ATYPICAL GAP JUNCTIONS IN THE CILIARY EPITHELIUM OF THE ALBINO RABBIT EYE

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Gap junctions are now routinely described between many kinds of cells and are thought to mediate electrotonic coupling by providing patent communicating channels between cells through which ions and other metabolites can pass (3, 17). The gap junction has an intercellular space of 20–40 Å as delineated by en bloc uranyl acetate staining and colloidal lanthanum tracing techniques in sectioned tissue (16). The substructure as seen by lanthanum tracing in en face sections through gap junctions (16) as well as by negative staining (2) is typically described as an hexagonal array of subunits having a diameter of about 80 Å and a center-to-center spacing of 90–100 Å. (See reference 7 for review.)

With the more recent utilization of freeze-fracture techniques it has been shown that not all gap junctions are morphologically identical. The major differences noted are the varying sizes of subunit particles, their density per unit area, and their arrangement. Such atypical gap junction substructures have been noted by examination of freeze-fracture preparations in vertebrates (1, 4, 6, 13, 15, 18) and invertebrates (11, 17). Study of lanthanum-treated tissue has in one case suggested two packing arrangements of subunits (10) and in another case suggested an unusual configuration of subunits which are more clearly defined in freeze-fracture preparations (1).

We also find gap junctions with an unusual...
substructure in the ciliary epithelium of the albino rabbit eye and are able to demonstrate this in conventional thin sections in lanthanum-treated tissue.

MATERIALS AND METHODS

Albino rabbits from birth to over 6 mo of age were anesthetized with sodium pentobarbitol. The ciliary body-iris was lifted from the eye into a primary fixative containing 2.5% glutaraldehyde, 1% LaOH, and 0.1 M Na cacodylate for 1 h. The final buffer rinse was carried out overnight after three shorter rinses in 0.1 M Na cacodylate containing 1% LaOH. Postfixation was carried out in 1% OsO₄ containing 1% LaOH for 1/2-1 h. Stock LaOH was prepared fresh each time by slowly adding 0.1 M NaOH to a 4% solution of lanthanum nitrate with constant swirling until a milky opalescence became apparent, usually at pH 7.6-7.8 (16). The tissue was embedded in Epon after dehydration. Thin sections were cut on a Porter-Blum MT2 microtome. Staining was done in lead salts followed by uranyl acetate in methanol or aqueous uranyl acetate followed by lead salts. Specimens were examined with an RCA-3G or an AEI 801 electron microscope.

OBSERVATIONS

Because of the embryological infolding of the optic cup, the single cell layers of the nonpigmented epithelium and the pigmented epithelium of the ciliary process are in apposition at their apices. The base of the nonpigmented epithelium faces the aqueous humor, and the base of the pigmented epithelium faces the stroma. Lanthanum tracing became apparent, usually at pH 7.6-7.8 (16). The tissue was embedded in Epon after dehydration. Thin sections were cut on a Porter-Blum MT2 microtome. Staining was done in lead salts followed by uranyl acetate in methanol or aqueous uranyl acetate followed by lead salts. Specimens were examined with an RCA-3G or an AEI 801 electron microscope.

In the lanthanum-treated tissue, the intercellular space in the gap junctions viewed in cross section is 30-40 Å (double arrows in Figs. 2 and 3). Hexagonal arrays appear in en face sections with a center-to-center spacing of about 105 Å. The unusual and striking feature of these gap junctions is the dark banding which regularly appears every three rows of subunits or facets. This dark banding represents an interruption of the facets so that we visualize the lanthanum-filled, enlarged extracellular space at regular intervals. The dark band is 210 Å wide or the width of two rows of facets.

DISCUSSION

Staehelin (18) demonstrated three types of gap junctions in the epithelium of the rat small intestine. Type I was the “normal” gap junction with 80–90 Å particles hexagonally arrayed and a center-to-center distance of 90–100 Å. Type II was closely associated with Type I, but had larger particles of 100–110 Å. Type III was randomly distributed away from Type I and II and consisted of small rectilinear arrays of only 4–30 particles with a center-to-center distance of 60–80 Å. Type III, however, was subsequently demonstrated in muscle fiber sarcolemmal which are separated by a thick basement membrane and is no longer considered to be a gap junction (14).

Peracchia (10, 11) has described two classes of subunit arrays in the lateral giant fibers of the crayfish. One set of globules sits in hexagonal array with a center-to-center distance of about 200 Å, and another set has disordered packing with a minimum center-to-center distance of about 125 Å. Peracchia and Dulhunty (12) have suggested that these packing arrangements are related to changes in permeability of the junction. After incubating crayfish ganglia and rat stomach in the presence of uncouplers, they find the arrays more closely and more regularly packed. Raviola and Gilula (15) demonstrated gap junction particles between photoreceptor cells of monkeys, rabbits, and turtles arranged in one or two rows with about 50 particles per row. The particles had a range of diameters from 80 to 140 Å and a center-to-center spacing from 80 to 180 Å. Typical hexagonal arrays were also present. Goodenough and Gilula (6) describe larger particles with a center-to-center distance of 95–120 Å surrounding the normal particles in hepatocytes after freeze-fracture of mouse livers which had been perfused with hypertonic solutions.

Albertini and Anderson (1) very recently demonstrated unusual arrays of particles between granulosa cells of the rabbit ovarian follicle. The granulosa cells of large Graafian follicles have gap junctions which, in freeze-fracture preparations, appear to be very similar to what we see in the ciliary epithelium. Their sections of the lanthanum-treated tissue suggest an unusual array of subunits, but do not clearly confirm the freeze-
FIGURE 1  Electron micrograph of a section of ciliary epithelium from an 8-day-old albino rabbit. Lanthanum has demarcated the gap junctions between the nonpigmented epithelium (NPE) and adjacent pigmented cells (PE) (small arrows). Typical finger-like processes protrude between adjacent cells (large arrows). PG = pigment granule. x 23,000.

fracture data demonstrating orderly interruptions between rows. Pinto da Silva and Gilula (13) also demonstrated by freeze-fracture a similar subpopulation of gap junctions in normal and transformed chick embryo fibroblasts which are not visualized in their lanthanum-impregnated cultures. Recently, Gilula (5) published an electron micrograph of a freeze-fracture preparation show-
FIGURE 2  Section of a portion of a gap junction between two cells. A perpendicular view through the junction is seen at the double arrow, with lanthanum filling the gap. A honeycomb arrangement of subunits is evident when a tangential to en face view of the junction is seen. The three arrows point to the dark banding which occurs every three rows of subunits. PG = pigment granule. × 64,500.

FIGURE 3  Lanthanum preparation similar to that in Fig. 2. The linear pattern of dark banding (small arrows) between the subunits or facets of the gap junction breaks up in this particular tangential section (at large arrow), due to the curvature of the junction at this point. Perpendicular view of the junction is shown at the double arrows. × 117,000.

FIGURES 4 and 5  In these sections, the pattern of dark banding every three rows of facets is clearly seen in the two en face profiles through gap junctions surrounding the finger-like processes of one pigment cell projecting into another. This dark banding (at arrows) represents an interruption of the subunits or facets so that we visualize the lanthanum-filled extracellular space at regular intervals. The dark bands measure 210 Å or the width of two rows of facets. In Fig. 5, the gap junction probably represents an en face profile of the tip of the projection of one cell into the other. Fig. 4 = × 210,000. Fig. 5 = × 135,000.
ing gap junctions in the ciliary epithelium of the rabbit. Particle-free "aisles" can be seen. However, the arrangement of the particles does not show the striking regularity that we find in the sections of lanthanum-treated tissue.

Decker and Friend (4) have described the formation of gap junctions during amphibian neurulation, showing a transition from zonulae occludentes to typical gap junctions. Various arrays and particle sizes are noted during this transition period, from rows of 85 Å particles to beaded rows and doublets to small patches of polygonally packed particles of 80-90 Å surrounded by larger (about 100 Å) particles. As a result of this developmental study, the investigators felt that all other atypical gap junctions may represent transition stages in the formation of new junctions. The possibility that the typical gap junction represents a "finished product" in all tissues, with ongoing assembly of new junctions whose stages of development are unique configurations of subunits, certainly exists. However, it is tempting to speculate that there are in fact different populations of gap junctions present at all times which could allow for greater specificity of function. The aqueous humor of the newborn rabbit is not of the same composition as it is in the adult, and one might expect some change in the junctions as the aqueous humor takes on adult characteristics. But we do not see any differences with maturation when tracers and thin sectioning techniques are used, for atypical gap junctions are present from birth through adulthood, which supports the theory that different populations of substructures exist concomitantly.

Electrical coupling and the transfer of ions and low mol wt substances have been studied in cells where the subunits of the gap junction are arranged more or less compactly (3, 5, 8, 9). At this time, it is difficult to assess the physiological significance of the differences in the packing of the subunit arrays in some gap junctions as in the rabbit ciliary epithelium, for example.

SUMMARY

Gap junctions are present between the nonpigmented and pigmented epithelial cells layers, as well as between the adjacent pigmented cells, but not between the nonpigmented epithelial cells which face the posterior chamber. The unusual feature of these gap junctions is a dark banding which regularly appears every three rows of subunits. This dark band is the equivalent width of two rows of facets.

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REFERENCES

1. ALBERTINI, D. F., and E. ANDERSON. 1974. The appearance and structure of intercellular connections during ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. J. Cell Biol. 63:234–250.
2. BENEDETTI, E. L., and P. EMMELOT. 1968. Hexagonal array of subunits in tight junctions separated from isolated rat liver plasma membranes. J. Cell Biol. 38:15–24.
3. BENNETT, M. V. L. 1973. Function of electrotonic junctions in embryonic and adult tissues. Fed. Proc. 32:65–75.
4. DECKER, R. S., and D. S. FRIEND. 1974. Assembly of gap junctions during amphibian neurulation. J. Cell Biol. 62:32–47.
5. GILULA, N. B. 1974. Junctions between cells. In Cell Communication, R. P. COX, editor. John Wiley & Sons, Inc., New York. 1–29.
6. GOODENOUGH, D. A., and N. B. GILULA. 1974. The splitting of hepatocyte gap junctions and zonulae occludentes with hypertonic disaccharides. J. Cell Biol. 61:575–590.
7. MCNUTT, N. S., and R. S. WEINSTEIN. 1973. Membrane ultrastructure at mammalian intercellular junctions. Prog. Biophys. Mol. Biol. 26:45–101.
8. PAPPAS, G. D., Y. ASADA, and M. V. L. BENNETT. 1971. Morphological correlates of increased coupling resistance at an electrotonic synapse. J. Cell Biol. 49:173–188.
9. PAPPAS, G. D., and S. G. WAXMAN. 1972. Synaptic fine structure-morphological correlates of chemical and electrotonic transmission. In Structure and Function of Synapses, G. D. PAPPAS and D. P. PURPURA, editors. Raven Press, Hewlett, N. Y. 1–43.
10. PERACCHIA, C. 1973. Low resistance junctions in crayfish. I. Two arrays of globules in junctional membranes. J. Cell Biol. 57:54–65.
11. PERACCHIA, C. 1973. Low resistance junctions in crayfish. II. Structural details and further evidence for intercellular channels by freeze-fracture and negative staining. J. Cell Biol. 57:66–76.
12. PERACCHIA, C., and A. F. DULHUNTY. 1974. Gap junctions: structural changes associated with changes in permeability. J. Cell Biol. 63(2, Pt. 2):263 a. (Abstr.).
13. PINTO DA SILVA, P., and N. B. GILULA. 1972. Gap junctions in normal and transformed fibroblasts in culture. Exp. Cell Res. 71:393–401.
14. RASH, J. E., L. A. STAHELM, and M. H. ELLISMAN. 1974. Rectangular arrays of particles on freeze-
cleaved plasma membranes are not gap junctions. Exp. Cell Res. 86:187–190.
15. RAVOLA, E., and N. G. GILULA. 1973. Gap junctions between photoreceptor cells in the vertebrate retina. Proc. Natl. Acad. Sci. U.S.A. 70:1677–1681.
16. REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. 33:C7–12.
17. SATIR, P., and N. B. GILULA. 1973. The fine structure of membranes and intercellular communication in insects. Ann. Rev. Entomol. 18:143–166.
18. STAHELIN, L. A. 1972. Three types of gap junctions in interconnecting intestinal epithelial cells visualized by freeze-etching. Proc. Natl. Acad. Sci. U.S.A. 69:1318–1321.