Brush Border Cytoskeleton and Integration of Cellular Functions

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The primary function of the intestinal epithelial cell is the absorption and transport of nutrients and electrolytes from the lumen of the gut to the organism's blood supply. The absorptive membrane on the lumenal surface of the cell—the brush border—has been the subject of intensive physiological and chemical study. Similarly, the exquisite array of actin filments and associated binding proteins that underlies this membrane is among the best-characterized actin-based cytoskeletal structures in nature. Consequently, one would think that the brush border is a system uniquely suited for the study of the cytoplasmic matrix and the integration of cellular functions. Future studies may prove this to be true, but at present, almost nothing is known about how the cytoskeletal apparatus of the brush border participates in absorption, or any other cellular function of the intestinal epithelial cell. Nevertheless, this subject invites speculation. We first will give an overview of the brush border cytoskeleton and then summarize results of several in vitro experiments that have provided a basis for speculation on the function of the brush border cytoskeleton in vivo.

Organization and Constituent Proteins of the Brush Border Cytoskeleton

A working model for the cytoskeletal apparatus underlying the brush border membrane is presented in Fig. 1. It is based on work from many laboratories (e.g., see references 4, 13, 50, 57, and 77), summaries of which can be found in recent reviews on the brush border cytoskeleton (5, 55, 59). This scheme is primarily based on studies using chicken intestinal brush borders, although it probably holds for the intestinal brush borders of other vertebrates, including mammals. For the sake of the discussion that follows, we will briefly review salient features of the two structural domains of the brush border cytoskeleton—the microvillus core and the terminal web.

MICROVILLUS CORE: The microvillus contains a bundle or core of 20–30 uniformly polarized actin filaments, the barbed ends of which are embedded in a dense plaque at the tip of the microvillus. This bundle extends the entire length of the microvillus and below it for one-fourth to one-third of its length into the apical cytoplasm of the cell. The portion of the core that extends below the microvillus is referred to as the core rootlet. In addition to actin, the microvillus core contains polypeptides of 110, 95 (villin), 80, 68 (fimbrin), 30 (tropomyosin), and 17 kdaltons (calmodulin). All these proteins have been purified, and to varying degrees their physical properties and actin-binding characteristics (when relevant) have been at least partially characterized. The localization of all these proteins has been established by light and/or electron microscopic immunolocalization techniques and/or by biochemical dissection procedures.

Villin (21), and probably fimbrin, are localized along the entire length of the microvillus core. Both villin (9, 51, 58) and fimbrin (3, 27) will crosslink purified actin filaments into bundles. Unlike those formed with villin, fimbrin-actin bundles are similar to the native microvillus core in that they are highly ordered, containing hexagonally packed, axially aligned filaments of uniform polarity (27, 52). This suggests that fimbrin is the "primary" bundler of the microvillus core. The function of villin in the core is probably related to its various calcium-dependent interactions with actin filaments, a topic that will be discussed in more detail below.

The 80-kdalton subunit (80K)[1] is a relatively minor component of the microvillus core, and, like villin and fimbrin, is localized along the length of the core (6). Antibodies to the 80K have been used to "stain" a variety of tissue culture cell types, and cross-reacts with a polypeptide of similar molecular weight in the mammalian brush border. However, no function (e.g., the 80K does not bind to actin as assayed by cosedimentation) for the 80K in the microvillus has yet been determined (6).

The 110-kdalton subunit (110K) (31) and probably most, if not all, the calmodulin associated with the cytoskeletal apparatus of the isolated brush border is localized exclusively to the portion of the microvillus core that underlies the membrane. The calmodulin of the microvillus is tightly bound to the 110K (32, 40). Several lines of evidence (discussed in more detail below) indicate that the 110K-calmodulin complex comprises the periodic, helically arranged bridges that connect the core laterally to the plasma membrane.

[1] *Abbreviations used in this paper:* C_0, critical concentration; 80K, 80-kdalton subunit; 110K, 110-kdalton subunit; QFDERR, quick-freeze, deep etch rotary replication; ZA, zonula adherens.

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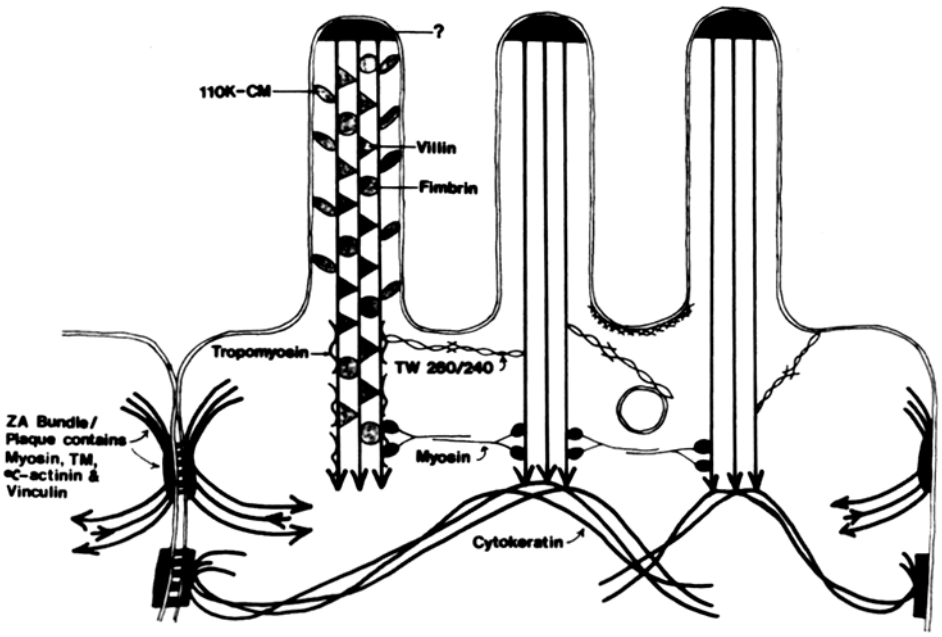

FIGURE 1 Working model for the brush border cytoskeleton. Reprinted from reference 55.

Whereas the "rootlet" end of the microvillus core lacks the 110K (and presumably calmodulin), tropomyosin is restricted to this portion of the core (8, 12). Brush border tropomyosin is of the shorter, nonmuscle type (8, 54, 57). Its function in the terminal web is not known, but it is in an excellent position to participate in the regulation of actin-myosin interaction in the brush border (see below; see also reference 12 for a review of nonmuscle tropomyosin and its possible functions in regulating actomyosin interaction in nonmuscle cells).

TERMINAL WEB: The apical cytoplasm of the cell, into which the core rootlets descend, is appropriately named the terminal web. The cytoskeletal apparatus within the terminal web can be subdivided into at least two distinct structural and biochemical domains, one of which we will refer to as the "interrootlet zone." The other is the junctional complex at the lateral margin of the cell (43). The interrootlet zone consists of a dense meshwork of filamentous (nonactin) material that interdigitates between, and presumably links together, the adjacent core rootlets. The structural organization of the interrootlet zone has been best visualized by Hirokawa, Heuser, and co-workers using the technique of quick-freeze, deep-etch rotary replication (QFDERR) (36–39). In the terminal web of the isolated chicken brush border (Fig. 2); there is a high concentration of filamentous strands of varying straightness and diameter that crosslink adjacent core rootlets. These filaments interconnect the core rootlets at various angles, and most appear to span the distance between only two adjacent core rootlets.

There is both immunological and biochemical evidence from experiments using isolated brush borders for the presence of at least two chemically distinct classes of crosslinking filaments in the interrootlet zone consisting of TW 260/240,[2] a spectrinlike protein (26, 28, 29, 36, 64), and myosin (7, 20, 33, 39, 61), respectively. The molecular organization of these two proteins within the interrootlet zone is not known, but we can make some guesses based on the morphology of the crosslinkers in situ and the structural properties of the purified proteins. For example, it is likely that the TW 260/240

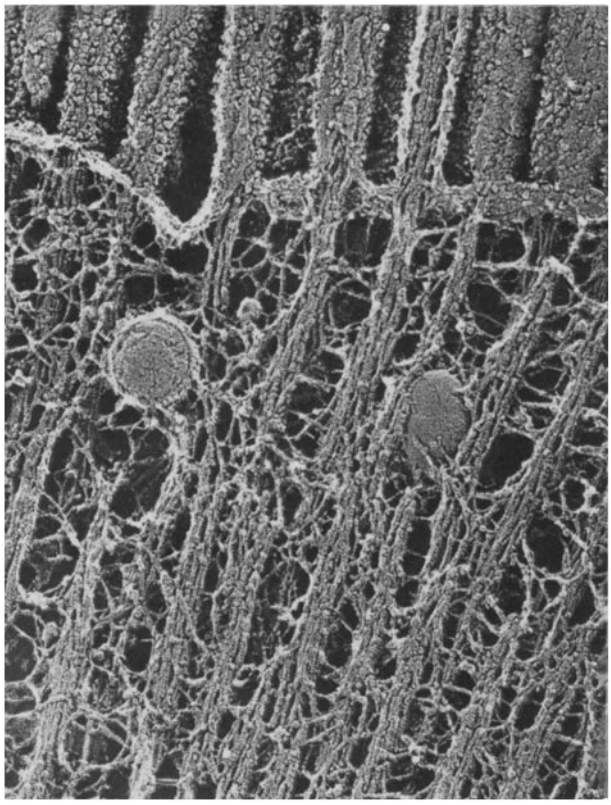

FIGURE 2 Terminal web region of an isolated brush border from chicken intestine visualized by quick-freeze, deep-etch technique. This micrograph is from a collaborative study with Dr. N. Hirokawa, Department of Physiology and Biophysics, Washington University Medical School, St. Louis, MO. × 95,000.

[2] TW 260/240 is a tissue-specific form of spectrin found only in fully differentiated intestinal epithelial cells of the chicken (26). It has not been conclusively determined whether mammalian brush borders contain a similar brush border-specific protein or fodrin (brain spectrin), a nonerythroid spectrin first purified from brain but also widely distributed in other nonmuscle tissues (26, 47).

crosslinkers are comprised of the tetrameric form of this protein. Like other nonerythroid spectrins (28, 64), purified TW 260/240 forms stable tetramers (two heterodimers, molecular weight ~1,000,000), which are potent crosslinkers of actin filaments in vitro (64). The morphology and dimensions of the TW 260/240 tetramer (250–260 × 2–6 nm) are similar to that of many of the interrootlet crosslinkers observed in situ in QFDE replicas. Similarly, Hirokawa et al. (36, 39), on the basis of the diameter and length of interrootlet crosslinkers identified as myosin-containing by electron microscopic localization techniques, have suggested that myosin is present as small oligomeric complexes. However, despite the clarity of the QFDE replicas, it has not yet been possible to determine whether the myosin filaments tagged by antimyosin are bipolar in organization. Recent findings on the morphology of myosin extracted from the interrootlet zone provide evidence for the presence of bipolar dimers (see below).

The distribution, relative to one another, of TW 260/240 (or its immunologically related equivalent in the mammalian brush border) and interrootlet myosin has not been clearly established. Hirokawa and co-workers (36, 39) have studied the distribution of these two proteins in mouse brush borders using immunolocalization techniques coupled with QFDERR. Their observations indicate that much of the spectrinlike filaments within the interrootlet zone are confined to the apical portion of the terminal web, directly beneath the plasma membrane, whereas the myosin-containing filaments are localized more basally along the core rootlets, as illustrated in Fig. 1. On the other hand, immunolocalization studies by Glenney et al. (29), who used antibody to TW 260/240, indicate that in the chicken brush border the spectrinlike protein is uniformly distributed along the entire length of the core rootlets. These findings may reflect a species difference, because the terminal web of the chicken brush border contains a much higher concentration of interrootlet filaments than that of the mouse brush border (cf. Fig. 2 and results discussed in references 36–39).

In our laboratory, we have taken a different tack in dissecting the distribution of these two proteins in the interrootlet zone by using a series of selective extraction procedures. Extraction of chicken intestinal brush borders with elevated salt results in solubilization of 60–80% of the TW 260/240 (reference 64 and Fig. 3) without removing myosin. Examination of the terminal web region in TW 260/240-depleted brush borders reveals an almost complete loss of interrootlet filaments in the region of the terminal web directly below the plasma membrane (Fig. 3). On the other hand, it is possible to selectively remove up to 80% of the terminal web myosin by ATP extraction of brush borders at 0°C (Fig. 4, *a* and *b* and reference 44). Electron microscope examination of the inter-rootlet zone in myosin-depleted brush borders reveals a lower density but a more or less uniform distribution of the remaining cross-filaments between the rootlets. Finally, extraction of both TW 260/240 and myosin by sequential treatment with salt then ATP causes an even greater loss of interdigitating filaments at all levels along the core rootlets (Fig. 4). These results suggest that in the chicken intestinal brush border, TW 260/240 is more or less uniformly distributed along the entire length of the core rootlets, in agreement with the immunolocalization studies of Glenney et al. (29), whereas myosin is not present in the region of the terminal web directly below the plasma membrane—a finding consistent with the observations of Hirokawa et al. (36).

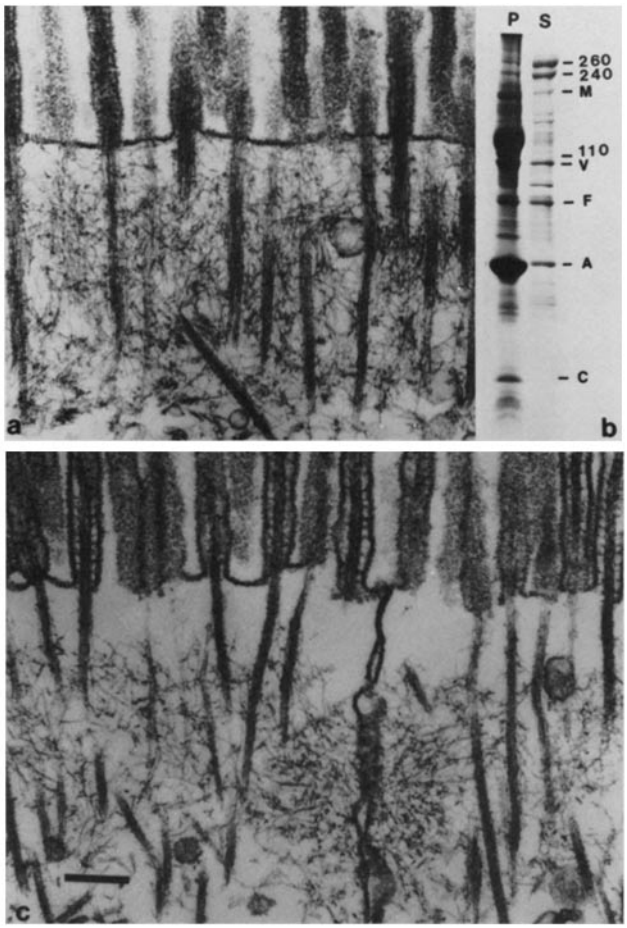

FIGURE 3 Salt extraction of TW 260/240 from the isolated brush border. (*a*) Terminal web region of a brush border in a preparation before extraction. Filamentous material interdigitating between the "rootlet" ends of microvillus core is present at all levels of the rootlets. (*b*) SDS polyacrylamide gel electrophoresis of a brush border preparation extracted with 0.3 M KCl after TW 260/240 isolation procedures (64). Pellet (*P*) and supernatant (*S*) fractions, loaded stoichiometrically, after centrifugation at 10,000 *g* are shown. Most of the TW 260/240 has been extracted, together with lesser amounts of villin (*V*), fimbrin (*F*), and actin (*A*). Myosin (*M*) and the 110K-calmodulin complex (*C*) have remained in the pellet fraction. (*c*) Terminal web region of a brush border in the salt-extracted pellet fraction depicted in *b*. Note the loss of interdigitating filaments (compared with *a*) from the apical zone of the terminal web directly beneath the plasma membrane. The junctional complex is intact, including the "contracting ring" of actin filaments associated with each side of the zonula adherens. From reference 64. Bar, 0.2 μm.

We have examined the morphology of the myosin which "falls out" of the terminal web in the presence of ATP at 0°C. The myosin in these ATP extracts, as visualized by low-angle rotary shadowing (71), is in the form of bipolar dimers (Fig. 5*a*) and some monomers (results not shown). The myosin molecules have the "kinked" conformation characteristic of unphosphorylated myosin in the presence of ATP (16). That this myosin is predominantly unphosphorylated is confirmed by urea-glycerol polyacrylamide gel electrophoresis (65, 69) of these extracts, a technique which resolves the phosphorylated and unphosphorylated forms of the 19-kdalton light

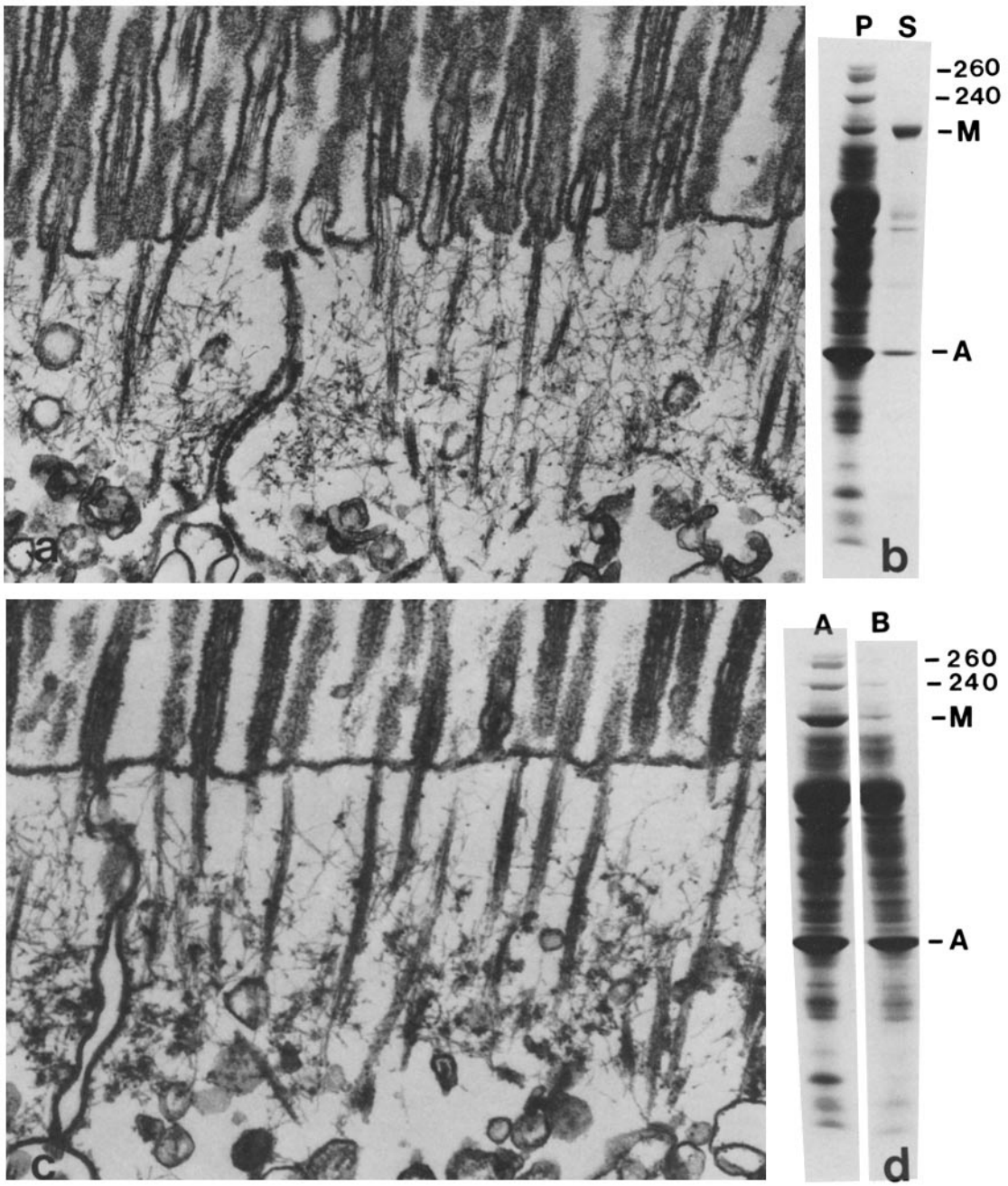

FIGURE 4 (*a* and *b*) ATP-dependent extraction of myosin from the terminal web. (*a*) Terminal web region of brush borders extracted with 2 mM ATP, 75 mM KCl, 2 mM $MgSO_4$, 10 mM imidazole, pH 7.5, at 0°C. Under these conditions, no terminal web contraction or phosphorylation of myosin occurs. This treatment results in a substantial loss of crosslinking filaments from the interrootlet zone at all levels of the core rootlets (compare with Fig. 3*a*). × 54,000. (*b*) SDS polyacrylamide gel electrophoresis of ATP-extracted brush borders visualized in *a*. Pellet (*P*) and supernatant (*S*) fractions (centrifugation at 10,000 *g*) after ATP treatment are shown. Much of the myosin (*M*) has been solubilized. (*A*) Actin. (*c*) Terminal web region of a brush border extracted first in elevated salt (as in Fig. 3), then with ATP at 0°C as in *a*, to remove both TW 260/240 and myosin. The density of interrootlet filaments is greatly reduced, but as in *a* and Fig. 3*c*, the ZA-associated bundles are retained within the junctional complex. (*d*) SDS of (*A*) control brush borders and (*B*) brush borders extracted sequentially in salt and ATP as described above. Note the reduced levels of both TW 260/240 and myosin in the double-extracted brush borders.

chain of myosin. The presence of these bipolar dimers, a configuration that has not been observed in preparations of purified myosin, raises the possibility that in situ interrootlet myosin is in a dimeric state or in some higher-order aggregate (e.g., side-polar filaments [15]) comprised of such dimers.

The junctional complex at the lateral margins of the terminal web also has an extensive cytoskeletal apparatus associated with it. The zonula adherens (ZA), which is subjacent to the zonula occludens, has associated with it a circumferential bundle of actin filaments. This bundle of actin filaments is associated with the cytoplasmic surface of the membrane by a dense beltlike adhesion plaque. Immunological studies

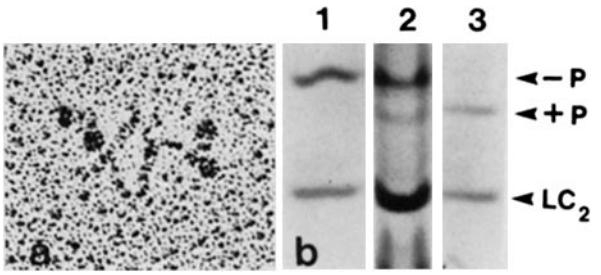

FIGURE 5 Ultrastructure and phosphorylation state of myosin released from the terminal web by treatment with ATP at 0°C. (*a*) Example of a myosin dimer visualized by low-angle rotary shadowing (71), which is the predominant form of myosin visualized in the 0°C-ATP extracts from isolated brush borders. The myosin molecules within the dimer have the kinked configuration characteristic of unphosphorylated myosin in the presence of ATP (16). (*b*) Urea-glycerol polyacrylamide gel electrophoresis (65, 69) of brush border myosin preparations. (*1*) Purified, unphosphorylated myosin. The two bands correspond to the unphosphorylated 19-kdalton light chain (*−P*) and the 16-kdalton light chain (LC_2). (*2*) Supernatant fraction of brush borders extracted with ATP at 0°C (see Fig. 4). The 19-kdalton light chain of the myosin in the ATP extracts is mostly unphosphorylated. (*3*) Purified brush border myosin phosphorylated by the addition of myosin light-chain kinase from smooth muscle. The position of the phosphorylated 19-kdalton light chain (*+P*) is indicated.

have shown this adhesion plaque to contain the actin-binding proteins vinculin and α-actinin (17, 23, 24). The ZA-actin bundle itself contains myosin (7, 20), filamin (7), and tropomyosin (7, 20, 24), as determined by immunolocalization studies. It is important to note that TW 260/240 is not present in the ZA bundle (29). The functional properties of the circumferential ring of actin filaments will be discussed in further detail below. Finally, associated with the macula adherens, is a characteristic network of 10-nm (cytokeratin) filaments that course throughout the basal level of the terminal web, often making direct contact with the core rootlets (22). These 10-nm filaments are lost during the isolation of brush borders from chicken but not mammalian intestine (22).

Functional Properties of the Microvillus Cytoskeleton

The only function of the microvillus core that has been well established is in maintaining the cylindrical form of the microvillus membrane. However, if this is the core's sole function, then this structure is a classic example of "overdesign," something which is rarely, if ever, found in nature. This is because the core contains proteins, villin, and 110K-calmodulin complex, which potentially convey to this actin bundle the ability to rapidly change its structural configuration, as well as allow it to participate directly in the absorptive functions of the cell. The properties of these proteins and some of the ideas that we and workers in other laboratories have suggested regarding the cellular functions of the microvillus core are described below.

VILLIN AND Ca^{++}-DEPENDENT REGULATION OF MICROVILLUS CORE STRUCTURE AND ASSEMBLY: At Ca^{++} concentrations below $\sim 10^{-6}$ M, the microvillus core is a stable, rigid structure. Above this Ca^{++} threshold, the filaments of the core are rapidly chopped up into short fragments, as first observed by Howe et al. (42) using preparations of demembranated microvillus cores. This phenomenon, termed core solation, is mediated by the Ca^{++}-dependent severing action of villin on the actin filaments of the core (25, 58).

Villin is a Ca^{++}-binding protein (three binding sites per molecule) that undergoes a marked conformational change when it binds this ion (34, 35). Villin is a member of a ubiquitous class of chemically distinct but functionally similar Ca^{++}-activated binding proteins that regulate the structure and assembly state of actin. Villin and its interaction with actin have been the subject of intensive study by numerous investigators, most extensively by researchers in K. Weber's laboratory (for reviews, see references 18 and 46). We will summarize here some of the key features of the interaction of villin with actin, which are helpful in discussing the possible function of this protein in vivo.

In the absence of Ca^{++} ($<10^{-7}$ M), villin has no detectable effect on either the nucleation stage or the elongation stage of actin polymerization (57, 74, 77). In the presence of Ca^{++}, however, (>1–5 μM), its effects on actin assembly include the following: (*a*) Villin accelerates the rate-limiting "nucleation" phase of actin assembly by forming stable oligomeric complexes with actin that nucleate polymerization (74, 77). (*b*) Villin "caps" the barbed, fast-growing end of the filament, thereby inhibiting the rate of filament elongation to that of the pointed, slow-growing end (1, 30). (*c*) By capping the barbed filament end, villin raises the critical concentration (C_0) for assembly to that of the pointed filament end (73, 74). In the presence of KCl and Mg^{++}, the C_0 of the pointed and barbed filament ends are ~0.1 and 0.6–0.8 μM, respectively (2). (*d*) The villin-dependent increase in C_0 saturates at very low villin-to-actin ratios (1:200–400) (73, 74). (*e*) The length filaments achieve at steady state is inversely proportional to the concentration of villin present (18).

Villin also affects preassembled actin filaments. As noted above, villin is a bundler of actin filaments in the absence of Ca^{++}. The effects of villin on F-actin in the presence of Ca^{++} include the following. (*a*) Villin also caps the barbed end of preformed filaments, thereby inducing net depolymerization of the filaments until a new steady state is achieved, reflecting the C_0 of the pointed ends (74). (*b*) As predicted for a barbed-end capper, villin inhibits the net rate of filament depolymerization, as measured by diluting filaments below the C_0 (73). This is consistent with the finding that the dissociation rate constant at the barbed end is greater than that at the pointed end (2, 66). (*c*) Villin rapidly decreases average filament length by directly severing monomer-monomer interactions within the filament (1, 77). (*d*) The severing action of villin requires somewhat higher Ca^{++} concentrations than that required for capping the barbed end (5–10 μM vs. 1 μM) (58, 76). Maximum severing activity requires Ca^{++} concentrations as high as 25–50 μM (76). (*e*) Tropomyosin blocks the severing of F-actin by villin (1, 57), which possibly explains the resistance of the rootlet portion of the microvillus core to Ca^{++}-dependent solation (57).

The Ca^{++}-dependent interaction of villin with actin provides the basis for speculation regarding the function of this protein in vivo. One possibility is that the Ca^{++}-dependent severing of the microvillus core mediates release of right-side-out vesicles from microvillus membranes. Experiments performed with either membrane-intact, isolated microvilli (60, 72) or whole brush borders (11) demonstrate that solation of the underlying core results in rapid vesiculation of the plasma

membrane along the entire length of the previously cylindrical microvillus. This vesiculation is not a direct effect of Ca^{++} on the membrane, because it does not occur if core solation is blocked by stabilization of the core filaments with phalloidin (60). Moreover, vesiculation of microvilli has been observed in vivo as a response to feeding (53). Perhaps the vesicles (which are right side out) released from microvilli serve to increase the levels of hydrolytic enzymes within the lumen, thus facilitating hydrolytic processing of food.

Another possibility is based on the observation that, at threshold Ca^{++} concentrations, the cutting action of villin on actin filaments is marginal (58). Perhaps at these threshold Ca^{++} concentrations in vivo, villin cuts filaments randomly along their length, thereby reducing the rigidity of the microvillus without inducing its complete disruption. Such a "softened" microvillus might then be expected to exhibit passive Brownian movements, which would increase the access of the lumenal contents to the spaces between tightly packed microvilli, thereby facilitating absorption. Alternatively, such softening of the microvillus might reduce the viscosity of the cytoplasm within the microvillus and consequently increase rates of diffusion for absorbed nutrients through the microvillus cytoplasm into the cell interior.

Villin may also play a role in regulating changes in microvillus length that occur in vivo, such as the transient shortening that accompanies fasting (53) or the gradual elongation of microvilli as the cell migrates up from the villus crypt. An increase in intramicrovillus Ca^{++} concentration to levels that would activate barbed-end capping by villin, but not severing, might cause a shortening of the core filaments by a loss of subunits from their pointed ends. Shortening of the core filaments and, as a result, of the microvillus as well would continue until a new steady-state equilibrium was reached, reflecting the higher C_0 for the pointed ends of the core filaments. For villin to play such a role in the regulation of microvillus length, the barbed, membrane-associated end of the core filaments should be accessible to monomer addition when the intramicrovillus Ca^{++} concentration falls below $\sim 10^{-6}$ M. This is plausible, because we have shown that, at least in vitro, elongation of core filaments by monomer addition onto the barbed, membrane-associated ends of the core filaments can occur without disruption of the apparent attachment of those filaments to the membrane at the tip of the microvillus (62). In addition, other factors would also be required if villin were to play a role in governing microvillus length and assembly in vivo. For example, there would have to be mechanisms for regulation of the available actin-monomer pool, for control of actin and other core protein synthesis, and, of course, for precise regulation of intramicrovillus Ca^{++} concentration.

CHARACTERIZATION OF THE LATERAL-BRIDGE PROTEIN, 110K-CALMODULIN COMPLEX, AND ITS POSSIBLE FUNCTIONS IN THE MICROVILLUS: The evidence that the lateral bridge is comprised of 110K-calmodulin complex is compelling but still indirect. Immunolocalization studies (31) have shown that the 110K, like the lateral bridge is confined to that portion of the core that is within the microvillus. As first demonstrated by Matsudaira and Burgess (49), the lateral bridges can be stripped from microvillus cores by treatment with ATP, a treatment that also results in a loss of 110K. That the 110K is a calmodulin-binding protein was first demonstrated using gel-overlay techniques (32, 40), which also were used to demonstrate that the 110K binds to calmodulin both in the presence and absence of Ca^{++}. Finally, a 110K-calmodulin complex (1:1–2 molar ratio 110K:calmodulin) has been purified that is stable in the presence and absence of Ca^{++}, and, like the lateral bridge, binds to actin filaments in the absence but not presence of ATP (41). However, our attempts to reconstruct lateral bridges by adding purified 110K-calmodulin to ATP-stripped microvillus cores have not been successful.

The interaction of 110K-calmodulin complex with the plasma membrane also is ATP sensitive. Treatment of membrane-intact microvilli (72) or brush borders (41) with ATP results in a loss of lateral bridges as well as solubilization of 110K-calmodulin. Another aspect of the interaction of 110K with the membrane has been revealed by the studies of Coudrier et al. (14), who have identified a 200-kdalton integral membrane protein in the microvillus membrane from pig brush borders that binds to porcine 110K. It will be important to determine whether the interaction of 110K with this integral membrane protein is ATP sensitive.

Given the effects of ATP on the interaction of 110K-calmodulin with the core and the membrane, it is not surprising that the 110K is, in fact, an ATP-binding protein (56) as determined by the photoaffinity labeling technique of Maruta and Korn (48). However, the 110K-calmodulin complex has no detectable ATPase activity under physiological conditions ($+/-Ca^{++}$) in the presence or absence of actin (41). We have also noted that the 110K is the substrate for a membrane-associated kinase (41), but what, if any, effect the phosphorylation state of the 110K has on its interaction with calmodulin, the membrane, or the microvillus core has not yet been determined.

The function of the 110K-calmodulin complex within the microvillus remains a complete mystery. One must even question the most obvious possibility, that it effects the attachment of the core to the membrane, because physiological levels of ATP are sufficient to dissociate this complex from both the membrane and the core filaments. Admittedly, the situation in vivo may be completely different from what we have observed in vitro. It is quite possible that the brush border we isolate and study retains only a skeleton of the real cytoskeleton present in the living cell.

The Ca^{++}-independent interaction of 110K with calmodulin is unusual and suggests that calmodulin functions within the microvillus in a bound state, perhaps acting as a calcium buffer within the microvillus cytoplasm. It is important to note here that such a buffering system might play a key role in the vitamin D-dependent transport of Ca^{++} (for a review, see reference 75) from the lumen by the intestinal epithelium. The close apposition of 110K-calmodulin to the membrane raises the possibility that the entire complex mimics the action of free calmodulin in regulating Ca^{++}-calmodulin-dependent enzymes within the microvillus membrane. In this regard, it will be important to determine whether 110K-calmodulin can function as an activator of calmodulin-dependent enzymes and whether displacement of calmodulin from the 110K occurs in the presence of competing binding proteins.

Brush Border Contractility—Evidence for Two Functional States of Myosin in the Terminal Web

There are potentially at least two distinct sites of myosin-actin interaction in the terminal web, reflecting the dual localization of myosin in the interrootlet zone and in the ZA

bundle. The numerous studies summarized below have implicated ZA-associated myosin in the ATP-dependent contraction of this filament bundle, a phenomenon analogous to ZA contraction in corneal epithelium as described by Owaribe et al. (63). However, the role of myosin found in the interrootlet zone is unclear.

In 1976, Rodewald et al. (67) reported an ATP-dependent contraction of the terminal web region of neonatal rat brush borders. Recently, we were prompted to further investigate this phenomenon because of the disarming discovery (42) that in our own studies on microvillar contraction (54) in isolated brush borders we were actually investigating a complicated artifact of Ca^{++}-dependent core solation coupled with simultaneous terminal web contractility (for a more complete discussion, see reference 60). In our recent studies, terminal web contractility was analyzed in sheets of brush borders interconnected by intercellular junctions (Fig. 6, *a* and *b*). We observed a Ca^{++}- and ATP-dependent constriction of the terminal web region in brush borders within these sheets (time-course, 1–4 min), resulting in a fanning out of each brush border's array of microvilli (45) (Fig. 6). Movements or shortening of microvilli were not observed. Ultrastructural studies indicated that the observed shape changes were most likely due to constriction of the ZA bundles, which in many instances completely disrupted the junctional region between adjacent brush borders (45) (Fig. 6*c*).

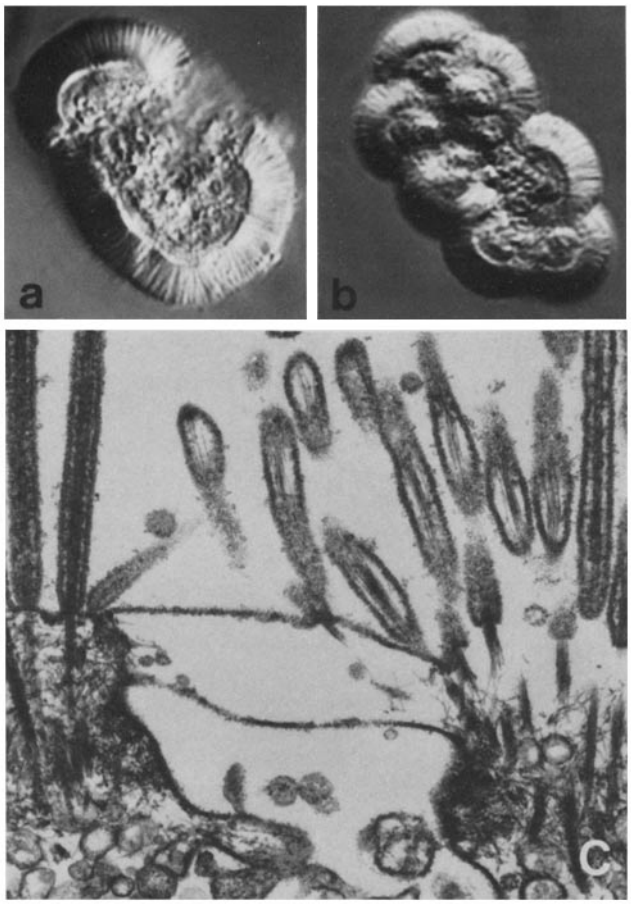

FIGURE 6 Ca^{++}- and ATP-dependent contraction of the terminal web in isolated brush borders. (*a* and *b*) Differential interference contrast light microscopy of sheets of brush borders connected by intercellular junctions, incubated for 10 min at 37°C in the absence of ATP (*a*) or in the presence of 2 mM ATP and 1 μM Ca^{++} (*b*). × 1,900. (*c*) Thin-section electron micrograph of the junctional region between two brush borders in a "contracted sheet" similar to that in *b*. × 39,000. *a–c* reprinted from reference 45.

At about the same time, essentially identical observations were made using preparations of glycerinated intestinal epithelial cells (10, 38), although in these preparations, no Ca^{++} sensitivity of contraction was observed. In addition, the ultrastructural studies of Hirokawa et al. (38) demonstrated that the ZA bundle contains myosinlike filaments that interdigitate between actin filaments of opposing polarities, a finding that establishes the possibility that ZA contractility is mediated by a sliding-filament-type mechanism.

The molecular basis for terminal web contractility in isolated brush borders has also been investigated (44, 45). The results of these investigations strongly implicate myosin as the force producer and suggest that the Ca^{++}-dependent regulation of contractility is conferred by a Ca^{++}-calmodulin-dependent, cytoskeletal-associated, myosin light-chain kinase. The evidence for this includes the following: (*a*) The addition of an antibody prepared against brush border myosin, which in vitro inhibits both actin-activated ATPase activity and thick filament formation, inhibits terminal web contraction in isolated brush borders (44 and footnote 3). (*b*) During contraction, the 19-kdalton light chain of brush border myosin is phosphorylated, the kinetics and extent of which parallel the time-course of terminal web contraction (45). (*c*) The drug trifluoperazine, an inhibitor of calmodulin functions, blocks both terminal web contractility and Ca^{++}-dependent phosphorylation of myosin (45).

The function of ZA contraction in vivo is not known, but there are a number of possibilities. The ZA bundles may function in maintaining tension between cells within an epithelial sheet or in providing force for the upward movement of cells from the crypt to the tip of the villus. One exciting possibility is that the constriction of the ZA bundle modulates the permeability of the zonula occludens. Finally, ZA contraction might squeeze the apex of the cell, causing slight fanning out of the microvilli and thus increasing access of the lumenal contents to the spaces between microvilli.

In our discussion of terminal web contractility, we have concentrated on the myosin associated with the ZA bundle. The extent to which interrootlet myosin participates in this contraction is not known, but recent results obtained in our laboratory provide evidence, outlined below, that (*a*) interrootlet myosin is not involved in, or at least not necessary for, terminal web contractility and (*b*) that the myosin involved in ZA contractility is more tightly associated with the brush border cytoskeleton than is interrootlet myosin. First, we have recently observed (44) that terminal web contraction occurs equally well in brush borders depleted of interrootlet myosin by preextraction with ATP at 0°C (Fig. 4). Second, the myosin still associated with the cytoskeleton after 0°C-ATP extraction remains associated with the brush border during contraction and becomes phosphorylated (44 and footnote 3).

These observations suggest the presence of at least two populations of myosin molecules, one tightly associated with the cytoskeletal apparatus, which is involved in ZA contractility, and another, more loosely associated population that is at least partially localized within the interrootlet zone. The

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function or functions of interrootlet myosin remains strictly a subject for speculation. For example, interrootlet myosin, particularly if it is in bipolar arrays as discussed above (Fig. 5), could generate microvillar movements of some kind, although there has been no solid evidence to substantiate earlier observations of such movement in vivo (68, 70). Perhaps interrootlet myosin provides tension between adjacent core rootlets in order to keep microvilli erect. It has also been speculated that interrootlet myosin, perhaps in concert with the spectrinlike protein TW 260/240 (or its immunological equivalent in the mammalian brush border), mediates the movement of endocytotic vesicles down or Golgi-derived vesicles up through the terminal web (36, 45, 60). So far, the only basis for such speculation is the presence of vesicles in apparent tight association with the core rootlets (36, 60) (Fig. 2). The possible role of myosin and TW 260/240 in membrane cycling is discussed in greater detail elsewhere (36, 60).

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