0031-6997/79/3004-0383$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 30, No. 4


# Gap Junction Dynamics and Intercellular Communication*

DANIEL A. GOODENOUGH

*Department of Anatomy, Harvard Medical School, Boston, Massachusetts*

## I. Properties and Structure of High Resistance Gap Junctions

Since the description of the gap junction (or nexus) in the late 1950s and 1960s as a site of intercellular communication between adjacent cells, a large amount of literature has accumulated documenting the anatomy and physiology of these low resistance pathways between almost all cell types (see reviews: 7, 9, 11, 17, 28, 36, 37, 50, 52, 53, 57, 58, 60, 61, 76, 78, 81). In addition to these studies, enriched preparations of gap junctions have been isolated, using selective detergent solubilization techniques, from mammalian liver (6, 27, 31, 35, 38, 45) from bovine lens (24, 39, 43), and from mouse myocardium (48).

The gap junction is a differentiated area of conjoined plasma membranes of adjacent cells that is specialized to facilitate intercellular diffusion of small metabolites through low resistance pathways. A general property of most gap junctions is the capacity to be switched rapidly from a low resistance to a high resistance state that effectively isolates a triggered cell from communication with its neighbors. This switching phenomenon is discussed in greater detail below; however, since aldehyde fixation is a potent stimulator of the low-to-high resistance switch (7), it should be noted that conventional studies of gap junction ultrastructure are descriptions of the high resistance state.

In thin sectioned specimens, the gap junction appears as an area where the apposed plasma membranes are aligned in parallel, separated by a 2-nm "gap," with an overall junctional thickness of 15 to 18 nm (Fig. 1). Lanthanum impregnation (70) and freeze-fracture electron microscopy reveal a characteristic substructure in the plane of the gap junctional membranes not apparent in thin-sectioned material. Figure 2 is an electron micrograph of freeze-fractured gap junctions between mouse hepatocytes, revealing a polygonal aggregation of uniformly-sized intramembrane particles that have been called "connexons" (36).

The two-dimensional lattice of connexons in hepatocyte gap junctions is visualized in the electron microscope to best advantage in micrographs of isolated, negatively stained material. Figure 3 is an electron micrograph of isolated mouse hepatocyte gap junctions negatively stained with 1% aqueous uranyl acetate. Uranyl acetate, sequestered in the 2-nm "gap," outlines those portions of the connexons located in the gap, and stains a central, hydrophilic domain in the center of each connexon. It is thought that this central domain is an end-on view of an aqueous channel that connects the conjoined cells' cytoplasms, although it is not possible to decide from these micrographs if this channel extends across the full thickness of the junction, from cytoplasm to cytoplasm.

Since the subunits (connexons) of the gap junction are characteristically found in a two-dimensional hexagonal lattice in the plane of the junctional membranes in chemically fixed tissue (70, 73) and in isolated preparations (6, 38, 44), it is possible to record low-angle X-ray diffraction patterns

* This research was supported by Grants GM18974, EY02430, and GM00231 from the National Institutes of Health; PCM77-13955 from the National Science Foundation; and 76-653 from the American Heart Association.

FIG. 1. At high magnification the gap junction in thin sectioned whole mouse liver appears as an intimate association of the apposed cells' plasma membranes, separated by a 2 to 3 nm "gap." The zonula occludens (tight junction) may be clearly distinguished from the gap junction in thin sections by the latter junction's uniform 15-nm thickness and lack of areas of fusion of the membrane outer leaflets.

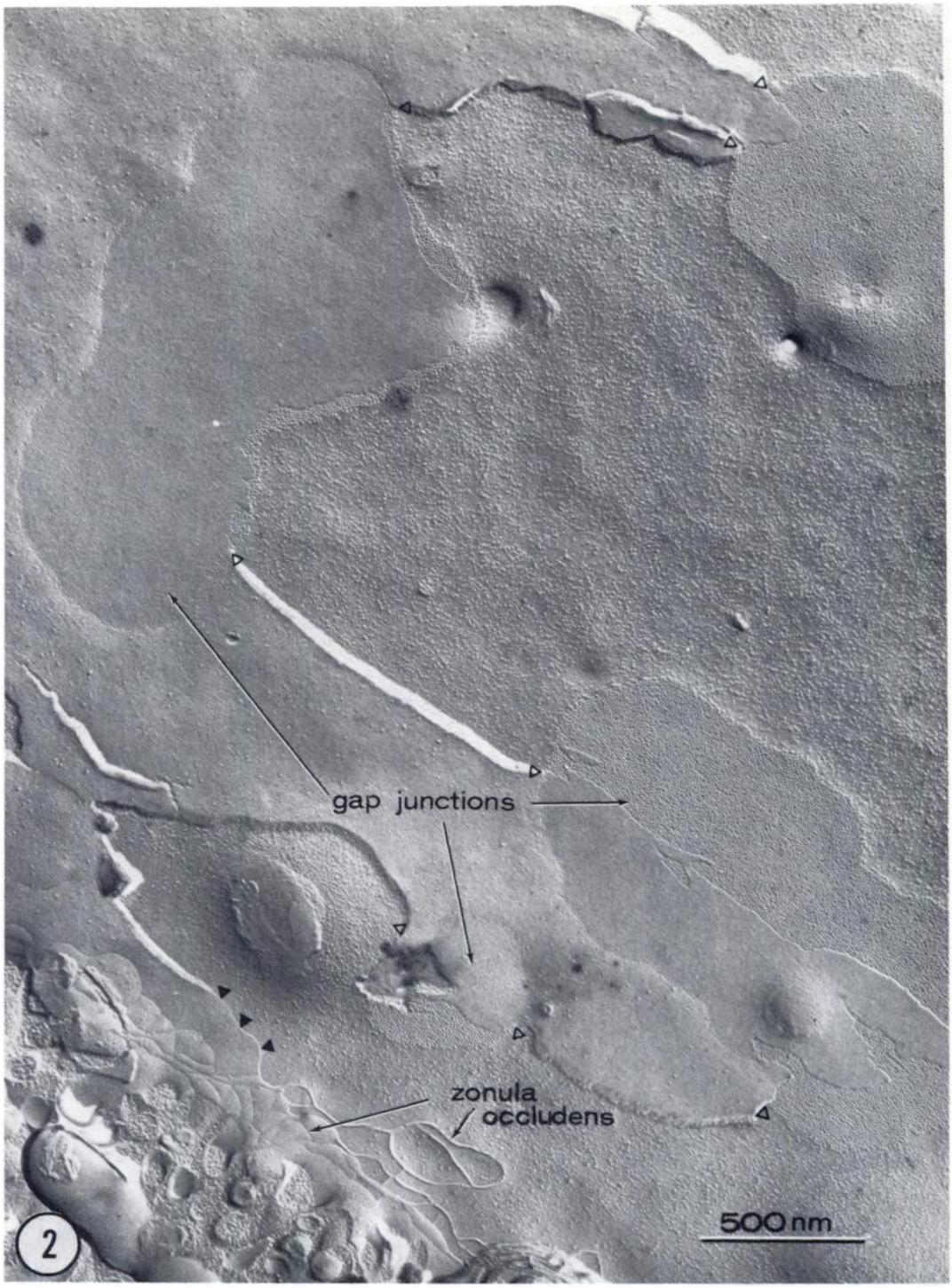


FIG. 2. In freeze-fracture replicas, the gap junction appears as a polygonal lattice of particles on the P-fracture face and a complementary lattice of pits on the E-fracture face, with a variable lattice constant in the range of 8.5 to 9.5 nm. Note in this replica of mouse hepatocytes the severe narrowing of the liver extracellular space at the gap junctions (*open triangles*) where the membranes approach to within 2 nm. The zonula occludens presents branching and anastomosing fibrils and grooves on the P- and E-fracture faces, respectively, and occupies an obligatory location immediately adjacent to the microvillus-engorged bile canaliculus (lumen under the figure number). The extracellular space also narrows dramatically at the zonula occludens (*closed triangles*).

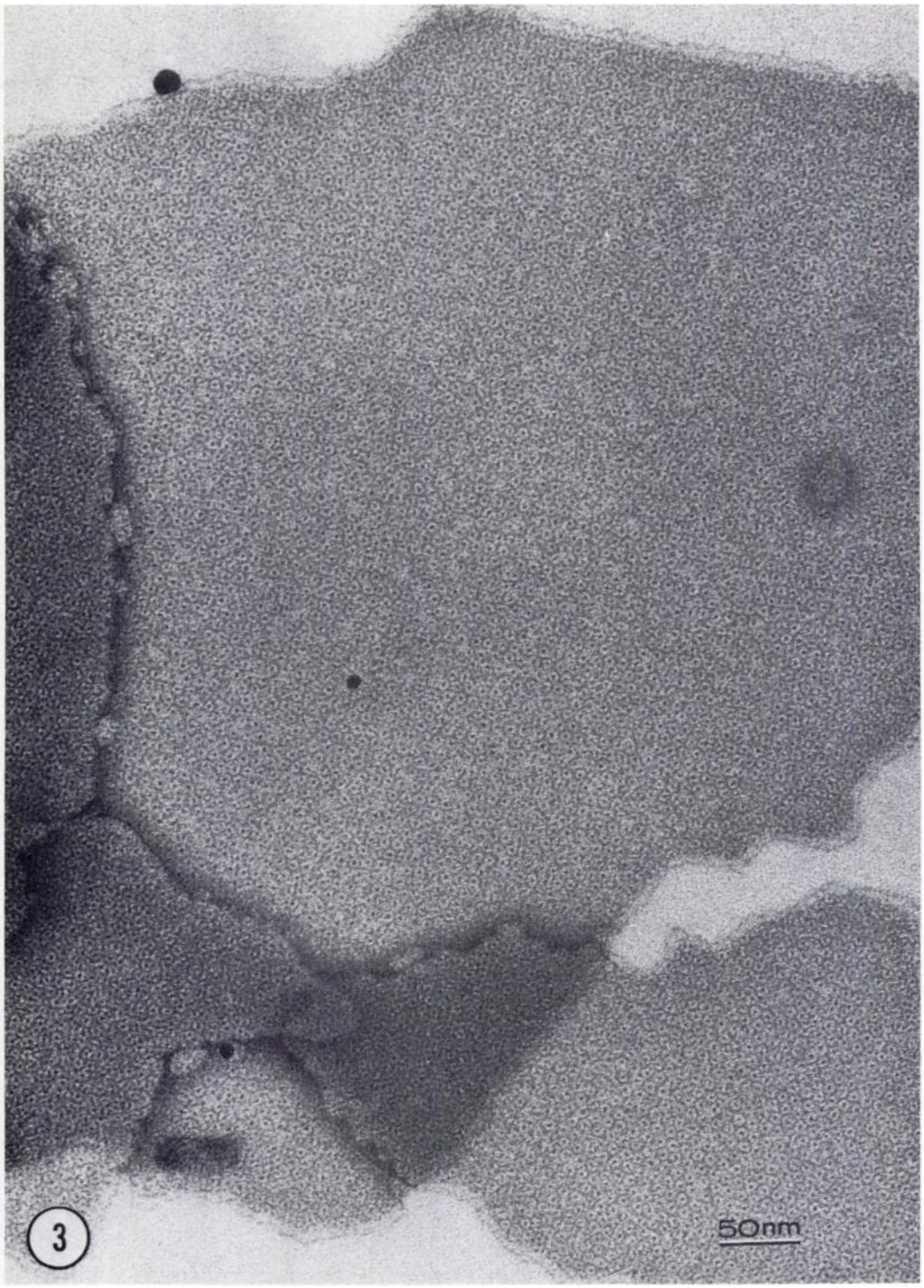


FIG. 3. Electron micrographs of isolated mouse hepatocyte gap junctions negatively stained with 1% aqueous uranyl acetate reveal the hexagonal lattice of connexons, here with an 8-nm lattice constant. Each stain-delineated connexon is pierced by a central dot or hydrophilic core. X-ray diffraction patterns suggest that this core may extend partly or all the way across the 15-nm thickness of the gap junction.

of isolated hepatocyte gap junctions stacked in oriented pellets by high speed centrifugation (16, 40, 41, 45, 56). These X-ray diffraction patterns have demonstrated that each connexon, presumably an intercellular channel in the high resistance state, is composed of a dimer of hexameric assemblies of protein subunits. The connexons in the high resistance state aggregate in the plane of the paired junctional membranes to form a $P6_m$ lattice. Figure 4 is an artist's conception of the arrangement of protein and lipid in the gap junction based on the integrated electron microscopy and X-ray diffraction studies of Caspar et al. (16) and Makowski et al. (56). Although the shapes of the protein subunits are fanciful, the drawing is a faithful representation of protein mass distribution across the junctional profile. Although direct connexon-connexon interactions are possible, the resolution of the data used for this structure determination does not permit direct observations, hence they are not drawn. The isolated gap junctions are certainly in the high resistance state, hence one would expect the central, intraconnexon channel to be closed at some level. The location and mechanism of channel closure are also below the resolution of the data available, thus at 18 to 20 Å resolution, the channel appears open.

Protein profiles, visualized by sodium dodecyl sulfonate (SDS)-polycrylamide gel electrophoresis, have been clouded by problems with proteolysis (35, 38) and low

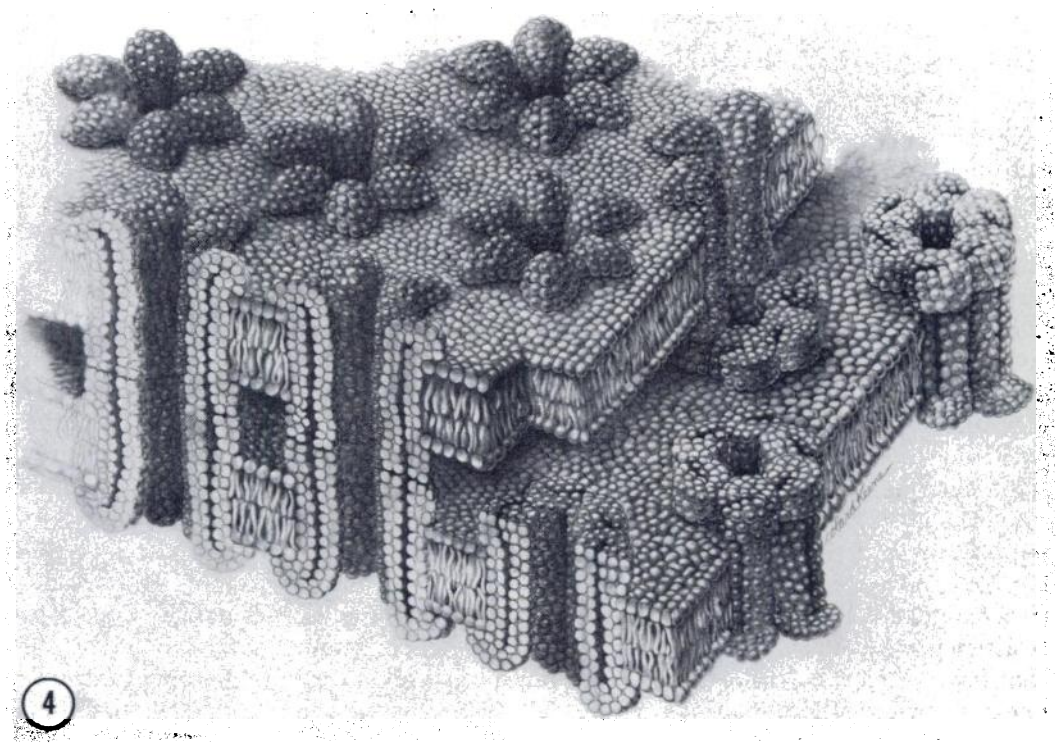


FIG. 4. An artist's (Sylvia Collard Keene) conception of the structure of the isolated, high-resistance mouse hepatocyte gap junction summarizing electron density data calculated from X-ray diffraction patterns coordinated with electron micrographs. The drawing summarizes 10 Å data from the gap junction electron density profile, and 25 Å data from electron density fluctuations in the plane of the junctional membranes. The shapes of the connexons are arbitrary but the relative protein mass at different junctional levels is drawn to scale. The lattice constant of this specimen is 87 Å. This figure is redrawn from the model of specimen E153 in Makowski et al. (56).

yields. To date, several laboratories have reported quantitatively principal polypeptides from mammalian liver gap junctions in the range of 27 kilodaltons (21, 31, 41) although there are reports of peptides at 34 kilodaltons (25) and 38 kilodaltons (20). From bovine lens, a principal polypeptide of 34 kilodaltons has been reported (24), while others resolve mainly a 27 kilodalton polypeptide (2, 13, 39). A principal polypeptide of 27 kilodaltons has also been reported in enriched preparations of gap junctions from mouse myocardium (48).

As noted by Bennett (7), the above data have been accumulated from gap junctions in the high resistance state, with the possible exception of lenticular junctions. Cell homogenization and fixation with aldehydes are potent stimulators of the low-to-high resistance switch, discussed in more detail below.

## II. Properties and Structure of Low-Resistance Gap Junctions

Observations by Goodenough and Gilula (42) and controlled experiments by Peracchia (62) suggest that the connexons are more tightly packed and crystalline in the membrane plane under experimental conditions that favor high resistance. These observations, however, were made with specimens fixed with glutaraldehyde, which has probably switched all junctions from low to high resistance. Recent experiments by Raviola et al. (69), using ultrarapid freezing techniques at liquid helium temperature, which avoid fixation and glycerination (46, 84), reveal that the connexons are in a highly fluid, disorganized morphology when frozen under conditions that favor a low resistance physiology. Under conditions that favor the low-to-high resistance physiological switch, the connexons aggregate with time in the membrane plane, and form the high-resistance crystalline morphology.

This pattern of morphological crystallization may be compared with recent electrophysiological experiments by Loewenstein et al. (54). These investigators were able to detect minute quantal steps in resistance between cells during channel formation and uncoupling, detected by use of a sinusoidal current input in one cell and a phase sensitive measuring system in the adjacent cell. The quantal steps were about 0.61 $\mu$V and suggest that each channel or group of synchronized channels behaves autonomously. If the control is at the level of single channels it would appear that each channel is independently switched. One can imagine that this switch is effected by a structural change in each gap junction connexon. In the open channel (low resistance) conformation, the connexons have little or no mutual affinity. As the connexons switch to high resistance, the resultant structural changes permit lateral crystallization of the connexons in the membrane plane. If true, the crystallization of the gap junction is a consequence of the individual connexon's switch to high resistance, and is not in itself causative.

It has been demonstrated by Rose and Loewenstein (74) in the midge that the high resistance switch is temporally closely in association with a rise in intracellular concentrations of calcium, and junctions may even show graded permeability changes at intermediate calcium levels (75). Turin and Warner (83) have demonstrated in amphibian embryos that the intercellular communication pathways close in response to lowered intracellular pH. Distinguishing between calcium and protons as the trigger is not a straightforward task, and at present there are no data to permit this distinction unequivocally.

A variety of extracellularly applied chemical stimuli have been reported to result in the cellular response of switching to high resistance. Unfortunately, none of these stimuli are specific inhibitors of intracellular communication, but rather have broad and complex effects on the biology of the cell. While many of the chemical stimulants of high resistance are nonphysiological, there are a few noteworthy exceptions where a loss of electrical coupling or a disruption of gap junction structure is effected by "natural" processes and thus reflects

more directly part of the cellular and tissue biology. Included among these exceptions are the uncoupling (switch to high resistance) of embryonic cells during development (10, 30, 34, 66, 77, 85), the rapid changes in granulosa cell junctions in response to estrogens, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (1, 59), the uncoupling of the oocyte at ovulation (1, 32), and acetylcholine-stimulated high resistance in the pancreas (47).

In the embryo, in addition to the hypothesis that gap junctions permit intracellular chemical gradients with positional information (86), the generalization emerges that some cells may uncouple at the time when different developmental pathways are to be pursued. In cell culture, however, heterotypic gap junctions are not uncommon between many differentiated cell types, suggesting that the uncoupling event may be a consequence and not a stimulator of differentiation. It is conceivable, however, that coupled cells may transiently uncouple during a critical moment of development, then reacquire the ability to reform communicating junctions. During this critical period of isolation, extracellular matrix material could then be interposed between the differentiated cells, denying further junctional interaction.

The presence of extracellular matrix between groups of different cell types does not universally imply that the cells are noncommunicating via low resistance pathways. As mentioned above, heterotypic junctional interactions are common in culture (26, 29, 51) and may exist in vivo. For example, angiotensin II, labeled with the electron microscope tracer horseradish peroxidase, is found localized on arterial endothelial cells of the intima, not on the smooth muscle cells of the media (72). Rhodin (71) has illustrated blunt endothelial cell projections that reach through fenestrae in the basal lamina and make intimate contact with the underlying smooth muscle cells. One can imagine, then, that the endothelial cells act as transducers for the pharmacological action of angiotensin II, passing the message to contract to the smooth muscle cells via an unknown second messenger through low resistance intercellular pathways.

## III. Information Passed through Gap Junctions

In nonexcitable tissues, the function of gap junctional communication is not clear. Numerous studies have demonstrated the passage of microinjected fluorescent molecules between conjoined cells [see table 2.1 in Bennett (8)]. Elegant studies by Simpson et al. (80) have defined 1000 to 1200 daltons as the upper limit of molecular weight that can pass through gap junction channels, suggesting a pore diameter of 10 to 14 Å.

In tissue culture, the classical phenomenon of "metabolic cooperation" (82) has been described, which is defined as a contact-dependent cellular sharing of small metabolite pools. Gilula et al. (33) demonstrated that metabolic cooperation is a phenomenon mediated by gap junctions, and studies in several laboratories (18, 19, 64, 65, 79) have demonstrated that only small biological molecules may pass intercellularly, in good agreement with the fluorescent tracer data.

The biological significance of metabolic cooperation is largely unknown. Recent innovative experiments by Lawrence et al. (51) have shown that cyclic AMP, released intracellularly as a second messenger in response to exogenously applied hormones, will pass through low resistance pathways to adjacent cells. These experiments demonstrate that intercellular communication plays a role in the amplification and coordination of the response of groups of cells to hormonal stimulation via second messengers.

The mammalian lens is comprised of an avascular cyst of highly differentiated cells that receives their nourishment from the aqueous humor, a specialized secretion product of the ciliary epithelium. The lens depends on diffusion of nutrient molecules, principally glucose, from the aqueous space in the posterior chamber of the eye into the

lens interior. Ultrastructural studies have revealed enormous numbers of gap junctions between the lens fibers (cells) (11, 12, 43), suggesting an important role for metabolic cooperation in lens cell biology, perhaps in the nourishment of inner cells via cytoplasmic diffusion of small molecules. An unusual feature of lenticular gap junctions is that the connexons in the membrane plane remain in a fluid morphology (43), reminiscent of gap junctions rapidly frozen under low-resistance physiological conditions (69). Isolated lenticular junctions remain in this fluid morphology (39). Attempts to crystallize the lenticular connexons in situ with high-resistance physiological conditions have so far been unsuccessful, with the exception of some lens fiber junctions in the chicken (Goodenough, in preparation). Electrophysiological studies by Andree (5), Duncan (22, 23), and Rae (67) have shown that surface injury to the lens causes a loss of the lens potential difference, that is, whole organ depolarization. Taken together, the morphological and electrophysiological data suggest that lenticular gap junctions, unlike those in other tissues, are refractory to the low-to-high resistance switch.

Lens gap junctions may be isolated (24, 39) and morphological examination reveals that the connexons remain disordered even following these disruptive procedures (43). Peracchia (63) has reported the crystallization of isolated lenticular gap junction connexons in whole membrane preparations in vitro by increased free calcium concentrations, in support of Loewenstein's hypothesis (53) that this divalent ion is central to the regulation of gap junction channel resistance, as reviewed above. Attempts to crystallize enriched preparations of isolated lens gap junctions quantitatively in this laboratory have not yet been successful (39), such that X-ray diffraction patterns of lens junctions have revealed no crystalline order, as they have for isolated liver gap junctions (16, 56). It is of interest to develop methods for quantitative crystallization of lenticular junctions in vitro, since this may allow investigation of the molecular events resulting in the low-to-high resistance switch.

In the lens, it is reasonable to hypothesize that the large numbers of gap junctions function in metabolite diffusion into the lens, as well as ion diffusion. The lens maintains a potential difference of −70 mV relative to the bathing solution (review, 68). The ion pumps in the lens are asymmetrically placed (49), and this results in a translens potential difference of 28 mV, anterior surface positive, with a short-circuit current of 30 $\mu$Å $cm^{-2}$ (14, 15). One may hypothesize that this physiology is only possible because of the numerous gap junctions in the lens, which allow more metabolically active cells at the anterior lens surface to pump ions not only for themselves, but also for less metabolically active cells in the lens interior.

In summary, although there are good data in a number of experimental systems that define pore sizes through gap junctions, an understanding of the biologically significant molecules used for intercellular communication is limited to cyclic nucleotides, ion balance in tissues, and possible metabolite diffusion between cells both in culture and in the lens. A situation similar to the lens may also exist in developing mammalian follicles, where oocytes have been demonstrated to be joined to surrounding granulosa cells by gap junctions (4, 32).

A sorely needed research tool is a method to interrupt gap junctional communication specifically so that its effects on cell and tissue biology can be observed. Alcala and Maisel (3) have reported a specific antiserum to the main intrinsic polypeptide of chick lens fiber plasma membranes, thought to be a specific gap junction polypeptide (2, 55). Once characterized and purified, these kinds of antisera may provide a valuable probe for gap junctions in cell and tissue physiology.

It is clear that cells comprising most tissues are connected by gap junctions, and that gap junctions are dynamic structures

capable of switching their resistance properties rapidly in response to a variety of stimuli. In order for groups of individual cells to behave in a functionally coordinated manner, such as responses to hormones and growth control (64), one could imagine that a communication system might be mandatory. Deciding whether gap junctions subserve these coordinating functions in tissues and organs must await further investigation.

## REFERENCES


1. ALBERTINI, D. F., AND ANDERSON, E.: The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. J. Cell Biol. **63**: 234–250, 1974.
2. ALCALA, J., LIESKA, N., AND MAISEL, H.: Protein composition of bovine lens cortical fiber cell membranes. Exp. Eye Res. **21**: 581–595, 1975.
3. ALCALA, J., AND MAISEL, H.: Specific antiserum to the main intrinsic polypeptide of chick lens fiber cell plasma membranes. Exp. Eye Res. **26**: 219–221, 1978.
4. ANDERSON, E., AND ALBERTINI, D. F.: Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. J. Cell Biol. **71**: 680–686, 1976.
5. ANDREE, G.: Über die Natur des transkapsularen Potentials der Linse. Pflügers Arch. Gesamte Physiol. Menschen Tiere **267**: 109–116, 1958.
6. BENEDETTI, E. L., AND EMMELOT, P.: Hexagonal array of subunits in tight junctions separated from isolated rat liver plasma membranes. J. Cell Biol. **38**: 15–24, 1968.
7. BENNETT, M. V. L.: Function of electrotonic junctions in embryonic and adult tissues. Fed. Proc. **32**: 65–75, 1973.
8. BENNETT, M. V. L.: Junctional permeability. *In* Intercellular Junctions and Synapses, ed. by J. Feldman, N. B. Gilula, and J. D. Pitts, pp. 23–36, Chapman and Hall, London, 1978.
9. BENNETT, M. V. L., AND GOODENOUGH, D. A.: Electrotonic junctions. Neurosci. Res. Program Bull. **16**: 373–486, 1978.
10. BLACKSHAW, S. E., AND WARNER, A. E.: Low resistance junctions between mesoderm cells during development of trunk muscles. J. Physiol. (London) **255**: 209–230, 1976.
11. BLOEMENDAL, H.: The vertebrate eye lens. Science **197**: 127–138, 1977.
12. BLOEMENDAL, H. A., ZWEERS, R., VERMOVEN, I. D., AND BENNEDETTI, E. L.: The plasma membrane of eye lens fibers. Biochemical and structural characterization. Cell Differentiation **1**: 91–106, 1972.
13. BROEKHUYSE, R. M., KUHLMANN, E. D., AND STOLS, A. H.: Lens membranes. II. Isolation and characterization of the main intrinsic polypeptide (MIP) of bovine lens fiber membranes. Exp. Eye Res. **23**: 365–371, 1976.
14. CANDIA, O. A., BENTLEY, P. J., AND MILLS, C. D.: Short-circuit current and active Na transport across isolated lens of the toad. Amer. J. Physiol. **220**: 558–564, 1971.
15. CANDIA, O. A., BENTLEY, P. J., MILLS, C. S., AND TOYOFUKU, H.: Asymmetrical distribution of the potential difference in the toad lens. Nature (London) **227**: 852–853, 1970.
16. CASPAR, D. L. D., GOODENOUGH, D. A., MAKOWSKI, L., AND PHILLIPS, W. C.: Gap junction structures. I. Correlated electron microscopy and x-ray diffraction. J. Cell Biol. **74**: 605–628, 1977.
17. COX, R. P. (editor). *Cell Communication*. John Wiley & Sons, New York, 1974.
18. COX, R. P., KRAUSS, M. R., BALIS, M. E., AND DANCIS, J.: Evidence for transfer of enzyme product as the basis of metabolic cooperation between tissue culture fibroblasts of Lesch-Nyhan disease and normal cells. Proc. Nat. Acad. Sci. U.S.A. **67**: 1573–1579, 1970.
19. COX, R. P., KRAUSS, M. R., BALIS, M. E., AND DANCIS, J.: Metabolic cooperation in cell culture. A model for cell-to-cell communication. *In* Cell Communication, ed. by R. P. Cox, pp. 67–95, John Wiley & Sons, New York, 1974.
20. CULVENOR, J. G., AND EVANS, W. H.: Preparation of hepatic gap (communicating) junctions. Identification of the constituent polypeptide subunits. Biochem. J. **168**: 475–481, 1977.
21. DUGUID, J. R., AND REVEL, J. P.: The protein components of the gap junction. Cold Spring Harbor Symp. Quant. Biol. **40**: 45–47, 1976.
22. DUNCAN, G.: Relative permeabilities of the lens membranes to sodium and potassium. Exp. Eye Res. **8**: 315–325, 1969.
23. DUNCAN, G.: The sites of ion restricting membranes in the toad lens. Exp. Eye Res. **8**: 406–412, 1969.
24. DUNIA, I., GHOSH, C. S., BENEDETTI, E. L., ZWEERS, A., AND BLOEMENDAL, H.: Isolation and protein pattern of eye lens fiber junctions. Fed. Eur. Biochem. Soc. Lett. **45**: 139–144, 1974.
25. EHRHART, J. C., AND CHAUVEAU, J.: The protein component of mouse hepatocyte gap junctions. Fed. Eur. Biochem. Soc. Lett. **78**: 295–299, 1977.
26. EPSTEIN, M. L., AND GILULA, N. B.: A study of communication specificity between cells in culture. J. Cell Biol. **75**: 769–787, 1977.
27. EVANS, W. H., AND GURD, J. W.: Preparation and properties of nexuses and lipid enriched vesicles from mouse liver plasma membranes. Biochem. J. **128**: 691–700, 1972.
28. FELDMAN, J., GILULA, N. B., AND PITTS, J. D. (editors): *Intercellular Junctions and Synapses*, Chapman and Hall, London, 1978.
29. FENTIMAN, I., TAYLOR-PAPADIMITRIOU, J., AND STOKER, M.: Selective contact-dependent cell communication. Nature (London) **264**: 760–762, 1976.
30. FURSHPAN, E. J., AND POTTER, D. D.: Low-resistance junctions between cells in embryos and tissue culture. Curr. Top. Develop. Biol. **3**: 95–127, 1968.
31. GILULA, N. B.: Isolation of rat liver gap junctions and characterization of the polypeptides. J. Cell Biol. **63**: 111a, 1974.
32. GILULA, N. B., EPSTEIN, M. L., AND BEERS, W. H.: Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. J. Cell Biol. **78**: 58–75, 1978.
33. GILULA, N. B., REEVES, O. R., AND STEINBACH, A.: Metabolic coupling, ionic coupling, and cell contacts. Nature (London) **235**: 262, 1972.
34. GINZBERG, R. D., AND GILULA, N. G.: A correlation between gap junctions and synaptogenesis in the developing chicken otocyst. J. Cell Biol. **75**: 38a, 1977.
35. GOODENOUGH, D. A.: Bulk isolation of mouse hepatocyte gap junctions. Characterizationof the principal protein, connexin. J. Cell Biol. **61**: 557–563, 1974.
36. GOODENOUGH, D. A.: Methods for the isolation and structural characterization of hepatocyte gap junctions. *In* Methods in Membrane Biology, ed. by E. D. Korn, vol. III, pp. 51–80, Plenum Press, New York, 1975.
37. GOODENOUGH, D. A.: The structure and permeability of isolated hepatocyte gap junctions. Cold Spring Harbor Symp. Quant. Biol. **40**: 37–43, 1976.
38. GOODENOUGH, D. A.: In vitro formation of gap junction vesicles. J. Cell Biol. **68**: 220–231, 1976.
39. GOODENOUGH, D. A.: Isolation of lens gap junctions. Biophys. J. **21**: 147a, 1978.
40. GOODENOUGH, D. A., CASPAR, D. L. D., MAKOWSKI, L., AND PHILLIPS, W. C.: X-ray diffraction of isolated gap junctions. J. Cell Biol. **63**: 115a, 1974.
41. GOODENOUGH, D. A., CASPAR, D. L. D., PHILLIPS, W. C.,

AND MAKOWSKI, L.: Preparation and characterization of highly ordered gap junction structure (abstract). J. Cell Biol. 79: 223a, 1978.
42. GOODENOUGH, D. A., AND GILULA, N. B.: Cell junctions and intercellular communication. *In* Membranes and Viruses in Immunopathology, ed. by S. B. Day and R. A. Good, pp. 155–168, Academic Press, New York, 1972.
43. GOODENOUGH, D. A., PAUL, D. L., AND CULBERT, K. E.: Correlative gap junction ultrastructure. Birth Defects: Original Article Series, 14: 83–97, 1978.
44. GOODENOUGH, D. A., AND REVEL, J. P.: A fine structural analysis of intercellular junctions in the mouse liver. J. Cell Biol. 45: 272–290, 1970.
45. GOODENOUGH, D. A., AND STOECKENIUS, W.: The isolation of mouse hepatocyte gap junctions. Preliminary chemical characterization and x-ray diffraction. J. Cell Biol. 54: 646–656, 1972.
46. HEUSER, J. E., REESE, T. S., AND LANDIS, D. M. D.: A preservation of synaptic structure by rapid freezing. Cold Spring Harbor Symp. Quant. Biol. 40: 17–24, 1976.
47. IWATSUKI, N., AND PETERSEN, O. H.: Pancreatic acinar cells: Acetylcholine evoked electrical uncoupling and its ionic dependency. J. Physiol. (London) 274: 81–96, 1978.
48. KENSLER, R. W., AND GOODENOUGH, D. A.: Isolation of the nexus from mouse myocardium. Circulation 58: Suppl. II, 53, 1978.
49. KINSEY, V. E., AND REDDY, D. V. N.: Studies on the crystalline lens. XI. The relative role of the epithelium and capsule in transport. Invest. Ophthalmol. 4: 104–116, 1965.
50. LARSEN, W. J.: Structural diversity of gap junctions. A review. Tissue Cell 9: 373–394, 1977.
51. LAWRENCE, T. S., BEERS, W. H., AND GILULA, N. B.: Transmission of hormonal stimulation by cell-to-cell communication. Nature (London) 272: 501–506, 1978.
52. LOEWENSTEIN, W. R.: Membrane junctions in growth and differentiation. Fed. Proc. 32: 60, 1973.
53. LOEWENSTEIN, W. R.: Permeable junctions. Cold Spring Harbor Symp. Quant. Biol. 40: 49–63, 1976.
54. LOEWENSTEIN, W. R., KANNO, Y., AND SOCOLAR, S. J.: Quantum jumps of conductance during formation of membrane channels at cell-cell junction. Nature (London) 274: 133–136, 1978.
55. MAISEL, H.: The nature of urea-insoluble material of the human lens. Exp. Eye Res. 24: 417–419, 1977.
56. MAKOWSKI, L., CASPAR, D. L. D., PHILLIPS, W. C., AND GOODENOUGH, D. A.: Gap junction structures. II. Analysis of the x-ray diffraction data. J. Cell Biol. 74: 629–645, 1977.
57. MCNUTT, N. S.: Ultrastructure of intercellular junctions in adult and developing cardiac muscle. Amer. J. Cardiol. 25: 169, 1970.
58. MCNUTT, N. S., AND WEINSTEIN, R. S.: Membrane ultrastructure at mammalian intercellular junctions. Prog. Biophys. Mol. Biol. 26J: 45–101, 1973.
59. MERK, F. S., BOTTICELLI, C. R., AND ALBRIGHT, J. T.: An intercellular response to estrogen by granulosa cells in the rat ovary; an electron microscopy study. Endocrinology 90: 992–1011, 1972.
60. PAPPAS, G. D.: Junctions between cells. Hospital Practice, August 1973, pp. 39–46, 1973.
61. PERACCHIA, C.: Gap junction structure and function. Trends Biochem. Sci. 2: 26–50, 1977.
62. PERACCHIA, C.: Gap junctions. Structural changes after uncoupling procedures. J. Cell Biol. 72: 628–641, 1977.
63. PERACCHIA, C.: Calcium effects on gap junction structure and cell coupling. Nature (London) 271: 669–671, 1978.
64. PITTS, J. D.: Junctional communication and cellular growth control. *In* Intercellular Junctions and Synapses, ed. by J. Feldman, N. B. Gilula, and J. D. Pitts, pp. 61–79, Chapman and Hall, London, 1978.
65. PITTS, J. D., AND SIMMS, J. W.: Permeability of junctions between animal cells. Intercellular transfer of nucleotides but not macromolecules. Exp. Cell Res. 104: 153–163, 1977.
66. POTTER, D. D., FURSHPAN, E. J., AND LENNOX, E. S.: Connections between cells of the developing squid as revealed by electrophysiological methods. Proc. Nat. Acad. Sci. U. S. A. 55: 328–336, 1966.
67. RAE, J. L.: The potential difference in the frog lens. Exp. Eye Res. 15: 485–494, 1973.
68. RAE, J. L.: The electrophysiology of the crystalline lens. Curr. Top. Eye Res. 1: 37–90, 1978.
69. RAVIOLA, E., GOODENOUGH, D. A., AND RAVIOLA, G.: The native structure of gap junctions rapidly frozen at 4°K (abstract). J. Cell Biol. 79: 229a, 1978.
70. REVEL, J. P., AND KARNOVSKY, M. J.: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. 33: C7, 1967.
71. RHODIN, J. A. G.: The ultrastructure of mammalian arterioles and precapillary sphincters. J. Ultrastruct. Res. 18: 181–204, 1967.
72. RICHARDSON, J. B., AND BEAULNES, A.: The cellular site of action of angiotensin. J. Cell Biol. 51: 419–432, 1971.
73. ROBERTSON, J. D.: The occurrence of a subunit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains. J. Cell Biol. 19: 201–221, 1963.
74. ROSE, B., AND LOEWENSTEIN, W. R.: Permeability of cell junction depends on local cytoplasmic calcium activity. Nature (London) 254: 250–254, 1975.
75. ROSE, B., SIMPSON, I., AND LOEWENSTEIN, W. R.: Calcium ion produces graded changes in permeability of membrane channels in cell junction. Nature (London) 267: 625–627, 1977.
76. SATIR, P., AND GILULA, N. B.: The fine structure of membranes and intercellular communication in insects. Annu. Rev. Entomol. 18: 143–166, 1973.
77. SHERIDAN, J. D.: Electrophysiological evidence for low-resistance intercellular junctions in the early chick embryo. J. Cell Biol. 37: 650–659, 1968.
78. SHERIDAN, J. D.: Functional evaluation of low resistance junctions: Influence of cell shape and size. Amer. Zool. 13: 1119, 1973.
79. SHERIDAN, J. D., FINBOW, M., AND PITTS, J. D.: Metabolic cooperation in culture: Possible involvement of junctional transfer in regulation of enzyme activities. J. Cell Biol. 67: 396a, 1975.
80. SIMPSON, I., ROSE, B., AND LOEWENSTEIN, W. R.: Size limit of molecules permeating the junctional membrane channels. Science 195: 294–297, 1977.
81. STAEHELIN, L. A., AND HULL, B. E.: Junctions between living cells. Sci. Amer. 238: 140–152, 1978.
82. SUBAK-SHARPE, H., BURK, R. R., AND PITTS, J. D.: Metabolic cooperation between biochemically marked mammalian cells in tissue culture. J. Cell Sci. 4: 353–367, 1969.
83. TURIN, L., AND WARNER, A.: Carbon dioxide reversibly abolishes ionic communication between cells of early amphibian embryo. Nature (London) 270: 56–57, 1977.
84. VAN HARREVELD, A., AND CROWELL, J.: Electron microscopy after rapid freezing on a metal surface and substitution fixation. Anat. Rec. 149: 381–397, 1964.
85. WARNER, A. E.: The electrical properties of the ectoderm in the amphibian embryo during induction and early development of the nervous system. J. Physiol. (London) 235: 267–286, 1973.
86. WOLPERT, L.: Gap junctions: Channels for communication in development. *In* Intercellular Junctions and Synapses, ed. by J. Feldman, N. B. Gilula, and J. D. Pitts, pp. 81–96, Chapman and Hall, London, 1978.