURE 6. Gap junctions are membrane domains concentrating connexons. *Top:* immunofluorescence reveals the punctate distribution of Cx36 (green) at discrete membrane domains of interacting cells. Note the absence of labeling in the regions of the cell membrane that face the extracellular milieu. *Bottom:* at the ultrastructural level, the membrane sites concentrating Cx36 are shown to comprise plaquelike aggregates of connexons (*left*, freeze-fracture electron microscopy), to feature a narrow extracellular “gap” between the 2 interacting membranes (*middle*, transmission electron microscopy after lanthanum impregnation), and to be labeled by antibodies against the COOH terminus of Cx36 (*right*, immuno-cryo-electron microscopy with protein A-gold labeling). Bar, 20 μm in *top panel* and 100 nm in *bottom panels*.

larization ($V > 40\text{--}60\text{ mV}$), and in some cases may also be activated by low divalent ion concentrations or under ischemic conditions (26, 96, 97, 200, 238, 417, 424, 462). These conditions raise the intriguing question of the physiological relevance of these structures, which is not disputed for pannexon hemi-channels (456, 474). A major concern is that the lack of opening of connexons at negative resting potentials is not consistent with the dye uptake observed under such conditions in many cell types (103, 456, 457, 474). Still, there is now evidence that connexin hemi-channels can open at negative potentials and in the presence of external divalent ions, e.g., in HeLa cells transfected for Cx43 (96). The opening of an unpaired connexon was originally expected to cause a rapid and deadly loss of cytosolic molecules. Recent evidence suggests that this is not necessarily the case, because hemi-channels are rare and open only briefly (54, 56, 58, 143, 150). The existence and relevance of these structures remain to be fully validated *in situ*. Hemi-channel opening is only seen when exceptionally large potential steps are established across the cell membrane (150, 456, 457, 508), which are unlikely to be observed *in vivo*. Future studies should document whether these unusual gating conditions only

reflect the artificial environment of the *in vitro* experiments.

E. Other Connexin Signaling Modalities

Until recently, connexins were thought to signal cells by their ability to form either gap junction channels or “hemi-channels” (200, 201, 211–213, 345, 456, 523), both of which result in changes of membrane conductance and permeability. However, experiments testing the deletion or the overexpression of individual connexins have revealed effects of connexins that cannot be easily accounted for by alterations in the function of these channels (456, 523). In some cases, these effects, or as it matters the lack of effects, could be accounted for by compensatory changes of other connexins, but other cases were not. Furthermore, deletion of a single connexin isoform has sometimes resulted in severe *in vivo* phenotypes, that could be ascribed to alterations in unsuspected biochemical pathways (90, 95, 581). These data indicate that, under certain conditions, connexins may signal cells by mechanisms that do not depend on either cell-to-cell channels or “hemi-channels” (FIG. 10).

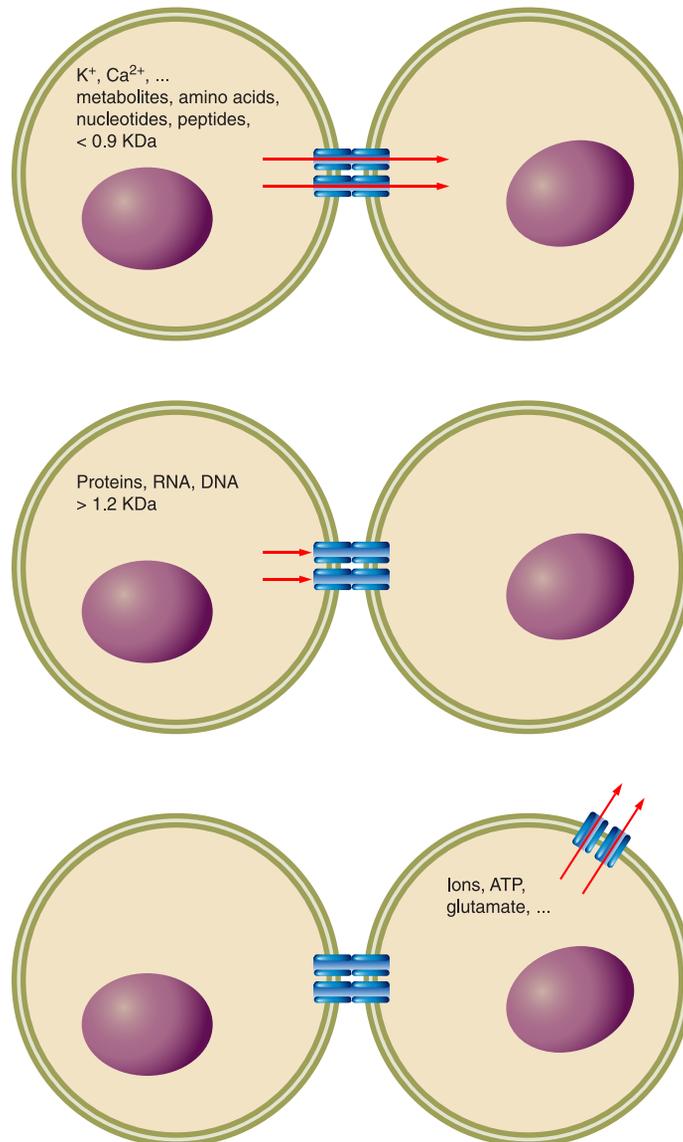


FIGURE 7. Connexin cell-to-cell channels and “hemi-channels” are permeable to ions and small molecules. *Top:* gap junctional channels are permeated by a variety of current-carrying ions and cytosolic molecules up to 900 Da. *Middle:* the channels, however, do not allow for the cell-to-cell exchange of macromolecules, such as proteins and nucleic acids. *Bottom:* connexin “hemi-channels” allow for the escape of cytosolic ions and signaling molecules into the extracellular space.

Two lines of research begin to investigate these mechanisms.

Transcriptome analysis of brain and inner ear stria vascularis has also revealed that many genes are altered after loss of individual connexins, implying that the protein may tightly and coordinately control the expression of multiple genes (90, 95, 230, 231, 363, 581), which contribute to a variety of branches of the mammalian transcriptome, and possibly of the cognate proteins. The apparently selective and coordinated effect of different connexins on the genome, with the expression of a number of genes being up- or downregulated in parallel, also provides for an efficient amplification of the connexin effect, which could explain why the functional loss of a single connexin can induce dramatic

phenotypes, in spite of the persistence of junctional coupling provided by other connexin isoforms (69, 110, 175, 263).

Several studies have documented that at least some connexin isoforms colocalize with a variety of other membrane and cytosolic proteins, and may directly interact with them, within multi-protein complexes (75, 115, 200, 211–213). Most of these proteins are tight junction-associated species, such as ZO-1, ZO-2, and ZO-3 (208), or are part of the cytoskeleton (307, 308, 380). Thus colocalization, and at least functional interaction, has now been documented between connexins (mostly Cx43) and actin, myosin, α -actinin, α/β -tubulin, vimentin, spectrin, vinculin, and numerous other less abundant cyto-

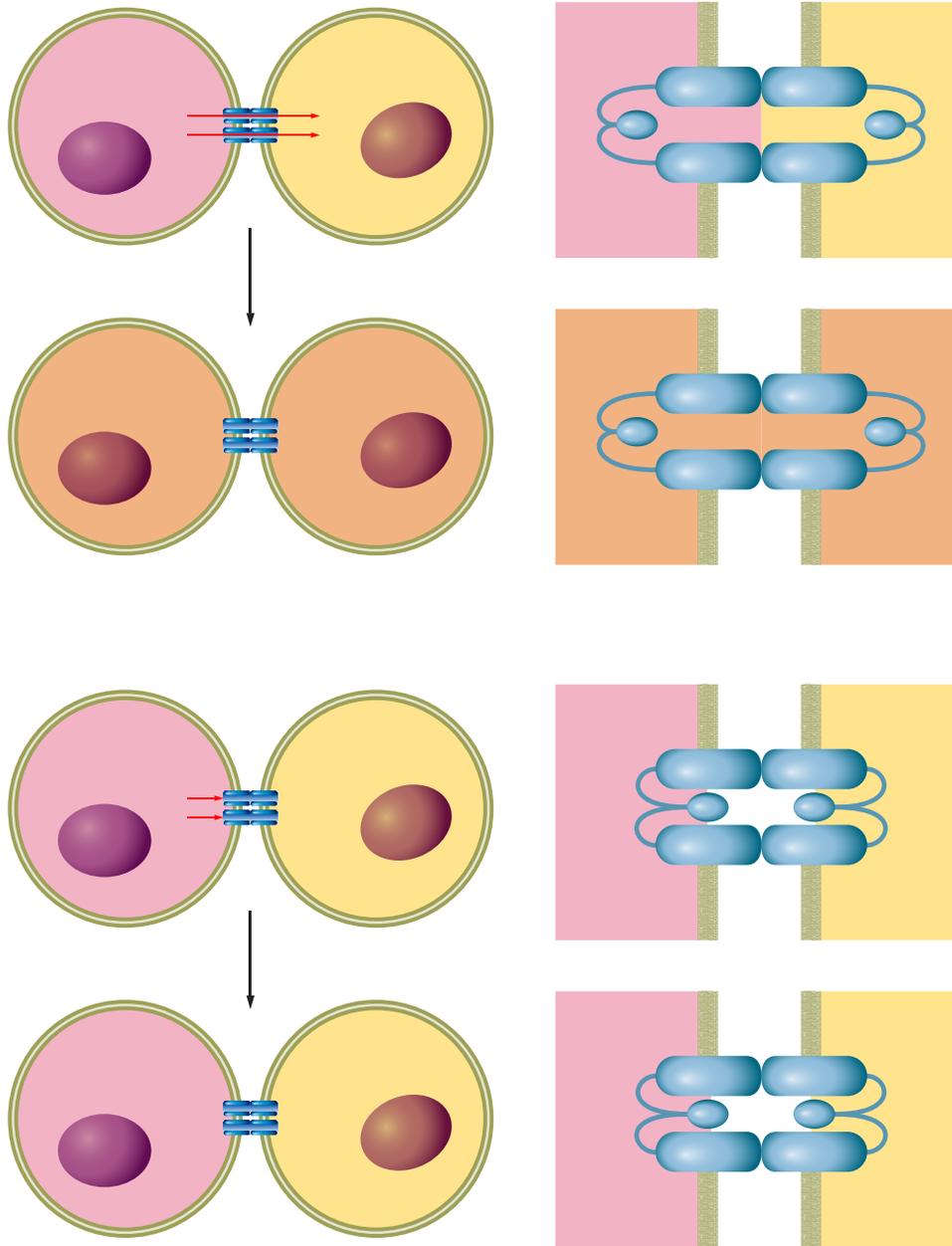


FIGURE 8. Functional gap junction channels abolish gradients of cytosolic molecules between the coupled cells. *Top:* electrochemical gradients direct the transfer of gap junction-permeant molecules from one cell to another coupled cell (*left*), when gap junction channels are in a functionally opened state (*right*). At steady state, the concentration of the exchanged molecule is similar in all coupled cells (second left panel from *top*). *Bottom:* such an equilibration is not seen between cells in contact, when the gap junction channels are closed. Current models suggest that gap junction channels close when a plug, linked by a flexible sequence, becomes located in the inner, restricted part of each paired connexin (*right*).

skeleton-associated proteins (380). The functional interactions between these proteins have been shown to be essential for the intracellular trafficking of the connexins and their incorporation into the cell membrane, since many vesicles transporting connexins follow the microtubule routes to reach the plasma membrane (483). Following insertion of individual connexins into lipid raft domains, the connexons are also moved together to form gap junction plaques by actin microfilaments (285). Surprisingly, perturbing the actin arrangement, or the asso-

ciated RhoA or debrin proteins, resulted in altered permeability and gating of established gap junction channels (61, 114, 133), implicating that the cytoskeleton further regulates the functioning of established connexin channels. Debrin and actin microfilaments are certainly implicated in the retrieval of gap junction plaques from the membrane by endocytotic internalization, and formation of the so-called annular gap junctions (61, 407). Functional connexin-cytoskeletal interactions are now also thought to account for biological effects of connexins

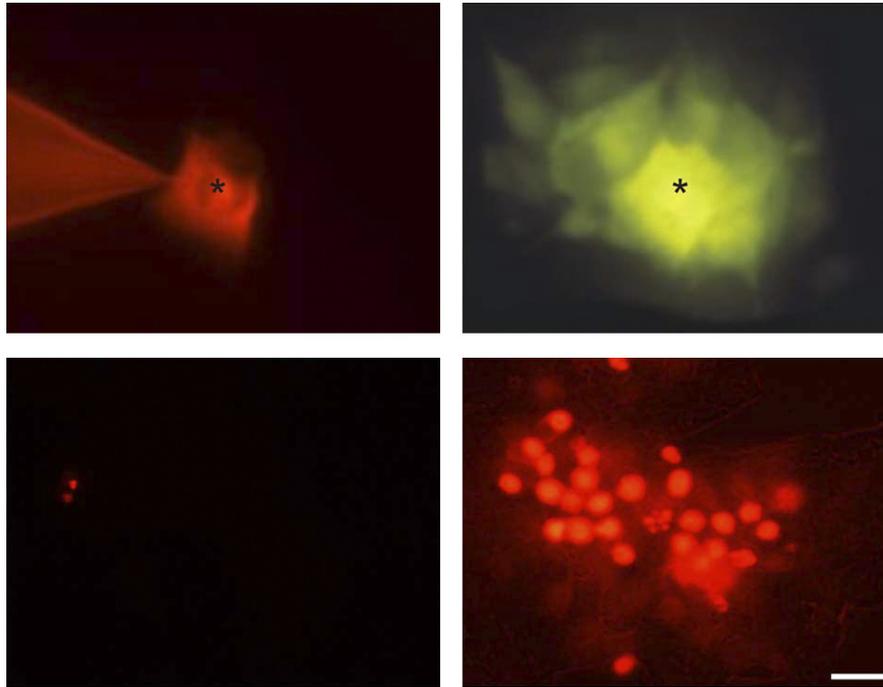


FIGURE 9. Functional connexin and pannexin channels allow for the passage of membrane-impermeant fluorescent tracers. *Top:* in cells expressing Cx43, one cell (asterisk) was simultaneously injected with ethidium bromide (*left*) and Lucifer yellow (*right*). While the former positively charged tracer was exchanged between only three coupled cells, the latter negatively charged tracer diffused into many more coupled cells, illustrating the permselectivity of Cx43. *Bottom:* MIN6 cells, which express pannexin 1 (Pnx1), do not spontaneously form channels and, thus, cannot incorporate ethidium bromide added to the culture medium (*left*). In contrast, this uptake was extensive after transfection of these cells for Pnx1. Bar, 10 μm in *top panels* and 20 μm in *bottom panels*.

that appear independent of their building connexin channels and “hemi-channels” (200, 201, 211–213), and which may provide for an integrated signaling pathway between the cell membrane and the nucleus. This signaling, which has not yet been fully unraveled, is expected to be enhanced by the recruitment in restricted cell domains of many interacting proteins. It is believed that some connexins may provide the scaffold for such an integration center. At any rate, connexin effects that are independent of cell coupling have been documented, such as resistance against apoptosis-inducing conditions (82, 222), possibly by interfering with the Ca^{2+} -induced changes in gene expression and caspase activation. Cell migration is another function modulated by connexins, in the absence of detectable coupling changes. Thus downregulation of Cx43 was shown to alter the migration of astrocytes (398), prenatal neural precursors (128, 152, 570), and neural crest cells (422, 576, 578), which correlated with alterations of multiple cytoskeleton and cytoskeleton-associated molecules. The proposed scenario is that Cx43 forms a scaffold for the integration of multiple signals that, via cytosolic intermediate proteins (ZOs, RhoA, . . .), converge to regulate the mechanical and the signaling functions dependent on cytoskeleton (133) and associated cell adhesion molecules (578). By altering the structural/functional organization of the cytoskeleton and cell adhesion molecules, connexins could

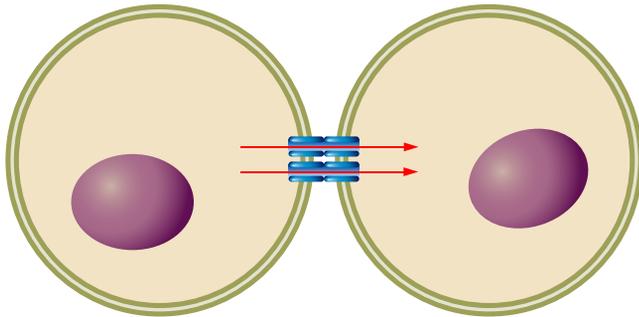
therefore alter their normal functions, thus leading to biological effects that may not involve alterations in connexin channels/hemi-channels. It has to be recognized that virtually all this information has been gathered in cells expressing Cx43 so that future studies should assess to what extent a similar scenario could be contemplated for other connexin isoforms, specifically, for connexins which, like Cx36, have a short COOH terminus, where most of the functional protein-protein interactions involving connexins appear to take place (200, 201, 211–213, 380). At any rate, the data indicate that connexin signaling functionally interacts and is integrated with the signaling provided by other cell communication mechanisms.

These observations further stress the necessity to understand the hierarchical relationships, and the possible cross-talk between the connexin-dependent signaling and that dependent on the other mechanisms of cell-to-cell communication that, collectively, ensure the proper function of a multicellular organism, in allowing individual cells to become integrated in a functionally coherent tissue. Loss of the coupling provided by Cx36 channels between the insulin-producing β -cells of pancreas, as a result of either cell dispersion (44, 45, 47, 241, 242, 452), pharmacological blockade of the channels (336), or inactivation of the *Gjd2* gene (421, 503, 568), results in

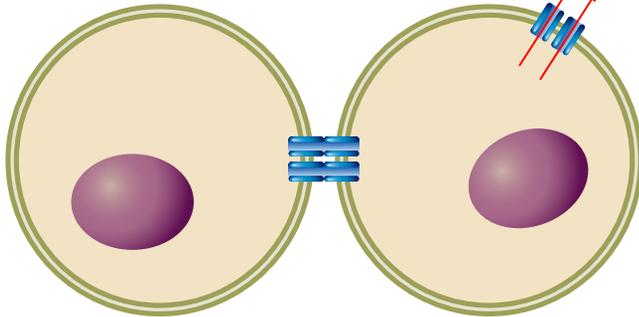
two apparently contradictory alterations of β -cell function, i.e., increased basal secretion and decreased glucose-stimulated release of the hormone. This dual regulation has biological sense, since it provides low amounts of insulin between meals and much higher levels of insulin immediately after food intake. The underlying molec-

ular mechanism likely involves β -cell signaling via an ephrin-A5 ligand/receptor mechanism, in which the forward signaling activated by the ephrin A receptor in the presence of low glucose levels inhibits basal insulin secretion, whereas the reverse signaling activated by the ephrin A ligand in the presence of high glucose concentrations stimulates insulin secretion (273). Strikingly, the effects of the ephrin A mechanism were prevented after interference with Cx36 mRNA, suggesting a cross-talk between the ephrin and the connexin-dependent pathways (273), and raising the question of whether the ephrin-attributed effects may actually be mediated by Cx36 changes or, conversely, whether effects so far attributed to Cx36 may have actually been due to altered ephrin signaling. An interaction between the signaling dependent on Cx36 and that dependent on E-cadherin has also been shown (63), providing evidence that connexins may simultaneously cross-talk with multiple signaling pathways.

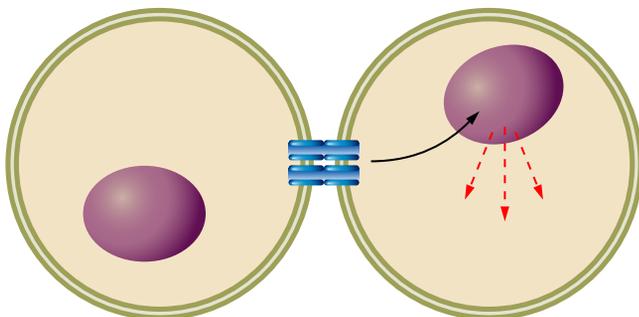
Cell-to-cell coupling



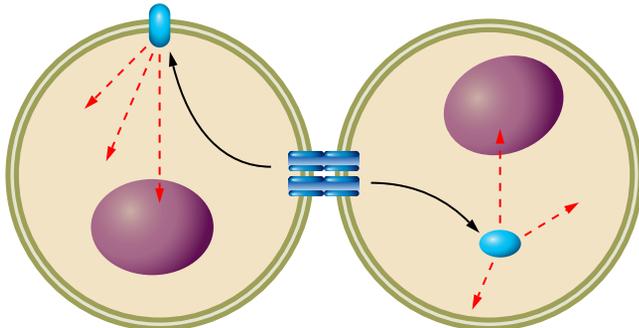
Paracrine intercellular communication



Regulation of gene expression



Cx-protein interaction



F. The Physiological Functions

As mentioned above, the formation of “hemi-channels” by connexins, under physiologically relevant conditions, is still a matter of debate. Therefore, it is uncertain whether any of the effects observed in experiments documenting hemi-channel changes were actually mediated by connexins or rather should be attributed to the coexpression of some pannexin, which is widespread (456, 508). At any rate, the release of ATP (273), glutamate (585), and epoxyeicosatrienoic acid, which could serve as paracrine signaling in many systems, has been repeatedly attributed to Cx43 hemi-channels (237, 246, 273).

In contrast, connexin channels have been largely implicated in a variety of functions (159, 227, 345, 504, 506, 509), including embryonic development, morphogenesis, and cell differentiation, as well as in the control of adult cell proliferation and migration, the functioning of muscle cells, hormonal transmission, electrical and mechanical synchronization, resistance to cytotoxic agents, compensation of enzymatic defects, transmission of trophic or deadly molecules, and secretion under both normal

FIGURE 10. Connexins signal cells in multiple ways. Connexons may pair to form intercellular (gap junctional) channels for cell-to-cell coupling, i.e., for the direct exchange between adjacent cells of current-carrying ions and low-molecular-weight metabolites (*top panel*). Connexons may also insert into a nonjunctional domain of the cell membrane, to form “hemi-channels,” which allow for the acute efflux of ATP, glutamate, and possibly other cytosolic signals into the extracellular space, thus allowing for paracrine signaling (*second panel from top*). Connexins may also signal cells by influencing the coordinated expression of selected genes (*third panel from top*). Eventually, connexins may signal cells via their interaction with other membrane and cytosolic proteins (*bottom panel*).

and pathological conditions (14, 53–55, 92, 110, 167, 507, 509). Whereas several of these functions have been attributed to connexins solely on the basis of circumstantial, correlative studies, a number of others have been documented after careful interference with selective connexins. Initially, most studies of gap junctions were conducted *in vitro*, using primary cells or transformed cell lines that were grown under conditions favoring or preventing cell contact and junctional communication. Subsequently, a number of the functions that were circumstantially inferred from these studies have been confirmed and/or extended using specific genetic tools, whereby selected connexins have been altered in genetically modified mice, including general and cell-specific knockouts, cell-specific overexpression of individual connexin isoforms, and knock-in replacement of one connexin species by another (121, 411, 569). The functional importance of connexins and junctional communication is also evident from the increasing number of human genetic diseases that have been associated with connexin mutations and pathogenic single nucleotide polymorphisms (144, 145, 146, 205, 327, 426, 569), as well as the growing list of acquired diseases in which a connexin participation is contemplated, if not thought to play a central role (TABLE 2). The following sections comment on some of the functions that have been identified *in vivo*.

1. Development

Connexins significantly contribute to the prenatal development, morphogenesis, and differentiation of many tissues. Thus deletion of the *Gja1* gene, which codes for Cx43, delays the migration of the neural crest cells that contribute to cardiac morphogenesis, leading to obstructed right ventricular outflow, impaired blood supply to the lungs, and perinatal death (312, 422). Even though no similar pathogenic role has as yet been demonstrated in humans, mutations of Cx43 have been reported in patients affected by oculo-dento-digital dysplasia, an autosomal dominant syndrome characterized by craniofacial and limb dysmorphology, spastic paraplegia, and neurodegeneration (396). Analogous mutations in other connexins result in specific genetic disorders, mostly involving the inner ear and the skin. Mutations of either Cx26 or Cx30, two connexin isoforms that are usually corecruited in the same gap junction plaques of skin keratinocytes and most inner ear cells, cause a variety of nonsyndromic congenital deafness and hyperkeratosis syndromes, highlighting the importance of the connexin signaling for tissue homeostasis, and stressing that, in this signaling, individual connexins have remarkably different roles (90, 102, 217, 326).

2. Neural synchronization

Connexins form the electrical synapses, *i.e.*, the sites where current-carrying ions allow for bidirectional neuronal interac-

tion (24, 54, 55, 347, 493–495). This electrical coupling, which is particularly frequent among the interneurons of several CNS regions during prenatal and early postnatal life, helps synchronize firing and γ oscillations, across neuronal and glial populations (24, 154). In turn, this synchronization is thought to be critical for major cognitive processes, including perception, memory, and learning (493–495). Of the many connexin isoforms that have been reported in the CNS (54, 493, 494, 495), only Cx36 has been definitively shown to form gap junction plaques between neurons (419). Interestingly, however, Cx36 knock-out mice are viable and do not exhibit major anatomical or functional alterations of the CNS (24, 110, 317). They also have normal motor coordination and behavior (110, 263, 327). Presumably, the absence of an obvious neurological phenotype implies some compensation for the loss of Cx36 (461). However, mice lacking Cx36 cannot synchronize the oscillatory activity of some neuronal populations (110, 317), or coordinate the agonist-induced response of others (24, 493–495). These findings, and the decreased expression of Cx36 observed in models of drug-induced neuronal firing (497), suggest that this Cx may be involved in the development of some forms of epilepsy. Consistent with this idea, a variant of the human Cx36 gene was found associated with the juvenile myoclonic form of epilepsy (205, 327). The mechanism whereby a base change, which does not affect the amino acid sequence of the cognate protein, results in disease, remains to be understood. A possibility is that the change affects the stability of Cx36 mRNA, thus resulting in altered levels of the protein (329). Genetic variations in other connexins have been implicated in other human diseases (144–146, 172). It is worth noting that the lack of Cx36 did not desynchronize all types of neurons, notably in regions where cells also express other connexin isoforms (54, 494). These findings show that, in spite of the overall similarity in sequence, topography and role in cell-to-cell communication, individual connexins fulfill specific roles that cannot be compensated by other isoforms. A similar conclusion was reached in a knock-in mouse model, in which the native Cx43 was replaced by the quite different Cx32, under control of the promoter of the former gene, in order for the ectopic protein to be expressed at the same sites and levels as the native one (194, 411). In this case, some tissues showed a normal function, but others did not, indicating that Cx32 could not fully compensate for the loss of Cx43, presumably as a consequence of the different permeability, conductance, and gating of the two connexins (200, 211–213, 554, 555).

3. Signaling

Cells devoid of appropriate receptors can respond to specific hormones, provided they contact cognate target cells in coculture (215, 293, 369). Most likely, this response is made possible by the transfer of second messengers that are generated within the receptor-bearing cells, into those cells that do not possess appropriate hormone receptors. While there is little doubt that such a transfer depends on the establishment of gap junctions between the receptor bearing and nonbearing cells

Table 2. Human diseases caused by connexin mutations and single nucleotide polymorphisms

Mutated Cx	Inheritance	Human Disease	Phenotype in Mutated or KO Mice
Cx26	Autosomal recessive	Deafness DFN1B	Deafness in cochlea-specific deletion
	Autosomal dominant	Deafness DFNA3	
	Autosomal dominant	Vohwinkel syndrome	D66H mutation = signs of Vohwinkel syndrome
	Autosomal dominant	Keratodermia-ichthyosis-deafness (KID) syndrome	
Autosomal dominant	Bart-Pumphrey syndrome	KO: prenatal death in general knockout; throphoblast alterations	
Autosomal dominant	Keratodermia and deafness		
Cx30	Autosomal dominant	Deafness DFNA3	KO: deafness
	Autosomal dominant	Keratodermia-ichthyosis-deafness (KID) syndrome	
	Autosomal dominant	Clouston syndrome	
Cx30.3	Autosomal dominant (rarely recessive)	Erythrokeratodermia variabilis	
Cx31	Autosomal dominant (rarely recessive)	Erythrokeratodermia variabilis	F137L mutation = signs of erythrokeratodermia variabilis
	Autosomal recessive	Deafness	
	Autosomal dominant	Deafness	
	Autosomal dominant	Deafness with peripheral neuropathy	
Cx26 + Cx31	Autosomal dominant	Digenic deafness	
Cx32	X-linked (dominant, recessive or intermediate)	Charcot-Marie-Tooth (X-linked)	Mild peripheral neuropathy, alterations in secretion of pancreatic enzymes, liver glycogenolysis, liver carcinogenesis
Cx36	SNP588	Myoclonic juvenile epilepsy	alterations in insulin secretion, altered retinal transmission, altered SNC ripples and interneurone coupling
Cx40	SNP132-213	Familial atrial standstill hypertension in men	KO: atrial arrhythmia KO: renin-dependent hypertension
Cx43	Autosomal dominant	Oculodentodigital dysplasia (ODDD)	G60S and I130T mutations = signs of oculodentodigital dysplasia Neonatal lethality, congenital heart defects, ventricular Arrhythmia, lens alterations
Cx46	Autosomal dominant	Zonula pulvurent cataract	Nuclear cataract
Cx47	Autosomal recessive	Pelizaeus-Merzbacher-like disease	
Cx50	Autosomal dominant	Zonular pulvurent cataract	Nuclear cataract and microphthalmia
	Autosomal dominant	Nuclear progressive cataract	
	Autosomal dominant	Nuclear pulvurent cataract	
	Autosomal dominant	Microcornea with cataract	

The lines in bold outline the alterations in the gene of mouse Cx36 and Cx40, which are the main topic of this review.

(293, 369), it is still uncertain which second messengers are implicated. cAMP has received much attention (108, 293, 369, 505, 514), and the participation of other common mediators of hormonal action, such as Ca^{2+} or IP_3 , cannot be excluded (78, 85–87, 443, 453). A comparable mechanism may be implicated in the suggested role of gap junctions in the in vitro enhancement of cell resistance to both viral infections and damage by cytokines (33, 148, 423). In this case, gap junctions may increase the buffer capacity of a tissue by in-

creasing the total cytoplasmic volume of the communication compartment, hence diluting any toxic agent.

4. Muscular contraction

Regulation of contractile activity and its synchronization over long distances is a prerequisite for the proper functioning of smooth muscles throughout the digestive, respiratory, urinary, vascular, and genital tracts. Although such

control depends on the proper cell-to-cell propagation of action potentials, which helps to synchronize cytoplasmic Ca^{2+} increases in multiple cells, the mechanism whereby such synchronization is achieved is still the subject of controversy (104, 575). Studies conducted on uterine and vascular smooth muscles have provided strong evidence that this synchronization involves gap junctional communication. The uterus exhibits spontaneous contractile activity which, in both pregnant and nonpregnant females, is maintained relatively quiescent up to labor, at which time it markedly increases in strength, duration, and coordination to permit effective parturition (157, 575). In several animal species, including humans, gap junctions and their constitutive connexins are sparsely represented among cells of prelabor myometrium (155–158, 528), a tissue which, at this time, also shows limited coupling, poor spatial propagation of action potentials, and asynchronous contractions (157, 158). Gap junctions as well as electrical and metabolic coupling markedly increase, due to a differential modulation of different connexin isoforms between uterine muscle cells during labor and delivery (35, 155–158, 386, 447, 528). At this time, action potentials and contractions spread across much larger distances, become synchronized, and intensify (575). In vitro and in vivo studies have shown that the gap junction, coupling, and contraction changes are all controlled by similar modifications in steroid hormones, prostaglandins, and their receptors (123, 155–158, 320, 386), a major indication for their likely endogenous regulation under physiologically relevant conditions. Moreover, transcription of Cx43 is upregulated by estrogen due to the presence of a specific responsive element on the promoter region of this gap junction gene (587).

Functional gap junctions have also been demonstrated between smooth muscle cells of arterioles as well as in both resistance and conduit vasculature (141, 197, 509), and numerous studies have documented the presence of Cx43 as well (36, 85, 141, 197, 509). Because vascular smooth muscle cells do not readily generate propagated action potentials (36, 85, 351), the coordination of contraction, relaxation, and other functions may be largely mediated by the intercellular exchange of second messengers, presumably through gap junctions (87).

5. Metabolic cooperation

Since the unraveling of a gap junction-mediated pathway by which cells equipped with hypoxanthine phosphoribosyltransferase permit the survival in cocultures of mutant cells deficient in this enzyme (524, 525), the mechanism of metabolic coupling has been shown to have multiple implications. Thus, at least in vitro, several other enzymatic and cofactor defects have been shown to be compensated through the direct cell-to-cell exchange of appropriate metabolites (218). That this compensation actually takes place in vivo has so far been shown in the lens, where connections between epithelial and lens fiber cells appear responsible for

metabolite supply throughout this avascular tissue (175). Clinical trials against tumors have begun in which retroviral vectors expressing the herpes simplex virus thymidine kinase gene HSV *tk* have been introduced into tumor cells (270). Infected cells expressing HSV *tk* are then killed by exposure to ganciclovir, a guanosine analog which is metabolized to a phosphorylated product by HSV but not mammalian thymidine kinase. This phosphorylated product is incorporated into nascent DNA molecules, stopping synthesis in proliferating cells. Remarkably, as few as 70% of the tumor cells need to be infected to make the whole tumor sensitive to ganciclovir (101). Conferral of ganciclovir sensitivity to uninfected cells is termed the “bystander effect,” which has been variably attributed to secretion of toxic factors, disruption of blood supply to the tumor in vivo, apoptosis followed by uptake of toxic metabolites, initiation of immune response in killed cells (253), and metabolic cooperation (31, 253, 270). There is compelling evidence that the 300-Da ganciclovir metabolite formed in HSV *tk*⁺ cells can be transferred to adjacent, noninfected cells, thereby killing them as well (31, 113). Moreover, the bystander effect is much reduced in cells deficient in gap junction expression (140) and, in vitro, is conferred upon expression of Cx32 or Cx43 in communication-deficient cells (129, 352), and in vivo after Cx37 transfection (558). In at least one model system, the expression of gap junctions between infected and noninfected cells was found to protect cells from ganciclovir-induced death (576). Also, whereas expression of gap junctions appears to mediate the bystander effect in ganciclovir/*tk*⁺ effects, other enzyme/drug systems do not appear to involve gap junctions (294).

6. Exocrine secretion

Connexins are also obligatory features of adult secretory cells of endocrine and exocrine glands, whichever the type of secretory product, the mechanism of its release, and the pattern of regulation by stimuli and inhibitors (342, 346, 347, 356, 367, 480). A large body of circumstantial evidence indicates that connexins, and/or the junctional coupling that these proteins mediate, are not necessary for secretion to occur but are required for its fine regulation, in terms of biosynthesis, storage, and release of many secretory products (36, 85, 142, 197, 509). Endocrine and exocrine cells have selected distinct, and to a large extent alternative, patterns of connexins to achieve this regulation (55, 338, 341, 347, 356, 480), via a differential transcriptional control of the promoters of various connexin genes (325, 438). If there is not a specific “endocrine” and “exocrine” connexin, there is certainly a highly conserved pattern of expression that varies in the two main types of secretory systems (341, 356, 480) (TABLE 3). When Cx32 was expressed ectopically, either by knock-in of Cx32 at the locus which codes for Cx43 (411), or by transgenic overexpression of the cognate cDNA in a cell type that only expresses Cx36 (79), altered function of the targeted exocrine and endocrine glands was observed, indicating that specific con-

Table 3. *The connexins of endocrine and exocrine glands*

Type of Secretion	Gland	Cell	Hormone	Connexin
Endocrine	Hypothalamus	Neurons	GnRH, CRH, TRH, GHRH, somatostatin, PIH, PRF, . . .	Cx36
	Pineal	Pinealocytes	Melatonin, serotonin	Cx26?, Cx32?
	Hypophysis (anterior)	Acidophil, basophil, and chromophobe cells	GH, prolactin, FSH, LH, TSH, ACTH, β -lipotropin	Cx43
	Neurohypophysis	Neurons of paraventricular and supraoptic nuclei	Oxytocin, vasopressin	Cx43
	Thyroid	Follicular cells C cells	T_3 , T_4 Calcitonin	Cx43, Cx32, Cx26 Cx43
	Parathyroid	Principal cells	Parathormone	Cx43
	Heart	Auricular myoendocrine cell	ANH	Cx43, Cx45
	Pancreas	β-Cells	Insulin	Cx36, Cx43, Cx50
	Kidney	Myoepithelial cells of afferent arteriole	renin	Cx40
	Adrenal	Medullary cells Cortical cells	Epinephrine, norepinephrine Corticoids, mineralocorticoids, androgens	Cx36 Cx43s
	Testis	Leydig cell	Testosterone	Cx43
	Placenta	Throphoblast cells	hCG	Cx43
	Ovary	Luteal cells	Progesterone	Cx43
	Placenta	Throphoblast cells	Chorionic gonadotropin, sommatomammotropin	Cx43
Pheromonal	Skin	Sebaceous cells	Several in sebum	Cx43
	Preputial glands (rodents)	gland cells	Several farnesenes	Cx43
Endocrine-exocrine	Ovary	Granulosa cells	Estradiol	Cx43, Cx37
	Liver	Hepatocyte	Biliary acids, bilirubin, glucose, proteins	Cx32, Cx26
Exocrine	Lacrymal glands	Acinar cells	Water, ions, peptides, (glyco)proteins	Cx26, Cx32
	Salivary glands	Acinar cells	Amylase, lipase, ions, mucins	Cx26, Cx32
	Mammary glands	Alveolar cells	Casein, lipids, ionsimmunoglobulins, other proteins	Cx26, Cx30
	Stomach	Parietal cells	Water, intrinsic factor, HCl, HCO_3^-	Cx26, Cx32
	Pancreas	Acinar cell duct cells	Amylase, lipase, and other digestive enzymes Water, bicarbonate, and other ions bicarbonate	Cx26, Cx32 Cx45?
	Prostate	Alveolar cells	Fibrinolysin, enzymes, peptides	Cx26
	Ovary	Granulosa cells	Water, ions, glycoproteins	Cx37
	Testis	Sertoli cells	Water, ions, inhibin, activins, . . .	Cx43

The lines in bold outline the situation of pancreatic β -cells and kidney renin-producing cells, which are the main topic of this review.

nexin patterns are required for proper *in vivo* secretion. In view of recent studies relating different connexin isoforms to different conductance, permeability, and regulatory characteristics of gap junctional channels (201, 211–213, 554, 555), it is most likely that this differential selection happened during evolution to match the requirements for specific gap junction-permeant molecules to the various needs of different secretory cell types. An additional regulatory event is indicated by the alterations in the levels of coexpressed connexins after manipulation of the expression of only one of the genes. Deletion of the basic helix-loop-helix transcription factor *Mist1* resulted in decreased transcription of the *Cx32* gene (438). It also resulted in decreased levels of *Cx26*, which were not paralleled by a change of *Cx26* mRNA, indicating differential transcriptional, translational, and/or posttranslational control of these two connexins, which are coexpressed by the acinar cells of several exocrine glands (74, 341, 356, 480). Deletion of the *Cx32* gene also resulted in altered membrane levels of *Cx26* (74), indicating that some stringent, still to be defined mechanism, links the expression of different connexins in various secretory cells (341, 347, 356, 480). At present, no human disease resulting from a severe secretory defect has been directly linked to either connexin dysfunctions or mutations. However, *Cx36* has been shown to modulate the survival of the insulin-producing β -cells of pancreas, and to protect them against the immune cytokines that induce β -cell apoptosis at the onset of type 1 diabetes (5, 265).

In all the multicellular exocrine glands studied so far (endocrine glands are discussed in sect. IV), secretory cells express *Cx32*, which is often coexpressed with *Cx26*, whichever the type of secretion they produce, whereas *Cx40*, *Cx43*, and *Cx45* connect the vessels as well as connective and ductular cells of the glands (341). Acinar cells form the exocrine portion of the pancreas (~99% of the adult gland volume) and secrete a mixture of ~20 (pro)enzymes. These cells are extensively coupled through *Cx32* and *Cx26* junctional channels (73–76, 341, 401, 402). Knockout mice lacking *Cx32* display a significant decrease in acinar cell coupling, which, however, was not abolished due to the persistent, though reduced, expression of *Cx26* (73). This change in connexin expression was associated with an increase in the nonstimulated secretion of amylase and in the circulating levels of this digestive enzyme, but did not affect the maximal stimulation of amylase secretion induced by acetylcholine (73, 338). The latter finding is congruent with the physiological uncoupling that this cholinergic neurotransmitter, which is the main natural stimulus for the release of pancreatic (pro)enzymes, induces in the acinar cells of control rodents (401, 402). Further studies have provided insights to explain how a neurotransmitter can stimulate amylase release, which implies the recruitment of increased numbers of secreting cells (43, 48), while causing uncoupling. Immediately after binding to acinar cell receptors, acetylcholine

induces in pancreatic acini waves of cytosolic free Ca^{2+} that propagate from cell-to-cell by a mechanism partly dependent on junctional channels (76), and leads to the recruitment of increasing numbers of actively secreting cells (43, 48). Thus *Cx32/Cx26* coupling is initially required for the spreading across pancreatic acini of the second messengers that are generated as a result of the agonist stimulation. This is physiologically relevant inasmuch as only some acinar cells directly contact a cholinergic terminal *in situ*, and, taken individually, are directly stimulated by acetylcholine *in vitro* (43). In this setting, coupling compensates for this native functional heterogeneity (41, 46), thus ensuring the stimulation of sufficiently large numbers of cells, while presumably retaining low the basal secretion of the system. Subsequently, however, when the Ca^{2+} increase induced by acetylcholine has reached its maximum (76, 401, 402), acinar cells become uncoupled. While the biological significance of this uncoupling remains to be established, the finding that the secretory alterations observed in *Cx32*-knockout mice, *i.e.*, increased basal release of amylase and unchanged maximal release of the enzyme during stimulation, are mimicked by an acute exposure of acini to uncoupling drugs, indicates that the physiological, acetylcholine-induced uncoupling depends on the gating of existing connexin channels, rather than on the levels of either the *Cx32* or *Cx26* proteins (76). Together, these studies have provided direct evidence that *Cx32*-dependent coupling is important for a normal basal secretion of pancreatic enzymes and that this function cannot be supported by *Cx26* channels. They further indicate that the coupling signaling is sufficiently important to affect the *in vivo* functioning of the exocrine pancreas (56, 77). The persistence of coupling at the beginning of this stimulation presumably allows for the transmission of a threshold signal produced in few acinar cells to all other cells of each pancreatic acinus, thus ensuring the functional recruitment of many additional secreting cells (43, 48). The subsequent uncoupling may be needed to terminate the agonist action, as indicated by the decrease in the junctional conductance of acinar cell pairs after the agonist-induced Ca^{2+} peak (76). However, it is equally possible that some of the endogenous molecules that permeate connexin channels may negatively control the secretion of enzymes produced by acinar cells. By hindering the intercellular exchange of these signals, uncoupling could then allow for a number of acinar cells to escape inhibition and enter activated secretion. Eventually, uncoupling may be needed to functionally isolate those acinar cells that are highly sensitive to secretagogues, to prevent the dilution of the agonist-induced second messengers into less sensitive cells of the acinus (43, 48, 77). It has indeed been shown that, when exposed simultaneously to a secretagogue, individual acinar cells show a rather variable secretory function and that this functional heterogeneity decreases as acinar cells aggregate and express connexins (43, 48). A similar situation may apply to the acinar cells of lacrimal glands. Thus individual cells of the major salivary glands show a heteroge-

neous function (475), become uncoupled during stimulation by cholinergic agonists (401, 402, 418, 454), and show inhibited secretion after drug-induced uncoupling (258). The specificity of this effect has not yet been tested using mice invalidated for individual connexins. In exorbital lacrimal glands, the uncoupling of acinar cells as a result of the invalidation of the Cx32 gene, selectively decreases their fluid secretion in response to low, but not to high doses of carbachol, and only in female mice (564).

G. The Pharmacology

So far, what has been learned about connexin biology has been mostly derived from genetic manipulations which, *per se*, complicate the elucidation of the physiological roles of connexins under physiological *in vivo* conditions. The genetic strategy has been imposed by the lack of drugs that would specifically and directly affect connexin channels. Thus we do not yet have molecules to acutely modify the conductance and permeability of connexin channels (453, 513), possibly because the portions of both the two-cell intercellular channels and the single-cell “hemi-channels” that would need to be targeted, are intracellular (FIG. 8). As it matters, we also do not have drugs to more chronically affect the expression and steady-state levels of connexins, without exerting several pleiotropic effects on other channels or endogenous key molecules and signaling pathways. These considerations contrast with over 2,000 reports on a large variety of molecules that indirectly affect connexins, the functioning of the channels/hemi-channels these proteins make, and/or the independent signaling pathways with which connexin signaling interacts. Thus it is not surprising that drugs affecting the intracellular ionic and metabolite concentrations, hence altering the electrochemical gradients which are the driving force for coupling, affect this event even though they may not directly affect connexins (380). Similarly, all drugs affecting cytoskeleton components and/or altering the phosphorylation of multiple proteins (9) would be anticipated to indirectly affect the number and/or function of connexin channels, in parallel to pleiotropic effects on many other important cell functions (290, 380, 450, 513).

Other drugs and reagents have been recently reported to have a more connexin-targeted action, as judged by a slow modulation of the gating mechanisms that controls the open/close state of connexin channels (118, 209, 450, 513). All these drugs (long-chain alkanols, volatile anesthetics, α -glycyrrhetic acid and derivatives, carbenoxolone, quinine and derivatives, polyamines, fenamates) induce an acute and usually reversible blockage of connexin channels. However, none of these “blockers” has a sufficient specificity for a given connexin (eventhough the dose-response curve may differ somewhat for certain connexin isoforms), which is a major drawback in most cell systems in which multiple connexins, each with a specific function, are coex-

pressed (200, 211–213). Also, these drugs certainly affect other types of channels, as well as unrelated proteins and membrane lipids (which is the case for the numerous lipophilic “blockers”), thereby preventing a definitive, unambiguous identification of those effects which may be solely attributable to connexin changes. Eventually, several of these drugs may not necessarily work, at least under the same concentration and exposure conditions, in all cell types. It is also intriguing that, so far, few connexin channel “openers” have been clearly identified (117, 119, 124, 206, 292, 432), which delays the development of future strategies aimed at developing a connexin-targeted therapy of several diseases, specifically in humans.

It is obvious that the search of molecules interacting directly with connexins, whether these are assembled in “hemi-channels” or cell-to-cell channels, is now a priority of the field. The identification of these drugs requires the development of new methods to rapidly and automatically screen many molecules for their effect on some bona fide connexin-dependent function (18). It will further require the investigation of the candidate drugs *in vivo*, under the conditions in which connexin signaling is integrated with, and likely interdependent on several other signaling mechanisms.

IV. ENDOCRINE GLANDS

A. When, Why, How

The appearance of chemical communication between cells predates multicellularity, as exemplified by the case of cAMP, which acts both as an autocrine signal on single-cell organisms, as well as a paracrine or endocrine signal on multicellular systems (29, 65, 177, 521). The way the same molecule came to act both on the cell that produced it, e.g., for metabolic purposes, and on other types of cells may have been an opportunistic process in which novel structures (receptors, effectors) and new domains (binding and catalytic sites) of an old signaling molecule developed by chance, leading to the regulation of new functions, such as cell growth and proliferation (29, 65, 177, 521). Still, such developments could not cope with the increasing complexification of multicellular systems, which progressively included increasing numbers of cells, differentiating in multiple directions (162, 250). Neither the direct mechanism, which only operates between closely apposed cells, nor most indirect mechanisms, which usually operate between nearby cells due to the rapid binding and/or degradation of the low amounts of signaling molecules that diffuse in the extracellular spaces, could coordinate the function of distant cells. Thus alternatives emerged to bring these molecules close to the target cells, even when these were quite distant from each others within the multicellular community (521). The way multicellularity solved this situation is by imposing the development of both nervous and endocrine systems (16, 20, 39, 40, 65, 177, 521).

The numerous similarities between the two systems, notably the use of several identical signal molecules and of similar signal molecule-receptor interactions in most taxa of the phylogenetic tree, suggest that the endocrine systems of glands and neurons evolved from a common ancestor, most likely a pheromone system of some unicellular whereby a gas or liquid product leaves a cell to affect another organism (16, 33, 34, 302–304, 433–436, 459, 521). Presumably hormones were born by chance modifications of common metabolites such as amino acids, amines, fatty acids, and cholesterol, and then resulted in the adaptation of target cells when this adaptation resulted in selective advantages (29, 177). With evolution, many of the prokaryotes, plants, invertebrates, and mammalian and nonmammalian vertebrates existing today have come to produce a variety of biogenic amine, peptide, and steroid molecules which, if different in chemical structure and site of production in different phyla, have similar functions, suggesting little evolutionary changes in the type of hormones in many organisms (16, 177, 521). In contrast, there has been a large diversification of the types of cells targeted by the signaling molecules, and, to a lesser extent, of the pathways that are hormonally controlled in these cells (16, 100, 177, 521). This explains how the same signaling molecule may have markedly different effects in different species (177). Thus recent work suggests that preexisting ligand-receptor systems could be bridged to form new regulatory hormonal networks (65). Multicellular invertebrates, such as sponges, hydra, and flies, produce hormonal peptides, quite similar to those of vertebrates, in a variety of individual secretory cells, which are dispersed in different body locations, including the brain in flies. However, these species lack the endocrine glands that are typical of vertebrates. As the latter multicellular organisms developed increasing sizes, the request for hormones increased in parallel, as the signal molecules had to be taken at larger distances and to more target cells. This necessity presumably pressured for the development of multicellular glands, where many cells would concentrate to produce one or several signaling molecules. There is no continuity of structure, function, or location between the endocrine glands which, embryologically originate from all three germ layers (ectoderm and endoderm for peptide-producing glands, mesoderm for steroid-producing glands). Also, there was little adaptation of their structure, as many endocrine glands are functionally and cytologically similar in different phyla, even when their spatial architecture has undergone major variations (29, 136, 177, 552), providing evidence for convergent evolution.

B. Hormone Secretion Is a Multicellular Process

It is apparent from the above that the phylogeny of endocrine glands recapitulates several steps of the evolution of multicellularity, notably the essential need for intercellular communication to ensure a proper, coordinated function of

the endocrine secretory cells that are vital for mammal survival. Secretion of all vital hormones is a multicellular process, resulting from the synchronized function of numerous endocrine cells, since no individual cell can produce, at any given time, the large amount of hormones that is required to ensure vascular transport across a large organism, and to regulate the numerous cells of several peripheral target organs. Hence, endocrine cells making a specific hormone should coordinate their activity with that of companion cells, especially if they all do not express the full pattern of transporters, receptors, and effector molecules that are required to couple the stimulus by an extracellular signal to the secretory response. When endocrine glands contain different cell types, producing agonistic or antagonistic hormones, such a coordination should further integrate the activity of these different cell types, which may not function synchronously and/or at the same rate (19, 71, 334, 335, 376). Eventually, since most hormonal secretions undergo regular fluctuations, as a function of circadian and other rhythms, and are affected by changes of the milieu intérieur, the extracellular milieu of the organism and the environment, this coordination should be continuously adapted, on a moment-to-moment scale, to provide a hormone output commensurate with the ever-changing needs of the organism.

Endocrine cells comply with these requirements, by using in parallel, though not necessarily simultaneously, all the indirect and direct forms of cell-to-cell communication discussed above (19, 71, 334, 339, 376) in patterns which differ, from gland to gland, with regard to the quantitative importance of each mechanism and its hierarchical position relative to the others. This organization results in a complex network of different regulatory systems, each with its own specificity, that largely overlap and, at times, interact with others to promote or decrease their influence. Most likely, this redundancy was pressure selected by the vital necessity of some of the hormones, e.g., insulin. Providing the cells that are making this peptide with many regulatory systems, presumably ensures that the biosynthesis, storage, and release of insulin are maintained within a life-sustainable range under most conditions, including when individual regulatory mechanisms may have been lost. In addition, the integrated network of distinct communication mechanisms, each resulting in quantitatively and qualitatively different effects, further provide the endocrine cells with a much more sensitive and graded way, in both temporal and spatial terms, to control their functioning, that would be possible using a single, on/off type of regulation.

In this context, there is now ample structural, biochemical, and physiological evidence that connexins are obligatory components of all vertebrate endocrine glands, in which these proteins mediate the electrical and metabolic coupling of hormone-producing cells (334, 335, 344, 356, 367, 480). The further realization that the pattern of connexins differs

in different endocrine glands, and that this tissue-specific distribution is conserved across vertebrate species, including humans, indicates that the connexin signaling may not be dispensable (334, 335, 343, 344, 356, 367, 478–480).

C. Different Endocrine Glands Express Different Connexins

The secretory cells of most endocrine glands usually express one connexin isoform of either the α or the γ group (FIG. 2, TABLE 3). Cx43 is the most widespread isoform. A few endocrine cells and most endocrine neurons have selected Cx36, whereas Cx40 is so far restricted to the renin-producing cells of kidney (FIG. 2, TABLE 3). With the notable exception of the thyroid, which embryologically develops as an exocrine gland and maintains throughout life an architectural organization reminiscent of this primordial exocrine function (16, 303, 343), no endocrine cell expresses Cx32, even though a few of these cells have selected Cx26, another connexin of the β group (FIG. 2, TABLE 3). Conversely, the secretory cells of exocrine glands usually express various combinations of β connexins, usually Cx32 (343). This different choice is due to a promoter control. Indeed, the 5' regulatory region of the human Cx36 gene features a region containing a conserved neuron-restrictive silencer element, which binds the NRSF/REST factor. NRSF/REST is widely expressed in most cell types, in which it represses the expression of neuronal genes, but insulin-secreting cells and neurons, which therefore express Cx36. In support of this mechanism, the ectopic expression of NRSF/REST reduces that of Cx36 in insulin-producing cells (324). Thus a differential transcriptional control of connexin genes accounts for the different expression of gap junction proteins by endocrine cells (343). Much less understood is the reason why other endocrine cells have selected different connexins, since this choice cannot be directly related to the embryological origin, the chronology of development, the architecture of the adult glands, the biochemical nature of the hormones produced, the rate of their secretion, and the control of their release and biosynthesis. A plausible explanation is provided by the findings that different connexins form channels with distinct conductance, permeability, and regulatory characteristics (30, 80, 169, 170, 201, 375, 554, 555). Thus it is likely that, during evolution, endocrine cells selected the sets of channels that, by favoring the intercellular exchange of specific signals while preventing the diffusion of others, better fitted the requirements of their own secretory machinery. As these requirements remain to be established, one has to start from the distribution of different connexin species in different glands, secreting either peptide, glycoprotein, steroid, or amine hormones.

1. Peptide-producing endocrines

The insulin-producing β -cells of pancreatic islets are coupled exclusively by Cx36 channels in rodents and humans

(111, 478, 479, 538). Several lines of evidence, discussed in detail in section V, indicate that this protein and/or the β -to- β -cell coupling it establishes is involved in the control of insulin secretion (19, 71, 334, 335, 339, 376). Such an involvement has been less investigated in other peptide-producing endocrine glands. Pinealocytes have been reported to be coupled by Cx26 channels, which are upregulated in vitro by norepinephrine, possibly to improve melatonin secretion (441). Hypothalamic neuroendocrine cells are coupled (328, 329, 532) by Cx36, the only connexin which has so far been convincingly shown to be expressed at neuronal gap junction plaques (373, 419). Electrophysiological studies testing gap junction blockers have indicated that this coupling is implicated in the control of the pulsatile release of several neuropeptides, including growth hormone releasing hormone (486), gonadotropin releasing hormone (553), and luteinizing hormone releasing hormone (535). Eventually, the endocrine cell types that form the anterior pituitary are coupled (137, 305, 359) via channels made predominantly of Cx43 and Cx26 (341, 580). This coupling increases the intercellular synchronization of Ca^{2+} transients (187). Ca^{2+} waves are also propagated throughout the entire gland via the network of folliculo-stellate cells, which are extensively coupled by Cx43 channels (137, 487). Seasonal changes in the expression of pituitary Cx43 have been associated with changes in prolactin secretion (556).

2. Glycoprotein-producing endocrines

The follicular cells of thyroid are coupled by Cx32-, Cx26-, and Cx43-made channels (188, 341). Cx32 and coupling are lost with passages in culture, together with the ability of the cells to form follicular structures (166, 180, 181, 364, 365). These alterations can be reverted by exposing cell monolayers to thyroid stimulating hormone (TSH) (365) or by transfection of communication-incompetent thyrocytes with Cx32, but not Cx43 (545). While these data implicate Cx32 in the morphogenesis of thyroid follicles, no obvious thyroid defect has been reported in mice knocked out for the Cx32 gene (219, 374). The involvement of connexins in thyroid secretion is supported by the observation that TSH stimulation increases the coupling of thyrocytes in a time- and concentration-dependent manner (364), whereas loss of coupling due to a Cx32 mutation reduces the release of thyroxine (180). Transfection of Cx32 in thyroid cell lines resulted in increased expression of the thyroglobulin gene (516), whereas loss or overexpression of Cx32 modulated the growth of the thyroid gland in vivo (414).

Cx43 connects the cells of placental cytotrophoblast, between themselves and with those of the syncytiotrophoblast (98, 99, 427, 571). The pharmacological blockade of gap junctions as well as the antisense interference with the Cx43 transcript uncouple cytotrophoblast cells, impairing their fusion and thus the formation of the syncytiotrophoblast (98, 99, 151). These alterations were associated with a de-

crease in both the expression of trophoblast-specific genes, including those coding for β -human chorionic gonadotropin and human chorionic somatomammotropin (98, 99, 151), and the secretion of the former placental hormone (151).

3. Steroid-producing endocrines

The endocrine cells of the adrenal cortex are coupled by Cx43 channels (343, 368, 370), particularly in the glucocorticoid- and the androgen-producing regions (107). In vitro experiments testing drugs thought to block Cx43 channels have documented an impaired secretion of cortisol from clusters of adrenal cells, but not single cells, stimulated by ACTH but not cAMP (366). In agreement with these findings, transfection of adrenal cells with a Cx43 antisense construct also resulted in the inhibition of ACTH-induced steroid secretion (389). The data are consistent with the observation that ACTH increases the expression of Cx43 both in vivo and in vitro (368). However, and unexpectedly, another gap junction blocker was reported to stimulate steroid production, via the activation of both an extracellular signal-related kinase and a calcium/calmodulin-dependent kinase, i.e., by two pathways distinct from the protein kinase A-dependent pathway, which normally controls steroidogenesis of adrenal cells (223).

Cells derived from the testosterone-producing Leydig cells of testis express Cx43 and are coupled in culture, until the cells reach confluence. Thereafter, Cx43 expression and coupling decrease, presumably as a result of activation of pathways dependent on PKA and PKC (171). A comparable decrease in coupling was observed during luteinizing hormone (LH)-induced stimulation of testosterone secretion, suggesting a tonic inhibitory influence of connexin signaling on the output of the steroid hormone (171).

Cells of the ovarian corpora lutea are also connected by Cx43 (252, 330). Pharmacological treatments enhancing this coupling increase progesterone secretion, whereas treatments uncoupling luteal cells decrease steroid release (179). Consistent with a relationship between connexin signaling and steroid production, the experimental inhibition of Cx43 expression decreased the LH-induced steroid secretion of luteal cells (251, 252). Strikingly, mice lacking Cx37, the connexin that forms gap junctions between oocytes and granulosa cells, develop numerous abnormal corpora lutea (489). The thecal cells of ovarian follicles, which derive from the endocrine differentiation of the granulosa layers, are also connected by Cx43 (438, 489). The expression of this connexin varies with the stage of follicle development, consistent with an involvement of connexins in the endocrine function of thecal cells (239, 254, 268).

4. Catecholamine-producing endocrines

Chromaffin cells of the adrenal medulla are joined by Cx36-made gap junctions (93, 111, 322), which account for the

cell-to-cell spreading of the $[Ca^{2+}]_i$ transients driven by action potentials (93, 322, 323). This spreading is enhanced after exposure of a few cells to nicotine, which also triggered the release of catecholamines, indicating that connexin signaling can amplify the secretion of epinephrine and norepinephrine induced by the synaptic activation of individual cells (322). Interestingly, both the pharmacological blockade of synaptic transmission as well as the surgical denervation of the adrenal glands resulted in increased coupling of chromaffin cells, an effect which was also observed in newborn rats at a time when the synaptic transmission of the adrenal gland had not yet fully matured (323). These results indicate that the Cx36-dependent signaling of chromaffin cells is tonically inhibited by cholinergic synaptic inputs (323).

5. Pheromone-producing endocrines

Pheromone-producing glands, the most likely phylogenetic precursors of vertebrate endocrine glands, express unusually high levels of Cx43 (341), like their human sebaceous gland counterparts (451). While this abundance would suggest some necessary role, in view of the quite short half-life of Cx43, no study has yet tested this possibility.

6. Connexins and hormone action

Most hormone targets are at a sizable distance from the gland that produces the endocrine products, usually preventing the establishment of connexin-dependent signaling between the secretory cells and the cognate effector cells. However, connexins may be involved in the cross-talk between these two cell types in the case of paracrine signaling, whereby an endocrine cell releases a hormone that acts on nearby cells. An example is provided by pancreatic islets, in which the insulin released by β -cells acts on receptors on the closeby, sometimes adjacent α -, δ -, ϵ -, and PP-cells, and, vice versa, glucagon and somatostatin act on receptors of β -cells (249, 393). These different islet cell types have been reported to be joined by gap junctions (382, 383), but to be exceptionally electrically coupled (247, 333, 336, 337, 346, 350, 415). To date, no study as yet provided direct evidence that this heterologous coupling is due to connexin channels, and modulates the endocrine function of pancreatic islets. Another challenging organ is the renal juxtaglomerular apparatus, the endocrine system which plays a central role in the control of blood pressure, and which comprises many interacting cell types (399). The renin-producing cells of the afferent arteriole, which are central players of this apparatus, share Cx40 junctions with both adjacent endocrine cell and endothelial cells (193, 561, 563). As discussed in section VI, rats made hypertensive after clipping of one renal artery showed a selective increase in kidney renin and Cx40, implying that the connexin is implicated in the control of renin secretion and/or in the vasomotor control of kidney vessels (193, 561, 563). Thus connexin signaling may inte-

grate the chemical and mechanical signals that concur to control blood pressure in this renin-dependent model. Since this model also results in changes of aortic Cx43 (191, 192, 197), we revisited it using transgenic mice in which the coding region of this connexin was replaced by that of Cx32 (411). As expected, wild-type and heterozygous mice, which showed a normal distribution of Cx43, became hypertensive as a result of increased plasma renin levels. In contrast, homozygous littermates, in which Cx32 had replaced Cx43, retained a normal blood pressure and control levels of circulating renin (194), providing additional support for a mechanism whereby altered connexin signaling between endothelial cells modifies the functioning of the renin-secreting cells. The case of the juxtaglomerular apparatus may not be unique. Thus, in the pituitary, the nonendocrine folliculo-stellate cells cross-talk with distant endocrine cells by establishing gap junctions that allow for a rapid and extensive propagation of waves of cytosolic calcium (137). This arrangement provides an efficient mechanism to orchestrate the function of the endocrine cells that are scattered throughout the gland (137).

Analysis of transgenic mice lacking one or two of the connexins that are normally expressed by endocrine cells have failed to reveal obvious alterations in the prenatal development, morphogenesis, and differentiation of endocrine glands (183, 189, 219, 537). However, altered growth of the endocrine pancreas was detected by morphometric analysis and measurements of hormone content in transgenic mice whose insulin-producing β -cells were selectively forced to ectopically express Cx32 (79). The data suggest a role of connexin-dependent signaling in the postnatal growth of pancreatic islet cells, a hypothesis verified in still another transgenic mice model in which β -cells were selectively induced to overexpress the native Cx36 (5, 265). Transfection of Cx32 in thyroid-derived, communication-incompetent cell lines markedly reduces their proliferation rate (545), whereas restoration of intercellular communication by stable expression of Cx43 did not modify the growth of the same cell types (147). This study also indicates that the slow-down of cell proliferation induced by Cx32 was accounted for by a prolonged G_1 phase, possibly mediated by proteins of the Cip/Kip cyclin-dependent kinase inhibitor family (147). Adrenocortical cells normally respond to ACTH stimulation by increasing steroid production and Cx43 expression, and by decreasing their growth (368). Pharmacological inhibition of their gap junctions produces the opposite effect, i.e., increases cell growth and decreases ACTH-stimulated steroidogenesis, without altering the levels of Cx43 (482). Furthermore, exposure of adrenal cells to a Cx43 antisense cDNA that reduced these levels also resulted in a decreased steroidogenic response, and in an increase in the cell growth rate (482). Thus, in different endocrine cells, connexins contribute to control hormone biosynthesis and release, as well as the size of the endocrine cell population. As yet, however, the relevance of

the later control for primary endocrine cell in vivo has only been shown in the case of pancreatic islets (79). Other experiments have revealed that early-passage cultures of rat thyroid cells, which express functional Cx32 channels, are more resistant to γ irradiation than later-passage cultures, which express a truncated, nonfunctional form of Cx32. Strikingly, this beneficial effect was not observed after proton irradiation (181). These observations may be related to those made on insulin-producing β -cells after exposure to drugs that experimentally reproduce the massive cell death observed in pancreatic islets at the onset of type I diabetes (5, 265). The data indicate a direct protective role of Cx36, Cx32, and Cx43 in vitro and, more importantly, provide the very first evidence that this protection operates also in vivo. Thus, whereas transgenic mice overexpressing either the native islet Cx36 or the islet ectopic Cx32, were fully protected against the cytotoxic effects of streptozotocin and alloxan, had a normal insulin content, and remained normoglycemic, transgenic mice lacking Cx36 were highly sensitized to the effects of the drugs and became rapidly hyperglycemic due to loss of most β -cells and pancreatic insulin (5, 265). Strikingly, this sensitization appears to reflect a gene-dosage dependence, inasmuch as heterozygous Cx36-KO mice showed many alterations intermediate between those of wild-type littermates expressing normal levels of the connexins and homozygous littermates lacking the protein (5, 265). Together, these data indicate that connexin channels significantly contribute to the resistance of endocrine cells to cytotoxic conditions, either by enhancing their resistance to damaging conditions and/or by favoring an efficient cell repair after the insult.

7. Hormonal control of connexin expression

Several hormones have been reported in a variety of models to affect the transcription, mRNA stability, translation and cytoplasmic trafficking of connexins, as well as the gating and regulation of the cell-to-cell channels formed by these proteins. The effects observed in different studies are variable, and sometimes opposite, presumably reflecting the essential influence of various cell types, physiological states at the time of the experiment, and different connexin patterns (334, 335, 344, 367, 368, 443, 514). Implantation of mammalian embryos in the uterus occurs in the so-called receptive phase, during which steroid hormones and local embryonic signals suppress the expression of endometrial Cx26 and Cx43. Later, connexin expression is locally reinduced by the implanting blastocyst, presumably as a result of factors secreted by the growing trophoblast (184, 185, 186). Ovariectomized rats treated with different ratios of 17β -estradiol and progesterone revealed a dose- and time-dependent regulation of the expression of Cx26 (the main connexin of the endometrial epithelium) and Cx43 (the prominent connexin of uterine stroma and myometrium) but not Cx32, indicating that hormonal ratios mimicking pregnancy conditions differentially regulate α - and β -type connexins (185). During preimplantation, this induction is

due to estrogens, via the activation of a pathway initiated at α -estrogen receptors. However, during the subsequent embryo implantation and decidualization, endometrial connexins are upregulated by an estrogen receptor-independent pathway, possibly implicating catechol estrogen, prostaglandin $F_{2\alpha}$, and interleukin- 1β (184). LH and follicle-stimulating hormone control the coupling of ovarian thecal cells, by regulating Cx43 expression at the transcriptional, translational, and posttranslational levels (178). Strikingly, the effects of the two gonadotrophins vary as a function of the developmental stage of the ovarian follicles (239). Throughout the estrous cycle, luteal cells are coupled by Cx43-made channels that are modulated by agonists and antagonists of cytosolic calcium, cAMP, and PKC (179). Thus posttranslational phosphorylation of Cx43 is likely to be relevant for the function of luteal cell channels, as it is the case in many other cell types (442, 444, 566). The levels of Cx43 increase with gestational age in the adrenal cortex of sheep fetuses, and cortex size is stimulated by ACTH, but not dexamethasone infusion (331). Under these conditions, the cellular distribution of Cx43 is also altered, possibly implicating connexin signaling in the long-term effects of ACTH, which are critical both for the maturation of adrenal glands and to initiate parturition (331). Administration of steroids to castrated rats resulted in different changes in the number of gap junctions connecting folliculo-stellate cells of the anterior pituitary, depending on the type of steroid tested and the sex of the animals (449). In a parallel study, hydrocortisone was further reported to delay gap junction formation, a change that was concomitant with an altered phenotype of folliculo-stellate cells (450).

This set of data provides compelling evidence that connexins participate to the function of endocrine glands, and that hormones control the expression of connexins. However, only in a few glands have experiments documented that connexins are necessary and sufficient to regulate major, selected aspects of endocrine gland function (344, 347, 356, 374, 480), including the biosynthesis, storage, and release of various hormones; the differentiation and distribution of the endocrine cells; and the proper action of the chemical messengers on their target cells (344, 347, 356, 367, 374, 401, 402, 480). Two systems in which such a comprehensive experimental approach has been undertaken are the insulin-producing β -cells of the endocrine pancreas and the renin-producing juxtaglomerular cells of the kidney cortex.

V. THE INSULIN-PRODUCING β -CELLS

Insulin is a peptide hormone secreted by the coordinated activation of numerous β -cells, which form the bulk mass of the endocrine pancreatic islets of Langerhans. Each islet is an ovoid mass of 50–600 μm in diameter, which comprises \sim 50–3,000 cells, of which β -cells represent \sim 60% in hu-

mans. The remaining cells are glucagon producing α -cells, somatostatin-producing δ -cells, pancreatic polypeptide PP-cells, ghrelin-producing ϵ -cells, endothelial cells, some fibroblasts, and some passengers lymphocytes and macrophages (49). In humans, about a million of such islets are dispersed in the pancreas, of which they collectively form the endocrine portion, which represents \sim 1% volume of the adult gland (49).

A. The Endocrine Function

Insulin secretion is a multicellular event resulting from the coordinated activation of the numerous β -cells that are dispersed in pancreatic islets. The collective output of these microorgans is pulsatile and undergoes cyclic fluctuations over time (25, 28, 166, 190, 204, 421, 534). Islets are separated from each other by connective tissue, and the acini of the exocrine pancreas, and their coordination thus depends on a large variety of hormonal and neural control. Thus insulin secretion is experimentally stimulated by gastric inhibitory peptide, thyrotropin-releasing hormone, glucagon-like peptide 1, β -adrenergic agonists, acetylcholine, cholecystokinin, gastrin-releasing peptide, and vasoactive intestinal polypeptide (27, 409, 515, 572). Conversely, inhibition is induced by corticotrophin-releasing factor, peptide YY, atrial natriuretic peptide, pancreastatin, α_2 -adrenergic agonists, galanin, neuropeptide 1, and somatostatin (27, 409, 515, 572). In vivo, the secretory response of β -cells, which is initiated by circulating nutrients, mostly glucose, is essentially modulated by gut hormones, specifically gastric inhibitory peptide, glucagon-like peptide 1, cholecystokinin, and somatostatin (27, 409, 515, 572). In vitro, pulsatility of insulin secretion is preserved in the absence of hormonal circulation (28, 166, 176, 515), further indicating that some intrapancreatic nervous system helps coordinate the individual islets. Since insulin pulses are altered by tetrodotoxin, but not by drugs blocking cholinergic and adrenergic receptors, this islet coordination presumably involves the interconnection due to postganglionic fibers autonomous ganglia (27). These ganglia, which serve both as pancreatic pacemakers and as integration centers (515), receive adrenergic, cholinergic, and peptidergic inputs from both the central and the autonomous nervous systems (515).

Under in vitro conditions which perturb, if not abolish both the hormonal and the nervous signaling that functionally interconnect pancreatic islets in situ, isolated islets of Langerhans still retain their main characteristic which is the ability to release graded amounts of insulin, as a function of nutrient stimulation, specifically by glucose (25, 28, 190, 204, 301, 421). Albeit quantitatively reduced and qualitatively modified, insulin release and biosynthesis are retained, indicating that intact islets are the functionally competent units of the endocrine pancreas (28, 515). Since each isolated islet releases amounts of insulin that largely exceed

the total hormone content of any individual cell, its proper functioning implies an adequate coordination of the β -cells which form its major mass, as indicated by the high degree of synchronization of both silent and burst periods of electrical activity (125, 333, 337, 350), and of glucose-induced Ca^{2+} transients between the β -cells of each islets (139, 190, 204, 534). Many signals flowing in the extracellular spaces of the islets (e.g., neurotransmitters released by extra-islet ganglia, K^+ , nitric oxide, and the four main islet hormones themselves) contribute to fine tune such a coordination (27, 249). While this signaling is important for modulating the stimulus-secretion coupling of β -cells, as shown by the significant changes in threshold, oscillation, and levels of insulin output observed between *in vivo* and *in vitro* studies (190, 204, 280), it is dispensable for glucose responsiveness. In contrast, loss of the mechanisms that operate between closely apposed β -cells results in severe alterations of this responsiveness.

Thus knockout of the gene coding for the Igf1 receptor of β -cells results in loss of glucose-induced insulin secretion (280), and a severe impairment of this function is also observed after interference with the EphA- and Fas-dependent pathways of β -cells (273, 471). Strikingly, several of these alterations are mimicked by loss of the physical contacts that β -cells establish within the islets of Langerhans (44–47, 241, 242, 301, 408, 410, 452, 469), as well as by the interference with surface molecules that mediate such contacts, including E-cadherin (63, 235, 579) and Cx36 (25, 63, 64, 72, 421, 503, 568). These findings support the notion that glucose-induced insulin secretion is critically dependent on the signaling mediated by β -to- β cell contacts. The further finding of Cx36 alterations in experiments testing the effects of the E-cadherin- (63, 64) and EphA-dependent pathways (273) further suggests that Cx36 may be a common partner of several signaling mechanisms operating within the islets, which possibly provides for their cross-talk and/or final, distal effects.

B. The Connexin and Coupling Pattern

Gap junctions between β -cells (FIG. 11) were identified more than 30 years ago using electron microscopy (383), and their function first indirectly suggested by two electrode recordings that documented synchronous waves of electrical activity induced by glucose in different cells of the same islet (25, 125, 247, 333, 336, 350). Further studies showed that *in vivo* these gap junctions typically comprise small numbers of connexons (341, 345) made only of Cx36 *in vivo* (65, 421, 478, 479, 538) within the islets of adult, control animals of all species investigated so far, whereas under certain *in vitro* conditions they may also contain Cx43 (91, 296, 309). Cx36 is a 321-amino acid protein with a long (99 amino acid) cytoplasmic loop containing an unusual stretch of 10 glycine residues (94) and a short cytoplasmic COOH-terminal region containing potential rec-

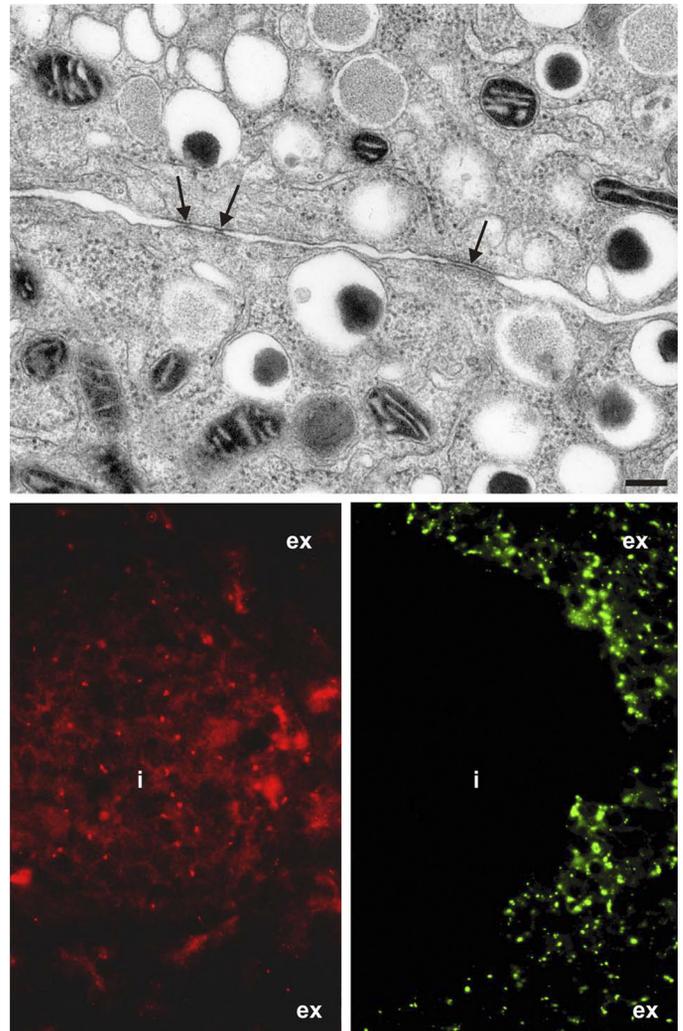


FIGURE 11. β -Cells are connected by gap junctions made of Cx36. *Top:* electron microscopy at the periphery of 2 β -cells, identifiable by their characteristic insulin-containing granules, shows the punctate contact of the apposed plasma membranes at gap junctions (*arrows*). At these sites, where connexon channels concentrate, the normal intercellular space is reduced to a narrow gap. *Bottom:* immunostaining of a control pancreas shows the punctate distribution of Cx36 in a pancreatic islet (i) but not in the surrounding exocrine acini (ex; *left*). In contrast, Cx32 is abundantly detected in the exocrine acini but not the islet (*right*), providing evidence for the tissue-specific distribution of the 2 connexins. Bar, 100 nm in *top* panel and 10 μm in *bottom* panels.

ognition sites for casein kinases I and II, cAMP-dependent protein kinase, and calmodulin-dependent protein kinase II (60), consistent with the finding that, at least under certain conditions, the function of Cx36 may be regulated by phosphorylation (3, 274, 550). Cx36 does not form functional cell-to-cell channels with other connexins (201), and the homotypic channels it makes differ from those made by other connexins by a small unitary conductance (10–15 pS) and minimal voltage sensitivity (201, 360, 512). These features account for the frequent, but small level of electrical coupling that has been documented between islet cell pairs monitored by dual patch-clamp recording (360), as well as by the electrophysiological recordings of rodent pancreatic

islets (125, 247, 333, 337, 350, 503). Thus loss of Cx36, after either homologous recombination of the *GJA9* gene (189, 421) or conditional deletion of the protein in β -cells targeted by an insulin promoter-driven Cre recombinase (568), results in the electrical uncoupling of β -cells, and in the loss of their normally exquisite synchronization of glucose-induced Ca^{2+} transients (25, 421). The weak modulation of Cx36 channels by transjunctional voltage is well adapted to ensure the electrical coupling of β -cells under both resting and stimulated conditions, which are associated with hyperpolarization and depolarization of the membrane potential, respectively (247, 333, 350). Cx36 channels are fairly permeable to positively charged tracers, but less so to negatively charged molecules (64, 70, 80, 201, 342, 415), indicating that they favor the cell-to-cell transfer of small cationic species. Still, these channels allow for the metabolic coupling of islet cells, as shown by the exchange of negatively charged endogenous molecules, such as phosphorylated glucose metabolites (269) and nucleotides (336). Most β -cells appear electrically coupled, as indicated by the rhythmical and synchronized bursts of electrical activity as well as by the coordinated Ca^{2+} oscillations that are observed during glucose-induced insulin secretion throughout intact islets (25, 247, 333, 337, 350). Despite this widespread electrical synchronization, the transfer of metabolites and membrane-impermeant tracers occurs only between small group of islets cells (19, 70, 79, 336, 342, 345, 346). This apparent discrepancy cannot be simply explained by a limited sensitivity of the tracer methods, inasmuch as the very same method allows for detection of extensive coupling throughout the islets of transgenic mice whose β -cells are forced to ectopically express Cx32 in addition to the native Cx36 (19, 79, 80). Together, the available data support the view that islets do not function as a single syncytium, but rather are made of the coordinated assembly of multiple, distinct territories of coupled β -cells. The exclusive expression in β -cells of Cx36, which only forms homotypic channels, provides the basis for a further selectivity in communication, allowing for β -cell exchanges of signals that should not reach, at least at the same time or rate, other types of islet cells. Such selectivity would be anticipated for proper islet functioning since β - and δ -cells usually function in parallel, whereas β - and α -cells function antagonistically (339, 356, 572).

C. Cx36 in Insulin Secretion

Several studies have documented that single β -cells show increased basal release of insulin, poor or no responsiveness to glucose, decreased basal expression of insulin, decreased proinsulin biosynthesis, and poor or no elevation in free cytosolic Ca^{2+} after glucose stimulation (44, 45, 168, 241, 301, 406, 408, 410, 452, 469, 470). Thus contact between β -cells rapidly restores some glucose responsiveness, in terms of insulin release, (pro)insulin biosynthesis, and

secretagogue-induced elevations in cytosolic free Ca^{2+} (45–47, 241, 242, 342, 345, 346). Aggregation also promotes the recruitment of secretory and biosynthetically active β -cells, hence decreasing the intrinsic, functional heterogeneity of single β -cells (44–47, 168, 241, 242, 301, 410). Comparable observations were made in pseudo-islet organoids that mimic the three-dimensional arrangement and size of native islets, as well as with much smaller clumps of β -cells, obtained by either partial disruption of islets or reaggregation of single islet cells (44, 45, 168, 241, 301, 406, 408, 410, 452, 469, 470). These data indicate that the entire islet architecture is not obligatory to retain glucose responsiveness and that the functional unit responsible for this critical β -cell feature is rather small. Consistent with this view, modeling and electrophysiological recordings have shown that only a few β -cells, in numbers similar to those that form the coupled territories delineated by gap junction-permeant tracers, need to interact to sustain the regularly pulsatile oscillations in electrical activity and Ca^{2+} transients that are observed in glucose-stimulated islets (247, 589). Thus some enhancement of the glucose responsiveness is already evident as two β -cells contact each other in pairs, but not when a β -cell establishes a heterologous contact with another islet cell type (44–47, 241, 242), indicating that the contact-dependent signaling that promotes secretion is dependent on some homologous surface moiety. The further observation that clustered β -cells are not recruited to secrete more than single cells in the presence of low glucose concentrations (44–47, 451) further indicates that, beside physical interaction, some functional event is required to account for the contact-dependent regulation of insulin secretion.

Similar conclusions were reached by exposing isolated islets (338) or intact pancreas (56) to drugs known to block gap junction channels (118, 134). Again, glucose-induced insulin secretion was found altered during islet cell uncoupling, in a way that was rapidly reversible after washout of the uncoupling drug, and that did not significantly affect the function of single cells (340). Three observations converge to indicate the specificity of this effect. First, the same drugs did not significantly affect the insulin secretion of single cells, consistent with the absence in these cells of functional Cx channels (340). Second, pairs of β -cells functioned during uncoupling as if they were made by two apposed but not interacting β -cells, i.e., secreted twice as much as the average single cell (340), and did not show the fourfold potentiation that is normally seen in control cell pairs (44–47). Third, perfusion of the intact pancreas with an uncoupling drug simultaneously induced an inhibition of insulin secretion and a stimulation of amylase secretion (56), consistent with the opposite regulation of the Cx36 channels of the endocrine islets and the Cx26-Cx32 channels of exocrine acini, during stimulation by natural secretagogues (356, 480). These

experiments suggest that gap junction channels are an important determinant in the contact-dependent mechanism that controls the glucose-induced stimulus-secretion coupling. Still, because the available blockers of gap junction channels may affect other types of membrane channels (200, 508), the interpretation of these results needed to be validated by alternative approaches. Thus insulin-producing cell lines that do not release insulin in response to a physiological stimulation by glucose, such as provided by an increase in glucose concentration from 1–2 mM to 5–8 mM, were found not to express connexins and to be uncoupled (63, 64, 557), whereas cell lines retaining at least some glucose responsiveness expressed Cx36 and were coupled, like primary β -cells (72). Strikingly, only MIN6 cells, and to a lesser extent some clones of INS1 cells, spontaneously express the Cx36 protein into junctional membrane plaques like primary β -cells, in keeping with their robust glucose responsiveness (63, 64, 70, 557). Together, these findings indicate that the expression of Cx36 and junctional coupling may be a prerequisite for a normal secretion of insulin, notably in response to glucose.

Transgenic mice forced to express Cx32 in β -cells, under control of the insulin promoter, had morphologically normal islets. However, they were glucose intolerant since they could not respond normally to glucose concentrations typical of a postprandial glucose load (79). They also showed only modest insulin release after a supra-physiological glucose challenge (79). These studies suggest that a large enhancement of β -cell coupling via a connexin that is not normally expressed in β -cells is deleterious for insulin secretion and glucose homeostasis. Cx36-null mice were also found to have morphologically normal pancreatic islets comprised of β -cells lacking gap junctions (421, 503). These cells did not show the normal intercellular synchronization of intracellular Ca^{2+} transients that is seen during glucose stimulation of native β -cells and, as a result, did not release insulin in a normal, pulsatile fashion (25, 421). Furthermore, islets lacking Cx36 showed increased basal release of insulin, explaining why no significant increase in hormonal output was observed when the pancreas was challenged by concentrations of glucose found postprandially in the plasma (421). The excessive basal secretion is consistent with the observation that uncoupled β -cells lacking Cx36 cannot be inhibited by the adjacent cells via the cell-to-cell diffusion of a hyperpolarizing, inhibitory current, an event which is readily observed *in situ* between the coupled β -cells of control mice (503). The alterations in insulin secretion observed in the Cx36-null mice are similar to those observed in prediabetic states and type 2 diabetics, indicating that Cx36-dependent signaling may be essential for proper regulation of insulin release (421). Similar observations have been made in clones of MIN6 cells that were depleted in Cx36 by an antisense approach (64).

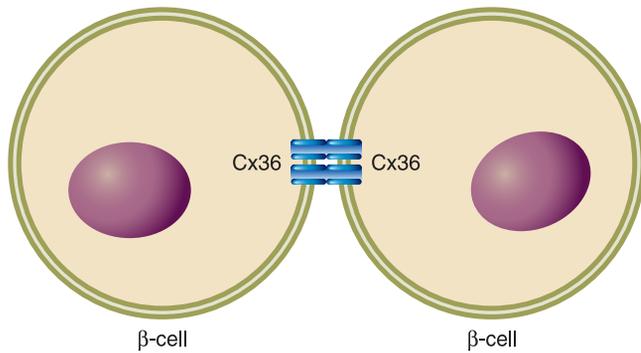
These cells allowed to further establish that the secretion control achieved by the Cx36 signaling cannot be mediated by other connexins or by E-cadherin (63, 70, 235), and that excess Cx36 is as deleterious for insulin secretion as the lack of the connexin (70, 295, 557). The results of these *in vivo* and *in vitro* studies show that Cx36-dependent signaling involves the intra-islet synchronization of glucose-induced oscillations in intracellular Ca^{2+} which, in turn, drive the oscillations in insulin output (25, 64, 421), and the intercellular diffusion of electrotonic currents, which control the on/off response of β -cells (FIG. 12).

D. Other Roles of Cx36

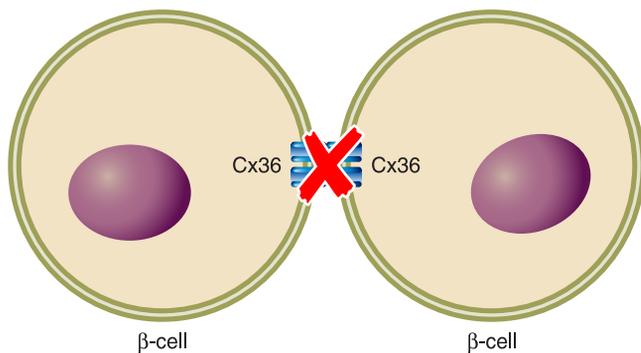
Recent observations have also identified the participation of Cx36-dependent signaling in the protection of β -cells against a variety of insults (4–6, 265), including the cytokines that are thought to induce β -cell apoptosis at the onset of the autoimmune attack of pancreatic islets that leads to type 1 diabetes (5, 265). Experiments on several lines of transgenic mice that feature different patterns of native (Cx36) or islet ectopic (Cx32, Cx43) β -cell connexins show that primary β -cells are protected by Cx36 against cytotoxic drugs and cytokines and, conversely, are sensitized to the pro-apoptotic action of these molecules in the absence of the connexin (5, 265). The mechanism of this Cx36-dependent protection remains to be fully elucidated. Present data show that it is partially explained by the extent of β -cell coupling and that it can be sustained by different connexin species (5, 265). Given that apoptosis is a major determinant of β -cell life (127), and that β -cell coupling is enhanced in rodent pregnancy (354, 484), a condition associated with a marked increase in β -cell proliferation and reduced β -cell apoptosis (161, 500), the data suggest that Cx36 may also contribute to the regulation of the β -cell mass (266). There is presently no evidence that Cx36 forms “hemi-channels” in insulin-producing β -cells (455), as it does for example in degenerating neurons (384, 385).

E. β -Cell Heterogeneity

Connexin channels permit a rapid, diffusion-driven, and bidirectional exchange of multiple molecules between coupled cells, a mechanism that would rapidly result in the equilibration of electrochemical gradients of cytoplasmic ions and molecules, in the absence of mechanisms sustaining their generation (200, 211–213, 347, 356). This implies that, compared with other mechanisms of cell-to-cell communication, connexin-dependent coupling may be particularly advantageous in tissues made of heterogeneous cells. Disparities in intrinsic structural and functional properties could conceivably result in the asynchronous function of individual cells,



Low basal insulin release
 High glucose-stimulated insulin release
 High expression of the insulin gene
 Intercellular synchronization of Ca^{2+} waves
 Intercellular diffusion of currents
 Recruitment of secreting cells
 Sensitivity to cytokines
 Ephrin signaling



INCREASED basal insulin release
 DECREASED glucose-stimulated insulin release
 DECREASED expression of the insulin gene
 DECREASED intercellular synchronization of Ca^{2+} waves
 NO diffusion of electronic currents
 DECREASED recruitment of secreting cells
 INCREASED sensitivity to cytokines
 NO ephrin signaling

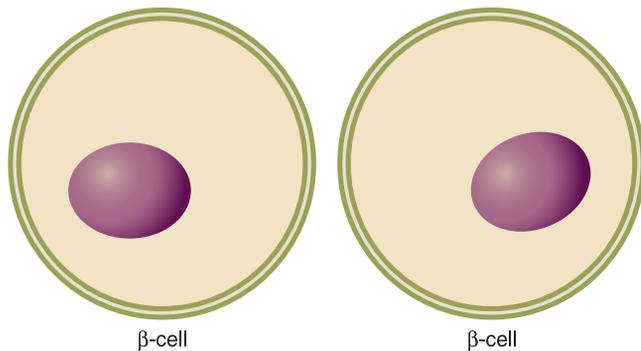


FIGURE 12. Effects of Cx36 signaling on pancreatic β -cells. *Top:* control β -cells natively express functional cell-to-cell channels exclusively made of Cx36. *Bottom:* deletion of Cx36 or blockade of the channels this protein forms results in alterations of the basal and glucose-stimulated insulin release, the recruitment of secreting cells, the expression of the insulin gene, the electrical synchronization of β -cells, their signaling by other pathways, and their resistance to cytotoxic conditions, including the exposure to diabetogenic cytokines.

and junctional coupling could decrease or correct these localized disbalances, thus permitting distinct cell subpopulations to function simultaneously and/or at the same rate (44–47, 64, 255, 322, 406, 421, 503). Several lines of evidence indicate that this is the case for pancreatic islets, in which β -cells differ in a number of structural, biochemical, and functional respects, including in terms of the biosynthesis, storage, and release of insulin (44–47, 64, 255, 322, 406, 410, 452, 589). For example, comparable levels of insulin are immunostained within β -cells of control, resting islets, but differ widely in different islet regions after a sustained period of glucose stimulation (517). At least some of these observations

cannot be attributed to a different environment within different regions of the islets, inasmuch as marked differences in insulin secretion are also readily observed *in vitro*. Thus individual β -cells simultaneously exposed to conditions of maximal stimulation by glucose or by other nonmetabolizable secretagogues feature marked differences in the ability to release insulin (44–47, 64, 255, 322, 406, 410, 452, 589), and retain this pattern for hours (168), in spite of similar electrophysiological changes indicating a comparable activation of the stimulus-secretion coupling machinery (501). This different behavior may be explained by a difference in the size of secreting and quiescent β -cells, and/or in quantitative

differences in their biosynthetic activity (45, 406), or levels of key proteins, including glucokinase (203, 236) and the sialylated form of N-CAM (248, 262).

Under such conditions, a mechanism dependent on cell contact allows for the recruitment of increasing numbers of secretory cells with both cell aggregation and the degree of stimulation (22, 44–47, 241, 242, 589). Thus, at the same time and in the very same environment, β -cell clusters release significantly larger amounts of insulin than single β -cells. Strikingly, when the cumulated insulin release is computed, taking into account the number of secreting cells and their individual insulin output, the resulting curve has a sigmoidal shape and threshold point, which are similar to those of the dose-response curve describing insulin release as a function of glucose concentration (46). The underlying mechanism somehow involves connexins, inasmuch as drugs blocking junctional channels and antisense constructs interfering with connexin transcription blocked the contact-dependent recruitment of secretory β -cells during glucose stimulation (338). Together, these experiments indicate that junctional coupling can counterbalance the asynchronous function of individual β -cells, of which it ensures the coordination of secretion, as well as the stimulus-dependent recruitment. The reason why β -cells are functionally heterogeneous, and why their asynchronous functioning may be inappropriate should be experimentally tested. Recent evidence indicates that, in many systems, cellular heterogeneity provides robustness to a population (8, 392). Lack of coupling prevents the sharing by β -cells of sparse ion channels that are critical for proper activation of the stimulus-secretion coupling (429). By inducing irregular oscillations of cytosolic Ca^{2+} and a basal, steady increase in the levels of this cation (79), β -cell uncoupling could also alter the expression of genes critical for secretion and/or control of β -cell apoptosis. Thus, in MIN6 cells, which show a glucose sensitivity and Cx36 expression like those of primary β -cells, loss of this connexin as a result of transfection of an antisense construct results in alterations of insulin secretion that are associated with altered expression of selected genes (63). It is further possible that some deleterious effects of β -cell uncoupling may be accounted for by the loss of the interactions between Cx36 and other membrane proteins, including those of the SUR1-Kir6.2, K_{ATP} -complex (429) and of β -cell tight junctions (307, 308). While the molecular composition of the latter structures remains to be unraveled in pancreatic islets, electron microscopy has clearly documented the close relationship between gap junction plaques and tight junction fibrils between rodent and human β -cells (342, 345, 383).

F. Cx36 and Diabetes

Type 1 diabetes results from an autoimmune attack selectively directed against pancreatic β -cells, which is thought to be mediated by increased levels of proinflam-

matory cytokines in the islet environment (127, 228, 229, 243, 549). The residual β -cell mass (reduced by $\sim 95\%$) becomes then insufficient to sustain the insulin demand of the organism. The reasons why some β -cells resist this attack and persist for years within the pancreas of patients remains to be understood (349). Transgenic mice overexpressing Cx36 showed protection of β -cells against cytotoxic drugs and cytokines that experimentally reproduce the massive cell death observed at the onset of type 1 diabetes (5, 265), whereas mice lacking Cx36 were sensitized to these insults and became rapidly hyperglycemic due to the apoptosis death of most β -cells (5, 265). Thus connexin channels may significantly contribute to the resistance of endocrine cells to cytotoxic conditions, either by enhancing their resistance to the damaging conditions and/or by favoring an efficient cell repair mechanism after the insult. The mechanism of this protection depends on cell-to-cell contact, is not due to loss of activity of the pro-apoptotic cytokines in the islet environment, and at least in vitro, also protects β -cells against these endogenous factors. Consistent with these findings, interleukin- 1β , interferon- γ , and tumor necrosis factor- α decrease the expression of the Cx36 protein by downregulating the transcription of the cognate gene (5, 265). Strikingly, changes in the expression of the Cx36 transcript have been detected in genome-wide scans of type I diabetes models (284, 420).

Type 2 diabetes is a heterogeneous disorder with a complex pattern of inheritance. Genome-wide scans have already detected linkage of diabetic phenotypes with several loci, including 15q14 (362) where the human Cx36 gene is located (21). While it is presently not known whether some Cx36 mutation/polymorphism may be pathogenic in the diabetic context, mice with an invalidated Cx36 gene feature alterations in insulin secretion (421, 503, 568) that are reminiscent of those that precede the development of overt diabetes in humans (e.g., loss of insulin oscillations) and, later, characterize the disease (e.g., increased basal release of insulin, failure to increase the insulin output in the presence of postprandial concentrations of glucose) (381, 413). These considerations, and the observation that Cx36 also forms gap junctions between human β -cells (478), raise the possibility that alterations in Cx36, in the cell-to-cell communications it establishes, and/or in the molecular signaling it controls, are involved in the pathophysiology of diabetes. At present, this possibility has not been tested due to the difficulty to access human β -cells.

One of the goals of understanding the physiology of β -cells is to develop better, targeted strategies for the treatment and cure of diabetes. One of the most promising therapeutic approaches is the transplantation of surrogate insulin-producing cells retaining the essential features of primary β -cells, including normal stimulus-secretion coupling of glucose-induced insulin release. Mouse embryonic stem cells differentiating towards a β -cell phenotype function

better after assembly into islet-like structures and acquisition of some neuronal traits (319). Cx36 is selectively expressed only by neurons and β -cells (356, 480), promotes the formation of three-dimensional organoid-like structures by β -cells (71), and correlates with the expression of insulin (66, 478), raising the possibility of a valuable, if not essential role of the connexin in the acquisition of β -cell characteristics. Interestingly, mouse ES cells, which so far have not been shown to be amenable to any production of the insulin protein, lack Cx36 (226, 574). Chronic exposure to high glucose levels, as expected in type 2 diabetics, negatively affects the expression of Cx36 in insulin-producing cell lines, due to a transcriptional downregulation, which is mediated by the presence of a highly conserved cAMP responsive element, referred to as CRE, in the promoter of the *Gjd2* gene (6). The same element is also repressed by high concentrations of palmitate, *in vitro* and *in vivo* (4). The effects of both glucose and palmitate are mediated by the cAMP-dependent overexpression of the inducible cAMP early repressor ICER-1, which adversely affects β -cell function and proliferation (1, 4, 6, 232, 233). If the same effect was to occur in human β -cells exposed to long-term hyperglycemia, a logical therapeutic measure would be to restore normal levels of Cx36. In this context, it is worth noting that at least one sulfonylurea, which efficiently stimulates insulin release from β -cells of type 2 diabetics, promotes the assembly of Cx36 channels and β -cell coupling (80, 340, 342, 345), in addition to its well-documented binding to the SUR-1 receptor, and the consequent activation of the Kir6.2 type K_{ATP} channels (10).

VI. THE RENIN-PRODUCING CELLS

Renin is a glycoprotein hormone produced by the epitheloid juxtaglomerular cells that form the media layer of the afferent arterioles, immediately before these small vessels enter the glomerulus of each nephron, to supply its capillary loops (69, 106). In a healthy human there are ~2.4 million sites of renin production dispersed throughout the cortex of the two kidneys, each made by a group of 20–40 endocrine cells. Together with the endothelial and smooth muscle cells of the nearby efferent arterioles, the epithelial cells of the macula densa region of the distal tubule, and the extraglomerular mesangium, renin-producing cells (FIG. 13) form the so-called juxtaglomerular apparatus (69, 106, 395).

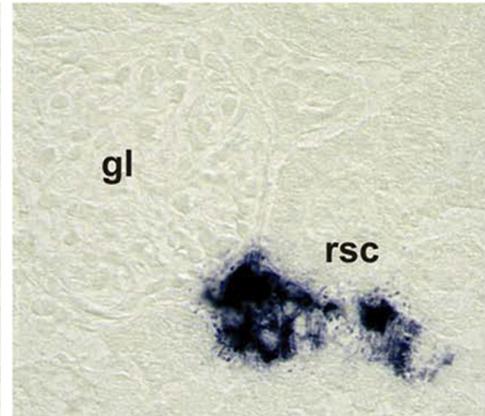
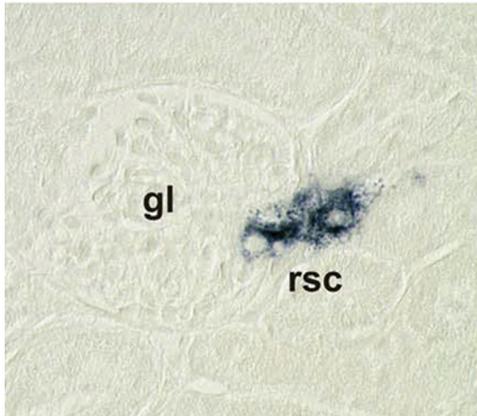
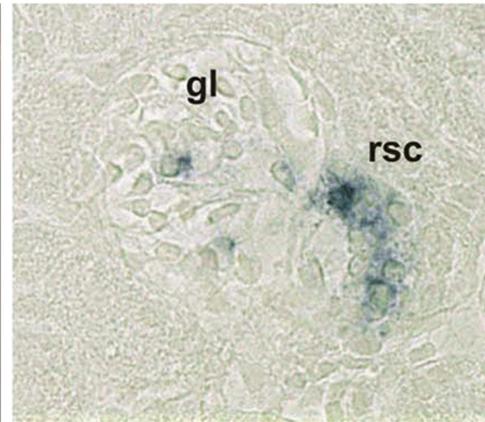
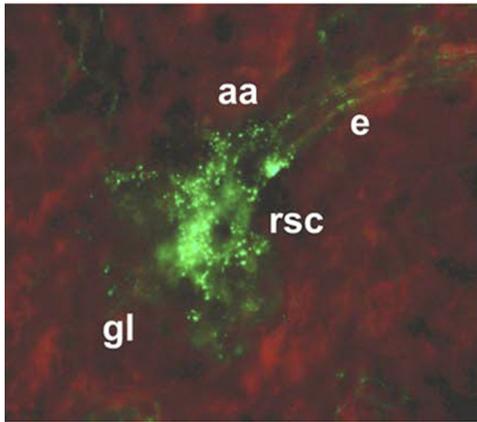
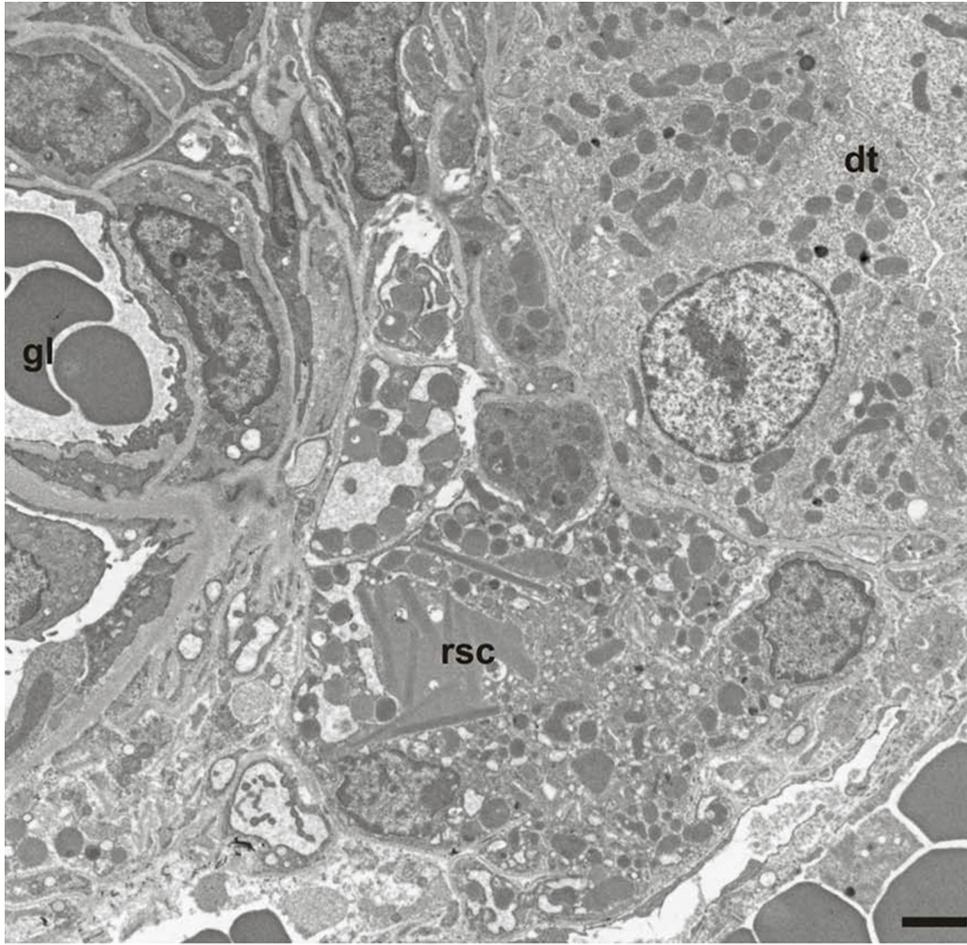
A. The Endocrine Function

Renin secretion is a multicellular event, resulting from the integrated function of the numerous cells that collectively form the juxtaglomerular apparatus. This system senses changes in both the hemodynamic characteristics of the kidney blood supply, mostly via the endothelial and smooth muscle cells of the afferent and efferent arterioles, and in the

concentrations of electrolytes in the lumen of the distal tubule, mostly via the epithelial cells of the macula densa. Accordingly, this multicellular apparatus regulates the release of renin to maintain fluid-electrolyte and blood pressure homeostasis, via the modulation of the renin-angiotensin system (69, 106, 395).

Thus, at the level of each nephron, the renin-producing cells are stimulated by a decrease in blood pressure and/or flow through both the afferent and efferent arterioles, which implies the presence of cells equipped with baro- or hemodynamic receptors, and their ability to transduce a mechanical information into a signaling to nearby companion and different cell types (69, 106, 395). This signaling presumably involves different types of direct and indirect cell communication mechanisms (22, 472), among which the connexin-dependent signaling has been shown to be prominent (69, 356, 472, 583). The renin-producing cells are also stimulated by a decrease in the levels of Na^+ and Cl^- in the ultra-filtrate of the nephron, which implies some signaling between the macula densa and the renin-producing cells to ensure the so-called tubuloglomerular feedback (69, 106, 395). Recent studies have indicated the likelihood of a prominent role of a connexin-dependent signaling across the mesangial cells (69, 356, 472, 582, 583). However, since the cells of the macula densa do not feature gap junctions, the participation of diffusible signals has also to be involved. Macula densa cells release ATP, probably via connexin “hemi-channels” (584), which activate purinergic receptors of mesangial cells, triggering a calcium wave that spreads from the macula densa to the renin-producing cells of the afferent arteriole, via the extraglomerular mesangium (403, 584). Prostaglandins also contribute to the initial paracrine step of the tubulo-glomerular feedback (32, 472). As in many other secretory cells, renin secretion is controlled by an interplay of several intracellular second messengers (472). However, and at variance with many other secretory mechanisms which are stimulated when the free cytosolic concentration of free Ca^{2+} increases (401, 402, 572), it is a lowering in the cytosolic levels of the cation that trigger renin secretion (281, 465, 466). Conversely, the renin-producing cells are locally inhibited by an increase in either blood pressure or blood volume and flow, as well as by elevated circulating levels of angiotensin II and Na^+ (32, 472).

The activity of the many units of the juxtaglomerular apparatus, which are dispersed throughout the kidney cortex, is further integrated by the sympathetic nervous system which, upon lowering of blood pressure, stimulates the renin-producing cells via β_1 adrenergic fibers (32, 472). It is also helped by the so-called “nephron coupling,” which is permitted by the end division of afferent arterioles into two or three branches, each reaching the glomerulus of a distinct nephron. Vasomotor signals retrogradely propagate from the vascular pole of one nephron to the branching sites, and



then, orthogradely, towards the other nephrons, via the gap junctions of the endothelial and/or the smooth muscle cells (67, 68).

Chronic stimulation of renin production induces an increase in the number of renin-producing cells within the afferent arterioles and, under certain conditions, also ectopically within the mesangium (276, 283). The recruitment of these ectopic cells may associate with altered differentiation of the renin cell lineage (477).

B. The Connexin and Coupling Pattern

Numerous gap junctions have been documented between most of the cells that form the juxtaglomerular apparatus, including the renin-producing cells, the endothelial and the smooth muscle cells of the afferent and efferent arteriole, and both intra- and extraglomerular mesangium, with the notable exception of the epithelial cells of the macula densa (149, 197–199, 282, 530, 563, 583). Electron microscopy showed that these junctions connected both similar, e.g., the renin-producing cells or the endothelial cells to companion cells, and different types of cells. Notably many junctions were found between the renin-producing cells and the endothelial cells of the afferent arteriole and the extraglomerular mesangium (531), as well as between the endothelial and the smooth muscle cells of this vessel (193, 197, 563). The renin-producing cells are joined to each other and to the nearby endothelial cells of the afferent arteriole by Cx40, lower levels of Cx37 (193, 438) and, at least prenatally, some Cx45 (199). This pattern is retained by the transformed cells of the renin-expressing line AS4.1, which also express Cx40, Cx37, Cx43, and Cx45 (440). Strikingly, however, renin-producing cells located outside the afferent arteriole only express Cx40 (193, 197, 199, 282, 283). The endothelial cells of the afferent arteriole are joined to companion cells by Cx40 and Cx37 (197, 563), whereas the smooth muscle cells of the vessel appear to express Cx45, and little Cx43 (198, 199, 563). The endothelial cells of the efferent arteriole also express Cx43, whereas no connexin has so far been shown in their media layer. The intra- and extraglomerular mesangial cells express Cx40 and Cx45 (198, 199, 460, 563, 583). Despite the lack of evidence of gap junctions in the macula densa, immunostaining for Cx37 has been reported in the basolateral membrane of macula densa cells (529).

Consistent with these observations, the renin-producing cells, and a transformed line derived thereof (440), have

been shown to be electrically and metabolically coupled (57, 199). Strong electrical coupling has also been demonstrated between mesangial cells, as well as between the endothelial and the smooth muscle cells of the afferent arteriole (220, 531). This coupling is implicated in the transmission along the afferent arteriole of vasomotor signals generated by the macula densa cells and which retrogradely reach the renin-producing cells to ensure the tubuloglomerular feedback (519, 559). Vasomotor signals propagating through the gap junctions of the endothelial and/or smooth muscle cells of the ramified ends of each afferent arteriole also account for the so-called “nephron coupling” (67, 68).

C. Cx40 in Renin Secretion

Inactivation of the Cx40 gene in mice results in a marked and sustained hypertension (7, 276, 277, 490). This phenotype is in part explained by a remarkable increase in both the synthesis and the release of renin (276, 561), and in part by peripheral vascular effects of the ensuing activation of the physiological renin-angiotensin system (7, 276, 277, 490, 561). With regard to renin secretion, a series of studies has now documented that Cx40 deficiency causes the loss of the normal feedback mechanisms whereby the circulating levels of angiotensin II, as well as an increased intrarenal blood pressure, normally suppress the expression of the renin gene and the release of the hormone at the level of the afferent arteriole (267, 283, 560, 561). Thus both renin transcription and, to a lesser extent, renin release are markedly stimulated, and the number of renin-producing cells increased, resulting in excessive circulating levels of the circulating hormone (267, 277, 560, 561). The inhibitory effect of the macula densa on renin secretion, which normally operates after a high salt intake, is also attenuated in Cx40-deficient mice, whereas the normal regulation by catecholamines is preserved (276, 277, 560, 561). Further studies showed that elevated levels of cyclooxygenase-2 and neuronal nitric oxide synthase also contribute to the excessive renin synthesis and secretion observed in the absence of Cx40 (277, 560), and that diminished circulating levels of angiotensin II, as a result of treatment with an inhibitor of angiotensin converting enzyme, clearly reduced renin synthesis and secretion in Cx40-deficient mice (276, 561). Parallel experiments in isolated kidneys further showed that perfusion of control organs with either a low extracellular calcium media or nonselective inhibitors of gap junctions reproduced the high renin output observed in Cx40-deficient mice, whereas the same conditions did not modify the

FIGURE 13. The juxtaglomerular epithelioid cells of kidney express Cx40. *Top:* electron microscopy at the periphery of a kidney glomerulus (gl) and distal tubule (dt) shows a cluster of juxtaglomerular epithelioid cells (rsc), identifiable by their characteristic polymorphic and electron-dense renin-containing granules. *Bottom:* immunostaining of a control kidney cortex for Cx40 (*top left*) reveals the distribution of this connexin in the endothelial cells (e) and the renin-producing cells (rsc) of the afferent arteriole (aa), which vascularizes the glomerulus (gl). The local expression of Cx40 is confirmed by in situ hybridization of the cognate transcript (blue; *top right*). In situ hybridization for renin shows that mice lacking Cx40 (*bottom right*) expressed much higher levels of renin mRNA (dark blue staining) than control littermates (*bottom left*). Bar, 3 μm in *top panel* and 25 μm in *middle and bottom panels*.

renin secretion from the kidneys of Cx40-deficient mice (561). The data point to an essential participation of both Ca^{2+} and gap junction channels in the mechanism leading to the altered renin secretion. The two factors may be tightly linked, inasmuch as other experiments have shown the intercellular spreading of calcium waves in cultures of juxtglomerular As4.1 line, which has a connexin expression pattern similar to native juxtglomerular cells (440), and have documented that these waves are dependent on ATP release, possibly mediated by connexin “hemi-channels” (529). Of note, however, the potential contribution of pannexins was not investigated in these experiments.

Given that renin-producing cells *in situ* also express Cx37 and Cx45, and that Cx40-deficient mice also feature alterations in the renal expression of Cx37 and Cx43 (276), the role of these connexin isoforms was tested in different genetic mice models. After a low-salt diet, with or without treatment with an angiotensin I-converting enzyme inhibitor, Cx37-deficient mice, which did not show altered renal expression of Cx40, Cx43, and Cx45 and did not feature hypertension, showed a normal stimulation of the expression of the renin transcript, and of renin secretion (451, 562). Contrasting with the observation in Cx40-deficient mice (283), animals lacking Cx37 also feature a normal localization of the renin-producing cells within the media of the afferent arteriole (562). These data demonstrate that Cx37 is dispensable for the proper positioning and function of the renin-producing cells. Even though the levels of Cx37 are somewhat decreased in Cx40-deficient mice (7, 277, 491), presumably indicating some interdependence between the expression of the two connexins, the data further indicate that the phenotype observed in the Cx40-deficient mice cannot be accounted for by the obligatory formation of heteromeric Cx37/Cx40 channels. Still, such channels may have some subtle regulatory function, inasmuch as a low-salt diet stimulated renin secretion more from Cx37-deficient mice than from wild-type controls (562).

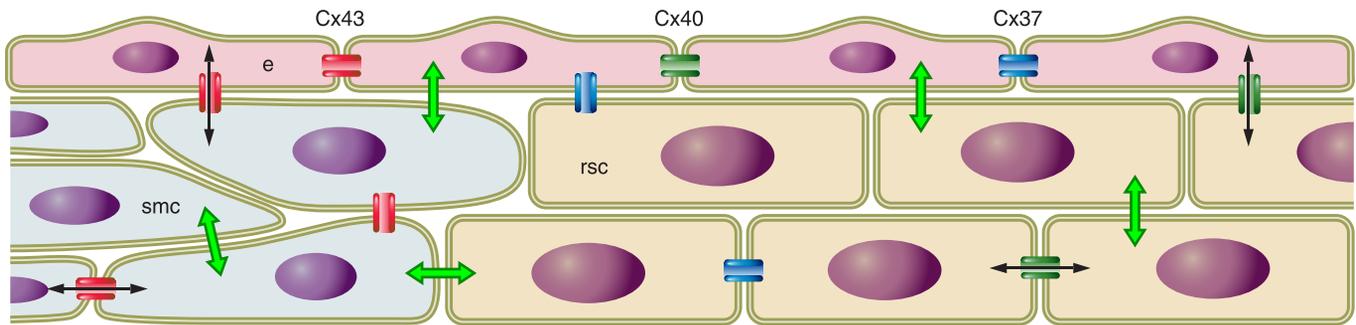
Conditional Cx45-deficient mice, which expressed enhanced green fluorescence protein instead of Cx45 in cells expressing nestin during development, featured hypertension and increased renin expression (199). *In vitro*, the vascular smooth cells of these Cx45^{fl/fl}:Nestin-Cre mice showed reduced propagation of mechanically induced calcium waves, thought to be normally transferred between cells connected by Cx45 channels (199), providing evidence that loss of this connexin also contributes to hypertension. Another set of experiments investigated mice in which Cx45 replaced Cx40, under control of the native Cx40 promoter (473). These knock-in mice featured plasma renin levels similar to those of wild-type controls, and a hypertension which was significantly less than in Cx40-deficient animals. Blockade of angiotensin II formation, and the unilateral hypoperfusion of one kidney, which do not modify the excessive levels of circulating renin and of hypertension

in Cx40-deficient mice, normally increased these plasma levels and blood pressure in the Cx45 knock-in animals (473). These experiments document that Cx45 can functionally replace Cx40 for at least some aspects of renin secretion, and indicate that loss of Cx40 does not impair the normal feedback control of renin secretion by either angiotensin II or the intrarenal pressure, which is lost in Cx40-deficient mice, provided Cx45 is still expressed. The implication is that renin biosynthesis and release are not dependent on the unique characteristics of Cx40 channels which have quite different biophysical and regulatory properties than those made of Cx45 (200, 201, 555).

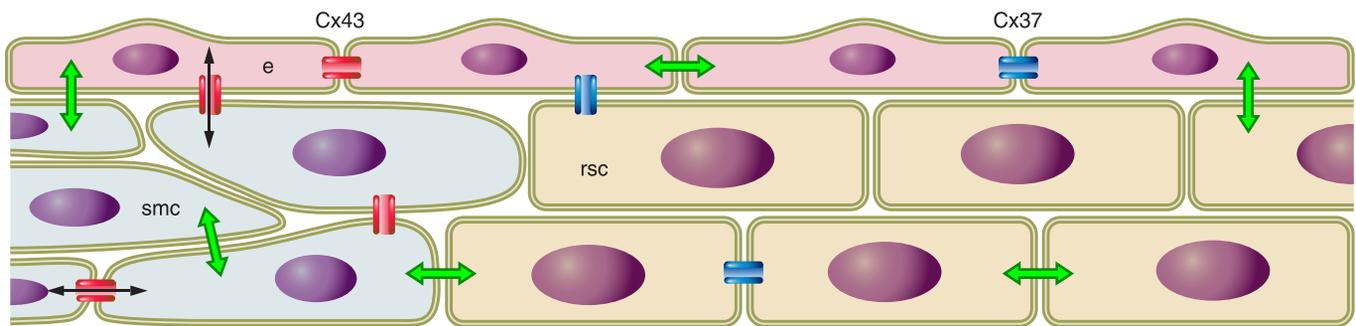
Given that, *in situ*, renin-producing cells also interact with vascular cells joined by Cx43 (194, 197), the influence of this connexin was analyzed using mice in which Cx32 replaced Cx43, under control of the native Cx43 promoter (411). This replacement reduced renin production and protected the mice from the hypertension that is normally induced by a decrease in the blood supply to one kidney or by a high-salt diet (194). Interestingly, the renal levels of Cx43 were higher in the hypertensive Cx40 null mice than in normotensive controls and, when the latter animals were made hypertensive by clipping one renal artery, the levels of Cx43 expression rose in the kidneys up to the levels observed in the Cx40 null mice (194). The contribution of Cx43 to the dysregulation of renin secretion implies either a cross-talk with Cx40 or the mediation of an extracellular signal generated in the endothelial and/or smooth muscle cells of the afferent arteriole. Both mechanisms are conceivable, inasmuch as Cx43 and Cx40 can form heteromeric channels, at least under certain conditions (160, 551, 554, 555), and that the close apposition of renin, endothelial and smooth muscle cells provides an ideal setting for paracrine interactions within the afferent arteriole.

The data from the gene invalidated and the knock-in mice models provide compelling evidence for a major role of Cx40 in the regulation of renin secretion. This tentative conclusion is further supported by the observation of increased expression of the Cx40 mRNA in the kidneys of control rats that were made chronically hypertensive by reducing the blood supply via clipping of a renal artery (193). This maneuver is known to significantly raise the levels of circulating renin, as a result of the decreased perfusion of the clipped kidney (69, 106, 395) (FIG. 14).

In summary, the existing data show that renin secretion is controlled by Cx40 signaling, and to a lesser degree by Cx45 signaling, in the cells that produce the hormone, as well as by a Cx43-dependent signaling that is generated in the nearby vascular cells (57, 58). The predominant role is played by Cx40, since Cx37 null mice (490, 491) and mice with a conditional deletion of vascular Cx43 are normotensive (310, 537). However, the interplay between the different connexins of the juxtglomerular apparatus finely tunes



Regulated renin secretion
 Control renin-containing secretory granules
 Normal, intra-arterial position of renin cells
 Control plasma levels of renin
 Control blood pressure



INCREASED dysregulated renin secretion
 Abnormal renin-containing secretory granules
 Abnormal, extra-arterial position of renin cells
 INCREASED plasma levels of renin
 INCREASED blood pressure

FIGURE 14. Effects of Cx40 signaling on renin-producing cells. *Top:* control renin cells (rsc) natively express functional cell-to-cell channels made of Cx40 and Cx37. These 2 connexins are found at the interface between renin-producing cells, as well as between these cells and the endothelial cells (e) of the afferent arteriole. These endothelial cells as well as the smooth muscle cells (smc) of the vessel also express Cx43. The endothelial, smooth muscle, and renin-producing cells further interact with each other via paracrine mechanisms (large double head arrows) *Bottom:* deletion of Cx40 alters renin release and storage, and the location of the renin-producing cells, which result in chronic hypertension.

the cross-talk between the multiple cell types which sense Na^+ fluid concentration, as well as blood flow and pressure, since Cx45-deficient mice are also modestly hypertensive (279). Furthermore, the hypertension of Cx40-deficient mice cannot be solely accounted for by the perturbed renin production, as the segmental vasoconstriction and altered vasomotion of small arterioles (109, 260, 490), which is induced in these mice by the normal activation of the angiotensin production as a result of the excessive renin levels, also plays a significant role. Thus blockade of either the angiotensin converting enzyme or the angiotensin II receptor AT_1 reduced, but did not normalize, the blood pressure of Cx40-deficient mice (277). The necessary participation of peripheral vascular factors is also supported by the observation that Cx40 knock-in Cx45

mice exhibit a modest hypertension, in spite of normal levels of plasma renin (473). In both cases, the vascular component of the hypertension triggered by the absence of Cx40 is probably due to an interruption of the cell-to-cell communication mechanism that normally controls the vasomotor tone (109, 260, 490).

D. Other Roles of Cx40

In the kidneys of hypertensive mice lacking Cx40, the renin-producing cells were more numerous than in the kidneys of control, normotensive littermates (277) and were no more located within the media of the afferent arterioles, but

rather around these vessels, and in nearby ectopic regions of the extraglomerular mesangium and periglomerular interstitium (283). This anomalous positioning was not noticed during embryonic life of the Cx40-lacking mice (283), after knock-in replacement of Cx40 by Cx45 (473) or in mice lacking Cx37 (562), raising the possibility that Cx40 is required for the correct postnatal morphogenetic patterning and positional information of renin-producing cells within the juxtaglomerular apparatus. These findings raise the intriguing possibility that the changes in renin secretion observed after Cx40 loss may not have directly resulted from the loss of Cx40-dependent coupling between the renin-producing cells and the other types of cells of the juxtaglomerular apparatus, but may be an indirect consequence of the altered microanatomical organization of this system. Renin-producing cells are normally recruited in increased functional numbers in response to hypotension, when they are found in multiple regions of the nephron (257, 318). Obviously, this homeostatic regulation is lost after loss of Cx40, since increased cell recruitment was observed in spite of a sizable hypertension (277). In this case, the renin-producing cells also featured smaller and more regular secretory granules than controls (277), further suggesting a shift in their differentiation pattern, as usually observed when cells of the renin lineage are not in their native niche, within the media of the afferent arteriole (477).

E. Cx40 and Hypertension

There are two major forms of hypertension, one due to altered functioning of the renin-angiotensin system, as a result of various kidney diseases and/or decreased renal blood supply (5–10% of the cases), and another due to alterations in renal and/or cardiac function, with or without increased peripheral vessel resistance (90–95% of the cases, referred to as “essential”). The finding that the distribution of connexins in the human kidneys, including the renin-producing cells (282), and vessels (197) is analogous to that observed in rodents, raises the possibility that abnormal expression or function of either renal or arterial connexins could be relevant for the development of chronic hypertension in humans.

On the renal side of the disease, the substantial and converging evidence summarized above has documented a major contribution of Cx40 in the experimental hypertension of rodents. Interestingly, two single nucleotide polymorphisms have been reported within the promoter region of the human Cx40 gene, which are associated with increased risk of hypertension in selected men populations (72, 144, 145, 146, 183). These polymorphisms are located close to sites that are thought to control the transcription of the Cx40 gene (144, 145). Furthermore, the Cx40 genotype featuring the pathogenic polymorphism also significantly increases the standing systolic blood pressure of normotensive individuals (145).

On the vascular side, blood pressure, blood flow, and shear stress all modulate the diameter of both large and resistance arteries, and the integration of these forces involves the gap junctions of endothelial and smooth muscle cells of the vessel walls (72, 141, 142, 197, 202). Coupling of these cells permits the rapid conduction of the electrical signals that generate vasomotor responses (142, 197). In three rat models of chronic hypertension, thickening of the aortic wall was associated with either increased [two-kidney, one-clip (2K,1C) model in which the 2 kidneys are left in situ with a clip inducing a unilateral stenosis of the renal artery, and DOCA-salt models] or decreased Cx43 expression (L-NAME model) by the smooth muscle cells of the media, depending on whether the vessels showed increased distensibility or not (7, 191, 192, 195–197), suggesting that connexins may contribute to regulate the elasticity of the vascular wall. The finding that Cx43 increases in both the 2K,1C and DOCA-salt models further suggests that the connexins can sense changes of intravascular pressure, whatever the levels of circulating renin, which was increased in the former but not the latter model (7, 191, 192, 195–197). Expression of Cx40 by the endothelial cells was also found increased in the aorta of 2K,1C animals (7, 195). Further studies in a genetic mouse model of hypertension have added complexity by demonstrating similar levels of Cx43 in some arteries of hypertensive and control rats, but decrease levels of Cx43, Cx40, and Cx37 in other vessels of the same animals (142, 197). Nevertheless, treatment of spontaneously hypertensive rats with inhibitors of the renin-angiotensin system normalized blood pressure and connexin expression (130, 183). Hypertension induced by inhibition of nitric oxide synthase appears associated with a decreased expression of Cx43 and Cx37, but not Cx40 (7, 142, 191, 192, 195–197). These apparently contradictory observations likely reflect the multiple functions played by different connexins in the vascular system, which may be so variable as to result in vasodilation of one vascular territory and vasoconstriction in another (142, 197). In the context of hypertension, they stress the necessity to carefully consider the complex contribution of several connexins in the regulation of vascular diameter and tone, notably in the resistance artery compartment, which is almost obligatorily involved in most forms of hypertension. It is worth noting that the peripheral vessel alterations are not necessarily independent from those that may occur in the renal juxtaglomerular apparatus, inasmuch as the expression of arterial Cx43 is selectively increased during renin-dependent hypertension, via an angiotensin II activation of the extracellular signal-regulated kinase and NF κ B pathways (7).

VII. CONCLUSIONS AND PERSPECTIVES

Connexin signaling has found its place among the numerous direct and indirect mechanisms for cell-to-cell communication, which cross-talk to ensure that every cell of a multicellular organism senses the activity of the other cells,

and regulates accordingly its own activity. This coordination is essential to provide the integrated functioning of the system as a whole, and to permit its dynamic adaptation to continuous internal and environmental changes. Connexin signaling appears particularly important in secretory systems, notably the multicellular glands that produce vital hormones. Thus direct experimental testing has now shown that specific connexins are required for the proper production and secretion of both insulin and renin. In turn, this knowledge forces us to address or revisit questions that may have been raised long ago, still remain unanswered. It further generates the many following questions that have not yet been comprehensively explored, and which may provide a stimulus for future research effort.

1) What is the molecular mechanism whereby connexins control endocrine cells?

Only in a few glands has this mechanism begun to be elucidated. Many of the endogenous molecules that permeate connexin channels (e.g., Ca^{2+} , K^+ , IP_3 , glycolytic intermediates, nucleotides) are also implicated in the control of secretion (69, 197, 281, 334, 335, 356, 401, 402, 522, 572), complicating the identification of the signals that may link changes in connexin-dependent communication to changes in endocrine function. In the endocrine pancreatic islets, stimuli-induced Ca^{2+} transients require connexin channels to become synchronized in different cells, and this synchronization is essential to provide the insulin oscillations that are observed in a normoglycemic and glucose-tolerant individual (190, 204, 534). Why an intercellular asynchrony of such transients is deleterious remains to be established. Conceivably, irregular Ca^{2+} oscillations could alter many cell events, including gene expression and apoptotic pathways. In this perspective, the parallel analysis of insulin- and renin-producing cells may provide interesting clues, inasmuch as increased intracellular Ca^{2+} stimulates insulin secretion (572), but decreases renin secretion (69, 281). Furthermore, secretory systems are just beginning to be evaluated for connexin functions that may not be mediated by cell coupling, but depend on the interactions of the junctional proteins with other membrane or cytosolic partners or specific transcriptions factors, as well as those that may involve connexins for the control of gene expression or the formation of “hemi-channels” contributing to the permeability of nonjunctional domains of the cell membrane (456, 508).

2) What is the cellular mechanism whereby the connexin-dependent signaling affects hormone secretion?

Connexin-dependent coupling is driven by diffusion, thereby achieving a rapid equilibration of ionic and molecular electrochemical gradients between coupled cells. It may be anticipated that, whenever the concentration of critical signal molecules reaches a threshold level for activation or

inhibition of an effector mechanism, the secretory function of gland cells is modified not only in the cell in which the ionic and molecular change first occurred, but also in all cells coupled to it. The coupling-induced equilibration of cytoplasmic molecules would also be expected to synchronize the secretory functions modulated by gap junction-permeant molecules in tissues made of functionally heterogeneous cells. Conversely, uncoupling and/or the expression of connexins resulting in the establishment of electrochemical gradients could prevent an excessive dilution of critical signals, thus permitting a proper activation of effector mechanisms. Several of these expectations have been experimentally verified in the pancreas, whose main secretory cells are functionally heterogeneous, and require coupling for recruitment, synchronization, and control of basal as well as stimulated secretion (43–48, 408, 410, 452, 469). We now need to understand which subpopulations of insulin- or renin-producing cells cross-talk with companion cells and/or with other cell types, notably endothelial cells, and why.

3) What is the hierarchical position of the connexin-dependent signaling?

Vital functions, including insulin and renin secretion, rely on several redundant and cross-talking control mechanisms, which ensure both the persistence of proper function when one mechanism is defective, and the fine tuning of its regulation to adapt to the ever-changing needs of the organism. The finding that chronic alterations of connexins are sufficient to reproduce *in vivo* defects of insulin secretion that are observed in type 2 diabetes (413), and renin alterations that characterize several forms of chronic hypertension (142, 197), indicates that connexin signaling plays a significant role in the network of mechanisms that control both endocrine pancreas and kidney. The reason why the connexin-dependent signaling may contribute to the overall regulatory network more prominently than other mechanisms remains to be understood, as is its hierarchical position among the pathways that allow individual cells to become integrated in a functionally coherent tissue. Also, interactions between connexin-dependent and connexin-independent pathways should be investigated. For example, the Cx36 signaling of pancreatic β -cells cross-talks with the signaling pathway that is dependent on both the ephrin ligand-receptor system (273), and the E-cadherin-dependent regulation (63, 235). In view of the overlapping distribution of connexins and pannexins in many cell types, of the similar, though not identical characteristics of the channels made by these proteins, and of the differential effects of various connexin isoforms have on the expression of selected genes (456, 508), this integration raises the question of which of these mechanisms actually initiates, sustains, and effects the changes in hormone secretion. Thus future studies should also investigate whether and how the pathways controlled by these distinct families

of channel-forming proteins cross-talk within endocrine glands.

4) What is (are) the physiological advantage(s)/disadvantage(s) of connexin signaling?

As compared with other forms of cell-to-cell communications, the intercellular communication mediated by connexins is unique in that it is driven by diffusion, and thus provides a direct mechanism to equilibrate ionic and molecular electrochemical gradients between coupled cells. In such a system, the increase of cytoplasmic ions or molecules less than ~900 Da into one cell may be followed by its diffusion-driven passage into nearby cells connected by functional gap junction channels. At steady state, this passage will lead to the equilibration of electrochemical concentrations on the two sides of the channels. If the resulting concentration reaches a threshold level for activation or inhibition of an effector mechanism, functioning will be modified not only in the cell in which the ionic and molecular change first occurred, but also in all other cells coupled to it. In this case, junctional coupling would result in the functional recruitment of cells that could not be directly activated (or inhibited) in the absence of junctional channels, or when such channels are temporarily closed. Experimental support for such a recruiting role of coupling has been obtained in different systems (46, 338, 348, 410, 446, 452). In some of these cases, it was further noticed that the activity of the coupled cells was actually improved compared with that which the same cells show when they function as single, independent units (43–48, 452). It is therefore likely that the equilibration induced by coupling also optimizes, at the level of a cell population, the threshold concentration of factors that are required for stimulation (or inhibition) of different effector mechanisms. Eventually, the coupling-induced equilibration of cytoplasmic constituents would be expected to synchronize those functions that are modulated by the factors exchanged through gap junctions. This expectation has also been clearly verified in some systems (8, 46, 338, 348, 392, 410, 446, 452). These considerations imply that junctional coupling may be of value in tissues made of cell subpopulations with substantial structural, metabolic, and functional differences. Although we usually assume, at least implicitly, that cells of a given type are all alike and function in the same way under different conditions, increasing evidence shows that this simplistic situation is unlikely to prevail in most tissues. On the contrary, significant structural and functional heterogeneity has been documented in all cell systems in which such diversity has been investigated (46, 338, 348, 410, 446, 452, 504, 506). Disparities in intrinsic properties will conceivably result in the asynchronous function of individual cells. By equilibrating ionic and molecular gradients between coupled cells, junctional coupling could correct these localized imbalances, thus permitting distinct cell subpopulations to function simultaneously and/or at the same rate. This synchro-

nization, however, may not necessarily extend throughout an entire tissue. Actually, in several embryonic and adult systems, coupled cells appear grouped in distinct communication territories, which may or may not be functionally linked to each other (80, 115, 245, 312–314, 342, 346, 437, 504, 506, 509, 565). At least in the case of cardiac muscle, this compartmentalization of intercommunicating cells is crucial for the proper functioning of the tissue (506). Even though several aspects of this scheme are still hypothetical, the overall picture provides a useful conceptual framework to think of specific contributions of coupling and of its potential advantages over other forms of cell-to-cell communication. We also need to appreciate what may be the deleterious consequences of an excess of connexins (79, 358, 557), notably with regard to the altered Ca^{2+} handling that is observed in secretory systems and that, conceivably, could negatively impact on the control of gene expression and cell apoptosis. As summarized above, renin secretion is controlled both by Cx40 signaling in the cells that produce the hormone, and by Cx43-dependent signaling that is generated in the nearby vascular cells. The data open the further intriguing possibility that specific effects of connexins be mediated at short distance by the paracrine action of some signal whose release is controlled by specific connexins, as recently documented in the inner ear (90, 194). The nature of this putative signal remains to be determined. In view of the finding that renin-producing cells communicate with nearby endothelial cells both via gap junctions and paracrine effects, it may also be worth investigating whether pancreatic β -cells also establish connexin-dependent interactions with the endothelial cells of pancreatic islets, which are not dispensable for proper islet formation and function (272).

5) Are connexins required for nonsecretory functions of endocrine cells?

The involvement of connexins and/or coupling in secretion does not rule out a further role of connexins in the control of the developmental growth and morphogenesis of glands, the differentiation of their secretory cells, and/or their renewal after birth. We have yet very limited information on the developmental expression of connexins and its control by transcription factors and/or epigenetic mechanisms. Furthermore, the finding that loss of Cx40 perturbs the spatial arrangement (283) and ultrastructural differentiation of renin-producing cells (277) suggest that some connexin signaling may be implicated in the migration and morphogenetic events that shape multicellular endocrine glands. Another unexpected finding was that loss of Cx36 resulted in increased cytokine-induced apoptosis of the insulin-producing β -cells, whereas overexpression of this connexin protected the islets against a variety of cytotoxic insults (5, 265). These findings raise the exciting possibility that connexins are implicated in controlling the life span of endocrine cells.

6) Do connexins contribute to endocrine diseases?

The involvement of connexins in the physiological secretion of endocrine glands, and the secretory alterations observed after the loss or blockade of specific connexins, raise the possibility that perturbed connexin signaling is involved in the pathogenesis of endocrine diseases. While there is yet no direct support for this possibility, increasing circumstantial evidence in animal models and human tissues calls for careful investigation of this possibility. In the type 1 form of diabetes, an autoimmune attack kills most pancreatic β -cells, leading to a residual mass insufficient to sustain the insulin demand of the organism. As mentioned above, experiments on a variety of transgenic mice expressing different levels of Cx36 have shown that Cx36 protects β -cells in vivo against molecules that experimentally reproduce the autoimmune attack seen at the onset of diabetes, and, conversely, that loss of Cx36 significantly sensitizes β -cells to these aggressive conditions, making the mice overtly diabetic (5, 265). In the type 2 form of diabetes, the β -cell mass is less severely reduced, but the residual insulin-producing cells can no longer release the hormone in response to increased levels of blood glucose. In humans, this defect is first heralded by the loss of the oscillatory release of insulin and, once fully established, is characterized by increased basal levels of the hormone and the inability of the pancreas to further increase its insulin output in response to a glucose challenge, leading to a sustained hyperglycemia. Most of these alterations were observed in rodents lacking Cx36 (421, 503), whereas sulfonylurea treatments (that stimulate the insulin release from the glucose-unresponsive diabetic β -cells) increase β -cell gap junctions and coupling (80, 341, 345). Together with the findings that prolonged exposure of insulin-producing cells to high concentrations of glucose and some fatty acids downregulates Cx36 expression (4, 6), and that the levels of Cx36 transcript correlate with those of insulin (66, 478), these observations raise the intriguing possibility that reduced levels of Cx36 are implicated in the pathogenesis and maintenance of the disease. Similarly, the finding that loss of Cx40 induces a severe hypertension in mice, which mimics several characteristics of the human dysfunction, raises the parallel possibility that decreased levels of Cx40, and/or altered Cx40 signaling is implicated in human hypertension. Studies investigating whether qualitative and/or quantitative alterations in connexin expression/function participate in a causal or consequential manner to the pathogenesis of type 1 and type 2 diabetes, and to hypertension, are now required in a variety of animal models. Obviously, these studies should be extended as far as possible to patients, at least by taking advantage of the genetic and molecular biology approaches that are applicable to humans. Strikingly, pathogenic single nucleotide polymorphisms have been identified in the both human Cx36 (205, 327) and Cx40 (145, 146, 182).

7) Could we take advantage of connexins for therapeutic approaches?

In view of the above, it remains to be investigated whether we could take advantage of connexin biology to develop innovative therapeutic approaches to diseases, primarily or secondarily due to dysfunctions of endocrine glands. This may involve identification of drugs targeted to specific connexins, which in turn implies the development of novel models for the high-throughput screening of candidate molecules (19). The quite limited availability of such molecules is presently a major limitation in testing the functions of connexins (118, 119, 210, 244, 358, 377, 412, 425, 450, 488, 499, 512, 513, 546, 548). Connexins will also be essential for the forthcoming implementation of cell therapies in which surrogate cells generated in vitro are used for the in vivo replacement of damaged cells. This replacement implies that the transplanted cells become functionally integrated within the host tissue, which in endocrine glands would presumably imply the development of appropriate connexin-dependent cell interactions. Strikingly, the embryonic stem cells and progenitor cells that are the basis of many such trials do not express many of the connexins found in adult differentiated endocrine cells (387, 388, 533, 574), including Cx36 and Cx40. This negative observation raises the exciting prospect that proper expression of these connexin isoforms may be feasible and instrumental to improve the function (391) of the insulin-producing β -cells of pancreas, and the renin-producing juxtaglomerular cells of kidney. Given the complexity of the connexin system, and the many cell functions in which connexins appear to be implicated, these challenges are formidable and exciting. They certainly are now timely and most needed to foster the development of innovative therapies of diabetes and hypertension disorders, in view of the exploding prevalence of these diseases.

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DISCLOSURES

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