

THE TERMINAL WEB

A Reevaluation of its Structure and Function

BARBARA E. HULL and L. ANDREW STAEHELIN

From the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309. Dr. Hull's present address is the Biology Department, Southern Connecticut State College, New Haven, Connecticut 06515

ABSTRACT

The apical cytoplasm of epithelial cells of the small and large intestines has been examined by freeze-etch techniques as well as conventional and high voltage electron microscopy of sectioned material to gain a better understanding of the fine structural organization of the terminal web region. In the small intestine the terminal web exhibits a distinct stratification caused by the association of different sets of filaments with the three members of the junctional complex. Individual filaments of this network are closely associated with the sealing elements of the tight junctions, the surface of the core microfilament bundles, and the intermicrovillar plasma membrane. This region of the terminal web is the apical zone. The adherens zone appears as a band of interwoven filaments of two different diameters extending across the cytoplasm at the level of the intermediate junction. Within this region of the terminal web, individual 60–70 Å actin-like filaments separate from the bundles of core microfilaments to interact with one another and with filaments of similar diameter from the zonula adherens. 100 Å tonofilaments also contribute to the adherens zone, presumably stabilizing the orientation of the actin-like filaments. The basal zone which underlies the adherens zone consists of closely interwoven bundles of tonofilaments that are anchored to and interconnect the spot desmosomes. Within the large intestine the cytoplasmic microfilaments form a looser and less clearly stratified network which nevertheless retains the same basic organization found in the small intestine. Transmembrane linkers appear to originate within the cytoplasmic plaques of the spot desmosomes, pass through the plasma membranes, and meet in a staggered configuration in the intercellular space; these linkers may thus mediate the actual mechanical coupling between the cytoskeletal networks of tonofilament bundles of adjacent cells. This integrated system of cytoplasmic filaments and intercellular junctions endows the apical cytoplasm with both the flexibility and the stability necessary for the normal functioning of the epithelium.

KEY WORDS terminal web · intestine · cytoplasmic filaments · intercellular junctions · high voltage EM · freeze-etch

Brush borders isolated from intestinal epithelial cells have served as a useful model system for studying nonmuscle motility. In 1971, Tilney and Mooseker (25) isolated actin from brush borders

of chicken intestinal epithelial cells. This prominent component of purified brush borders displayed the same electrophoretic mobility as muscle actin and polymerized under the same conditions required for the transformation of globular actin to filamentous actin. Since the core filaments of the microvilli bind heavy meromyosin, they are presumably composed of filamentous actin. After removing the plasma membranes from isolated brush borders using Triton X-100, Mooseker (9, 10) demonstrated that upon addition of Ca^{++} and ATP the bundles of core microfilaments rapidly moved into and through the terminal web, the network of filaments enmeshing their bases. Rodewald, Newman, and Karnovsky (17) isolated brush borders from the small intestine of neonatal rats; those brush borders which retained their surface plasma membrane would contract into small spheres in the presence of ATP and Mg^{++} or Ca^{++} . At the electron microscopic level this contraction represented a pinching in of the brush borders in the region of the intermediate junction, but did not appear to involve a shortening of the microvilli. Both Mooseker and Tilney (11) and Rodewald et al. (17) developed models postulating that the observed motility was the result of an interaction of actin filaments with myosin molecules in the terminal web region in a mechanism analogous to the actomyosin interaction of striated skeletal muscle.

To evaluate the plausibility of these motility models, it is necessary to determine whether they are consistent with the three-dimensional organization of the filaments composing the terminal web in the intact epithelium. In one of the few recent morphological studies centered primarily on the terminal web region, Brunser and Luft (2) used a variety of fixation techniques to examine the terminal web region of the absorptive cells of the rat small intestine. They observed that the bundles of core microfilaments terminated within a granular matrix which contained few discrete filaments other than occasional tonofilaments; they postulated that this region was actually composed of a web of fine branching filaments that they had been unable to resolve. According to Mukherjee and Staehelin (12) the attachment of this matrix of filaments to the microvillar membranes serves to stabilize mechanically the cell apex, permitting the isolation of brush borders as structurally coherent units (8).

To gain a better understanding of the structural organization of the terminal web region, we have

examined the apical cytoplasm of the intestinal epithelium of *Xenopus laevis*, the South African clawed toad, by both conventional and high voltage electron microscope techniques. By using the one million volt electron microscope that allows examination of sections up to 1 μm in thickness with high resolution, we hoped to be able to follow the paths of individual filaments over greater distances than is possible in the thin (800–1,200 Å) sections that are required for conventional transmission electron microscopes. In developing a general model for the organization of the terminal web and the associated intercellular junctions, we have examined corresponding structures in the mammalian small intestine as well.

MATERIALS AND METHODS

Tadpoles (stages 56 and 57, Nieuwkoop and Faber [14]) and newly feeding toads used in this study were obtained by mating pairs of adult *X. laevis* according to the methods of New (13). The tadpoles were raised to metamorphosis on a diet of bakers' yeast, while the newly metamorphosed toads were fed kidney or liver every 3–4 d. The intestinal tract was removed in one piece from animals that had been anesthetized with ether. After the tissue was fixed in 2% glutaraldehyde in 0.12 M Na cacodylate for 1½ h, it was rinsed with five or more changes of 0.12 M Na cacodylate and then postfixed for 1 h in 2% aqueous OsO_4 . After en bloc staining of the tissue in ½% aqueous uranyl acetate for 24 h at 4°C, the tissue was dehydrated slowly through a graded acetone series and embedded in Epon-Araldite. For the high voltage electron microscope, quarter-micron-thick sections were cut with glass knives and picked up on slot grids coated with 0.7% Formvar. The thick sections were stained for 2–3 h in saturated aqueous uranyl acetate at 60°C and poststained for 15 min in lead citrate (16). Both sides of the grids were heavily carbon-coated before the sections were examined in a JEM 1000 operating at 1,000 KV. Thin sections cut from the same blocks were stained for 30–60 min in saturated aqueous uranyl acetate at 60°C and poststained in lead citrate for 10 min, before being examined in a Philips EM 300 operating at 60 KV. The membranes and cytoplasmic filaments were particularly well-stained in both the thin and thick sections prepared in this manner. To determine cross-sectional diameters of the microfilaments, negatives were projected at a final magnification of 100,000 and measurements made directly on the projected image.

Brush borders were isolated from the tadpole small intestine by an adaptation of the method of Mooseker and Tilney (11). The small intestines from four tadpoles (stage 55) were dissected out into frog Ringer's plus 5 mM Mg^{++} and placed on ice while the luminal contents were teased out. The intestines were then homogenized

for 10 s at speed 30 in a VirTis homogenizer (VirTis Co., Inc., Gardiner, N. Y.) in 5 ml of homogenization medium (4 mM EDTA, 1 mM EGTA, 10 mM imidazole buffer, pH 7.3). The brush borders were pelleted at 1,475 g for 10 min. The pellet was washed once in 60 mM KCl, 5 mM MgCl₂, 1 mM EGTA, 10 mM imidazole buffer (pH 7.3), fixed for 1 h in 2% glutaraldehyde in 0.12 M Na cacodylate, and processed for transmission electron microscopy as described above.

Adult albino rats from the Charles River Breeding Laboratory were anesthetized with chloroform. Isolated ligated loops of the proximal region of the small intestine were injected either with a 30% glycerol-Ringer solution or with a 2% glutaraldehyde solution in 0.07 M phosphate buffer (pH 7.2). Small pieces of the epithelium prefixed with glutaraldehyde were removed after 15 min and then infiltrated with a 2% glutaraldehyde-30% glycerol-Ringer solution for 1 h before they were cut into cubes <1 mm³ and rapidly frozen on copper discs in Freon 12 held at -150°C. The specimens treated initially with glycerol were removed after 30 min and then immediately frozen. Freeze-fracture replicas were prepared as described by Hull and Staehelin (4) on a Balzers BA 360M freeze-etch apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.).

RESULTS

When quarter-micron sections through the brush border region of the small intestine are viewed in the high voltage electron microscope (Fig. 1), the most striking feature of the terminal web is its organization into three distinct layers. Each layer appears to link up with cytoplasmic surface components of a specific junction within the junctional complex. For these reasons it is useful to subdivide the terminal web into the following regions: the apical zone, the adherens zone, and the basal zone.

The apical zone (*Ap*, Fig. 1, small intestine; see also Fig. 2, large intestine) is found at the level of the tight junction (Figs. 3 and 4) and consists of lightly-staining filamentous material that surrounds and probably connects to the bundles of core microfilaments emerging from the bases of the microvilli. Underlying the apical zone is the adherens zone (*Ad*, Figs. 1 and 4), which links up with the zonula adherens or intermediate junction (Figs. 3, 4, and 5). Most of the 70 Å core filaments seem to terminate within this darkly staining layer of filaments, which also contains some 100 Å filaments. Immediately below the adherens zone is the basal zone (*Ba*, Fig. 1), composed primarily of bundles of tonofilaments that attach to the plaques of the spot desmosomes (Figs. 3, 4, and 5). In contrast to the thick-section

images of the terminal web region of the small intestine, those of the large intestine fail to reveal a distinct stratification of the filamentous layers (compare Figs. 1 and 2), even though the same types of filaments and the same types of junctional elements are present. Closer examination of this region reveals that the difference is largely because of the absence of a sharply delineated adherens zone in the large intestine, since the bundles of core microfilaments terminate within a broader band than in the small intestine.

The Apical Zone Region

The close association of cytoplasmic filaments with each of the three members of the junctional complex is quite striking in thick sections viewed with the high voltage electron microscope (Fig. 6). Farquhar and Palade (3) first demonstrated the close relationship of the 50–70 Å filaments and the 100 Å tonofilaments to the zonula adherens and spot desmosomes, respectively. The association of cytoplasmic filaments with the tight junction membrane is prominent only in thick sections. In thin sections, however, the filamentous coating of the tight junction membranes can be resolved into a network of 70 Å filaments (Figs. 4 and 7).

Within this network, pairs of filaments, one from each of the adjoining cells, appear to insert at specific points along the membranes of the tight junction (arrowheads, Fig. 7). Several other filaments of this meshwork appear closely opposed to the plasma membrane at the point of membrane fusion indicated by the arrow in Fig. 7. Filaments of this meshwork intimately associated with the membranes of the tight junction appear continuous with the 70 Å filaments of the somewhat looser network that crosslinks the adjacent bundles of core microfilaments throughout the apical web region (Figs. 4, 7, and 8). These network filaments occasionally seem to connect to small darkly-staining plaques associated with the surface of the bundles of microfilaments (Fig. 8), suggesting an attachment of the network filaments to the core microfilament bundles. A prominent layer of fuzzy material characteristically underlies the intermicrovillar membrane areas of the absorptive cells of the small intestine of *Xenopus* (Figs. 1, 8, 9, and 10). Since the intermicrovillar membrane regions of *Xenopus* frequently appear invaginated (Figs. 4 and 8), this darkly staining material may be important in the pinocytotic activity of the membrane regions it underlies. In Fig. 8 it can

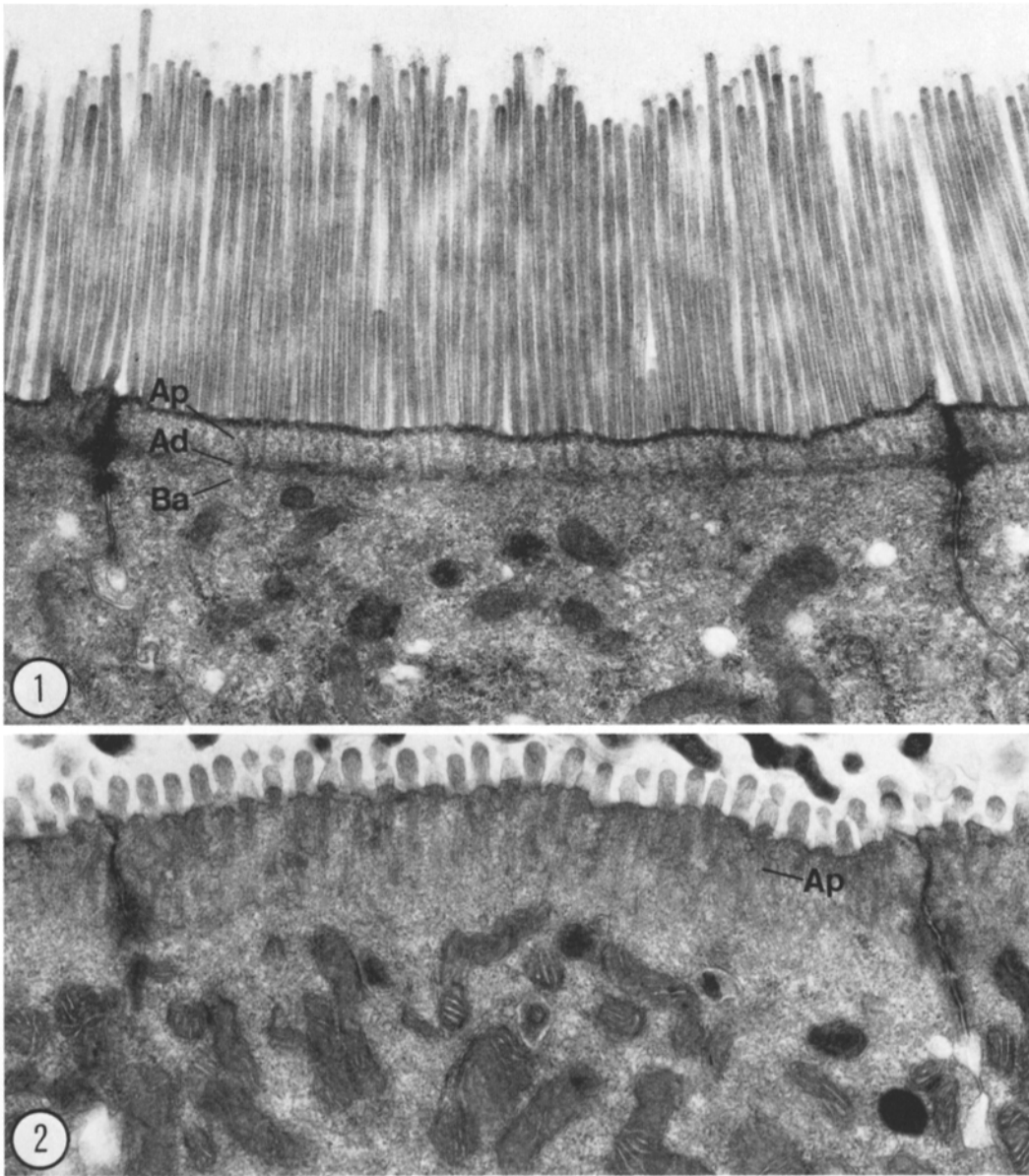


FIGURE 1 A high voltage electron micrograph illustrating the stratified organization of the small intestine of *X. laevis* after metamorphosis. The closely packed microvilli appear to stand on a base of darkly staining material that extends across the width of the cell. The bundles of core microfilaments pass through this material into the terminal web region. In the cytoplasm at the level of the tight junction, these core microfilaments are surrounded by the weakly staining material of the apical zone (*Ap*). The core microfilaments terminate within the adherens zone (*Ad*), the prominent band of filaments that extends across the cytoplasm at the level of the intermediate junction. Another network of filaments underlies the adherens zone to form the basal zone (*Ba*). $\times 16,000$.

FIGURE 2 In this thick section of the large intestine of *X. laevis*, the terminal web region lacks the prominent stratification which characterizes that in the small intestine. The bundles of core microfilaments can barely be discerned from the background matrix within the apical zone (*Ap*). No prominent band of filaments extends across the cytoplasm at the level of the intermediate junction, indicating the absence of a compact, well-defined adherens zone. $\times 18,000$.

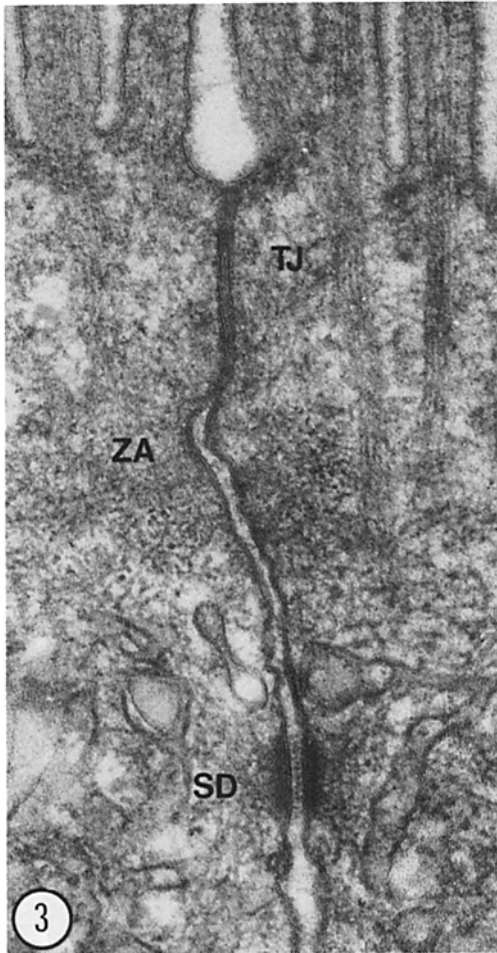


FIGURE 3 The close relationship between the organization of the junctional complex and that of the terminal web in the small intestine is illustrated in this thin-section electron micrograph. In the cytoplasm at the level of the tight junction (*TJ*), the core microfilaments appear as compact bundles enmeshed in a matrix of 70 Å filaments. Mats of 70 Å filaments line the membranes of the zonula adherens (*ZA*) or intermediate junction, and the bundles of core microfilaments become more diffuse in the adherens zone. Numerous tonofilaments course through the plaque of the spot desmosome (*SD*); some of these tonofilaments enter the adherens zone, while others form the basal zone. $\times 80,000$.

also be seen that the filaments of the apical zone attach to this fuzzy material.

The Adherens Zone Region

The core microfilaments, which form discrete bundles within the apical web region, become more diffuse at the level of the intermediate

junction (Figs. 4 and 8). Individual filaments fray out from the bundles of core microfilaments within the adherens web region to reorient parallel to the free surface of the cell (Figs. 8 and 9); frayed microfilaments from adjacent bundles appear to overlap one another (Fig. 8). Bundles of core microfilaments located immediately adjacent to the junctional complex sometimes bend away from the mat of filaments associated with the intermediate junction (Fig. 10); these core microfilament bundles thus enter the plane of the adherens web as a group. In addition to the 70 Å filaments arising from the bundles of core microfilaments, the adherens web is also composed of another group of 70 Å filaments that appear to originate at or near the membranes of the zonula adherens and extend into the cytoplasm in a plane parallel to the free surface of the cell (Fig. 11). These adherens zone filaments either may represent direct continuations of the zonula adherens filaments that form a band running parallel to the lateral plasma membranes (reference 3; Figs. 5 and 12) or they may compose a distinct set of filaments that forms within the zonula adherens region and extends directly into the cytoplasm. In the former case, one could hypothesize that some of the filaments of the zonula adherens bend away from the membrane to enter the adherens web. Tonofilaments in small bundles or as individual strands also form part of the adherens zone region (Figs. 4, 9, and 11); these tonofilaments appear to extend apically from the spot desmosomes and loop through the adherens zone.

The Basal Zone Spot Desmosomes and Tonofilament Systems

The bundles of 100 Å tonofilaments that contribute to the terminal web region extend apically from the many spot desmosomes that link the lateral plasma membranes of adjacent epithelial cells. As first demonstrated by Kelly (5), the tonofilaments do not arise or terminate within the cytoplasmic specializations of the spot desmosomes; instead they loop through the fine filamentous mesh composing the peripheral surface of cytoplasmic plaques of the spot desmosomes (Figs. 4 and 13). Within the basal zone, the bundles of tonofilaments are organized in the form of a coarse meshwork, which forms a platform-like structure underlying the adherens zone and the frayed bases of the core microfilament bundles (Figs. 1, 4, and 5).

In reexamining the fine structure of the spot

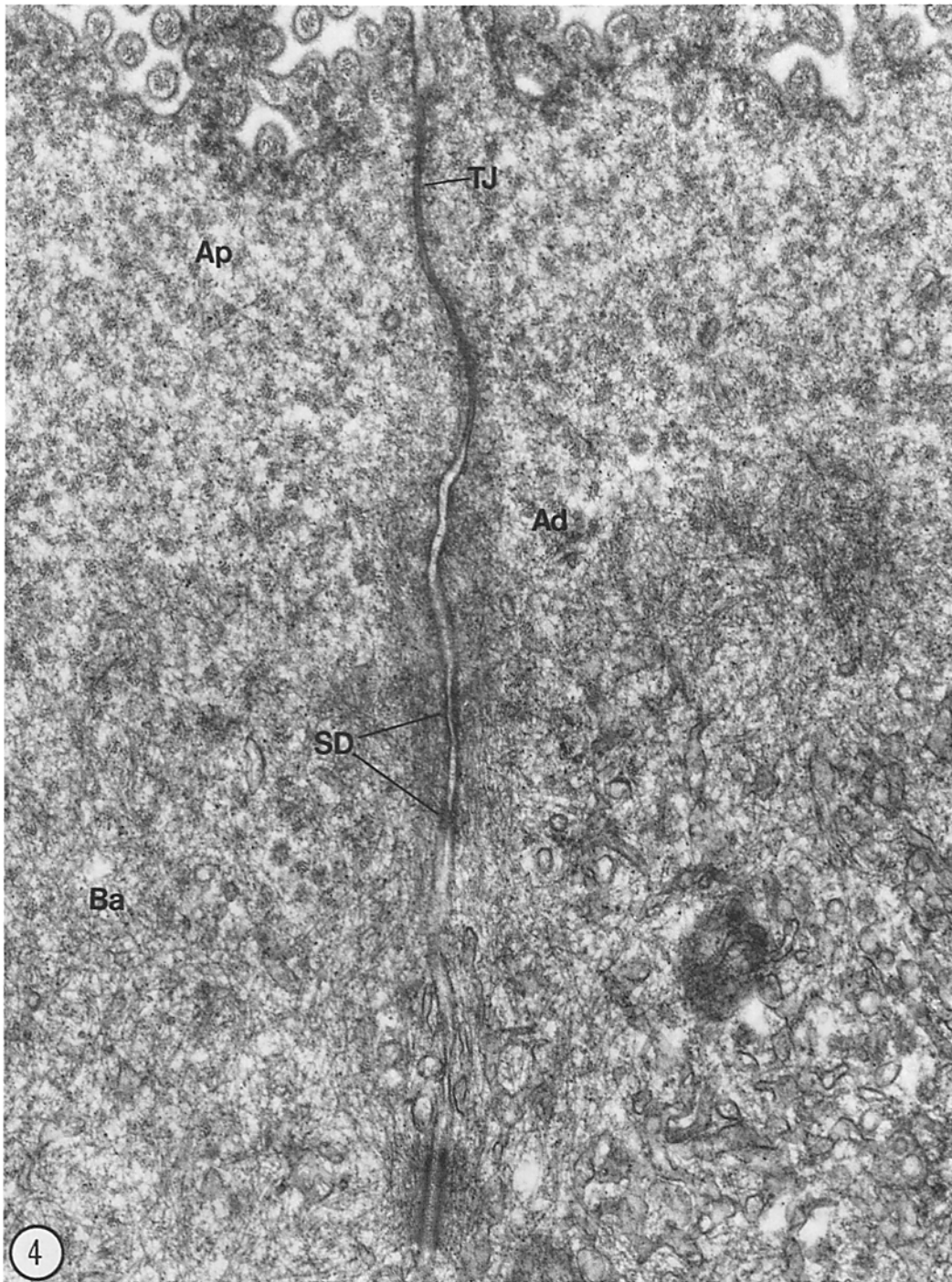


FIGURE 4 Electron micrograph of an oblique section through the terminal web region of the small intestine. The bundles of core microfilaments appear as clusters of darkly staining, cross-sectioned filaments. At the level of the tight junction (*TJ*), the bundles of core microfilaments are surrounded by a network of fine filaments. Tonofilaments extend from the spot desmosomes into the adherens zone (*Ad*), enmeshing the interacting groups of actin-like filaments. Other bundles of tonofilaments form the coarse meshwork of the basal zone (*Ba*). *Ap*, apical zone; *SD*, spot desmosome. $\times 46,000$.

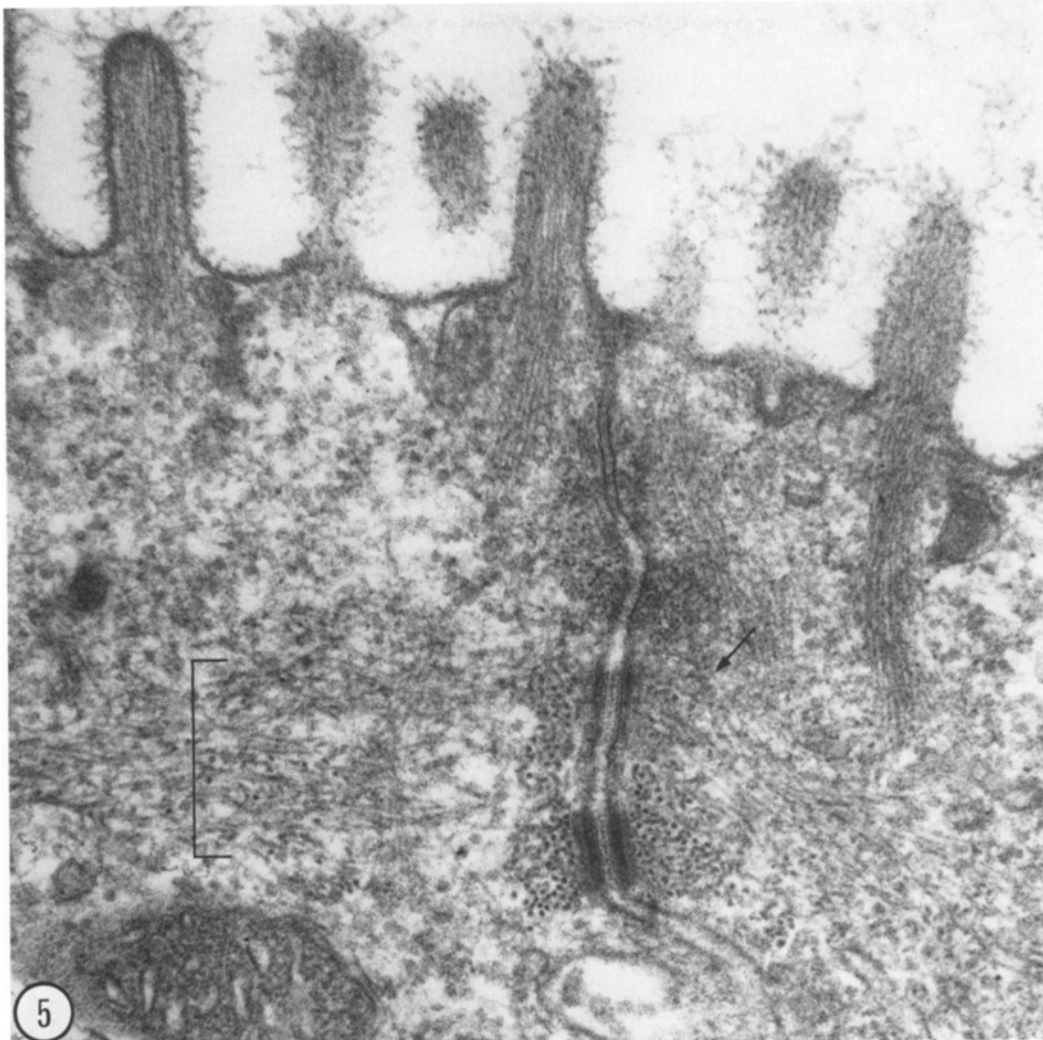


FIGURE 5 Interaction between the junctional complex and the terminal web region in the large intestine. As shown in this thin section, the bundles of core microfilaments terminate at the level of the spot desmosomes, where they mix with filaments that extend basally from the intermediate junction (arrow). The adherens and basal zones together form a broad network of mixed filaments extending across the cytoplasm at the level of the spot desmosomes; this broad band of filaments is well illustrated in the region indicated by the bracket. $\times 80,000$.

desmosomes we have found that the cytoplasmic plaques serve not only as attachment structures for the tonofilaments but also as attachment sites for short filamentous structures that interlink the adjacent cells across the intercellular space (Figs. 13, 15, 16, and 17). In suitable thin sections, these short filaments seem to originate on the membrane side of the cytoplasmic plaques and pass through the plasma membranes into the intercellular space (Figs. 13 and 15); the intercellular filaments from two adjacent cells do not

meet directly, but form a staggered array interconnected by other components that bisect the intercellular space (Figs. 14 and 15). Rayns et al. (15) have observed a similar set of staggered filaments within the intercellular space of spot desmosomes in guinea pig cardiac muscle. That these filaments are indeed transmembrane linkers is supported by images of freeze-fractured spot desmosomes. In slightly etched preparations of cross-fractured mammalian desmosomes, filaments can be seen in the intercellular space that appear to extend across

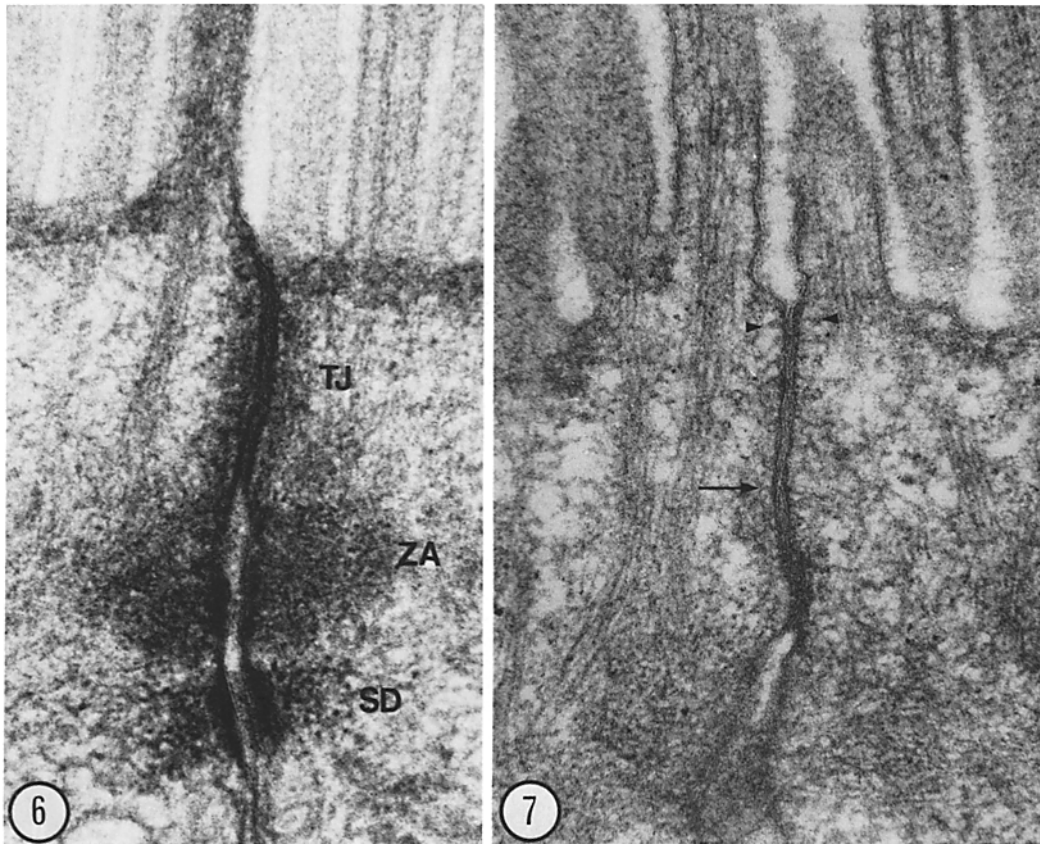


FIGURE 6 The junctional complex linking the absorptive cells of the postmetamorphic small intestine as visualized in a quarter-micron thick section viewed in the high voltage electron microscope. Prominent mats of filaments are associated with the cytoplasmic surfaces of the membranes of each of the three members of the junctional complex: the tight junction (*TJ*), zonula adherens (*ZA*), and the spot desmosomes (*SD*). $\times 90,000$.

FIGURE 7 Organization of the apical zone in the small intestine. The arrowheads point to the first of the pairs of 70 Å filaments that insert into the plasma membrane in the region of the tight junction. These filaments compose part of the filamentous network which links the bundles of core microfilaments to each other and to the membrane of the tight junction at the point of membrane fusion indicated by the arrow, suggesting that they are interacting directly or indirectly with the tight junction sealing elements. $\times 100,000$.

the plasma membranes (arrowheads, Figs. 16 and 17).

In both Figs. 16 and 17 the central stratum appears as a row of closely spaced granules to which the transmembrane filaments are attached. As first demonstrated by Staehelin et al. (22) and confirmed by McNutt and Weinstein (7), Staehelin (21), and Kelly and Shienvold (6), fracture faces of plasma membranes in the region of spot desmosomes reveal plaques of particles (Fig. 18). The hypothesis that many of these irregularly-shaped particles may represent filaments extend-

ing through the plasma membranes in the region of the spot desmosome is supported by the fact that the transmembrane filaments of the desmosome seen in Fig. 17 appear to end in particles on the fracture face of the lower plasma membrane.

The bundles of tonofilaments associated with spot desmosomes extend into the terminal web region as well as into the more basal areas of epithelial cells, where they form a network enveloping the cytoplasm. As illustrated in the high voltage electron micrograph shown in Fig. 19, bundles of tonofilaments loop from one desmo-

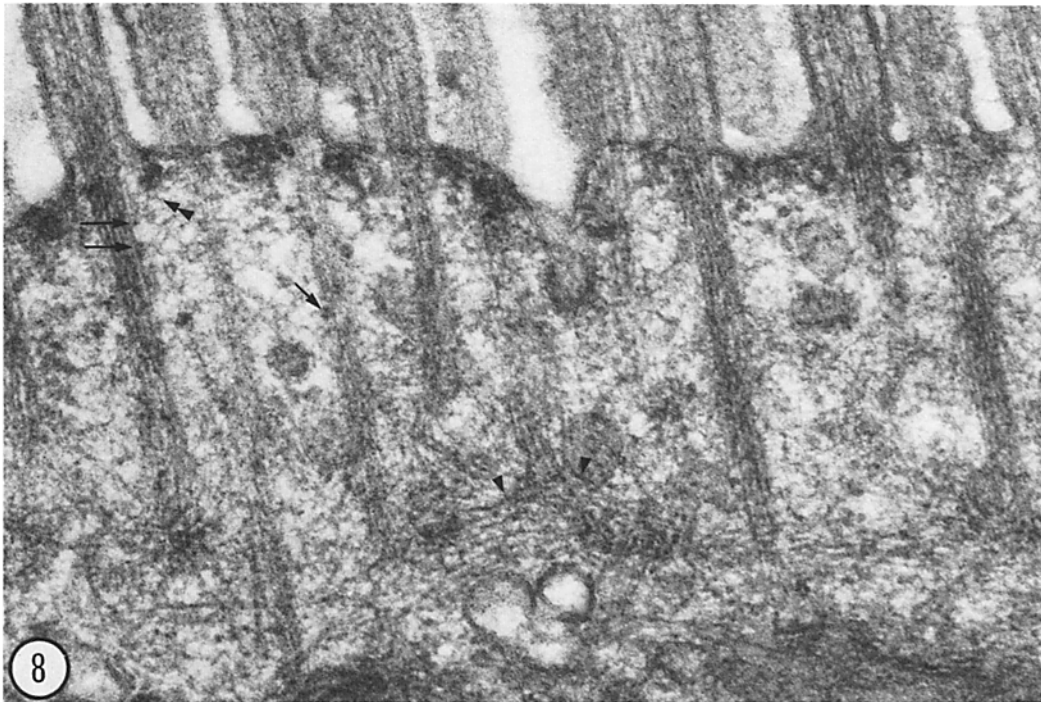


FIGURE 8 Thin-section electron micrograph of the terminal web region in the premetamorphic small intestine. Within the apical zone the bundles of core microfilaments are cross-linked by a network of 70 Å filaments. The actual linkage between the core microfilament bundles and the 70 Å filaments appears to be mediated by darkly staining patches of material on the surfaces of the bundles (arrows). Other filaments from this network connect to the darkly staining material underlying the intermicrovillar plasma membrane (double arrowheads). Individual filaments (arrowheads) separate from the bundles of core microfilaments within the adherens web region, overlapping one another in the manner predicted by microvillar motility models (10, 16). $\times 80,000$.

some to the next, paralleling the plasma membrane. A similar looping of tonofilaments along a more apical-basal direction is seen in Fig. 4, which also shows other bundles of tonofilaments extending away from the spot desmosomes to cross the interior regions of the cytoplasm. In this manner, one spot desmosome may serve as an anchoring structure for several sets of tonofilaments which extend into different regions of the cell, thereby providing button-like links between a number of tonofilament bundles in adjacent cells.

Our model summarizing the organization of the cytoplasmic tonofilament network and its interrelationship with the filaments of the terminal web region is illustrated in Fig. 20.

DISCUSSION

The cytoplasmic filaments in the terminal web region of the absorptive cells of both the small and large intestines display a high degree of structural

organization. The stratification of the filament interactions in the small intestine reflects the spatial organization of the junctional complex, permitting the division of the terminal web into three distinct regions: the apical, adherens, and basal zones (Figs. 1 and 3). In the large intestine the filaments of the adherens and basal zones merge to form a broad band extending across the cytoplasm at the level of the zonula adherens and spot desmosomes (Figs. 2 and 5).

Our observations on the organization of the cytoplasmic filaments of the terminal web region in the intact cell are largely consistent with the current models of microvillar motility (11, 17) which were based on observations of isolated brush border preparations. Mooseker and Tilney (11) proposed that the core microfilament bundles consist of polarized molecules of filamentous actin that are attached to the membranes at the tips of the microvilli by molecules of α -actinin. To pro-

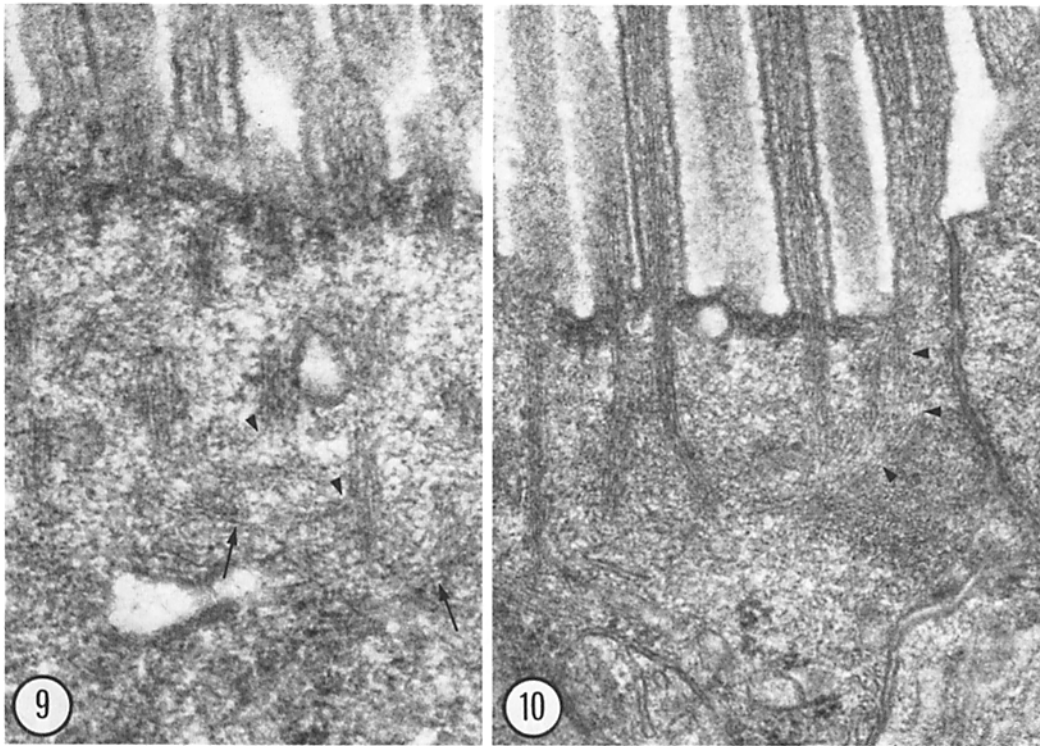


FIGURE 9 Organization of the adherens zone. In this thin section of the larval small intestine, many filaments can be seen to fray out from the bundles of core microfilaments (arrowheads) and enter the plane of the adherens zone. Tonofilaments (arrows) loop through the adherens zone, potentially stabilizing the orientation of the actin-like filaments. $\times 70,000$.

FIGURE 10 Bundles of core microfilaments lying adjacent to the junctional complex bend away from the cytoplasmic mat of the intermediate junction and enter the adherens zone as a group (arrowheads). $\times 53,000$.

mote contraction of the microvilli, the individual actin filaments splay out from the bundles of core microfilaments within the terminal web region; filaments from adjacent bundles interact with a common myosin polymer and slide toward one another in a mechanism analogous to the actomyosin interaction of striated skeletal muscle. Rodewald et al. (17) additionally postulated that actin-like filaments from the intermediate junction interacted with individual core microfilaments by means of common myosin molecules. This interaction could create an isometric tension within the terminal web region, restricting the movement of the sliding microfilaments toward the cell surface and promoting the contraction of the microvilli.

We have observed that within the apical zone the compact bundles of core microfilaments are surrounded and cross-linked by a loose meshwork of 70 Å filaments (Figs. 4 and 8). At its margins

this meshwork merges with a dense mat of filaments that is closely apposed to the membranes of the tight junction (Fig. 6). The pairing of filaments from adjacent cells and the close association of filaments with the region of membrane fusion shown in Fig. 7 both suggest that these filaments are closely associated with the sealing strands of the tight junction network, as these are the only structures held in common by the closely apposed membranes in the region of the tight junction (see review, reference 21). The apical zone meshwork also appears physically linked to the intermicrovillous membranes and the surface of the bundles of core microfilaments (Figs. 7 and 8). At present, it is not known whether the 70 Å filaments of the apical zone are composed of filamentous actin, but their disposition suggests that they play a stabilizing rather than a motile role. If the splayed core microfilaments do indeed slide relative to one

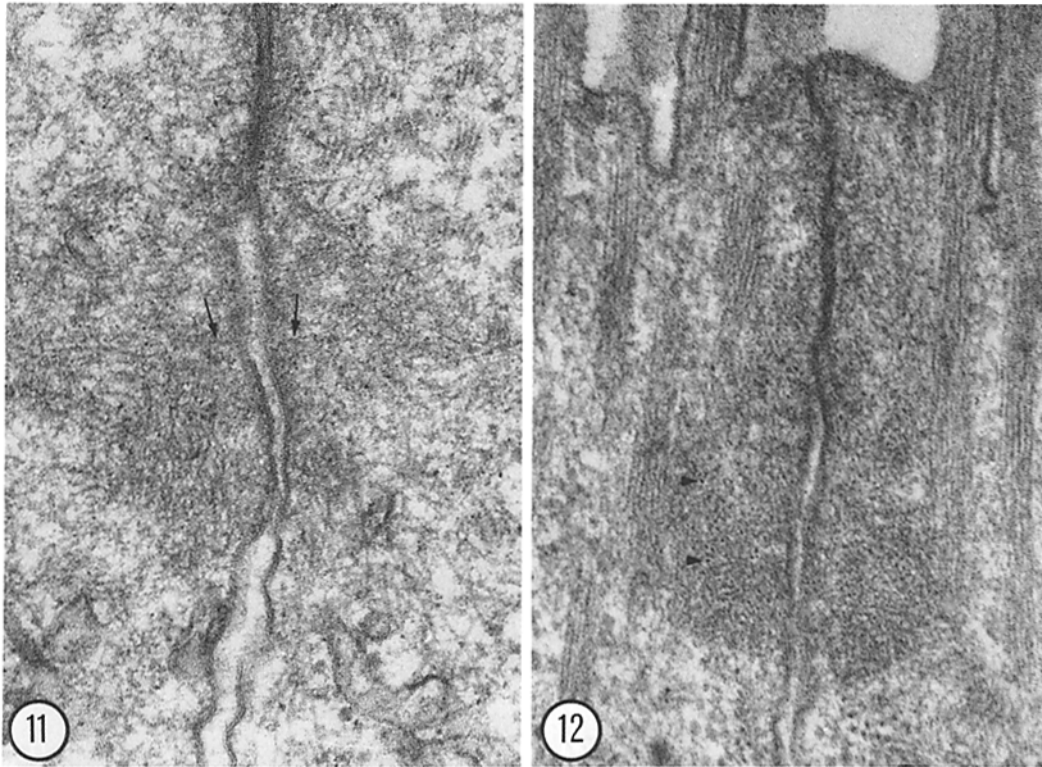


FIGURE 11 Several of the 70 Å filaments (arrows) appear to originate at or near the membranes of this zonula adherens and extend laterally into the adherens zone. The thicker tonofilaments in the lower portion of this micrograph belong to the basal zone. $\times 80,000$.

FIGURE 12 Cross-sectioned zonula adherens. The actin-like filaments form a clearly delineated band that closely parallels and adheres to the plasma membranes. The arrowheads point to cross-sections of individual filaments. $\times 75,000$.

another (11, 17), the cross-linking of the bundles of core microfilaments within the apical zone would promote the in-phase movement of adjacent microvilli. In addition, the attachments of the apical zone meshwork to the tight junction and intermicrovillous membranes would reinforce the physical integrity of the brush border membrane and prevent it from separating from the underlying cytoplasm as a result of microvillar movements. If the two groups of 70 Å filaments found within the apical zone (the core microfilaments and the meshwork filaments) are both composed of filamentous actin, the differences in their association with cytoplasmic proteins such as tropomyosin and alpha actinin could regulate their differing motile properties.

Within the adherens zone, the interactions among the diverse cytoplasmic filament systems are quite complex. Individual 70 Å filaments fray

out from the bundles of core microfilaments, thus enabling pairs of filaments from adjacent bundles to overlap one another in a manner consistent with the hypothesis that they are interacting with a common myosin aggregate (reference 11; Figs. 8 and 9). In accord with the observations of Rodewald et al. (17) on isolated brush borders, 70 Å filaments associated with the zonula adherens also enter the plane of the adherens zone, permitting their interaction with filaments from the bundles of core microfilaments (Fig. 10). From our thin sections we have not been able to determine whether the 70 Å filaments extending from the zonula adherens region and leading into the adherens zone represent filaments that initially run parallel to the plasma membrane of the zonula adherens and then bend abruptly to enter the zone, or whether they represent a distinct set of filaments originating close to the junctional mem-

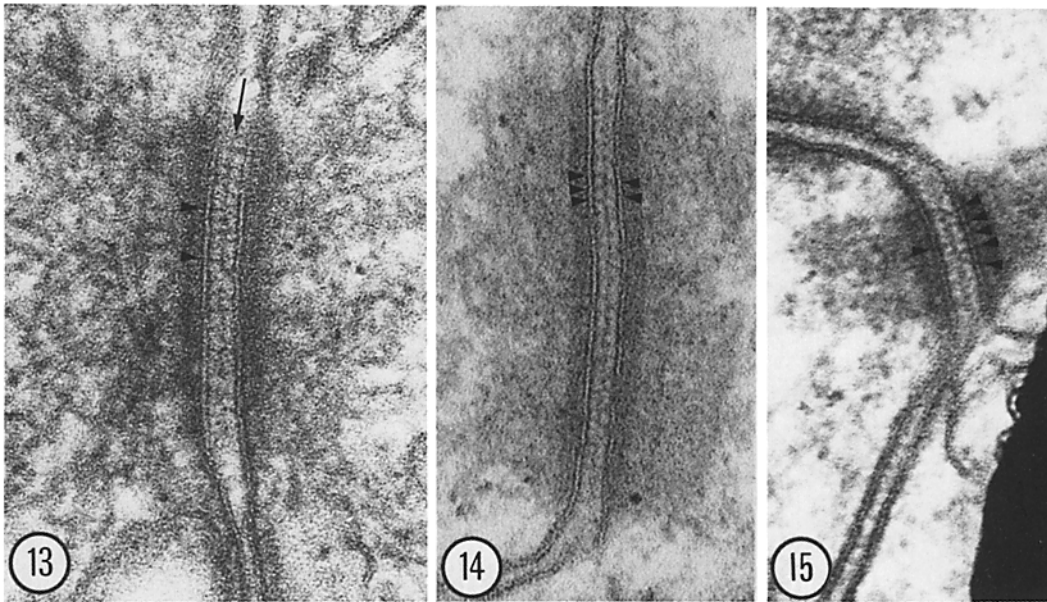


FIGURE 13 Spot desmosome from the tadpole small intestine showing the transmembrane linkers (arrowheads) which originate within the cytoplasmic plaque and pass through the intercellular space. A central stratum (arrow) appears to bisect the intercellular space. $\times 165,000$.

FIGURE 14 The intercellular space of this spot desmosome from the rat intestine is occupied by a central stratum that is connected to the adjacent plasma membranes by a series of alternating side arms (arrowheads). $\times 165,000$.

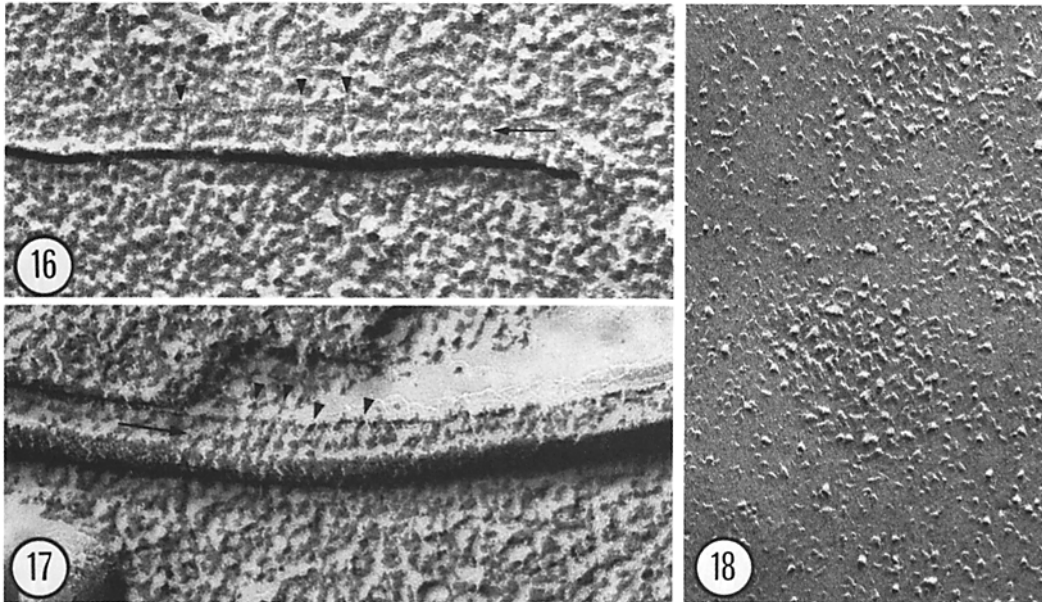
FIGURE 15 Thin-sectioned spot desmosome from the rat intestine depicting a central stratum with alternating side arms (arrowheads), some of which appear to extend across the plasma membranes. $\times 165,000$.

brane. As proposed by Rodewald et al. (17), these zonula adherens-associated filaments might serve to produce an isometric tension within the terminal web, thereby promoting the shortening of the microvilli. In addition, the organization of the actin-like filaments within the zonula adherens in the form of a girdle would permit the circumferential contraction of the apical cytoplasm. This kind of contraction could be important to close the holes formed in epithelial sheets when degenerated cells are sloughed.

100 Å tonofilaments surround the bases of the bundles of core microfilaments and loop through the network of 70 Å actin-like filaments of the adherens zone (Figs. 4, 9, 11, and 20). These tonofilaments are therefore in a position to prevent the sliding of the splayed core microfilaments toward the microvilli, restraining the actin-like filaments to move within the plane of the adherens zone. Thus, the restraining action of the tonofilament bundles could ultimately insure that the

sliding action of the actin-like filaments result in a shortening of the microvilli. Bundles of tonofilaments also form the basal zone, the tensile network that underlies the sheet of intermixed fibers of the adherens zone (Figs. 4, 11, and 20). This basal zone serves as a platform for the rest of the terminal web, and possibly restricts the movement of cytoplasmic organelles into the apical cytoplasm. Mooseker (10) reported that the best contraction of microvilli occurred in brush borders which still retained remnants of the underlying cytoplasm. In our preparations of isolated brush borders, we have found that cytoplasmic components remained associated with isolated brush borders only when they are trapped within an extensive meshwork of tonofilaments. Thus, the experimental observations of Mooseker (10) support our theory that the network of tonofilaments within the terminal web plays an indirect, but significant role in the contraction of microvilli.

The terminal web of the large intestine is less



FIGURES 16 and 17 Cross-fractured spot desmosomes linking adjacent absorptive cells in the rat small intestine. In these slightly etched preparations, filaments appear to pass through the plasma membrane (arrowheads) and connect to the central stratum (arrow) in the intercellular space; these filaments correspond in location and disposition to the transmembrane linkers shown in thin section in Figs. 13-15. $\times 165,000$.

FIGURE 18 P face of the plasma membrane in the mammalian small intestine illustrating the appearance of a spot desmosome in a freeze-fractured membrane. The desmosomal plasma membrane is characterized by a patch of irregularly-shaped particles. The degree of plastic deformation of these particles suggests that they may represent cross-fractured filaments that extend through the plasma membrane. $\times 120,000$.

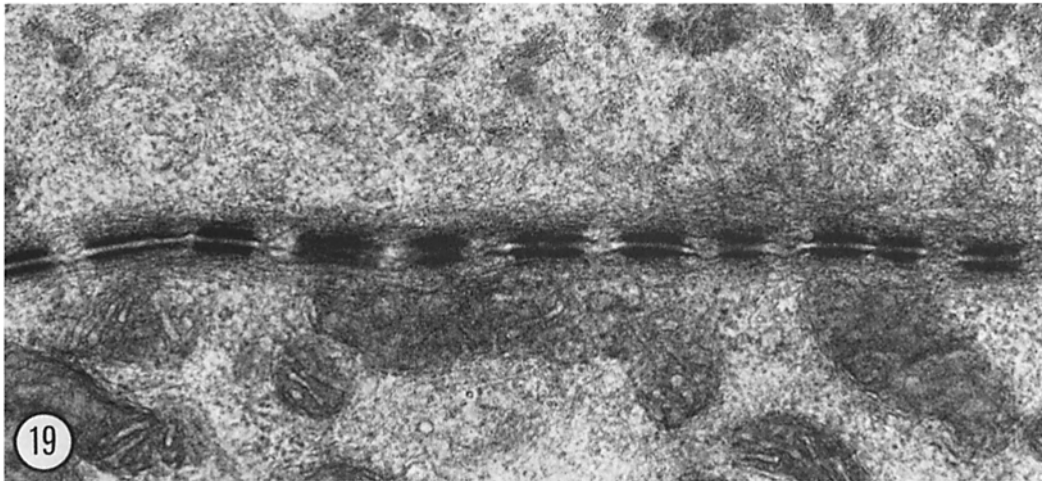
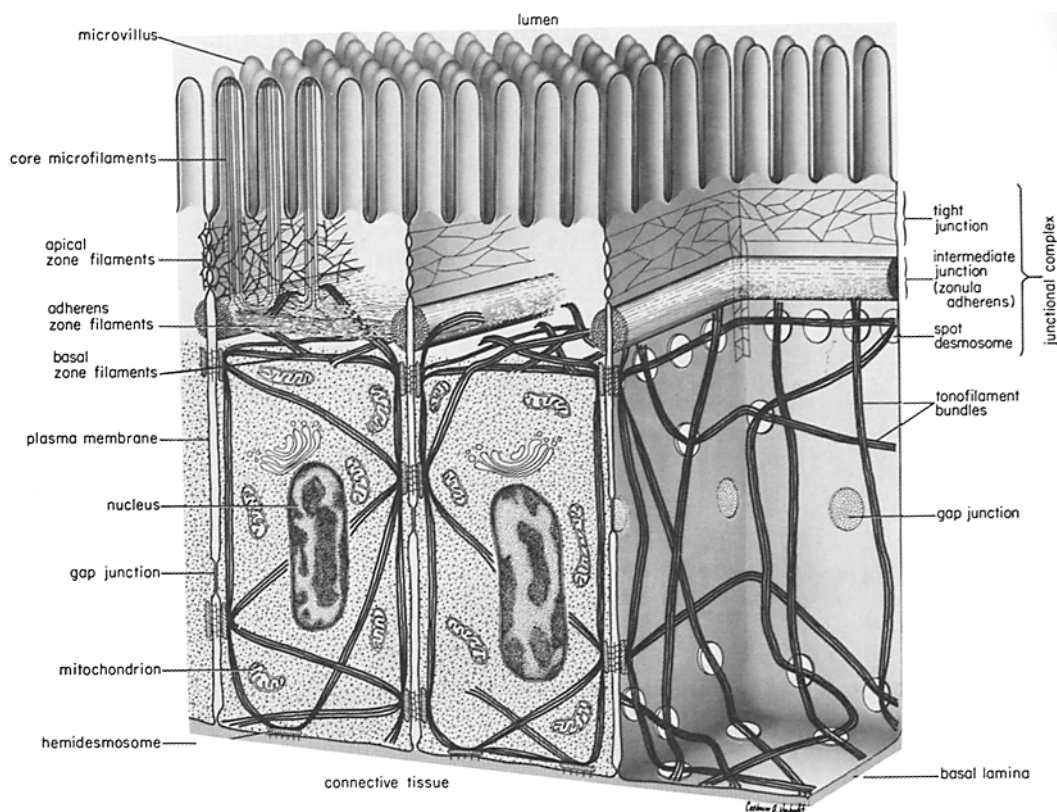


FIGURE 19 Band of spot desmosomes extending across the cytoplasm just below the region of the terminal web in the larval large intestine. This thick section passes obliquely from one cell to its neighbor, exposing a row of eleven spot desmosomes linking the two adjoining plasma membranes. Bundles of tonofilaments line the plasma membranes, looping between adjacent spot desmosomes. $\times 45,000$.



Schematic Diagram of Columnar Epithelial Cells

FIGURE 20 Organization of the cytoplasmic filaments in columnar epithelial cells. This diagram illustrates the association of the filaments with tight junctions, intermediate junctions (zonulae adherentes), spot desmosomes and hemidesmosomes.

tightly organized than that of the small intestine. This looseness in the organization of the terminal web in the large intestine is consistent with the observed flexibility of the tight junction networks linking these cells (4). The ability of the apical cytoplasm to stretch in response to tensional stresses would permit a significant expansion of the lumen of the large intestine, allowing the passage of large, hardened feces. On the other hand, the high degree of structural organization that we have observed in the terminal web of the small intestine would tend to further stabilize the form of the apical cytoplasm of these absorptive cells. At present, we cannot say whether the observed variations in the organization between the small and large intestines signify differences in the mode of microvillar motility between these two regions of the gut.

While progress has been made in isolating and

biochemically characterizing spot desmosomes (19, 20) and tonofilaments (23, 24), the structural organization of the spot desmosome and the nature of its association with the cytoplasmic network of tonofilaments remains hypothetical. We suggest that the bundles of tonofilaments are attached to the cytoplasmic plaques by a network of fine filaments such as the one proposed by Kelly (5) and observed by Skerrow and Matoltsy (19) to coat the cytoplasmic plaques of isolated epidermal desmosomes. Other fine (70 Å) filaments serve as transmembrane linkers, originating within the cytoplasmic plaque, passing through the plasma membrane, and linking up in a staggered configuration in the center of the intercellular space (Figs. 13, 15, 16, and 17). The central stratum or lamina observed in thin sections would be obtained by the superimposition of the elements that join the transmembrane linkers. We

feel that these filaments represent genuine transmembrane linkers rather than structural artifacts induced by our techniques. The filaments observed in the cross-fractured junctions (Figs. 16 and 17) exhibit a precise orientation across the intercellular gap and possess a uniform thickness along their length. In addition, they lie exactly in the plane of fracture and show up clearly only after slight etchings. The collapse of plastically deformed membrane or cytoplasmic components into the cross-fractured junctions would tend to form structures displaying great variability in size and orientation and would be visible without etching. The particles found within the fracture face of the membrane plaques of spot desmosomes have irregular sizes and shapes, and the form of many of these particles suggests that they may have been subjected to a considerable amount of plastic deformation during the fracturing process. Such extensive plastic deformation of the intramembrane particles is consistent with the idea that they actually represent filaments extending through the plasma membranes within the region of each spot desmosome. This notion is further strengthened by the observation that the transmembrane filaments of the desmosome seen in Fig. 17 appear to end in particles on the fracture face of the lower plasma membrane. The disposition and organization of transmembrane filaments that we have observed in our thin section images of spot desmosomes correspond very well with these freeze-fracture observations.

The occurrence of linkers within the intercellular space of spot desmosomes has been reported in several epithelia (1, 15). In addition, studies of desmosomes and hemidesmosomes of newt epidermis using freeze-fracture and tannic acid-glutaraldehyde fixative techniques (6, 18) have suggested that the short filaments which originate within the cytoplasmic plaque of these junctions extend through the cytoplasmic leaflet of the plasma membrane to terminate somewhere within the plane of the membrane. Correlating our thin-section (Figs. 13 and 15) and freeze-fracture (Figs. 16, 17, and 18) evidence with the data previously published on these fine desmosomal filaments (1, 6, 15, and 18) leads us to the conclusion that one class of filaments serves both the role of mediating the intercellular adhesion and the role of physically linking the cytoplasmic plaque to the plasma membrane. As suggested by Borysenko and Revel (1), variations in the interactions of the transmembrane linkers within the intercellular space could

explain the differences in the susceptibility of desmosomes in different epithelia to dissociation by EDTA and trypsin. These transmembrane linkers provide a direct mechanical coupling between the tonofilament networks of adjacent cells within an epithelium, permitting the distribution of a mechanical stress acting on one or a few cells to the tissue as a whole. The tonofilament bundles which underlie the plasma membrane and form a cytoskeletal network (Fig. 20) would thus provide epithelia with much of their tensile strength.

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