DEVELOPMENT OF CELL JUNCTIONS IN SEA-URCHIN EMBRYOS

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SUMMARY

The development of cell junctions in sea-urchin embryos has been investigated using thin sections, lanthanum-tracer and freeze-fracture techniques. Three types of desmosomes are present: belt desmosomes and spot desmosomes, which attach cells to each other, and hemi-desmosomes, which attach cells to the basement membrane. Two types of septate junctions are present: the straight, unbranched, double-septum septate, which is present in epithelial cells throughout embryogenesis, and the pleated, anastomosing, single-septum septate. The latter is formed only on cells that have invaginated to the interior of the embryo to form the digestive tract. The pleated junctions are shown to replace the straight junctions that were originally present before the cells migrated to the interior. It is suggested that these pleated septates may be specialized for digestive processes, since they are developed just prior to feeding and are retained in the adult intestine. Tricellular junctions, which join the bicellular junctions of three adjoining cells, have been identified in the embryo and in the adult intestine. Evidence for the presence of gap junctions was not obtained, but there are indications of their presence.

INTRODUCTION

The sea-urchin embryo has been used as a model system for studies of cell adhesion during development. A number of early studies on the reaggregation of dissociated cells were carried out by Giudice and his coworkers (reviewed by Giudice & Mutolo, 1970) and Spiegel & Spiegel (1975). More recent studies have involved the putative roles of surface antigens (McClay, Chambers & Warren, 1977; McClay & Chambers, 1978; McClay & Marchase, 1979); cell surface carbohydrates (Oppenheimer, 1978); membrane-bound glycoprotein synthesis (Schneider, Nguyen & Lennarz, 1978; Heifitz & Lennarz, 1979) and a number of extracellular matrix components (Spiegel & Spiegel, 1979) including fibronectin (Spiegel, Burger & Spiegel, 1980) and laminin (Spiegel, Burger & Spiegel, 1983). However, cell junctions, which are major structural components involved in cell adhesion, have received very little attention in the sea-urchin embryo.

The sea-urchin embryonic septate junction was first described in the blastula by Balinsky (1959) and Endo (see Dan, 1960), and further studied by Wolpert & Mercer (1963). Chang & Afzelius (1973) observed septate junctions as early as the four-cell stage in Arbacia punctulata and Gilula (1973) described their development in Strongylocentrotus purpuratus from early blastula to late gastrula. These studies were made using electron-microscope observations of thin sections. Gilula's study also involved the use of the freeze-fracture technique. Currently, studies of cell junctions
are carried out using freeze-fracture and lanthanum impregnation methods in addition to conventional thin-section techniques. Using these methods, Green (1978, 1981) and Green, Bergquist & Bullivant (1979) have identified two kinds of septate junctions in sea-urchin adult tissues: the straight, unbranched, double septum type in the tube foot and the pleated, anastomosing, single-septum type in the stomach.

In this paper we describe the appearance and development of six types of cell junctions in the sea-urchin embryo. A seventh type may also be present. In addition, we have studied cell junctions in the adult sea-urchin intestine in order to compare them with those in the embryo. Our results indicate that the straight, unbranched, double-septum septate junction found during early development is gradually replaced by the pleated, anastomosing, single-septum septate junction in the pluteus digestive system, prior to feeding. This correlates with our observation that the anastomosing septate is the type of junction found in the adult intestine.

MATERIALS AND METHODS

Embryos and adults of two species, *Arbacia punctulata* and *Strongylocentrotus drobachiensis*, were used. Adult *Arbacia* were collected by the Supply Department of the Marine Biological Laboratory, Woods Hole, Mass., during the summer months and maintained in running sea water. Adult *Strongylocentrotus* were collected at Kittery, Maine, during the winter months and maintained in Instant Ocean aquaria at 4°C.

Gametes from *Arbacia* were collected by electrical stimulation and fertilized eggs were cultured at 21°C. All procedures using *Arbacia* material were carried out at 21°C except where otherwise noted. Gametes from *Strongylocentrotus* were collected by injection with 0.5 M-KCl and fertilized eggs were cultured at 8°C. All procedures using *Strongylocentrotus* material were carried out at 8°C except for the lanthanum studies. Since lanthanum, in the solutions used for tracer studies, precipitates below 12°C this procedure was carried out at 15°C. Other exceptions are noted below.

Embryos were examined at several different developmental stages. Results obtained with both species, whether from embryos or adult tissues, were the same and will be discussed without reference to the species used. Lanthanum fixation procedures were adapted from those used by Lane, Skaer & Swales (1977) and Green, Bergquist & Bullivant (1979). Freeze-fracture methods were adapted from those used by Ruben, Allen & Travis (1981).

Conventional fixation

Embryos at various stages and adult intestine were fixed in 2% glutaraldehyde plus 0.5% paraformaldehyde in 75% Woods Hole formula artificial sea water (MBLSW) buffered at pH 7.8 with 0.05 M-sodium cacodylate. Embryos and adult tissue were fixed for 1 h and rinsed in MBLSW buffered at pH 7.8 with 0.025 M-sodium cacodylate. Post-fixation was carried out for 20 min at 4°C in 1% osmium tetroxide buffered at pH 6.0 with 0.1 M-sodium phosphate. Specimens were rinsed three times with cold distilled water and stained *en bloc* with 0.5% uranyl acetate for 1 h in the dark at 4°C. This was followed by dehydration through the ethanol series and propylene oxide with subsequent embedding in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100CX electron microscope.

Lanthanum incubation

Embryos at various stages were fixed for 1 h in the glutaraldehyde/paraformaldehyde solution referred to above, containing 1% colloidal lanthanum hydroxide, prepared from lanthanum nitrate. The solution was changed three times during the course of fixation. Up to this point, *Arbacia* embryos were processed at 21°C and *Strongylocentrotus* embryos at 15°C. Specimens were then put into 0.1 M-sodium phosphate buffer (pH 7.8) for 4 h at 4°C, followed by two rinses in 0.1 M-sodium cacodylate buffer (pH 7.8), at 4°C. Postfixation, for 3 h at room temperature, was in 1% osmium tetroxide in 0.1 M-sodium cacodylate buffer (pH 7.8), containing 1% lanthanum hydroxide.
Specimens were rinsed in buffer, dehydrated through the ethanol series and propylene oxide, and embedded in Epon. Thin sections were examined without further staining.

Adult urchins were injected with lanthanum hydroxide fixative, either through the oral opening or into the coelomic cavity. Fresh lanthanum fixative was injected two to three times over the course of a 2h fixation period. Up to this point, Arbacia were processed at 21 °C and Strongylocentrotus at 15 °C. This was followed by injection of cold 2 % glutaraldehyde in 0.1 M-phosphate buffer (pH 7.8), keeping the animals at 4 °C during fixation for 1 h. The urchins were then placed in buffer at 4 °C and the intestines were removed and left overnight in buffer at 4 °C. Intestinal tissue was cut into small pieces and postfixed, dehydrated and embedded as described above for embryos. Thin sections were examined without further staining.

A second lanthanum procedure involved the incubation of unfixed material to allow the uptake of ionic lanthanum to occur under in vivo conditions (Lane, Skaer & Swales, 1977). Embryos were collected by gently spinning an embryo suspension in a hand centrifuge. The sea water was replaced by a solution of lanthanum hydroxide in MBLSW (pH 7.8). Concentrations of lanthanum varied from 0.01 to 0.1 mm and incubation times varied from 15 min to 1 h. The embryos were then fixed in 2 % glutaraldehyde in 75 % MBLSW buffered with sodium phosphate at pH 7.8, with two changes of cold fixative over a 2 h period. Specimens were rinsed twice in cold buffer. Postfixation, en bloc staining, dehydration and embedding were carried out as described above for conventional fixation. For controls, samples of live embryos were incubated in lanthanum solutions for 1 h and then transferred to MBLSW. The embryos continued to develop normally through the pluteus stage.

Adult intestinal tissue was also incubated, without prefixation, in lanthanum solutions as described above for embryos. This was followed by fixation for 1 h at room temperature and an additional 1 h in fresh fixative at 4 °C. After transferring the tissue to cold buffer, it was carefully cut into small pieces and left in buffer overnight at 4 °C. Postfixation, en bloc staining, dehydration and embedding were carried out as described above for conventional fixation. Thin sections were examined without further staining. Both methods of lanthanum fixation gave similar results in embryos and adult intestinal tissue.

Freeze-fracture

Embryos and adult intestine were fixed in 2 % glutaraldehyde plus 0.5 % paraformaldehyde in 75 % MBLSW buffered with 0.05 M-sodium cacodylate (pH 7.8) for 1 h. Specimens were rinsed two to three times over a 15 min period in 0.1 M-sodium cacodylate buffer (pH 7.8) at 4 °C, followed by 25 % glycerol made up in the buffer, for 2 h at 4 °C. Embryos, on Balzers gold pedestals, and adult tissue on Balzers gold, deep-well pedestals, were quick frozen in liquid propane at −190 °C. They were then fractured in a Balzers 300 device, etched for 1 min at −100 °C and 5 × 10⁻⁷ Torr (1 Torr = 1.33 Pa) and shadowed at 45° with a 24–27 Å platinum/carbon film followed by backing with a 80–90 Å carbon film. The replicas were floated onto Clorox containing 10 % NaCl for about 12 h, transferred to 50 % Clorox/10 % NaCl solution for 4 h, then to distilled water for 4 h (Ruben et al., 1981). The replicas were then picked up on carbon-coated grids and examined with a JEOL 100CX electron microscope.

RESULTS

Desmosomes

Fig. 1, of a late blastula stage, shows the outer cell surfaces surrounded by the extracellular matrix or hyaline layer. The apical edges of adjoining cells show two types of junctions. The most apical is the belt desmosome, or zonula adhaerens, which is seen as dense intracellular plaques on opposite edges of adjacent cells. Closely following the desmosome is the septate junction, which has a ladder-like appearance. (Details of septate junction structure will be discussed below.)

Figs 2 and 3 show the basement membrane lining the blastocoel cavity of a late blastula. Fig. 2 shows spot desmosomes composed of small, dense, intracellular
plaques, which are found at various points along the cell membranes of adjoining cells. Fig. 3 shows hemi-desmosomes, which attach cells to the basement membrane and appear as a single, dense, intracellular plaque with no opposite plaque on the basement.
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membrane. Both spot and hemi-desmosomes are localized or focal junctions as compared to belt desmosomes, which encircle the cells.

Fig. 4 is a higher magnification of the belt desmosome, which shows that, in addition to the dense intracellular plaques on adjoining cells, there is some extracellular, less dense material between the plaques. Unlike the desmosomes in adult intestine (Fig. 9), sea-urchin embryonic desmosomes do not have a network of microfilaments emanating from the desmosomal plaques. This observation holds true for all three types of desmosomes.

The development of belt desmosomes begins as early as the four-cell stage. The dense plaques are not always seen in the apical corners of cells in thin sections, indicating that the desmosome has not yet encircled the cells. By the 16-cell stage, the plaques are denser and thicker, but the desmosome does not become a true zonula adhaerens, surrounding each cell, until the early blastula stage. At this time also, the hemi-desmosomes appear, since the basement membrane is now seen lining the newly formed blastocoel.

Spot desmosomes are already numerous at the four-cell stage and seem to be more common in very early stages than during later embryonic development. Fig. 5 is a high magnification of a spot desmosome in a four-celled embryo. In this figure, it appears as though two additional spot desmosomes may be in the process of forming. In these locations, there is increased intercellular density as compared to adjacent electron-lucent areas.

In freeze-fracture replicas, the desmosomal membrane usually appears smooth, with no intramembrane particles. This method, therefore, yields no additional information as to its structure. Occasionally, however, one finds small clusters of particles of irregular size that could be spot desmosomes (Fig. 12).

Septate junctions

The straight, unbranched septate junction is present throughout embryogenesis, from the four-cell stage to the pluteus. Its appearance in thin sections is of ladder-like septa that vary somewhat in thickness and are about 7–12 nm thick (Fig. 6). The extracellular dense material, seen between the desmosomal plaques in Fig. 4, extends

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Figs 1–4. These micrographs are of late blastula stage embryos.

Fig. 1. Outer cell surfaces of two adjoining cells showing belt desmosome (black arrowhead) and septate junction (open arrowhead). Extracellular matrix (ecm) surrounds cells and several microvilli (ntv) are seen within the ecm. ×26 300.

Fig. 2. Blastocoel cavity (bc) in interior of embryo is lined with basement membrane (arrowheads); spot desmosomes are indicated by arrows. ×13 650.

Fig. 3. Blastocoel cavity (bc) in interior of embryo is lined with basement membrane (arrowheads); hemi-desmosomes are indicated by arrows. ×13 650.

Fig. 4. High magnification of junctional area showing desmosome and septate junction with extracellular dense material between desmosome (black arrow) and between the septa (white arrows). ×130 000.

Fig. 5. Four-cell stage with spot desmosomes (black arrows) and intercellular dense areas (open arrows) where additional spot desmosomes appear to be forming, with electron-lucent areas in between. ×77 000.
Figs 6–8. Straight septate junctions of mid-gastrula stage (Figs 6, 7) and pluteus stage (Fig. 8) embryos.

Fig. 6. Tangential section of conventionally fixed thin section showing septa to be unbranched, i.e. crossing entire intercellular space without crossing or linking to another septum. ×91 000.

Fig. 7. Tangential section of lanthanum-impregnated thin section showing septa to be double-layered and smooth. ×91 000.

Fig. 8. Freeze-fracture replica showing 8–11 nm particles in rows on E face (EF) and corresponding grooves on P face (PF). Apical end of cell is at the bottom of micrograph. mv, microvilli. ×91 000.
into the interseptal spaces of the septate junction. The number of septa tends to
increase during development, starting with two to four septa in the four-cell stage and
reaching as many as 20 in the pluteus. Fig. 6 is a tangential section of a mid-gastrula,
which shows that the periodicity of the septa is not very uniform. Interseptal distances
can vary from about 12 to 25 nm. This figure also shows that the septa are unbran-
ched; i.e., each septum crosses the entire intercellular space without crossing or
linking to any other septum.

Using the lanthanum-tracer method, two additional properties of this type of sep-
tate junction can be seen (Fig. 7). The septa appear as straight, flat lines, in contrast
to the pleated septa described below. Furthermore, what appears to be a single
septum, as seen in Fig. 6, is actually composed of two thin septal sheets that are very
close together, forming a double septum (see also Figs 21, 22). As Green (1981) has
shown, each half of this double septum can be between 2 and 5 nm wide. They can
be touching or have a space between them up to 3 nm wide. This results in a variation
in the total width of the double septum between 7 and 13 nm. The lanthanum tracer
method has shown that these straight, unbranched, double-septum septates are
present at least as early as the 16-cell stage.

In freeze-fracture replicas, the straight septate junction is seen as rows of 8—11 nm
particles on the E face (Fig. 8). Corresponding grooves are seen on the P face, which
are in register with the E face particles. Fig. 8 is a replica of a pluteus stage, but particle
rows and grooves are seen as early as the 16-cell stage. The replicas also indicate that
the straight septate does not extend very far in a basal direction but remains rather
localized at the apical cell border. The exception to this is in the area of tricellular
junctions, which will be described below.

A second type of septate junction found in the sea urchin is the anastomosing septate
junction, which was first described by Green (1978) in the adult sea-urchin stomach.
Fig. 9 shows a junctional complex at the apical end of adult intestinal cells impreg-
nated with lanthanum. It should be noted that in both types of lanthanum-
impregnation experiments, the penetration of lanthanum is slowed down but not
completely blocked by the apical junctional complex. Lanthanum is concentrated
mostly in the junctional area, but can be seen to penetrate to the basal end, including
the basement membrane.

The most apical junctions are the belt desmosomes with dense intracellular plaques
and microfilaments emanating from them. The desmosomes are followed by the
septate junctions, which are accentuated by the lanthanum tracer. At higher magnif-
ication, these septates are seen to be made up of pleated, single-layered septa in an
anastomosing pattern (Fig. 10). The adult anastomosing septate extends basally to
about one-third of the length of the cells.

The pleated, anastomosing septate junction found in adult tissue has a very different
appearance in freeze-fracture replicas compared to that of the straight, unbranched
septate junction found in the embryo, as seen in Fig. 11. The intramembrane particles
are smaller, from 6—9 nm; they are found on the P face instead of the E face and they
form a network, or anastomosing pattern rather than rows. Complementary grooves,
which are very shallow, are seen on the E face. This junction has a well-defined edge
Figs 9–10
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or border, which contrasts sharply with the smooth membrane of the desmosomal area. These features are seen more clearly in Fig. 12, a higher magnification of an area of Fig. 11. The particle cluster seen just apical to the border of the anastomosing septate may be a spot desmosome, as judged by its location and the irregular sizes of the particles. Occasionally, we have seen what appears to be a remnant of a straight septate junction in adult intestine, showing one or two particle rows.

The presence of a pleated, anastomosing septate junction in the embryonic digestive system is shown in the pluteus stage in Fig. 13. The lanthanum-impregnated junction is similar to that found in the adult intestine shown in Fig. 10. The septa are single-layered, pleated and form an anastomosing pattern (Fig. 13). Freeze-fracture images show that the intramembrane particles are 6–9 nm in diameter and fracture onto the P face (Fig. 20). The junctions in the pluteus embryo, as seen in both the lanthanum and freeze-fracture images, are not as well formed as those seen in the adult. This indicates that further development and maturation of the junctions takes place beyond the pluteus stage, during metamorphosis when the organism is actively feeding. The increase in the length of the septate in the adult as compared to the embryo lends support to this possibility.

The transition from straight, unbranched, double-septum to pleated, anastomosing, single-septum septate junction takes place only in those cells that have invaginated to the interior of the embryo to form the embryonic digestive system. Not all of the cells undergo this transition at the same rate since several stages can be seen within the same embryo. The transition process appears to involve the loss of the straight septate junction and its replacement by the newly formed pleated septate junction. It should be mentioned, however, that the straight septate junctions are retained in the outer epithelial cells of the embryo throughout development.

The first indication that anastomosing septates are forming appears when the intercellular space begins to show an increase in intercellular dense material and fewer ‘ladders’ of the straight septates. This is shown in the invaginated cells of a mid-gastrula in Fig. 14. The intercellular density could be due to the breakdown of the straight septate junctions or to the fact, observed earlier, that the formation of junctions may be accompanied by a localized increase in intercellular density.

The transition in which the straight, unbranched junction is replaced by the pleated, anastomosing junction can be followed in freeze-fracture replicas. Fig. 15 shows straight septates of epithelial cells of a mid-gastrula stage that have just invaginated. The E face particles and P face grooves are still aligned in rows. Fig. 16 shows an invaginated cell in which there are fewer P face grooves and these are

Figs 9-10. Pleated septate junctions of adult intestine that have been impregnated with lanthanum.

Fig. 9. Apical belt desmosomes with microfilaments (arrowheads) are followed by septate junctions, which are darkly stained with lanthanum. Bicellular junctions (black arrows); tricellular junctions (open arrows). ×8580.

Fig. 10. Higher magnification of an area of Fig. 9 shows septate junctions to be composed of pleated, single-layered septa in an anastomosing pattern. Arrowheads show closely packed diaphragms of tricellular junction. ×91 000.
Figs 11-12
Fig. 13. Lanthanum-stained thin section of pluteus digestive tract showing pleated, anastomosing, single-septum septate junction. ×44 600.

Fig. 14. Invaginated cells of mid-gastrula showing increased intercellular density (arrowheads) associated with transition from straight to pleated septate junctions. ×14 000.

becoming discontinuous. In Fig. 17, the further disorganization of the straight septate is shown. The E face particles are no longer well-aligned in rows and the P face grooves are more disarrayed than in Fig. 16. In Fig. 18, it can be seen that the P face particles begin to accumulate toward the apical end of the cell and the P face grooves of the

Figs 11–12. Freeze-fracture preparations of pleated, anastomosing septate junctions of adult intestine. Apical ends of cells are at the bottom of micrographs.

Fig. 11. The 6–9 nm particles on the P face (PF) form a network or anastomosing pattern. Complementary shallow grooves are seen on the E face (EF). Tricellular junctions appear as ridges containing a double row of particles on the P face (black arrowheads) and a groove on the E face with a double line indicating the complementary pits of the double-particle rows (white arrowheads). ×28 370.

Fig. 12. Higher magnification of an area of Fig. 11 shows anastomosing pattern of P face particles and E face grooves. Apical to the sharp border, in the desmosomal area, is a cluster of particles of irregular size that may be a spot desmosome (arrow). ×91 000.
straight septate are not seen. In Fig. 19, the concentration of P face particles toward the apical end is increasing and the E face no longer contains the particles of the former straight septate. It does, however, show the shallow grooves of the new pleated septate. In Fig. 20, the newly formed septate appears as a band of P face particles with a well-defined apical border. This band of particles is beginning to form the network pattern characteristic of the anastomosing septate junction. As mentioned earlier, the new junction is confined to the apical region in the pluteus. Subsequent maturation and extension of the junction basally, as seen in the adult intestine, probably occurs.

Figs 15–20
following the pluteus stage; i.e., during metamorphosis when the organism begins to feed.

**Tricellular Junctions**

A tricellular junction is one in which the bicellular junctions of three adjoining cells meet. Fig. 9 shows several examples of bicellular junctions converging to form tricellular junctions in the adult intestine. Lanthanum impregnation shows that the tricellular junction is composed of a series of diaphragms, as illustrated in the adult intestine shown in Fig. 10. The structure of the diaphragms is difficult to distinguish because they are so close together. In the embryo, however, they are further apart and the details of diaphragm structure are more clearly visible, depending on the angle of sectioning, as seen in Fig. 21. Each diaphragm is made up of a central vesicular part with thin arms that connect it to the three bicellular junctions, as described by Graf, Noirot-Timothee & Noirot (1982).

The freeze-fracture image of the adult tricellular junction appears in Fig. 11 as a long ridge on the P face, containing a double row of particles. The complementary structure is seen on the E face as a long, shallow groove with a double row of pits. The ridge and groove, with the accompanying rows of particles and pits, comprise the tricellular junction in this tissue.

The embryonic tricellular junction is shown in lanthanum-tracer (Fig. 22) and freeze-fracture (Fig. 23) images. In Fig. 22, the septa are oriented parallel to the cell apices at the apical end of the junction, but tend to become oriented parallel to the juncture line as they proceed basally. In addition, the septa appear to converge from both sides toward the juncture line in the centre in this micrograph. This lanthanum image can be compared to the freeze-fracture image in Fig. 23, in which the E face particle rows appear to converge from both sides toward the deep vertical groove or furrow. The P face shows a ridge with shallow grooves converging toward it. As in the adult, the vertical groove and ridge identify this structure as a tricellular junction.

**Fig. 15-20. Freeze-fracture preparations of invaginated cells showing transition from straight to pleated septate junctions.** In order to retain the consistency of cell orientation for transition stages, all figures are mounted with the apical side up. Smooth desmosomal area of membranes and microvilli indicate the apical end of the cell. Figs 16 and 20, therefore, are mounted upside down with respect to the angle of shadowing, i.e. from top to bottom instead of the reverse.

Fig. 15. Straight septate junctions of cells that have just invaginated; E face (EF) particles and P face (PF) grooves are still aligned in rows. ×26 000.

Fig. 16. Fewer P face grooves are seen and some are discontinuous. ×91 000.

Fig. 17. Further disorganization of the straight septate; E face particles are no longer well-aligned in rows and P face grooves are in disarray. ×91 000.

Fig. 18. P face particles begin to accumulate toward apical end of cell and P face grooves of the straight septate are not seen. ×40 000.

Fig. 19. Concentration of P face particles toward apical end is increasing and the E face no longer contains particles of the former straight septate, but contains shallow grooves of the newly forming pleated septate. mvt, microvillus. ×28 000.

Fig. 20. The newly formed septate appears as a band of P face particles with a well-defined apical border, which is beginning to form the network pattern characteristic of the anastomosing septate junction. ×48 860.
Gap junctions

Our evidence for the presence of gap junctions in sea-urchin embryos is not convincing because of the difficulty of obtaining clear data from either lanthanum tracer thin sections or freeze-fracture replicas. However, there are indications of their presence, which should be mentioned since it is usually assumed that embryos have gap junctions at some stage during development in order to carry out intercellular communication.

As early as the two-cell stage, microvilli between the two blastomeres form focal close membrane appositions. At the four-cell stage, neighbouring blastomeres form extensive close membrane appositions, but at higher magnifications, these areas do not give the usual seven-layered appearance associated with gap junctions. The use of lanthanum tracer did not show the hexagonal array of junctional areas seen in tangential sections of gap junctions. Freeze-fracture replicas show very small clusters of irregularly spaced particles and individual particles on the E face, which are 12–14 nm in diameter (Fig. 24). These particles are larger than those forming the septate junctions and are in the size range of gap junction particles described for other tissues (Lane & Swales, 1980). Complementary P face pits have not been observed, probably due to the small size of the particle clusters, their irregular arrangement or the angle of shadowing. Some of the particles appear to have a small pore in the centre, but this effect could be due to carbon scattering. A recent study of electrical coupling of blastomeres in sea-urchin embryos, at the two, four and eight-cell stages, showed that blastomeres are electrically coupled during the first half of the division cycle, but are uncoupled during the latter half (Dale et al. 1982). Further studies, therefore, will be needed to establish whether or not gap junctions are present in the sea-urchin embryo.

Discussion

Sea-urchin eggs are fertilized externally and become surrounded almost immediately by a thick layer of extracellular matrix, which is further encased by a fertilization.
membrane. The fertilization membrane serves as a protective shell. The extracellular matrix maintains cell organization in the embryo during the various events of morphogenesis (Dan, 1960; Gustafson & Wolpert, 1967; Spiegel & Spiegel, 1979). The embryos are sessile during early development up to the early blastula stage. At this point, they have developed cilia and subsequently hatch out of the fertilization membrane to become swimming embryos throughout the rest of embryogenesis (i.e. through the pluteus stage). The timing of development of the desmosomes can now be fitted into this sequence of events. It is at the early blastula stage that belt desmosomes are fully developed and attach cells to each other at their apical ends. At this stage also, the inner blastocoel cavity has formed, with the concomitant formation of the basement membrane. The hemi-desmosomes are then found attaching the basement membrane to the basal cell surfaces at this time. It appears, therefore, that as soon as these two types of desmosomes are fully developed and functional, hatching of the embryo from the fertilization membrane takes place. Spot desmosomes may be temporary adhesive devices that are most prevalent during very early development and tend to decrease in number as the belt and hemi-desmosomes increase and mature.

The original study by Gilula (1973) of the development of septate junctions in sea-urchin embryos is corroborated by our observations. Freeze-fracture replicas show rows of E face particles and complementary P face grooves. Using the most recent technique of lanthanum impregnation, we are able to add some details to the general outline presented by Gilula (1973). These septate junctions are of the straight type, i.e. not pleated, and each septum is actually a double-layered septum. In addition, the septa are unbranched, i.e. not linked to other septa, but crossing the entire intercellular space. The details of this type of septate junction were first described by Green (1981) in echinoderm tube feet cells. Using all three techniques, we have found that straight, unbranched, double-septum septate junctions are present throughout development from the four-cell stage to the pluteus, not just from the early blastula on, as stated by Gilula (1973). After gastrulation begins and cells move inward to form the digestive system, the straight septates are found on the outer epithelial cells only.

The septate junction we have described in the sea-urchin embryo as straight, unbranched and having a double-layered septum, does not appear to fit the criteria presently used for defining septate junctions as either smooth or pleated. Although the septa are straight rather than pleated, they produce a clear ladder-like image in thin sections, which is characteristic of pleated junctions. Smooth junctions, on the other hand, contain more electron-dense material in the intercellular space, so that the septa are usually not visible in thin sections. In addition, columns between septa, which are characteristic of smooth septate junctions, were not observed. It has been proposed that this junction should be called the echinoderm double-septum septate junction (Green, 1981). Since it was necessary to compare various features of this junction with the pleated junction in the same organism, it seemed clearer in this context to use the contrasting terms; i.e., straight versus pleated and unbranched versus anastomosing, as well as double-septum versus single-septum, and abbreviated as straight versus pleated.
Freeze-fracture and lanthanum studies have enabled us to define more accurately the deep vertical groove or furrow described by Gilula (1973) as a nucleation site for the rows of particles. The deep vertical furrow on the E face has a complementary ridge on the P face and the rows of intramembrane particles and complementary grooves converge toward them (Fig. 23). Fristrom (1982) has pointed out that where a cell meets two adjacent cells, it usually forms an angle or corner. This results in a difference in the density of the shadowing material so that there is an abrupt change in shadow density where three cells meet. This is clearly illustrated in Fig. 23, where a change in shadow density is seen on both sides of the vertical groove and the vertical ridge. This is also analogous to the vertical groove and ridge in the adult (Fig. 11), which also show abrupt changes in shadow density and have been identified as tricellular junctions in other invertebrate cells (Graf et al. 1982; Noirot-Timothée, Graf & Noirot, 1982). The lanthanum image in Fig. 22, which shows the septa converging toward a central juncture line, supports the freeze-fracture image in Fig. 23. In addition, the lanthanum image in Fig. 21 clearly shows the diaphragms of the tricellular junction, as described by Graf et al. (1982). It is now possible, therefore, to identify the vertical groove or furrow and vertical ridge, seen in freeze-fracture images of the sea-urchin embryo, as embryonic tricellular junctions, rather than as nucleation sites for junction formation as suggested by Gilula (1973).

It has been suggested that the embryonic origin of the tissue determines the type of septate junction formed (Noirot-Timothée & Noirot, 1980). Cells that are of ectodermal origin are said to form pleated septates and cells of endodermal origin are thought to form smooth septates. This hypothesis is considered controversial because, in Malpighian tubules, both pleated and smooth junctions are found in the same junctional complex (Dallai, 1976). In the sea-urchin embryo, an analysis can be made of the embryonic origin of straight versus pleated junctions. The prospective fate of the blastomeres is already determined at the 16-cell stage (Hörstadius, 1939). The eight mesomeres will form ectoderm, the four macromeres will form endoderm and the four micromeres will form mesenchyme cells. At the 16-cell stage, all the blastomeres are joined by straight septates and all the descendants of the 16-cell stage also have straight septates until gastrulation occurs. At this time, the cells that invaginate to form the embryonic digestive system will lose their straight septates and will replace them with pleated septates. These cells are descendants of macromeres and, therefore, endodermal in origin. The cells that remain on the outside of the embryo to form the body wall are of ectodermal origin and retain the straight septates. All the cells of the embryo, therefore, have the same type of junction during early development, before there is a changeover in the digestive tract cells at a later stage of development. If the embryological origin of a junctional type is to be ascertained, therefore, it would be appropriate to indicate the stage of development one is starting with as a frame of reference, in addition to the germ layer from which the tissue is derived. In the case of the sea urchin, after gastrulation has taken place and the germ layers are formed, it can be said that the straight junctions are of ectodermal origin and the pleated junctions are of endodermal origin.

Our finding that the structure of the pleated septate is similar in both the adult
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Intestine and embryonic digestive tract is analogous to the situation found by Lane & Swales (1982) in a study of the development of septate junctions in the central nervous system of the locust. They found that in thin sections and in freeze-fracture images, the structure of the pleated septates was identical in adults and hatchlings. Furthermore, the structure of the straight septate in adult sea-urchin tissue (Green, 1981) appears to be similar to that of the straight septate that we have observed in sea-urchin embryonic cells. These findings indicate that the type of junction found in a particular type of adult tissue is, indeed, determined during embryological development. For this reason, the study of the development and structure of junctions in embryos could add to our fundamental understanding of both their origin and roles.

It has been proposed by Wood & Kuda (1980) that the smooth septate may be a phase in the development of the pleated septate. A similar analysis could be made in the case of the sea-urchin embryonic junctions, but with the opposite conclusion. In the sea-urchin embryo, the straight septate is unbranched and has a double-layered septum; it also has larger intramembrane particles that fracture onto the E face. In contrast, the pleated septate is anastomosing and has a single-layered septum; it also has smaller intramembrane particles that fracture onto the P face. These pronounced differences, therefore, indicate that the straight does not give rise to the pleated septate, but rather that the straight junction is replaced by the pleated junction, at least in the digestive system of the sea-urchin embryo.

It is more likely that the type of junction formed is related to its function and it is generally agreed that junctions play an adhesive role. Their structure and location make this highly probable. With regard to the desmosomes, we have observed that the focal spot desmosomes are numerous as early as the four-cell stage and continue to be seen frequently during early development. After the belt and hemi-desmosomes are fully matured at the early blastula stage, the spot desmosomes occur less frequently. It appears, therefore, as though spot desmosomes may be accessory structures that serve principally until the belt and hemi-desmosomes are well developed. We have also observed that, in the embryo, all three types of desmosomes lack the network of microfilaments associated with desmosomal plaques. In the adult intestine, however, the microfilament networks are found associated with the plaques of belt and hemi-desmosomes. It is possible that the microfilaments enhance adhesivity and lend support and stability to the greatly elongated adult intestinal cells.

The adhesive role of septates is also generally agreed upon and is illustrated by a simple dissociation experiment carried out by Dan (1960). When the body wall of the pluteus embryo (with straight, unbranched septates) was completely dispersed, the digestive tract (with pleated, anastomosing septates) was preserved intact and continued active peristalsis for a while. Since both types of cells have belt desmosomes, the greater adhesivity of the digestive tract cells must have been due to the interlocking network of the anastomosing septates.

It should be pointed out, however, that in addition to junctions, the embryo has other mechanisms to help ensure cell adhesivity and, thereby, to maintain its organization. These include the fertilization membrane, which is shed after the belt and hemi-desmosomes are fully developed; the extracellular matrix, which maintains cell
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organization during cell movement, changes in cell shape and other events associated with morphogenesis (Dan, 1960; Gustafson & Wolpert, 1967; Spiegel & Spiegel, 1979) and the glycoproteins, fibronectin and laminin (Spiegel et al. 1980, 1983), which are known to have adhesive properties (Wartiovaara, Leivo & Vaheri, 1980; Hynes & Yamada, 1982).

In addition to their adhesive role, septate junctions may also act as permeability barriers (Staehelin, 1974). The latter role is held in question because lanthanum studies show that the inward movement of tracers is slowed down but not completely blocked, as was also observed in our material. Wood & Kuda (1980) have presented a hypothesis to explain how septates could function as permeability barriers, which could be very pertinent in the case of sea-urchin embryos. They suggest that the interseptal space contains substances, such as glycosaminoglycans (Noirot & Noirot-Timotheé, 1967; Dallai, 1970), which have large numbers of anionic groups that can bind cations. These bound cations could then reduce the movement of ions through the junction due to repulsive charges (Skaer, Harrison & Lee, 1979). The septa, therefore, could serve as scaffolding for the intercellular substances that act as ion-trapping mechanisms. It is not yet certain if the septa themselves also aid in the barrier function. This would depend on their molecular structure, which could be a fenestrated or lattice-like arrangement that could allow molecules to go through.

In earlier studies we have used lanthanum hydroxide, Alcian blue and ruthenium red to show that the extracellular matrix surrounding the sea-urchin embryo is composed of proteoglycans, glycosaminoglycans and collagen-like striated fibrils (Spiegel & Spiegel, 1979). These same components were found in the basement membrane (Spiegel et al. 1983), a structure that is known to be a selective, semipermeable barrier (Kefalides, Alper & Clark, 1979). In addition, we have carried out immunofluorescence studies on live and fixed sea-urchin embryos, using antibodies to fibronectin and laminin. These large glycoproteins were found to be present on outer cell surfaces, between cells and in the basement membrane (Spiegel et al. 1980, 1983). The intercellular dense material associated with junctional structures (Fig. 4) must therefore include fibronectin and laminin. The sea-urchin embryo is thus amply supplied with the appropriate intercellular matrix materials that could act as ion-trapping mechanisms. These findings lend support to the Wood & Kuda (1980) hypothesis that septate junctions can function as permeability barriers, probably due primarily to the intercellular matrix. Since the matrix between the septa has some of the same components as the basement membrane, perhaps the septate junctions will also be found to function as selective, semipermeable barriers. Such selectivity could be a function of differences in types and/or concentrations of matrix substances. Structural differences in the type of septate, i.e. straight, pleated, unbranched, anastomosing, etc., could also be relevant to selectivity with regard to the distribution of matrix within the septal scaffolding.

Another point to be made in support of this role is the change in junctional type that occurs in a specialized tissue, the digestive tract, at a particular time in development, just prior to feeding. Furthermore, the intestine retains this junctional type into adulthood. The pleated, anastomosing septate in sea urchins, therefore, may be
specialized for a barrier role associated with digestive processes, whereas the straight septate is not. The latter may play a barrier role with regard to the external environment of the embryo, i.e. sea water. These differences in junctional structure and function tend to support the idea that septates may function as selective, semipermeable barriers. Finally, it should be mentioned that tight junctions were not found in sea-urchin embryos; therefore, the barrier function appears to apply to the septates.

Another possible role for septate junctions in developing systems is to permit cell rearrangements to take place, as in invagination in sea-urchin embryos, which involves the breaking of old contacts and the establishment of new ones, as described by Fristrom (1982). This model of Fristrom's also allows the transepithelial barrier to be maintained during the rearrangement of cells.

Our recent studies of cell adhesion in the sea-urchin embryo have focused on the role of a number of glycoproteins in cell adhesion during development. Since junctions are also involved in cell adhesion, we undertook this study to see what types of junctions are present in the embryo, when they appear and how they develop. Our goal is to attempt to define the relative roles of cell junctions and the intercellular glycoproteins in cell adhesion. It is of great interest, therefore, that septate junctions and intercellular glycoproteins may cooperate not only in cell adhesion, but also as permeability barriers. Perhaps this type of cooperation, in addition to the examples mentioned earlier, ensures a greater survival potential for the embryo.

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