TIGHT JUNCTIONS BETWEEN CELLS IN THE EARLY CHICK EMBRYO AS VISUALIZED WITH THE ELECTRON MICROSCOPE

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Once embryonic cells have begun a course of somatic differentiation, they are characterized by a remarkable degree of adhesiveness which can be disrupted by trypsin in calcium-poor solutions (1–3) and which seems attributable in part to desmosomelike specializations, the zonula adhaerens and macula adhaerens (4–5). Another cell contact specialization, one which has received little attention from embryologists, is the so-called tight junction which has been reported to take the form of a zonula, macula, or fascia occludens in mature tissues (4, 10). Potter, Furshpan, and Lennox (6) have recently discovered that electrical coupling between embryonic cells appears prior to overt cell differentiation in the squid embryo. A preliminary report by Sheridan (7) demonstrating low electrical resistance between cells in chick embryos shows that a similar phenomenon exists in developing vertebrates. In view of the possibility that tight junctions are involved in electrical conductance between cells (6, 8) and because of the relation to Sheridan's work, we are publishing a preliminary account of observations made over the past few years on the fine structure of cell contact specializations in the primitive germ layers and developing tissues of the chick embryo.

MATERIAL AND METHODS

Chick embryos from the four somite stage through the period of formation of the definitive organ anlage (Hamburger-Hamilton stages 4–18) were dissected from the yolk and fixed with the vitelline membrane intact for 15 min at 25°C in 2.5% glutaraldehyde with 4.0% paraformaldehyde and 0.075% $CaCl_2$ at pH 7.5 in 0.1 M cacodylate (9). The tissues were washed briefly with two or three changes in 0.1 M Na cacodylate and postfixed in 1.2% osmium tetroxide in collidine buffer at 4°C for 1 hr. After a brief wash in dilute HCl (pH 5.0), the whole embryos were immersed in 0.5% uranyl acetate solution in collidine buffer for 1½ hr at 25°C. The uranyl acetate solution was prepared by adding an appropriate amount of uranyl acetate to a solution of collidine at pH 6.1 (10). Following treatment with the uranyl acetate, the tissues were dehydrated in alcohol, flat-embedded in Araldite (R. P. Cargille Laboratories, Inc., Cedar Grove, Illinois), and sectioned in transverse and longitudinal planes for study in the light microscope and in the electron microscope (Siemens Elmiskop I, RCA EMU 3 F).

RESULTS

At the beginning of incubation the chick embryo already has two germ layers, the epiblast (presumptive ectoderm) and the hypoblast (presumptive endoderm). By 20 hr of incubation (39°C), the primitive streak is well delineated and formation of the primary mesoderm (mesoblast) is underway. The manner in which these cells migrate from the streak and reaggregate later into the epithelial primitive mesoderm will be the subject of a longer paper (11). At the stage (5 to 9 somites, 25 to 35 hr) which most interests us here, the presumptive notochord, mesoderm, and neural ectoderm are in apparent physical continuity, as visualized in the light microscope, not only in the region of the primitive streak and Hensen's node (Fig. 1), but also for some distance anterior to the node (Fig. 2). In the electron microscope it is difficult to tell where one tissue ends and the next begins. In the area of junction between the neural plate and notochord anterior to Hensen's node (arrow, Fig. 2), the cells are in close contact at many points along their adjacent surfaces. The points of contact may be short and often are located in regions where narrow (0.2 μ in diameter) cell processes abut on one another. Such cell processes extend from presumptive notochord to mesoderm (Fig. 1), from notochord to endoderm,

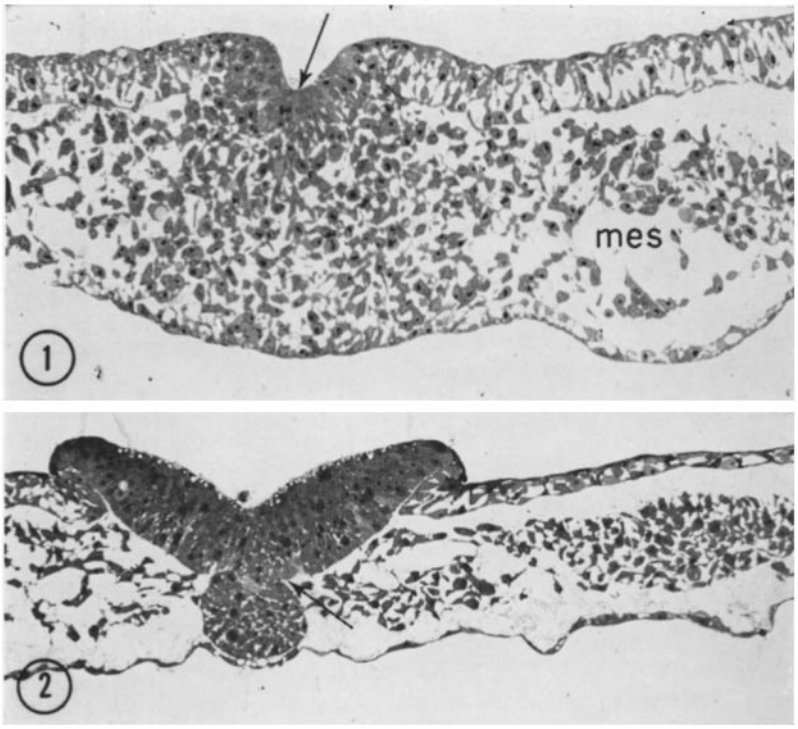


FIGURE 1 Light micrograph showing in cross-section the region of Hensen's node in a Hamburger-Hamilton (15) stage 6 embryo. Along the primary embryonic axis the epiblast invaginates to form a groove (arrow). The cells of the presumptive ectoderm are attached along their free surface (arrow) and lateral surfaces by primitive tight junctions. Cells streaming from the base of the epithelial epiblast contact the hypoblast (presumptive endoderm) and move laterally as the so-called mesoblat (*mes*). The mesoblast (presumptive mesoderm) establishes new close contacts with the overlying ectoderm and underlying endoderm which may play a role in guiding the movement. × 150.

FIGURE 2 Light micrograph showing in cross-section the region anterior to Hensen's node in a Hamburger-Hamilton (15) stage 10 embryo. The medullary plate is still in close contact (arrow) with the presumptive notochord, thus revealing the common origin and similar nature of the tissue which subsequently gives rise to the neural tube and notochordal process. The close and tight junctions between the cells of the medullary plate and presumptive notochord are, in a sense, then, all within the same tissue. At this stage and level of the embryo, the mesoblast has formed a distinct mesenchyme which is beginning to reorganize itself into an epithelium (somite, nephron, celomic mesoderm). Mesoderm is still in contact with the presumptive notochord and medullary plate, but contacts with lateral ectoderm and endoderm are broken as the extracellular matrix forms. × 150.

and from mesoderm to both ectoderm and endoderm.

Higher magnification electron micrographs illustrate better the degree to which the cell membranes are in actual contact in these areas of close apposition (Fig. 3 to 6). The intercellular space in adult tissues is rarely narrower than 200 A, except in areas of specialized junctions (4). Regions where the plasma membranes of adjacent cells are separated by less than 100 A will be called "close junctions." Following terminology that is already established (4, 10), we shall use the term "tight junction" for an area of apposition in which no extracellular space is visible. In a tight junction (zonula occludens macula occludens) the outer leaflets of adjacent membranes seem to be fused and appear as a single line (4). Whereas close junctions are common between cells and cell processes in all the tissues in the vicinity of the primitive streak, node, and presumptive notochord,

(11), tight junctions are found only after a careful search. They are probably difficult to detect because of their small size. They seem to be "punctate", that is, they seem only to occur as small foci on the cell surfaces (Figs. 3 and 4). The plasma membranes are 70 A in thickness and appear trilaminar in almost all cases (Figs. 3 and 4). The line along which they fuse in well-developed tight junctions is dense, as in the myelin sheath (12), zonula occludens (4), and related instances of membrane "coalescence" (Figs. 5 and 6). Well-developed tight junctions were never seen in the young tissues in and near the primitive streak and Hensen's node, but are common within the tissues, even the mesenchyme, at later stages (Figs. 5 and 6). Well-developed tight junctions were never seen between mesodermal cells and ectoderm or endoderm.

At later embryonic stages the tight junctions that develop on the lateral surface of the cells in each definitive tissue may be longer than 0.2 μ, and those on the free surface of the ectoderm, endoderm, and mesoderm seem to form true zonulae, in the sense that they probably extend completely around the luminal junctions of the cells. Other contact specializations, the zonula adhaerens and macula adhaerens (desmosome), are not present in the young tissues, but they do appear subsequently in the embryonic tissues (5, 11).

Another difference between the younger embryonic tissues and the more mature ones has to do with the development of the basement lamina (basement membrane). This zone of condensed glycoprotein appears in older embryos as a definitive sheet, $\backsim$ 1000 A thick, under the ectoderm and the endoderm and around the neural tube, notochord, and epithelial mesodermal derivatives (somite, nephron, mesothelium). In the younger embryo, however, basement laminae are incomplete or absent and it is for this reason, perhaps, that mesenchymal cell processes are able to contact the overlying and underlying epithelia so closely. The neural plate and presumptive notochord originate together at the primitive streak and can be regarded, in the stages that have been studied for electrical coupling (7), as a single tissue, which only later will become subdivided by basement laminae. The close and tight junctions between mesenchymal cell processes and ectoderm or endoderm must have formed secondarily, and in this case the presence or absence of basement membrane material along the epithelia could well determine the sites of contact and thus direct the pattern of adhesion of the mesoblast to the epiblast and hypoblast.

DISCUSSION

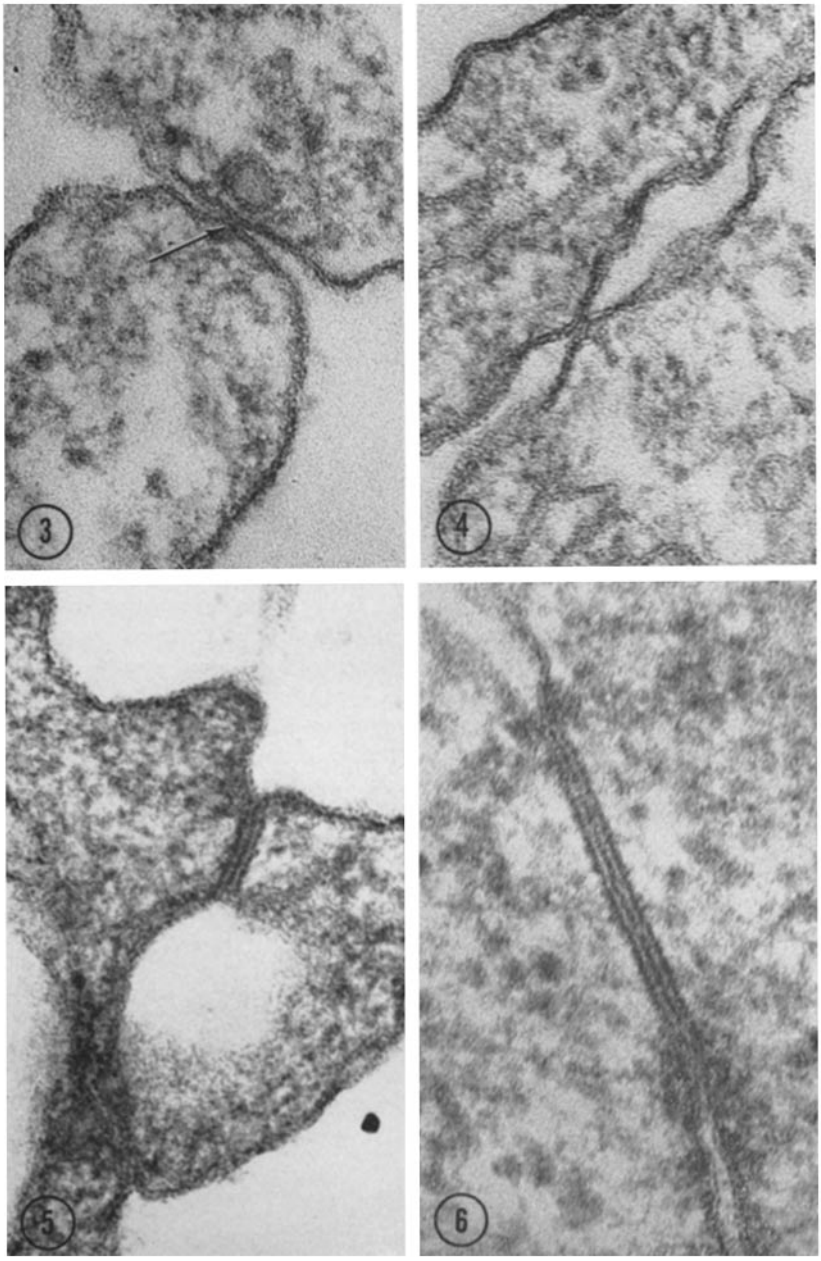
It is tempting to conclude that the minute tight junctions observed within the primitive tissues of the young chick embryo represent the sites of lowered cell to cell electrical resistance which have been reported by Sheridan (7). The possibility that the close junctions we observed (11) could

FIGURE 3 Electron micrograph showing an area of close junction between two mesoderm cells in a region near the primitive streak in a stage 4 embryo. At one point (arrow), the plasma membranes of the adjacent cells make a tight contact. × 140,000.

FIGURE 4 Electron micrograph showing another punctate junction in the mesenchyme (stage 4). Again, the adjacent plasma membranes are apparently fused in a small focal area. Such tight junctions seem to be more common between cells of the same type in a single tissue, but they also occur between mesoderm and ectoderm and between mesoderm and endoderm during the stage of migration of the mesoblast. × 165,000.

FIGURE 5 It is easy to imagine that the smaller focal junctions develop into the more sizeable tight junctions shown here at the free surface of the medullary plate (stage 6). Similar junctions can be noted on the free and lateral surfaces of cells in the ectoderm and endoderm as well as in the notochord and mesoderm when the latter become definitive tissues. × 175,000.

FIGURE 6 Tight junctions which form true zonulae (as opposed to the punctate junctions and macula occludens illustrated above) are present in all of the definitive epithelial tissues of the stage 18 embryo. Fasciae occludentes (10) occur not only in the epithelial germ layers, but also in true mesenchyme as shown here in the migrating sclerotome. × 230,000.



be sites of lowered electrical resistance also must be borne in mind. The tissues of the chick embryo that have been examined for electrical coupling to date (7) can be assumed to have contained at least focal tight junctions in the epithelium of origin (e.g. neural plate-presumptive notochord), whereas junctions between mesoderm and ectoderm or endoderm have to be secondary (new) contacts. It would be interesting to look for electrical coupling in the latter cases, because close junctions, rather than tight junctions, predominate in such areas of secondary tissue apposition in the young embryo.

In seeking to understand the significance of the electrical coupling phenomena described in embryos, it is important to keep in mind the fact that tight junctions and electrical coupling are not exclusive properties of developing systems. Focal obliteration of the extracellular space has even been described in adult epithelia (10). Moreover, the tight junctions that are best developed in the chick embryo occur within definitive tissues at the more advanced stages of development. Their principal function may have to do with the coordination of the activities of the cells within a single tissue rather than activities between tissues. Certainly, it seems unlikely that interacting tissues (13), separated by a space larger than 0.2 μ, will be in electrical continuity. As in the case of the zonula occludens and macula occludens, the development of the zonula adhaerens and macula adhaerens parallels the differentiation of the definitive tissues. We might most profitably use adult tissues to study the function of these contact specializations in tying together the cells of a single tissue.

What is special to the embryo, of course, is the problem of creating cellular diversity. The first series of events leading to the production of diverse cell types in the chick seems to begin with the creation of the primary germ layers by migration of cells away from the primitive streak. In this regard, it is interesting to note that numerous close junctions, as well as occasional focal tight junctions, are observed between the mesoblast and ectoderm or endoderm during the period of migration of the mesoblast from the primitive streak. It is tempting to think that the adjoined areas guide the movement of the mesoblast and represent areas of contact inhibition (11, 14). These tissues have not yet been studied for electrical coupling (mesoderm-ectoderm, mesoderm-endoderm). Control of the migratory activities of cultured cells, however, clearly seems related to cellular adhesion (14) and perhaps also to electrical coupling (6). It seems probable, then, that further study of the distribution of cell contacts and electrical coupling among the primitive tissues will lead to a better understanding of developmentally significant tissue movements which occur in the early embryo.

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