FUNCTIONAL SIGNIFICANCE OF THE VARIATIONS IN THE GEOMETRICAL ORGANIZATION OF TIGHT JUNCTION NETWORKS

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ABSTRACT

Using freeze-fracture techniques, we have examined the morphology of tight junction networks found along the length of the alimentary tract of *Xenopus laevis* before and after metamorphosis. We have developed the hypothesis, based on these observations, that the geometrical organization of the network is determined by the stress-induced shape changes normally experienced by the cells linked by the network. Consistent with this theory, tight junctions can be classified into two distinct types of network organization which differ in their response to normal and experimentally induced stress conditions: (*a*) loosely interconnected networks which can stretch or compress extensively under tension, thereby adapting to stress changes in the tissue; and (*b*) evenly cross-linked networks which retain their basic morphology under normal stress conditions. The absorptive cells of the large intestine as well as the mucous cells of the gastrointestine or stomach are sealed by the first, flexible type of tight junction. The second type of junctional organization, the evenly cross-connected network, is found between absorptive cells of the small intestine and ciliated cells of the esophagus, and reflects in its constant morphology the relative stability of the apical region of both of these cell types. Networks intermediate between these two types arise when a cell which would normally form a loosely interconnected network borders a cell which tends to form a more evenly cross-linked network, as is found in the esophagus where ciliated and goblet cells adjoin. Despite the change in the animal's diet during metamorphosis from herbivorous to carnivorous, the basic geometrical organization of the networks associated with each tissue of the alimentary tract remains the same.

Since the initial description of tight junctions by Farquhar and Palade (5) as regions of apparent membrane fusion which serve as intercellular permeability barriers, many investigators have attempted to define in greater detail the structural components that invest the junctional membranes with their unique physiological properties (see review, reference 18). In high resolution images of thin sections of epithelial cells the tight junction, or zonula occludens, appears as a series of punctate fusions rather than a continuous line of membrane fusion (8). Additional structural information about

the zonula occludens has been derived from freeze-fracture replicas. Freeze-cleaving plasma membranes in the region of the tight junction (3, 8, 17) reveals a network of anastomosing ridges which usually remain with the protoplasmic face (PF) and a corresponding series of grooves which are accordingly observed on the extracellular face (EF) (formerly, A face and B face, respectively; for new nomenclature see reference 2). The punctate fusions seen in thin sections actually correspond to cross-sections of the components which form the ridges seen in freeze-fracture replicas and which bridge the two adjacent membranes. Thus, these ridges are the structural equivalents of sealing strands which provide physical barriers that reduce or restrict the intercellular passage of solutes and ions across epithelial tissues. A systematic study of fixed and unfixed preparations supported the theory that each sealing strand is actually composed of a double row of tightly packed particles, one row being contributed by each of the two adjacent plasma membranes (17).

The electrical resistance across an epithelium has been used as a quantitative measure of the impermeability of tight junctions to solutes and small ions (9). Claude and Goodenough (4) demonstrated a rough correlation between the physiological "tightness" of the junctions of a given tissue and the number of sealing strands comprising the networks. So-called "leaky" junctions, such as those found in the proximal convoluted tubule of the mammalian kidney, have junctions composed of only one or two strands, while the physiologically "tight" tight junctions, such as those which seal the amphibian urinary bladder, have 4–11 strands.

Tight junctions vary from tissue to tissue not only in the number of sealing strands, but also in the geometrical organization of the networks that are formed by the cross-linking of the individual strands. Both the extent to which the component sealing strands are interconnected and the direction in which the resultant linkage groups are oriented relative to the cell surface appear to be characteristic for tight junctions of a given tissue. For example, in the small intestine the network is highly cross-linked with no preferential orientation of the strands (18, 17, 19). In contrast, the networks connecting pancreatic acinar cells are more loosely organized with individual strands extending relatively long distances parallel or perpendicular to the cell surface (8). Even within a given tissue, the packing of the individual strands and the general orientation of the network can vary with the physiological state of the tissue, as in mouse mammary gland before, during, and after lactation (14). Pitelka and her co-workers (14) postulated that cell shape changes might be involved in the observed variations in the patterns of tight junction networks.

The zonula occludens has a meshlike structure which is embedded within a fluid membrane matrix (15, 16); therefore, it should be able to respond to stress by stretching or compressing, much like a loosely woven fabric or a concertina (19). As in a fabric, the extent to which a given network pattern can change shape under tension depends to a large extent on how closely it is woven or interconnected. Therefore, we hypothesized that the geometrical pattern of a tight junction network from a particular epithelium would be governed by the degree of flexibility needed in that region of the plasma membrane. Cells undergoing extensive cell shape changes would require flexible junctions, while those retaining a fairly constant shape could be linked with networks having a more stable pattern.

To test this hypothesis and to evaluate the flexibility of different network geometries, we have examined the tight junctions of the alimentary tract of *Xenopus laevis* before and after metamorphosis. The simplicity of the structural organization of the larval tract compared with the mammalian digestive system has proven particularly convenient for this study. While this tube is divided into distinct functional regions, the esophagus, gastrointestine, and large and small intestines, no sphincters disrupt the continuity of the lumen. If tight junction geometry reflects functional shape variations of the epithelial cells the tight junctions link, the network pattern can be expected to vary along the length of the tract. Since very little connective tissue or smooth muscle underlies the epithelium of the larval tract and neither the small nor the large intestine possesses the villi or the plicae circulares characteristic of the mammalian gut (see Results), stretching regions of the tube before fixation can be expected to affect the epithelial cells and permit a rough test of the flexibility of the potentially different network patterns. Furthermore, the epithelia of the alimentary tract can be readily subjected to functional stresses by varying the amount of food to which the animal is exposed in a manner similar to the

conditions which one would normally expect the animal to encounter. By correlating any resultant cellular changes with the concomitant variations in tight junctional morphology, one can attempt to determine the ability of certain network patterns to respond to normal and experimentally induced changes in tension. In addition, the larval epithelium degenerates during metamorphosis and is replaced by a new adult epithelium in which the stomach is enclosed by sphincters and the small intestine forms folds; this reorganization accompanies the changes in the animal's diet from herbivorous to carnivorous. Changes in the geometry of the tight junction networks would be expected if this extensive reorganization involved significant alterations in the stresses to which the cells are subjected. On the other hand, if the cells in the corresponding epithelia before and after metamorphosis retained their basic functional characteristics, their tight junctions should closely resemble each other. Thus, the gastrointestinal tract of *Xenopus laevis* represents a potentially very useful system for correlating functional changes in cell shape with the geometrical organization of their tight junctional networks.

MATERIALS AND METHODS

Both the tadpoles, usually stages 56–58 (12), and the newly metamorphosed toads used in this study were obtained by mating adult *Xenopus laevis* after injecting them with chorionic gonadotropin (Sigma Chemical Co., St. Louis, Mo.) according to the methods of New (11). The tadpoles were raised on a diet of yeast until metamorphosis and the small toads were fed kidney or liver every 3–4 days. Each animal to be examined by freeze-fracture techniques was anesthetized with ether, its abdomen opened, and a small amount of fixative (2% glutaraldehyde in 0.12 M Na Cacodylate, pH 7.3) was pipetted into it. The digestive tract from the esophagus to the large intestine was removed intact and immersed in the fixative for 15 min to 1.5 h. The shorter fixation time permitted the exposure of larger expanses of membrane faces while giving adequate preservation, so we used that method exclusively in the later phases of the work. The tissue was then cut into smaller pieces and transferred either to 0.12 M Na cacodylate buffer or to fresh fixative, and an equal volume of 60% glycerol in the buffer or fixative, respectively, was added gradually over a 30–40-min period. The tissue was left in the 30% glycerol for an additional 40 min before small pieces were placed on copper disks and frozen rapidly in liquid Freon 12. The tissue was fractured at −104°C in a Balzer's apparatus equipped with a platinum gun (Balzer's High Vacuum Corp., Santa Ana, Calif.). After the Pt/C replication, the tissue was digested away with chlorine bleach and then chromic acid. The replicas were examined with a Philips 300 electron microscope.

To experimentally stretch the tissue, short lengths of the small and large intestine were removed from tadpoles, placed in the buffer, pulled taut along their length, and pinned to a wax-covered petri dish. The dish was then flooded with several changes of fixative and left for 15 min. After rinsing with buffer, the tissue was processed for freeze-cleaving as described above.

Changes in the distribution of mucous vesicles in the surface epithelial cells of the gastrointestine were promoted by starving or overfeeding tadpoles before removing their intestines. For the starving experiments, the tadpoles were placed in small beakers of tap water containing no yeast for periods of 2–4 days. For the overfeeding experiments, animals were placed in beakers of water containing a high concentration of yeast which was never completely consumed, permitting the tadpoles to feed continuously. Under normal feeding conditions in our laboratory, the supply of yeast added daily to the water in which the animals were kept was exhausted by the next morning; thus the animals could not usually eat constantly.

Additional samples of the intestinal material were prepared for examination using light microscopy or scanning electron microscopy by first fixing them for 1–1.5 h in 2% glutaraldehyde in 0.12 M Na cacodylate (pH 7.3). The fixative was rinsed out with four changes of the buffer (0.12 M Na cacodylate) and the tissue was postfixed for 40–60 min in 1% OsO_4 in 0.12 M Na cacodylate. After two to three rinses in distilled water, the material was dehydrated in a graded series of acetone. For thick sectioning the tissue was then embedded in Epon-Araldite. 0.75-μm thick sections were cut with glass knives in a Porter-Blum MT-2 (Dupont Instruments, Sorvall Operations, Newtown, Conn.) and stained with toluidine blue for examination by light microscopy. For scanning electron microscopy the blocks of tissue were dried according to the critical-point method with liquid CO_2 in a Sorvall apparatus. After shadowing with carbon and gold or gold-palladium, the tissues were examined with a Cambridge S4 stereoscan electron microscope.

RESULTS

Network Patterns of Tight Junctions in the Gastrointestine of Xenopus laevis Tadpoles

The long tube which comprises the alimentary tract of *Xenopus* tadpoles is lined by epithelial cells that are regionally specialized to carry out their particular functions, among these the secretion of mucus, digestion of food, transportation of nutrients, and absorption of water. The simplicity of the organization of this tube is apparent in the

cross-section of the larval small intestine shown in Fig. 1 A. The columnar epithelial cells, which comprise a major portion of the wall of the small intestine, show no extensive outfoldings or indentations, but rather form a nearly regular circle. At a higher magnification (Fig. 1 B) the columnar absorptive cells with their extensive brush border can be seen to overlie an extremely thin rim of connective tissue and smooth muscle. Relatively few goblet cells can be found in the small intestine although they are numerous in the esophagus. The organization of the large intestine (Fig. 1 C) closely resembles that of the small intestine, although the columnar absorptive cells are shorter, with a less well-developed brush border. Again, the epithelial cells are not organized into villi and the region of connective tissue and smooth muscle is relatively narrow.

When the columnar absorptive cells lining the small intestine are freeze-cleaved, an evenly cross-connected tight junction meshwork is revealed (Fig. 2). This network closely resembles those that have been described for the mammalian small intestine (18, 17, 19). 11–14 strands can be measured from the apical to the basal side of this network (Fig. 2), although in tight junctions found in the small intestines of some tadpoles, we have found as few as 7. These strands appear as a series of short ridges or furrows which meet at acute angles to form a tightly knit network of similar polygons which show no predominant orientation with respect to the cell axes. Physically stretching

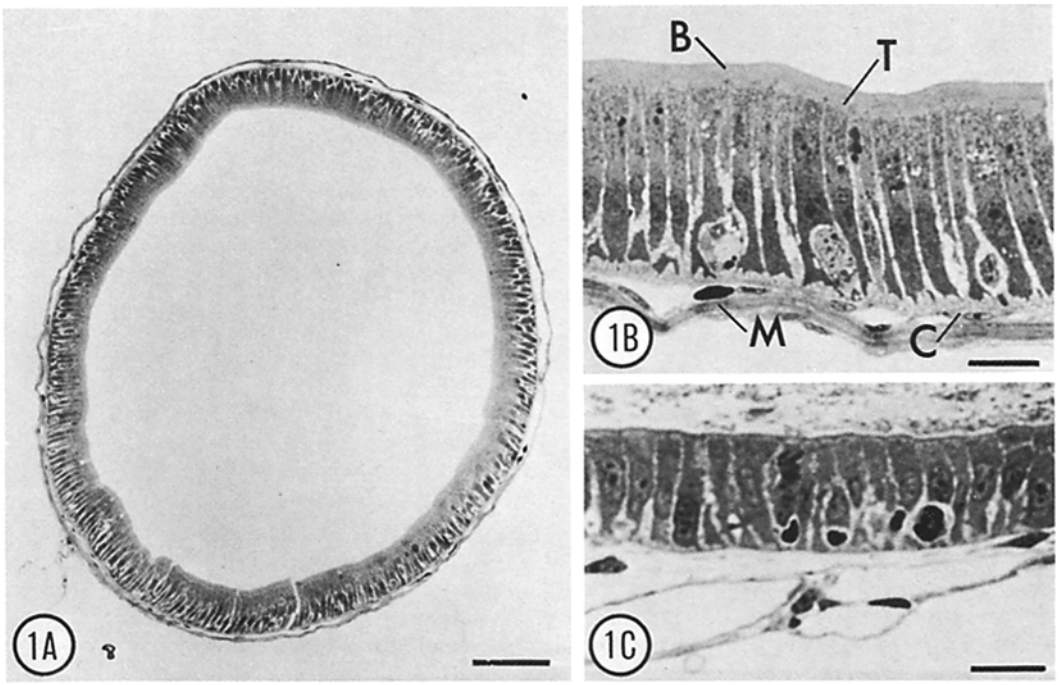


FIGURE 1 Light micrographs depicting the simplicity of the organization of the larval small and large intestines of *Xenopus laevis*. A, Cross-section through the small intestine of a tadpole, stage 57. Villi and crypts are both absent and there is no obvious region of undifferentiating stem cells. The small intestine appears as a regular thin-walled tube. Bar denotes 0.1 mm. × 100. B, An enlargement of a section of the small intestine shown in Fig. 1 A illustrating the general organization of the tissues of the small intestine. Columnar absorptive cells with their well-developed brush borders (*B*) and terminal web region (*T*) line the lumen. A very thin layer of connective tissue (*C*) and smooth muscle (*M*) underlie the epithelium. Bar, 16 μm. × 600. C, Large intestine of a stage 56 tadpole. Although the columnar absorptive cells of the large intestine appear much shorter than those of the small intestine, the basic organization of the tissue is conserved. The rim of connective tissue and smooth muscle is also relatively narrow. The layer of bacteria and yeast which clings to the surface of the cells is quite difficult to remove completely without damaging the cells and is presumably trapped within a coating of mucus. These surface contaminants can also be seen in the scanning electron micrographs shown in Fig. 5 A, B. Bar, 16 μm. × 600.

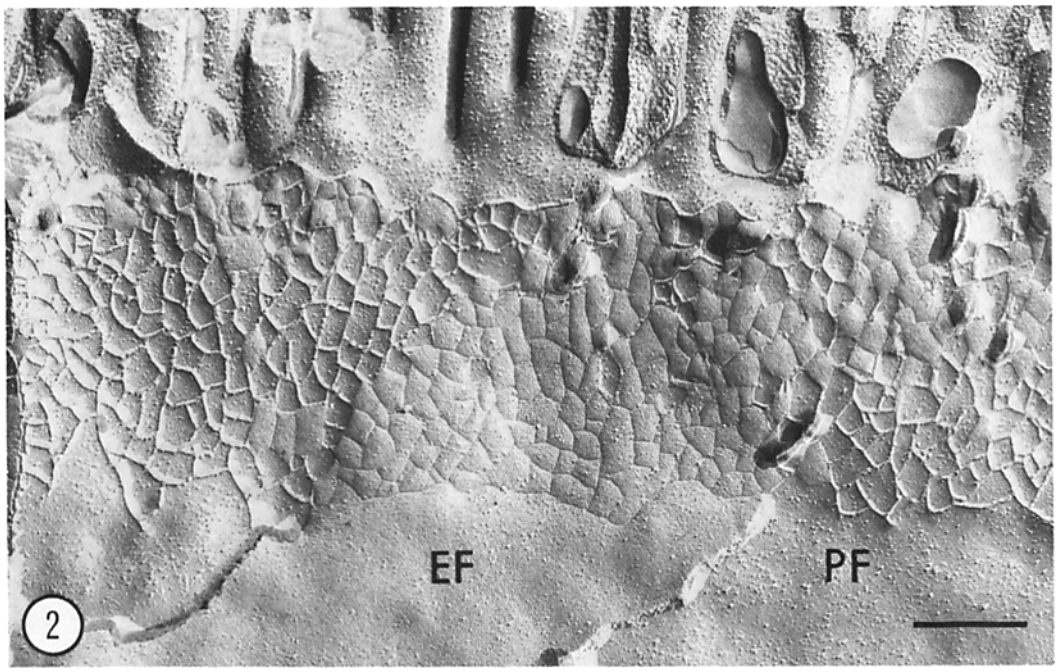


FIGURE 2 An evenly cross-linked tight junction network found between the absorptive cells of the small intestine of a *Xenopus laevis* tadpole, stage 57. Short ridges or grooves on the protoplasmic (PF) or extracellular (EF) faces, respectively, intersect at acute angles to form a series of adjoining, similarly shaped polygons which display no predominant orientation relative to the cell surface. Bar denotes 0.25 μm in the electron micrographs unless marked otherwise. × 60,000.

the tissue before its fixation has *no* noticeable affect on the geometrical organization of the junctions.

Tight junctions found in the tadpole large intestine differ from those in the small intestine in several significant respects. In the large intestine junction shown in Fig. 3, the sealing strands form only a few polygons with acute angles similar to those found in the small intestine. Instead, long ridges take curving paths roughly parallel to the cell surface and sloping gently away from it. These long strands meet at both acute and obtuse angles and are interconnected by short ridges. The resultant enclosed, elongated regions vary greatly both in shape and in area. Superimposed over the curviness of the individual strands is a waviness of the network as a whole, both in the apical-basal direction as evidenced by the changing orientation of the limiting strands, and in space as the network reflects the gentle undulations of the lateral plasma membrane.

The large intestine absorbs water from the waste products that are temporarily stored in its lumen before they are extruded. This dehydration process occasionally leads to the formation of large, hardened fecal pellets that distend the lumen around its circumference. To mimic roughly this kind of natural stress acting on the large intestine, we pulled the tissue taut before fixing it. Tight junctions found between cells which have been subjected to this stress exhibit a change in the overall orientation of the network when compared to junctions from control animals (compare Fig. 4 with Fig. 3). Now the long strands are primarily oriented parallel to the cell surface and the enclosed regions are more nearly rectangular. Both the tight junction and the membrane within which it lies appear planar, giving the impression that the junction has been stretched along its long axis and has become oriented along the line of force. Also supporting this notion is the reduction in the amplitude of the waves formed by the apical and basal strands. To obtain a rough estimate of the theoretically maximum extent to which the large intestine junction can be stretched, we measured the curved distance between two points of the basal

strand of the junction shown in Fig. 3 and compared it with the straight line distance between the same points. The straight line distance is only about 60% of the curving distance, indicating that the junction can stretch by as much as 40%.

To demonstrate further that we were able to affect the morphology of individual epithelial cells of the large intestine by stretching the tissue before fixation, we used scanning electron microscopy to examine the luminal surface of the tissue. In the unstretched large intestine (Fig. 5 A) the surface of the epithelium appears quite flat, comprised of randomly oriented, polygonally shaped cells. If the tissue is stretched before fixation (Fig. 5 B), the surfaces of the cells protrude into the lumen, appearing as rows of distended cells. The borders

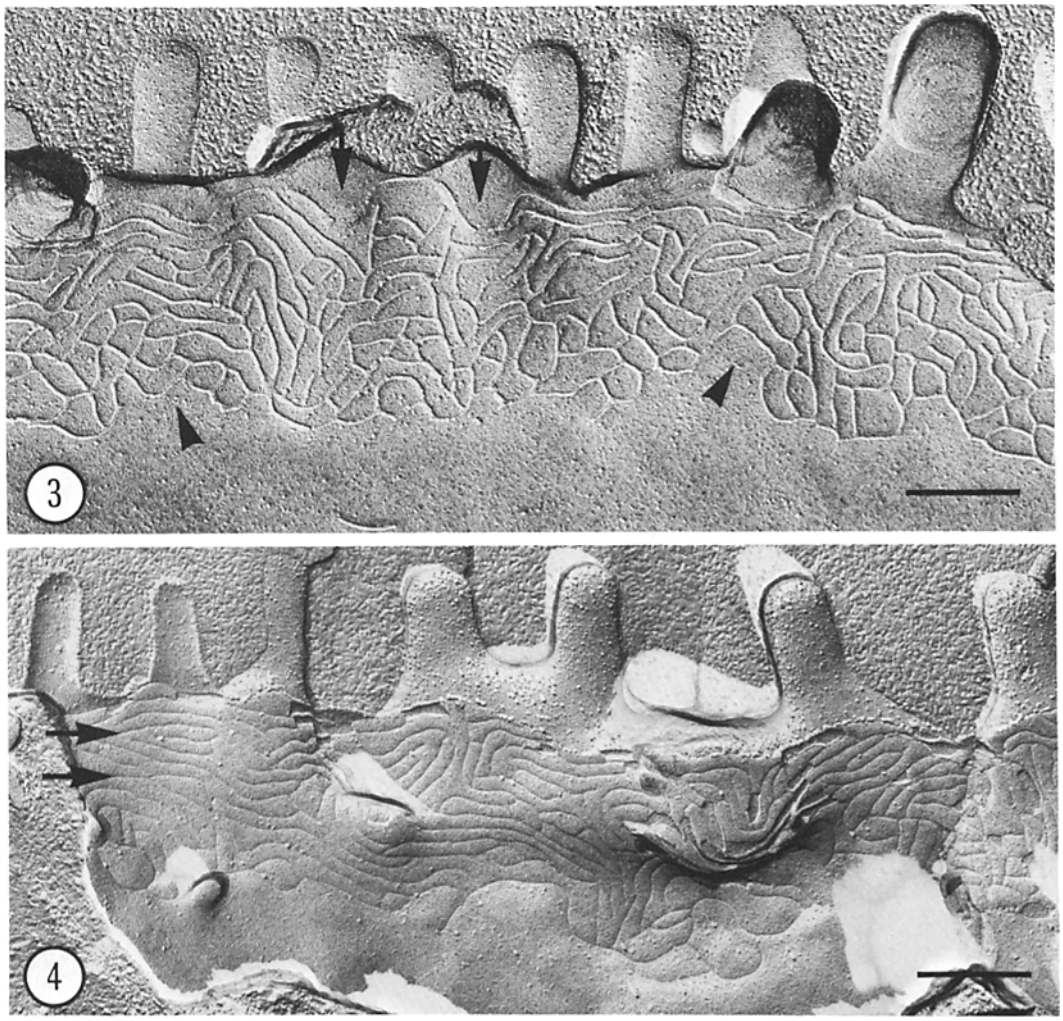


FIGURE 3 A loosely interconnected zonula occludens from the large intestine of a control tadpole, stage 58. Particularly characteristic of this network is the irregularity in the shape of the enclosed regions formed by the intersection of the long, waving strands with shorter strands. Both the apical and basal ridges curve extensively (arrowheads), as the curvature of the network reiterates the undulation of the membrane itself. × 60,000.

FIGURE 4 Elongated network from an experimentally stretched tadpole large intestine. The long grooves (arrows) have become oriented nearly parallel to the cell surface and the interstrand spacing now appears highly regular (compare with Fig. 3). Some waviness in the apical and basal strands remains, but the network as a whole appears stretched. × 60,000.

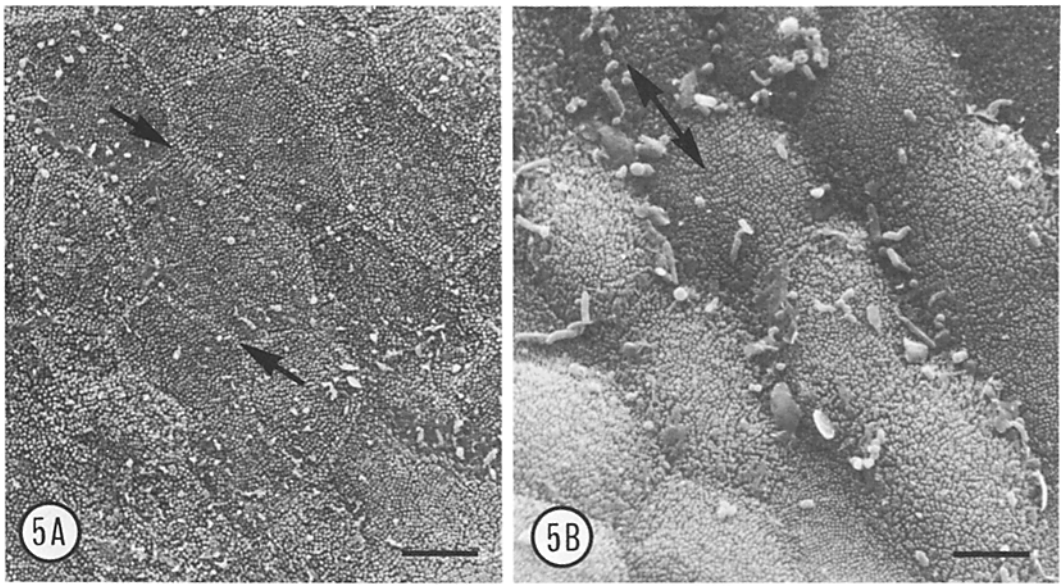


FIGURE 5 A Scanning electron micrographs of the luminal surface of the large intestine of a stage 56 tadpole. The cell surfaces are quite flat and the cell borders (arrows) form polygons which vary greatly in size, but show no overall orientation. Bar, 3.5 μm. × 2,800. B, The luminal surface of a larval large intestine which had been stretched before its preparation for scanning electron microscopy. The cell surfaces tend to protrude into the lumen and some of the borders seem elongated along the axis shown by the double-headed arrow. This distortion of the cell shape compared with that shown in Fig. 5 A indicates that we were able to affect the epithelial cell morphology by stretching the tissue, presumably along the axis shown by the arrow. Bar, 3.5 μm. × 2,800.

of the cells also seem somewhat distorted, showing a tendency to be elongated along the apparent line of force (arrow, Fig. 5 B). This distortion of the cell morphology supports the hypothesis that we were indeed able to stretch the cells, causing a reorientation of the tight junctions as shown in Fig. 4.

Although structurally continuous with the small intestine, the premetamorphic gastrointestine exhibits yet another type of tight junction network which is found the mucus-secreting cells (Figs. 6, 7). Typically, as seen in Fig. 8, the junction consists of a large number of gently curving sealing strands (15–23 or more) that extend roughly parallel to the cell surface and to each other to form a gently undulating network. The individual strands tend to have relatively few interconnections. Fig. 6 shows a typical mucous cell from a control animal that would exhibit a tight junction pattern similar to that illustrated in Fig. 8. It is important for this study that only one layer of mucous vesicles lies in the apical region of the cytoplasm immediately beneath the gently undulating surface plasma membrane. After 2 days of starvation (Fig. 7), the apical region of the cell has been distended by the accumulation of about four layers of mucous vesicles. In general, the greater the number of vesicles present, the more distended does the cell surface appear. The lateral plasma membranes from starved animals (Fig. 7) are much straighter than those of the control in Fig. 6, showing that the stress on the distended cell surface has been partly transmitted to the lateral membranes as well. Since this natural expansion of the cell surface also involves the tight junction region, it is not surprising that this stretching is reflected in the morphology of tight junctions from such cells (Fig. 9). In the expanded state of the network, the grooves and ridges become quite evenly spaced, with very few touching any others along their length. The separation of the strands and their orientation parallel to and away from the cell surface indicate that the forces acting on the membrane as the vesicles build up are much like those on a balloon being inflated. The strands curve very little and the network pattern seems more angular than in the control.

Placing a tadpole in a consistently high concen-

tration of yeast results in a decrease in the average number of mucous vesicles relative to that found in the apical cytoplasm of surface mucous cells in normal, control animals. The tight junctions of such cells have a more compressed appearance than those found in control or starved animals (compare Fig. 10 with Fig. 8 or Fig. 9). The ridges are very closely packed and tend to run parallel to the long axis of the cell, rather than parallel to the cell surface, as found in controls. Both the apical and basal strands of the network curve extensively, suggesting that the network as a whole is undulating. Indeed, the tight junction appears either laterally compressed or as if some tension had been released.

There is a normal variation in the number of mucous vesicles per cell and all replicas show a corresponding distribution in the degree of stretching or relaxation of the junctions. However, by starving or excessively feeding an animal, one can obtain an increase in the proportion of the junctional morphology shown for each treatment.

Tight Junctions Found in the Larval Esophagus

The esophagus of *Xenopus laevis* tadpoles contains two distinct types of epithelial cells: ciliated cells and goblet cells (6, 7). The ciliated cells do not accumulate any secretory product, but probably serve to help propel food along the digestive tract in the absence of a well-developed musculature (7). The tight junction network which is found in such a ciliated cell (Fig. 11) closely resembles in its geometrical organization the meshwork found in the small intestine (Fig. 1). Short strands interlink to form adjoining polygons which vary more in area than those of the small intestine network. Goblet cells, on the other hand, accumulate large vesicles containing mucus and their apical cyto-

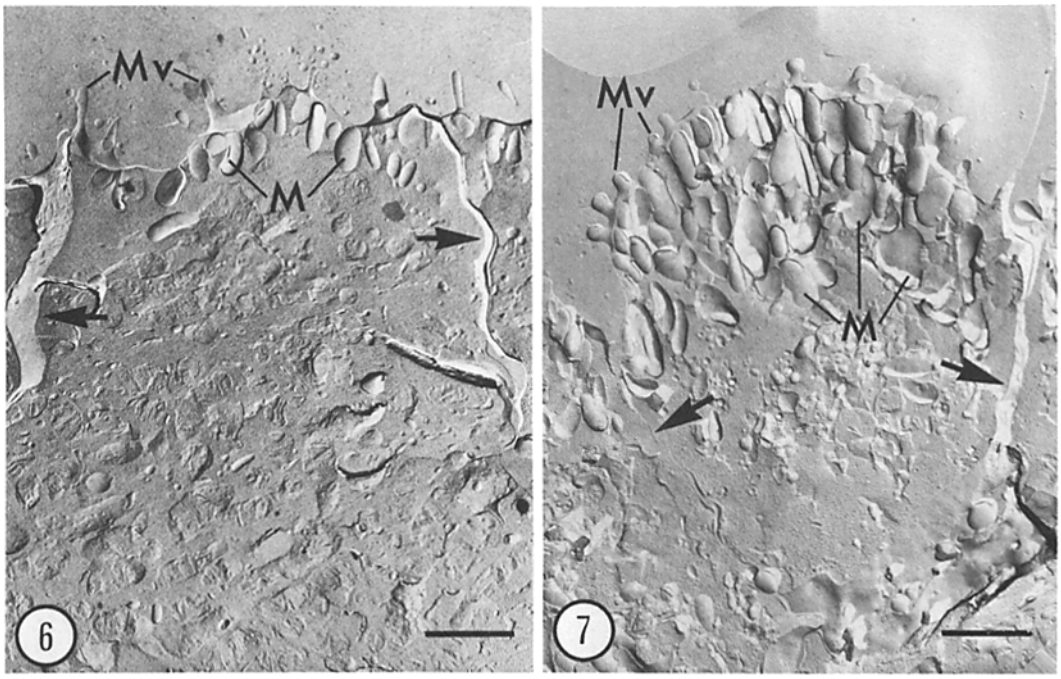


FIGURE 6 The apical cytoplasm of a mucous epithelial cell from the gastrointestine of a normally fed tadpole (stage 57). Only about one layer of smooth-surfaced mucous vesicles (*M*) lies immediately below the surface plasma membrane which displays narrow, elongated microvilli (*Mv*). In this cell the lateral plasma membrane curves gently (arrows). Bar, 1 μm. × 12,000.

FIGURE 7 In this micrograph of a gastrointestinal mucous cell from a stage 57 tadpole that had been starved for 2 days, approximately four layers of mucous vesicles (*M*) distend the apical region of the cytoplasm. Only short, stubby microvilli (*Mv*) extend into the lumen, and the lateral plasma membrane (arrows) follows a nearly straight path (compare with Fig. 6). Bar, 1 μm. × 12,000.

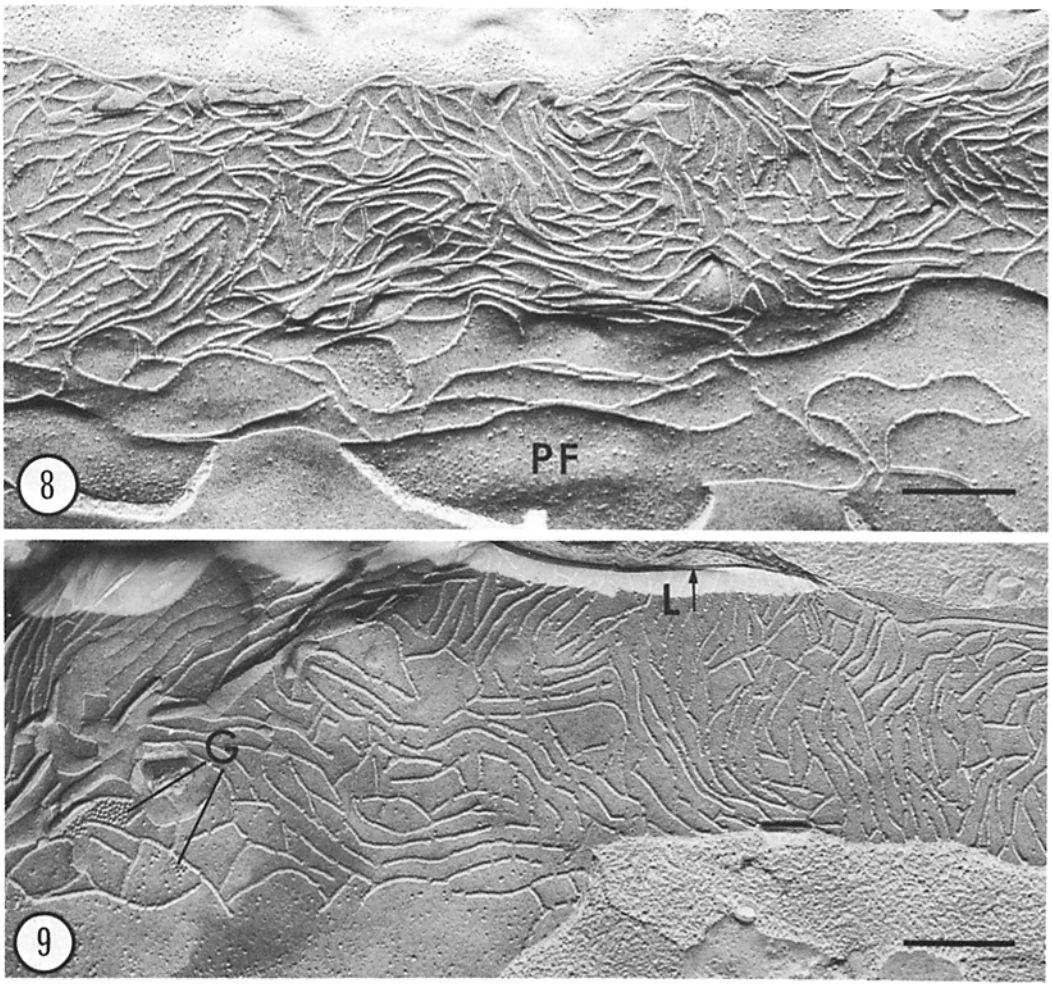


FIGURE 8 This micrograph shows the extensive, gently curving junctional network found between mucous cells of the gastrointestine of a stage 56 tadpole. The strands are interconnected infrequently and curve gently along paths roughly parallel to the cell surface. *PF*, protoplasmic face. × 60,000.

FIGURE 9 Zonula occludens from the gastrointestine of a stage 57 tadpole which had been starved for 4 days. The ridges comprising this network are more widely spaced, their paths nearly straight, and their angles of intersection larger than in the network shown in Fig. 6, indicating that this network has been stretched by the accumulation of mucous vesicles. Small gap junctions (*G*) can be seen between some of the tight junction ridges. *L*, lumen. × 60,000.

plasm often projects extensively into the lumen. Therefore, these cells should possess tight junctions with flexible networks having a low level of cross-linking, similar to those of the functionally related mucous cells of the gastrointestine. Rather than forming islands of functionally related cells in the esophagus, these goblet cells are typically surrounded by ciliated cells. Fig. 12 shows a junction formed between these two cell types, in which the PF face of the junction is continuous with the underlying ciliated cell and the EF face is contributed by the overlying goblet cell. This network pattern represents a compromise between the evenly cross-linked network of the ciliated cells (Fig. 11) and the more extensive, more loosely interconnected junction found between the analogous mucous cells of the gastrointestine (Fig. 6). The sealing strands form larger, more irregularly shaped polygons than those in the ciliated cell (Fig. 11) and this lower level of cross-linking has

permitted the network to expand with the build-up of vesicles in the goblet cell, for most of the strands appear widely separated. Thus, when these two cell types adjoin, whose cell apices are normally subjected to differing functional stresses, the resultant pattern seems to be a compromise between the relatively stable pattern of the ciliated cells and the more flexible network of the mucus-secreting cells.

Tight Junctions of the Digestive Tract of the Adult Toad

During metamorphosis the epithelial cells of the larval gastrointestinal tract degenerate and a group of underlying cells proliferate to form a new epithelium (1, 13). Consequently, the stomach becomes well defined in its shape and is separated from the rest of the tract by sphincters. The epithelium of the small intestine folds up to form the folds characteristic of the adult toad small intestine. Despite this extensive reorganization of the digestive tract which accompanies the change from a herbivorous to a carnivorous diet, we have found many similarities in the tight junctions of corresponding tissues of the digestive tract before and after metamorphosis.

In the tight junctions of the postmetamorphic small intestine (Fig. 13), short ridges and furrows meet to form small, acute-angled polygons, very similar to those of the tadpole small intestine (Fig. 2). This evenly cross-linked meshwork is composed of five to eight sealing strands, within the range of the number of sealing strands found in the small intestine of different tadpoles. In the large intestine of the toad (Fig. 14), the network is formed by long strands which undulate gently and are interconnected by short strands, very similar to the corresponding tadpole junction (Fig. 3). Similarly, the curviness of the individual strands and of the network as a whole both provide the meshwork with the potential to stretch circumferentially to accommodate large fecal masses.

The stomach of the metamorphosed *Xenopus* toad is structurally organized much like that of

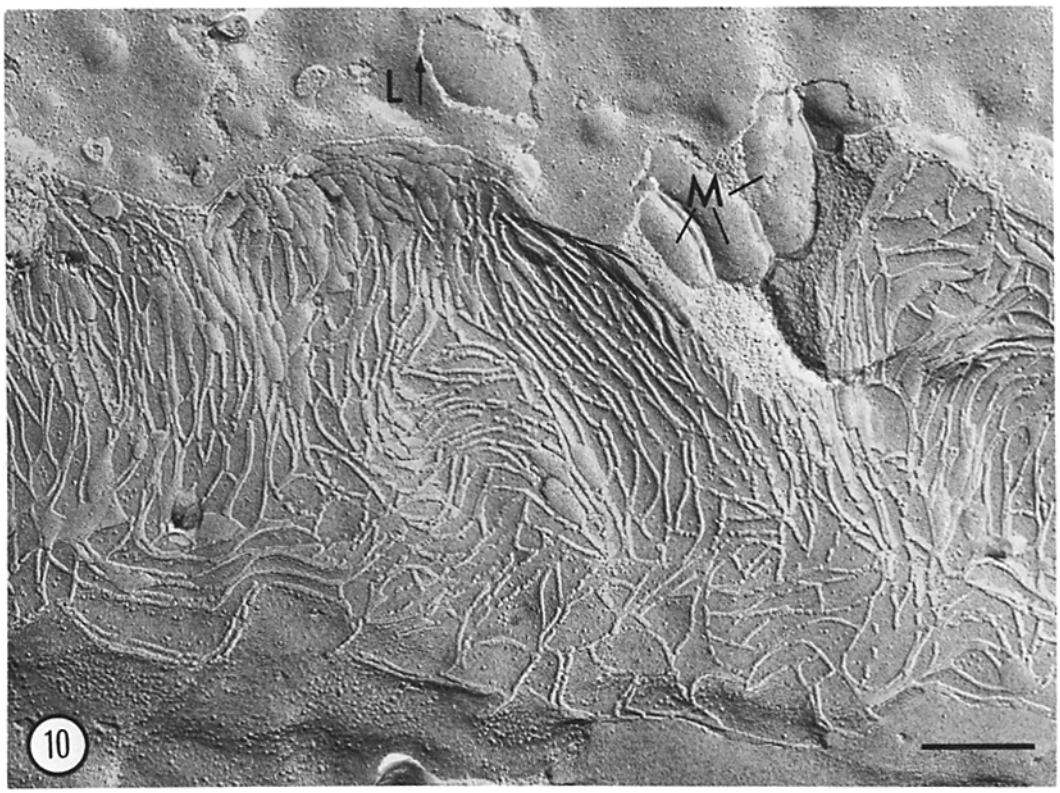


FIGURE 10 Tight junction from the gastrointestine of a well-fed tadpole (stage 57). In this network the ridges lie very close together and both the apical and basal strands curve extensively, giving the network a very compressed appearance (compare with Figs. 6 and 9). *M*, mucous vesicles. *L*, lumen. × 60,000.

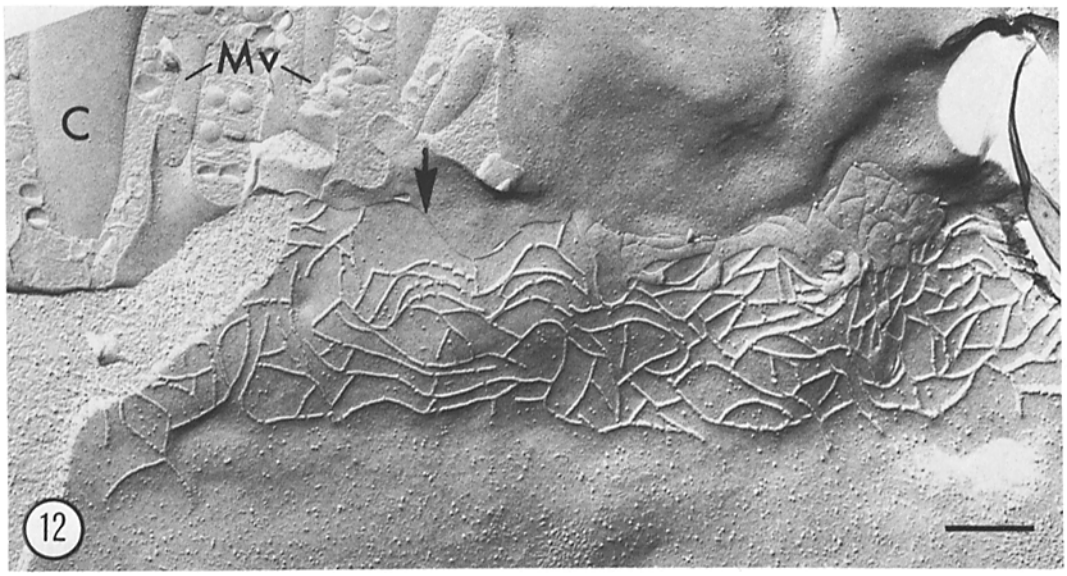


FIGURE 11 A highly cross-linked tight junction meshwork found between two ciliated cells of the larval esophagus (stage 53). Branching microvilli are found on both the underlying (Mv_1) and the overlying (Mv_2) cells. Cilia (*C*), recognizable by their larger diameter and smooth membrane faces, also extend into the lumen. Although the polygons enclosed by the intersecting ridges vary more in area and form than in the small intestine (Fig. 2), the basic geometrical organization of the two networks is very similar. × 50,000.

FIGURE 12 This micrograph shows a mixed tight junction network formed between a goblet cell and a ciliated cell of the larval esophagus (stage 53). The underlying cell, which contributes the PF face of the junction may be recognized as the ciliated cell due to its smooth-surfaced cilia (*C*) and its microvilli (*Mv*). The EF face of the junction is continuous with the surface plasma membrane of the goblet cell which characteristically extends deep into the lumen. The resultant junction has an intermediate level of cross-linking. The surface expansion of the goblet cell membrane seems to have pulled the apical strand of the network towards the lumen (arrow). × 50,000.

Rana pipiens (12). The surface mucous cells, upon which this study concentrated, line the luminal surface of the stomach. These cells closely resemble the corresponding ones found in the tadpole gastrointestine (Fig. 6), except that after metamorphosis they contain many layers of mucous vesicles filling the apical cytoplasm, and the number of vesicles does not vary significantly among recently fed and 3- or 4-day starved animals. The tight junctions found between mucous cells of recently fed toads (Fig. 15) are organized much like those of the tadpole gastrointestine (Fig. 8). In this junction many of the strands are oriented parallel to the long axis of the cell, but the similarity in structure to that of the gastrointestinal junction indicates that it could stretch extensively if the mucous vesicles should build up abnormally during a prolonged fast.

Thus, the tight junction networks found along the length of the digestive tract of *Xenopus laevis* vary both in the extent to which the individual strands are cross-linked and in the responsiveness of the meshwork to changing physical stresses. The different geometrical organizations and their potential orientations are summarized in two diagrams (Figs. 16 and 17) that are based on tracings of the networks in representative micrographs.

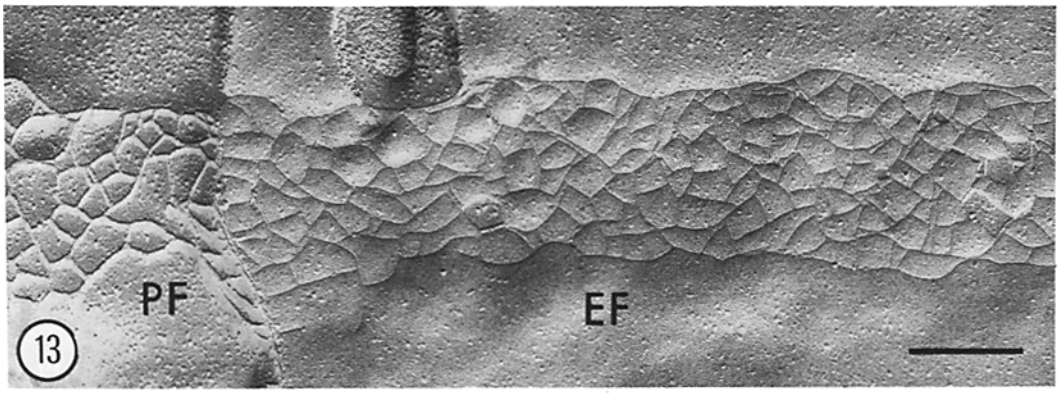
DISCUSSION

Epithelial cells which are linked by tight junctions can be undergoing extensive changes in shape in response to intracellular accumulation of secretory products, to developmental changes in the morphology of the cell, or to extracellular forces such as the contraction or expansion of the tissue as a whole. The presence of a rigid, beltlike tight junction encircling the cells would tend to restrict severely the extent to which the apical lateral plasma membrane could stretch or flex under these stresses without disrupting the seal. Therefore, the maintenance of the transepithelial gradient of solutes or ions under tension would be promoted if the tight junction network could stretch or compress within the fluid membrane matrix. The basic meshlike pattern of the zonula occludens would seem to give the network a certain degree of intrinsic flexibility (19), although modification of the general pattern would probably be necessary to adapt the meshwork optimally to the specific functional stresses of the tissue in which it is found. In particular, the network found between cells undergoing large, recurring shape changes would need greater adaptability than the network connecting cells which maintain a fairly constant shape.

By examining tight junctions from the esophagus, the gastrointestine or stomach, and the small and large intestines under normal and experimentally induced stress conditions, we have shown that variations in the organization of the networks are indeed related to the kinds of stresses to which the tissues are naturally subjected. These studies have demonstrated that tight junctions with low levels of cross-linking are found in tissues subjected to forces which change extensively, whereas highly cross-linked tight junctions are observed in tissues normally subjected to more nearly constant tension. The columnar absorptive cells of the small intestine do not accumulate any secretory product and their apical regions exhibit a nearly constant shape which is reinforced by the network of filaments from the microvilli, the terminal web, and those connected to the intermediate junction. The tight junctions found between these cells before and after metamorphosis (Figs. 2, 13, and 17 A) are composed of short strands connecting to form a meshwork which is composed of regular polygons showing no overall orientation and which does not respond significantly to stretching. Ciliated cells of the larval esophagus also have regular, evenly cross-linked tight junction networks (Figs. 11 and 16 B), reflecting the stability of the apical region of these cells, which is filled with the basal bodies of the cilia and their associated fibrillar components.

In the large intestine of both the tadpole (Fig. 3) and the adult (Fig. 14), in contrast, the network is loosely interconnected, allowing the network to expand in length when the tissue is stretched before its fixation (Figs. 16 C, D). This ability of the junction to stretch circumferentially seems to be of functional significance, since mechanical stretching occurs whenever large, hardened feces accumulate in the lumen and move toward the cloaca. Thus, the tight junction of the large intestine seems to be optimally designed to adjust to its normal stretching-contraction cycle.

Variations in interstrand spacing and the overall orientation of the sealing strands can be obtained in a more natural way in the loosely interconnected networks linking the mucous cells of the larval gastrointestine (Fig. 8) by varying the feeding conditions of the tadpole. The rate at which the mucous vesicles are discharged seems to depend on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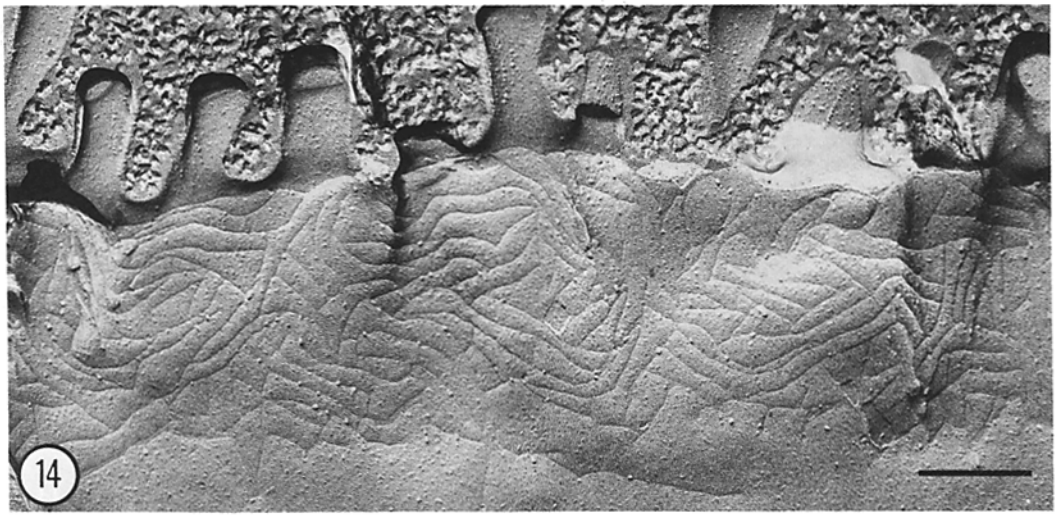
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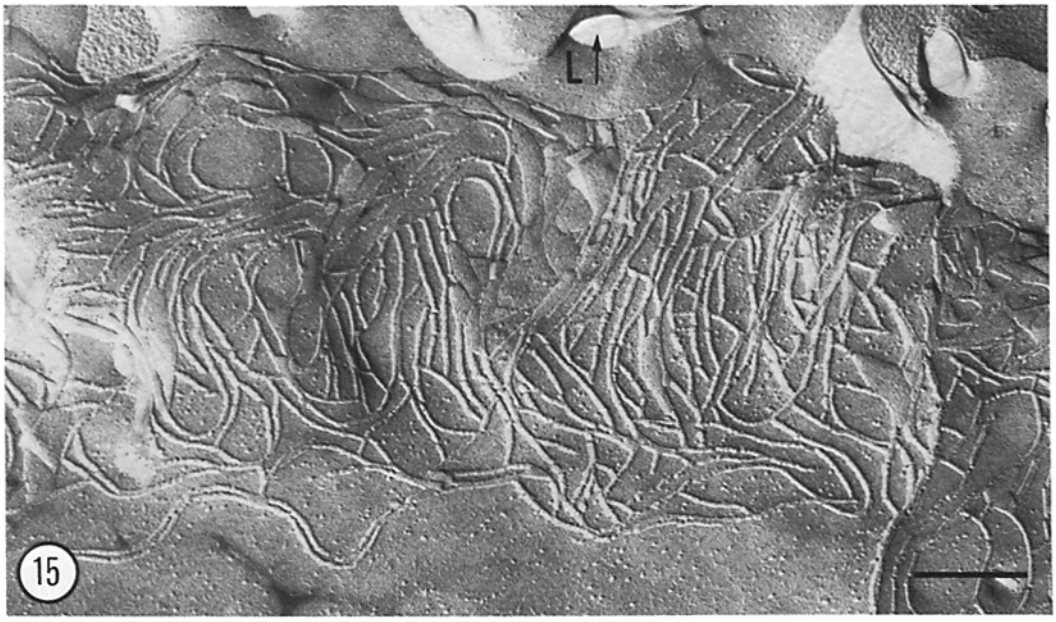
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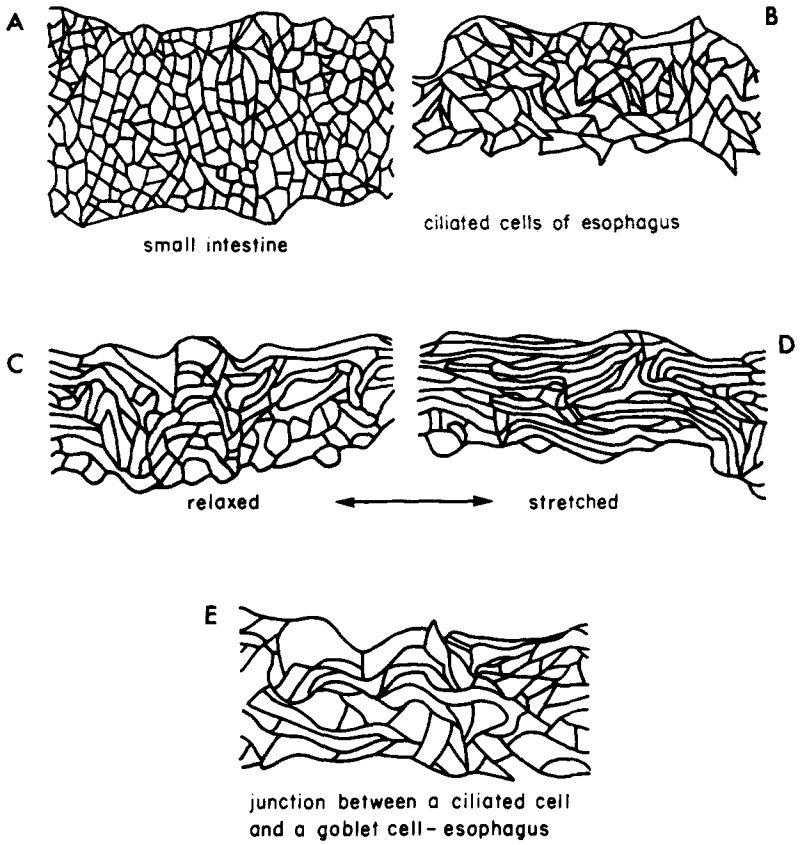

FIGURES 16 and 17 Tracings of representative tight junction micrographs enlarged to the same magnification and summarizing the basic network patterns found along the length of the alimentary tract of *Xenopus laevis* before and after metamorphosis.

FIGURE 16 This diagram illustrates in a schematic way the two basic types of geometrical organization that we have found for tight junction networks. The first type, the stable, evenly interconnected pattern composed of fairly regularly shaped polygons, seals the absoptive cells of the small intestine (A) and the ciliated cells of the esophagus (B). The second pattern, the loosely interconnected junction, which is found between the absorptive cells of the large intestine, permits the network to stretch when placed under tension (compare C and D). The mixed junction shown in E, which displays an intermediate level of cross-linking, forms when a ciliated cell adjoins a mucus-secreting goblet cell.

FIGURE 13 An evenly cross-linked tight junction network from the large intestine of the postmetamorphic toad. In its basic geometrical organization this network closely resembles the corresponding junction of the tadpole small intestine (Fig. 2), with its array of closely adjoining, similar polygons. *EF*, extracellular face. *PF*, protoplasmic face. × 60,000.

FIGURE 14 This micrograph illustrates the elongated, loosely interconnected network of the large intestine of the adult toad. As in the corresponding larval tight junction (Fig. 3), the long strands curve gently both parallel to the cell surface and sloping gently away from it. × 60,000.

FIGURE 15 PF face of a tight junction found between surface mucous cells of the stomach of a newly feeding toad. Despite the reorganization of the stomach during metamorphosis, the network bears a striking resemblance to the junction formed between the gastrointestinal mucous cells of premetamorphic tadpoles (Fig. 8). The close alignment of the long strands and their general orientation parallel to the long axis of the cell suggests that the network has become slightly compressed. *L*, lumen. × 60,000.

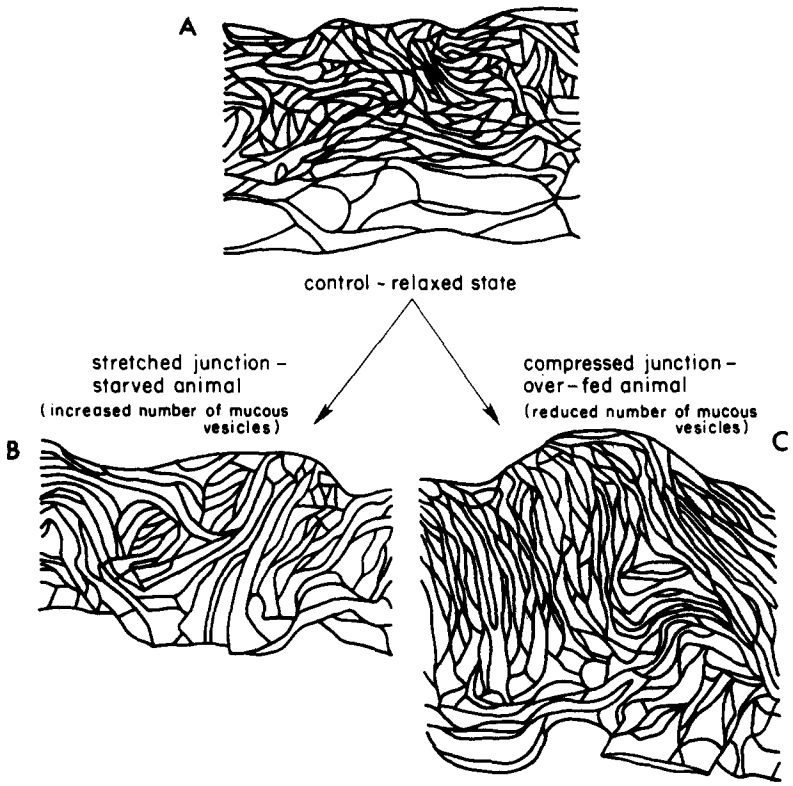

FIGURE 17 Diagram illustrating the stress-related adaptability of loosely interconnected junctions found between the mucous cells of the gastrointestine. In the normally fed, or relaxed state (A) the interstrand spaces are highly variable, while in the expanded state (B) the strands are well separated and fairly evenly spaced and the junction as a whole is narrower. The network shown in (C) has very narrow interstrand spacings and is deeper than either the relaxed (A) or expanded (B) network. These two characteristics both indicate that this network from an overfed animal has been extensively compressed. The changes in the number of sealing strands among the junctions presumably reflects the normal variation in number seen among different animals.

the feeding activity of the animal, with large numbers of the vesicles building up when the animal is starved (Fig. 6 and 7). These changes in vesicle population cause changes in the surface conformation of the cells which in turn stretch or compress the tight junction networks (Figs. 9 and 10, respectively). Thus, in the starved animals the increase in the interstrand spacings and the orientation of the strands parallel to and away from the cell surface reflect the balloon-like expansion of the cell surface. In an overfed animal, the close alignment of the sealing strands and their orientation parallel to the long axis of the cell stem from the release of tension on, or the compression of the apical region of the cell as the number of mucous vesicles per cell decreases below normal levels. We cannot rule out the possibilty that the mucous cells are forming new, static network patterns under the different physiological conditions to which they have been exposed. However, since we have shown that the large intestine junction can respond passively to changes in tension, as its long strands reorient along the lines of tension, we tend to favor the interpretation that the changes in the form of the mucous cell tight junctions reflect passive responses of the network to changes in the stresses acting on the plasma membrane in that region. Thus, we postulate that the low level of interconnection of the sealing strands linking the mucous cells of the gastrointestine permits the cells to accommodate to the changing stress field associated with the accumulation and release of the secretory vesicles (Fig. 17).

Corresponding stress-related variations in the

tight junctional morphology are not as apparent in the junctions found in the stomach of the adult toad because the mucous cells usually contain large numbers of vesicles which do not vary significantly in number during the normal feeding cycle. Presumably, since the network pattern is conserved during metamorphosis, the junctions in the stomach can accommodate any changes in the cell morphology which might occur during a prolonged fast or heavy, continuous feeding.

Despite the change in feeding habits associated with metamorphosis, the tight junctions of the stomach and small and large intestines (Figs. 15, 13, and 14, respectively) retain the basic geometrical organization found in the corresponding larval tissues (Figs. 8, 2, and 3, respectively). This constancy supports the hypothesis that the basic functional conditions of these tissues are quite similar before and after metamorphosis.

If the geometrical organization of the tight junction is intimately connected with the functional stresses occurring in the cells, then it is important to consider what type of junction forms between two cells which do not normally undergo the same kinds of shape changes. The esophagus contains ciliated cells intermixed with goblet cells. Ciliated cells form evenly cross-connected tight junctions with like cells (Fig. 11), while the analogue of the goblet cell, the surface mucous epithelial cell, has a larger, more loosely organized network to allow for apical expansion of the cytoplasm during the accumulation of secretory mucous vesicles (Fig. 8). In the mixed junctions of the esophagus (Figs. 12 and 16 C) the degree of interconnection and the number of strands are intermediate between the two types of junctions, permitting a somewhat greater flexibility of the junction than in the case of the evenly cross-connected networks linking two ciliated cells. Occasional ciliated cells also extend into the anterior region of the amphibian stomach (13) and into the anterior region of the larval gastrointestine. In these regions as well one can find occasional compromise junctions which have more cross links and fewer elongated strands than in regions which have no ciliated cells. These intermediate junction forms support the premise that both cells contribute information leading to the formation of a specific, geometrical network pattern between them.

Variations in the organization of tight junctions have been described in other cell types as well. The zonula occludens of the mammalian small intestine (8, 17, 19) closely resembles the type we have described in the *Xenopus* small intestine: a highly ordered network functioning as a stable intercellular permeability barrier. Although the tight junction network in the mouse liver (3, 10) is fairly evenly cross-linked, the areas enclosed by the strands are more irregularly shaped than those in the small intestine, presumably to permit small shape changes in the bile caniliculus. The urinary bladder is a storage vesicle which must periodically stretch and relax, so it should be expected that its tight junctions would have few cross-connections, permitting the network to elongate or shorten in response to changing stresses on the tissue. This type of network with long, flexible sealing strands interconnected by short strands has been demonstrated both for the frog urinary bladder (reference 4, Fig. 1) and for the mammalian bladder (Staehelin, unpublished results). In the latter case, the stretching-contraction cycles have been shown to result in network morphology changes similar to those described above in the large intestine.

Pancreatic acinar cells accumulate zymogen granules, and their tight junctions (reference 8, Figs. 2 and 3) appear as highly flexible structures with relatively few cross-connections, which would permit the junctions to respond to cycles of cell shape changes similar to those observed in the mucous cells of *Xenopus* gastrointestine. Tight junctions of the epididymis also show a great variation in their general orientation and have relatively short strands linking the many long ones (reference 8, Figs. 18–20). This morphological flexibility probably enables the junction to maintain its transepithelial sealing capacity when the tissue is stretched during the accumulation and passage of sperm. Epithelial cells in the mouse mammary gland also undergo extensive changes in cell shape which are associated with lactation (14). The tight junctions found in these cells resemble the loosely interconnected junctions of the *Xenopus* large intestine and show variations in orientation during the lactation cycle.

In summary, we would like to postulate that the differences in the geometrical organization of tight junctional sealing elements of different tissues are of functional significance. In particular, we propose that the network geometry is related to the cyclic changes in the stress field to which the cells in a given tissue might be exposed. To this end, the sealing elements are arranged and interconnected

so that they can maintain their sealing function and not be disrupted during the normal stretching-contraction cycles of a given tissue. Thus, junctions which have long sealing strands that interconnect infrequently can adapt more readily to the changing conformation of the cell membranes in which they lie than those with a greater amount of cross-linking. The first type of tight junction tends to be found in tissues which expand and contract, as well as between cells which accumulate a secretory product with a concomitant distension of the cell surface. An evenly cross-linked network, on the other hand, is characteristically found in tissues in which the forces acting on the plasma membrane are in a more stable equilibrium, reflecting a reduced need for flexibility. Since tight junction geometry reflects the potential cell shape changes of a tissue, its organization in different tissues may provide a useful tool for probing previously unsuspected changes in the morphology of the apical regions of epithelial cells.

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