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Dynamic control of cell-cell adhesion and membrane-associated actin during food-induced mouth opening in *Beroë*

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SUMMARY

We used rhodamine-phalloidin and ultrastructural methods to follow dynamic changes in adhesive cell junctions and associated actin filaments during reversible epithelial adhesion in the mouth of the ctenophore *Beroë*. A cruising *Beroë* keeps its mouth closed by interdigitated actin-coated appositions between paired strips of cells lining the lips. The mouth opens rapidly (in 0.2-0.3 s) by muscular action to engulf prey (other ctenophores), then re-seals after ingestion. We found that the interlocking surface architecture of the adhesive cells, including the actin-coated junctions, rapidly disappears after food-induced opening of the mouth. In contrast, forcible separation of the lips in the absence of food rips the junctions, still intact, from the surfaces of the cells. The prey-stimulated loss of adhesive cell junctions and associated actin cytoskeleton is one of the most rapid changes in actin-based junctions yet observed. This system provides unique experimental advantages for investigating the dynamic control of reversible cell adhesions and membrane-associated actin filaments.

Key words: cell adhesion, actin cytoskeleton, *Beröe* mouth

INTRODUCTION

Intercellular junctions, appositions and their associated actin cytoskeleton play essential roles in cell-cell adhesions. In most differentiated tissues the cells are relatively immobile and the junctions between them fairly stable. In moving cells of embryos, inflammatory responses or tumors; however, bonds between cells are made and broken and remade again, i.e. cell adhesions are transient and reversible (Trinkhaus, 1984; Osborn, 1990).

The study of dynamic cell-cell adhesions has been hampered by several technical difficulties (Curtis and Lackie, 1991; Trinkaus, 1984), particularly the asynchronous and heterogeneous distribution of these transient adhesions among groups of moving cells. We recently discovered a novel system of reversible cell adhesion that overcomes some of these difficulties. Certain beroid ctenophores swim mouth forward in search of prey with their mouth tightly closed by adhesion between paired strips of epithelial cells on opposing lips (Tamm and Tamm, 1991b). The epithelial cells adhere by interdigitated appositions of their plasma membranes which are lined by a dense filamentous coat (Tamm and Tamm, 1991b). When prey is encountered, cell-cell bonding is rapidly broken as the ctenophore opens its mouth to eat, and is readily reformed after feeding.

Here we use this new controllable system of reversible cell adhesion to follow structural changes in the adhesive cell junctions and underlying actin cytoskeleton during mouth opening. Our results demonstrate one of the most rapid disappearances of cell junctions and associated actin cytoskeleton yet observed, and provide a unique system for investigating molecular mechanisms of dynamic cell adhesions and actin rearrangements in cells.

MATERIALS AND METHODS

Animals

Beroë sp. was obtained locally in the fall, 1991, and also collected near St. Augustine, FL in late December 1991, and shipped to MBL. This unidentified species is similar to *Beroë* sp. collected at Woods Hole in fall 1985, and is called *Beroë* sp. (Gloria) (Tamm and Tamm, 1988). This beroid was used in our previous work on mouth adhesion (Tamm and Tamm, 1991b); it has a flattened body with a wide mouth and ridged comb rows. *Beroë* were maintained in aquaria of running sea water and fed *Mnemiopsis.*

Feeding behavior

Individual *Beroë* were placed in a bowl of sea water to which a *Mnemiopsis* was then added. Feeding behavior was recorded with a CCD video camera and Nikon macrolens mounted above the bowl, using a VHS recorder and field counter. Responses were analyzed by still-field playback; video fields were photographed from the monitor on Kodak Tech Pan 35 mm film (Fig. 6).

Experimental conditions

Closed mouths

Beroë were relaxed in isotonic 6.75% $MgCl_2$:sea water (1:1). The closed mouth was cut off with a dissecting scissors, then fixed for rhodamine-phalloidin staining or electron microscopy (below).

Pulled-open mouths

Beroë were either kept in sea water or relaxed first in isotonic $MgCl_2$:sea water. Closed mouths were removed with scissors, and one or both corners were cut off. The two adherent lips were then peeled apart with forceps, starting from one end. Pulled-open lips were used for living or fixed (rhodamine-phalloidin or EM) observations.

Food-opened mouths

Beroë in sea water were fed *Mnemiopsis* larger than themselves. Immediately after the *Beroë* opened its mouth to engulf the prey, the mouth was cut off and fixed for fluorescence or electron microscopy, or used for living observations.

Light microscopy of living lips

Freshly removed pulled-open (in sea water or Mg-relaxed) and food-opened mouths were trimmed into lip pieces and mounted on slides for DIC microscopy using Zeiss optics. Photographs were taken with an Olympus OM-2N camera on Kodak Tech Pan film using an Olympus T32 flash tube inserted in the light path.

Rhodamine-phalloidin staining

Pulled-open and food-opened mouths were fixed in 3.7% formaldehyde, 0.15 M NaCl, 0.2 M sodium cacodylate (pH 7.7), 0.1% Triton X-100 for approx. 30 min at room temperature, and washed in 0.3 M NaCl, 0.2 M sodium cacodylate (pH 7.7) for several hours, during which time the lips were trimmed into smaller pieces. Lip pieces were incubated in 0.33 μM rhodamine-phalloidin (Molecular Probes, Eugene, OR, USA) in buffer wash for 1-2 h, then washed in buffer and mounted on slides. Closed mouths were fixed as above, or with 1% Triton in fixative, or without Triton in the initial fixative followed by fixation with Triton. The latter treatment preserved lip adhesion, and required that lips be pulled apart later. In some cases, 0.5% glutaraldehyde was added to the formaldehyde fixatives. Slides of rhodamine-phalloidin-stained lip pieces were observed with Zeiss TRITC epi-fluorescence or DIC optics using a 25×/0.8 objective. Photographs were taken with an Olympus OM-2N camera on T Max 32000 film at ASA 1600 using Kodak HC-110 (dilution B) developer.

Electron microscopy

Pulled-open and food-opened mouths were fixed within 30 s after opening. Standard fixation was in 2.5% glutaraldehyde, 1% paraformaldehyde, 0.15 M NaCl, 0.2 M sodium cacodylate (pH 7.7), 0.01 M $CaCl_2$ for 1-2 h at room temperature. Lips were washed in 0.3 M NaCl, 0.2 M sodium cacodylate for several hours, during which time tissue was trimmed into smaller pieces. Lip pieces were post-fixed in 0.5% OsO_4, 0.1 M sodium cacodylate, 0.38 M NaCl at 0°C for 10-15 min, then washed in distilled water and treated with 1% aqueous uranyl acetate overnight at 4°C. Some mouths were fixed in a simultaneous glutaraldehyde-paraformaldehyde-osmium fixative as described previously (Tamm and Tamm, 1985). Closed mouths were fixed mainly by the simultaneous method. Tissue was dehydrated in ethanol, embedded in Araldite and thick- and thin-sectioned, and grids were viewed in a Zeiss 10CA EM at 80 kV in the MBL Central Microscope Facility.

RESULTS

Adherent lips: electron microscopy

We found previously that *Beroë* sp. Gloria keeps its mouth closed by adhesion of apposed strips of epithelial cells lining the inside of the lips (Tamm and Tamm, 1991b; Fig. 1). Sections through fixed closed mouths showed that the stomodaeal epithelium is markedly thicker in the adhesive strips because the cells are more columnar in this zone (Tamm and Tamm, 1991b; see also Fig. 4). The two adherent epithelial strips make numerous close contacts interrupted by vacuolar intercellular spaces (Tamm and Tamm, 1991b). At regions of close contact, the adjoining cell surfaces are folded and interdigitate. The apposed plasma membranes run parallel with a uniform separation of about 15 nm (Tamm and Tamm, 1991b) (Fig. 2). The intercellular space frequently contains dense material, which often appears periodically arranged (Fig. 2). The adherent plasma membranes are lined by a dense cytoplasmic coat, 15-30 nm thick, in which numerous 6-8 nm diameter microfilaments are embedded; the microfilaments typically run parallel to the overlying membranes (Fig. 2). These appositions of the stomodaeal adhesive cells are morphologically similar to transient adhesions formed between moving cells in embryos and in culture (Trinkaus, 1984).

The vacuolar spaces between the adherent surfaces vary in size, but typically appear 1-3 μm in width (Tamm and Tamm, 1991b). The plasma membranes in these widely separated regions do not bear cytoplasmic coats, and so are readily distinguishable from regions of close apposition. The spaces are considerably smaller than the vacuoles or craters described on the surface of pulled-apart living adhesive strips (Tamm and Tamm, 1991b). As we will show, the latter represent artifacts of cell rupture.

The epithelial cells within each adhesive strip are linked laterally by belt junctions encircling their apical ends (see Fig. 8). The junctional membranes make a series of focal, very close appositions and are lined by cytoplasmic coats

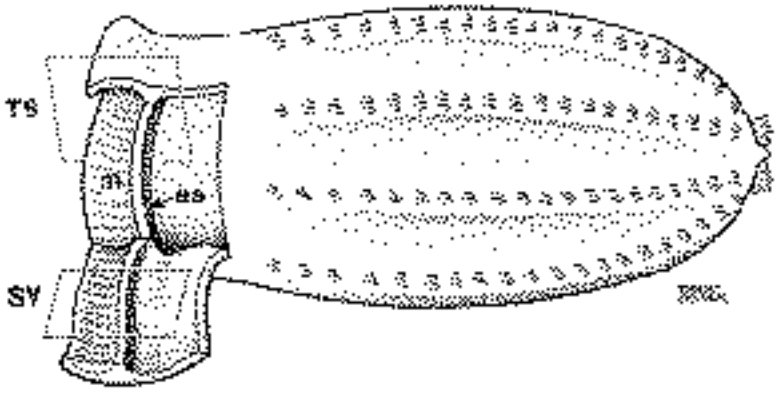


Fig. 1. Peel-away view of location of epithelial adhesive strips (as) inside the mouth of *Beroë*. The planes of section (or view) shown in the figures are: transverse sections (TS) through adhesive strips in Figs 4, 5 and 8; tangential or surface views (SV) of the strips in Figs 3 and 7. m, band of macrocilia. The relative sizes of the macrociliary field and adhesive strips are exaggerated for clarity. Four meridional rows of comb plates are shown extending from the aboral statocyst (right) towards the mouth. The animal may be up to 6 inches long.

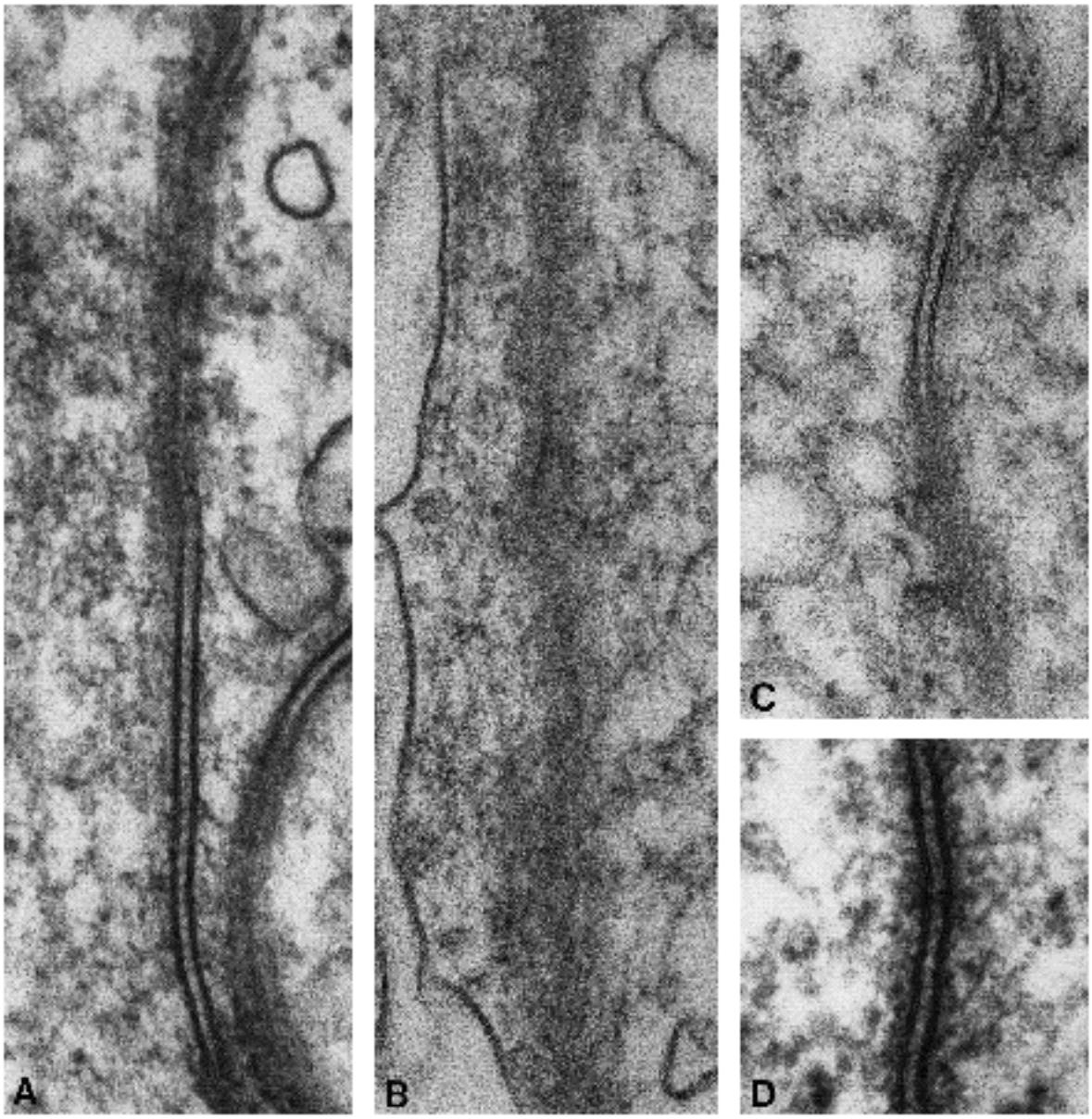


Fig. 2. Electron micrographs of membrane appositions of adherent epithelial cells in closed mouths. Numerous microfilaments run parallel to one another (vertically in figure) in the cytoplasmic coat underlying the membranes. The filaments are particularly evident in oblique or tangential views of the junctions (B; A, upper; C, lower). The ~15 nm intercellular space is often filled with flocculent material that sometimes appears periodically disposed (A, D). (A) ×115,600. (B) ×112,800. (C) ×113,600. (D) ×143,100.

containing microfilaments (Hernandez-Nicaise et al., 1989). The unique morphology of these apical belt junctions in ctenophores does not allow them to be readily identified as a type of septate desmosome, tight junction or gap junction (see Hernandez-Nicaise et al., 1989).

Rhodamine-phalloidin staining

To view the actin staining pattern of adherent epithelial strips, it was necessary to separate them. We used several methods and from the range of images obtained have been able to reconstruct the adherent state in situ.

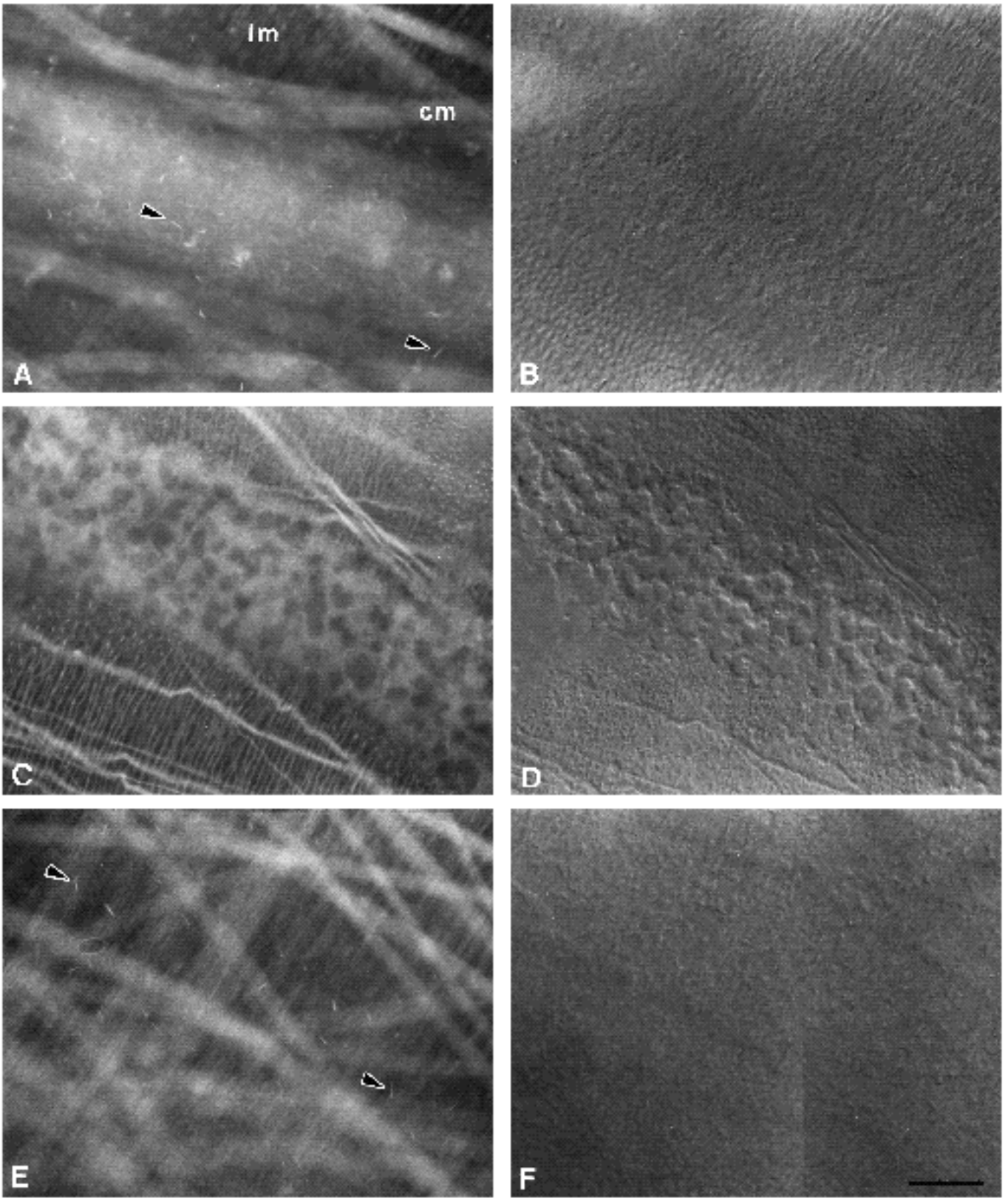
Most intact

Treatment of Mg-relaxed closed mouths in formaldehyde + 0.1% Triton usually results in separation of the lips in fixative. After incubation in rhodamine-phalloidin, some lips show a distinct zone of fuzzy surface fluorescence that is brighter than the surrounding epithelium and coincides with the location of the adhesive strips (Fig. 3A,B). Localization of F-actin to the epithelial adhesive strips confirms ultrastructural evidence (Fig. 2) for actin microfilaments in the dense coat lining the membrane appositions.

Scattered over the surface of the adhesive strip, but not elsewhere on the stomodaeal epithelium, are conical projections that stain brightly with rhodamine-phalloidin (Fig. 3A). These so-called actin pegs arise from presumed sensory cells that also bear onion-root cilia (Tamm and Tamm, 1991a), and were identified previously in adhesive strips by

electron microscopy (Tamm and Tamm, 1991b). Such cells are found in the epidermis of many ctenophores (Horridge, 1965; Hernandez-Nicaise, 1974; Tamm and Tamm, 1991a), and probably function as chemo- and mechanoreceptors (Tamm and Tamm, 1991a). The actin pegs therefore provide a convenient marker for the location of the adhesive strips in rhodamine-phalloidin-stained lips.

Corresponding DIC views of the surface of the adhesive strips do not reveal a tract of densely packed large vacuoles and craters, as described previously in peeled apart living



lips (Tamm and Tamm, 1991b). Instead, the surface of the adhesive strip appears slightly rough or pebbly, and essentially devoid of large vacuoles or craters (Fig. 3B). This actin staining and DIC appearance probably represent the structure of adherent epithelial strips in situ.

Less intact

In other closed mouths that opened in formaldehyde + 0.1% Triton, rhodamine-phalloidin staining reveals occasional dark 'holes' on the surface of the adhesive strip. These non-fluorescent, actin-poor areas appear in matching DIC images as 3-10 μm diameter craters, similar to those described previously in pulled-apart adhesive strips on living lips (Tamm and Tamm, 1991b).

We find further changes in the surface appearance of the adhesive strips in Mg-relaxed closed mouths which are (1) treated with formaldehyde + 0.1% Triton and remain closed in fixative, and then are pulled apart subsequently, or (2) treated with formaldehyde + 1% Triton and separated in fixative. In both cases, rhodamine-phalloidin staining shows a brightly fluorescent adhesive strip punctuated by numerous non-fluorescent holes, which correspond to vacuoles and craters in matching DIC images. The fluorescent intensity of the net-like actin staining is greater than that of the uniformly fluorescent adhesive zone described above. We find a similar 'holey' pattern of rhodamine-phalloidin staining in the adhesive strips of Mg-relaxed closed mouths when they are pulled apart before being placed in fixative (formaldehyde + 0.1% Triton). These images evidently represent varying degrees of disruption of initially adherent epithelial strips by mechanical separation.

Least intact

The most extreme disruption of adherent epithelial strips occurs in closed mouths that are fixed initially in formaldehyde ± glutaraldehyde without Triton to preserve lip attachment, followed by fixative + 0.1% Triton (which still maintains lip adhesion), and then are pulled part. Rhodamine-phalloidin staining shows large holes and gaps in the actin pattern of the adhesive strip (Fig. 3C). Matching DIC images show corresponding tears and craters on the epithelial surface of the strip (Fig. 3D). This pattern is even more disrupted than the epithelial strips of pulled-apart living lips (Tamm and Tamm, 1991b). The densely packed vacuoles or craters seen by DIC in pulled-apart living adhesive strips (Tamm and Tamm, 1991b) are therefore not genuine structures, but artifacts of ripping apart the attached cell layers.

Fig. 3. Rhodamine-phalloidin (A, C, E) and DIC(B, D, F) images of epithelial adhesive strips on the inner surface of the lips (surface view, Fig. 1). The adhesive strips run diagonally from upper left to lower right in each picture. (A, B) Intact state. (A) The surface of the adhesive zone shows uniform diffuse actin staining and is marked by brightly fluorescent actin pegs (arrowheads). Note fluorescence of belt-like circular muscles (cm) and narrow longitudinal muscles (lm) in the mesoglea. (B) The surface of the adhesive strip appears slightly rough. (C,D) Pulled-apart lips. (C) Large dark holes are present in the fluorescent adhesive strip, corresponding to craters and vacuoles on the epithelial surface in DIC views (D). Mesogleal muscles under the adhesive strip are out of focus and appear discontinuous because the protuberant strip (see Fig. 3A) has been pushed into the body wall by pressure from the coverslip. (E, F) Food-opened lips. (E) Actin fluorescence has completely disappeared from the surface of the adhesive zone, yet the actin pegs remain bright (arrowheads). The pattern of circular and longitudinal muscles is evident in the background. (F)The adhesive surface appears similar to that of the surrounding epithelium. Bar, 50 μm.

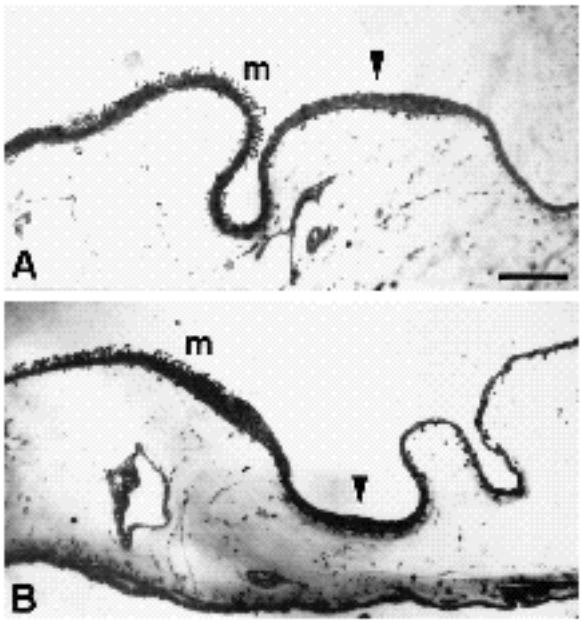


Fig. 4. Toluidine blue-stained thick sections, showing lips with epithelial adhesive strips cut transversely (see Fig. 1). The macrociliary field (m) is at the left. (A) Pulled-apart lip. The thickened adhesive strip protrudes as a ridge from the stomodaeal surface (arrowhead). (B) Food-opened lip. The adhesive strip is retracted into the mesoglea (arrowhead). Bar, 100 μm.

Pulled apart lips: EM

Transverse thick sections through pulled-apart lips show the adhesive strips protruding as ridges from the stomodaeal surface (Fig. 4A). Electron micrographs show pieces of extra plasma membrane and cytoplasm attached to the surfaces of pulled-apart adhesive strips (Fig. 5). These cortical remnants, 'untimely ripped' from the missing strip, consist of intact appositions between the adherent plasma membranes with their dense filamentous coats, as well as intervening vacuolar spaces (Fig. 5A). In other regions the surface of the strip is ruptured and the plasma membrane appears missing (Fig. 5B), evidently the result of being torn off and stuck to the other strip.

These observations explain the rhodamine-phalloidin staining pattern of separated or pulled-apart lips: the net-like actin fluorescence represents remnants of close appositions and their filamentous coats from *both* strips, hence its brighter fluorescence compared to that of intact single strips. The dark non-fluorescent holes represent areas where the appositions were ripped off and retained by the missing strip.

Pulling apart the adhesive strips in the absence of food does not therefore separate the membrane appositions nor cause their filamentous cytoplasmic coats to disappear.

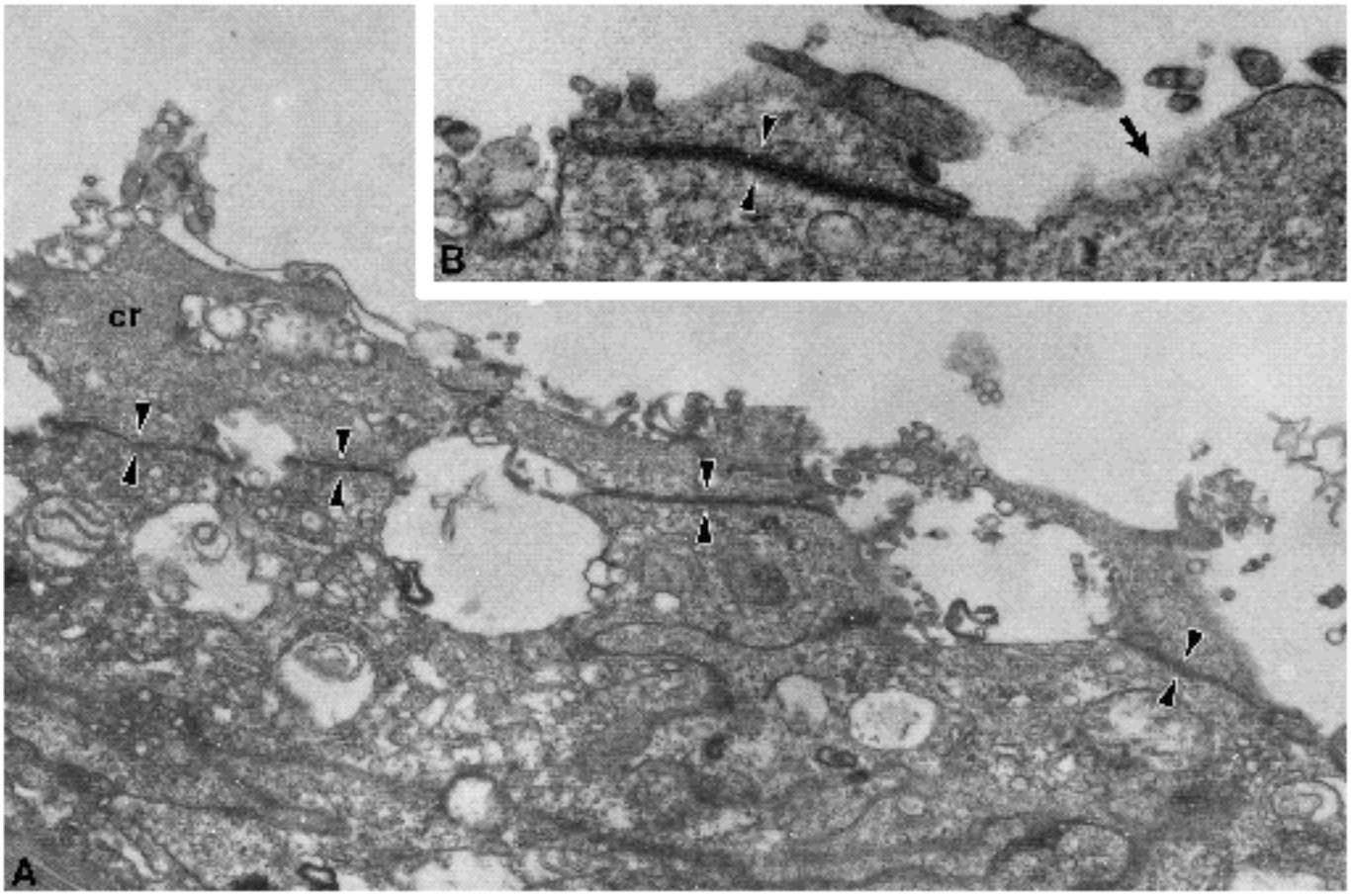


Fig. 5. (A) Electron micrograph of a pulled-apart adhesive strip, showing intact appositions (paired arrowheads), vacuolar spaces and cytoplasmic remnants (cr) ripped off the missing strip (transverse plane, Fig. 1). ×15,100. (B) Higher magnification of a ripped-off portion of an apposition on a pulled-apart strip, showing the cytoplasmic coats of the apposed plasma membranes (arrowheads). In an adjacent region, the plasma membrane of the adhesive cell appears missing (arrow). ×43,300.

Instead, the cell-cell appositions remain intact and are physically torn from the epithelial cells on both strips. Measuring the force required to separate the adhesive strips thus would not indicate the strength of cell-cell adhesion, but that of rupturing the complex surface architecture of the cells and breaking them apart. Our findings emphasize the need for caution in interpreting measurements of the force of 'cell-cell adhesion' by forcibly breaking up adhesions (see also Trinkhaus, 1984; Curtis and Lackie, 1991).

Food-opened lips

Behavior

Contact of prey (*Mnemiopsis*) with any region of the lips of *Beroë* triggers a local muscular separation of the lips followed by rapid peeling apart of the lips and full opening of the mouth; the entire response takes 0.2-0.3 s. Prey-induced mouth opening is considerably faster than mouth opening induced by artificial stimuli, such as egg white (Tamm and Tamm, 1991b). As the mouth opens, the stomodaeum rapidly expands by muscular action to suck in the prey (Fig. 6; Horridge, 1965; Tamm, 1982). *Beroë* can completely swallow a *Mnemiopsis* as large as itself in 1-2 s. The mouth then quickly closes and the lips re-seal (Fig. 6).

In order to obtain prey-opened lips before they re-adhere, we used *Mnemiosis* larger than the *Beroë*. Mouth opening is just as fast as with smaller prey, but the *Beroë* cannot engulf the entire *Mnemiopsis*. Instead the opened lips spread over as much of the prey as possible before slowly constricting to bite off a piece (see also Swanberg, 1974). We cut lips off such *Beroë* in the act of engulfing larger prey for the following experiments.

Living DIC

In contrast to pulled-apart lips (Tamm and Tamm, 1991b), the adhesive surface of food-opened lips is not marked by numerous large vacuoles or craters. Instead the surface of the adhesive strip contains many small pits and scattered round mounds, giving the strip epithelium a pebbly appearance in DIC views (Fig. 7).

Rhodamine-phalloidin staining

Lips fixed immediately after prey-induced opening show no rhodamine-phalloidin fluorescence of the adhesive strips (Fig. 3E). The actin pegs scattered over the strip epithelium stain brightly, however, marking the location of the strip. Underlying muscle fibers and ciliary actin bundles (Tamm and Tamm, 1987) are also brightly fluorescent, confirming

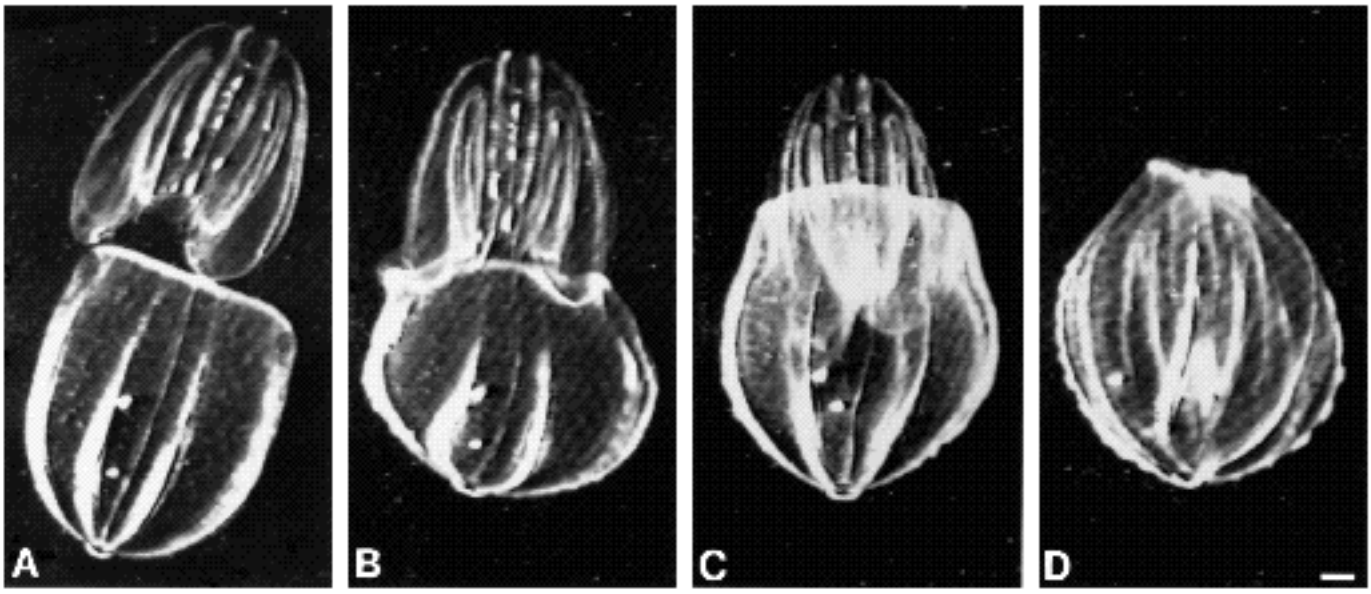


Fig. 6. *Beroë* (lower) eating a *Mnemiopsis* (upper). (A) The wide lips of *Beroë* touch the lobes of *Mnemiopsis.* (B) The mouth opens fully and the stomach cavity expands to begin ingestion. (C) Half the *Mnemiopsis* is swallowed and is visible inside the stomach of *Beroë.* (D) The entire *Mnemiopsis* is engulfed and *Beroë* closes its mouth to swim away. Bar, 5 mm.

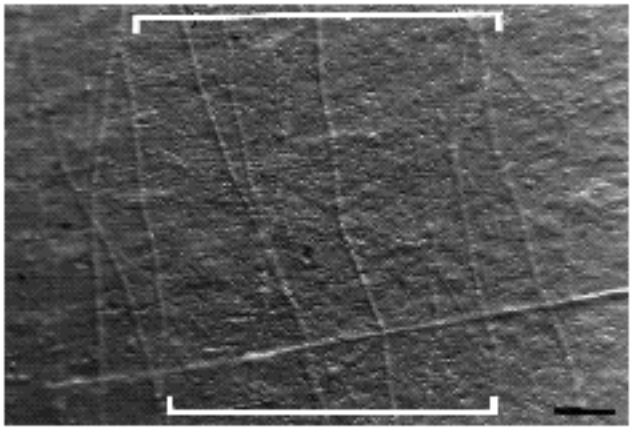


Fig. 7. DIC surface view of the adhesive strip (running vertically between brackets) in a food-opened mouth. The epithelial surface of the strip contains many small pits but few large craters. Underlying circular muscle fibers are slightly out of focus. Bar, 50 μm.

normal actin staining in these preparations. In matching DIC images, we find no large vacuoles or craters on the surface of the adhesive strips (Fig. 3F), in contrast to the appearance of pulled-open lips (above). These results show that the cortical actin of the adhesive strips disappears after prey-induced opening of the mouth.

EM

Transverse thick sections through food-opened lips fixed within 30 s after opening show the adhesive strip withdrawn into the mesoglea (Fig. 4B). This retraction of the adhesive strip epithelium is evidently part of the muscular mouth opening response triggered by prey.

Electron microscopy of adhesive strips in food-opened mouths shows no evidence of disruption or tearing of the cell surfaces. The plasma membranes appear smooth and intact, without any sign of close appositions and filamentous cytoplasmic coats (Fig. 8). This agrees with the absence of rhodamine-phalloidin staining in the adhesive strips of food-opened mouths (above).

The apical surfaces of the cells appear rounded, and often bulge above the rings of apical belt junctions which remain intact (Fig. 8). This change in shape of the apical end of the adhesive cells may reflect unfolding of apposed plasma membranes after loss of their underlying actin cytoskeleton. The retention of the apical belt junctions emphasizes the selective nature of prey-induced loss of cell junctions.

DISCUSSION

We used cytochemical and ultrastructural methods to follow dynamic changes in cell junctions and their associated actin cytoskeleton during reversible epithelial adhesion in the mouth of *Beroë.* Rhodamine-phalloidin staining confirmed that the filamentous cytoplasmic coat lining the apposed plasma membranes of adherent epithelial cells contains actin. Actin filaments and other cytoskeletal polymers provide mechanical stiffness and support for maintaining the asymmetric shape of cells and cell processes (De Rosier and Tilney, 1984; Elson, 1988; Jamney, 1991). The actin filaments underlying the adhesive cell membranes likewise must serve mechanically to stabilize and strengthen the highly folded interdigitating surfaces of these cells. The reinforced interlocking of the cell cortices probably fastens the paired epithelial strips together like a jigsaw puzzle, regardless of possible molecular interactions between the plasma membranes themselves (i.e., cell adhesion molecules, see below).

We showed that this complex surface architecture is rapidly lost when the mouth opens suddenly to ingest prey:

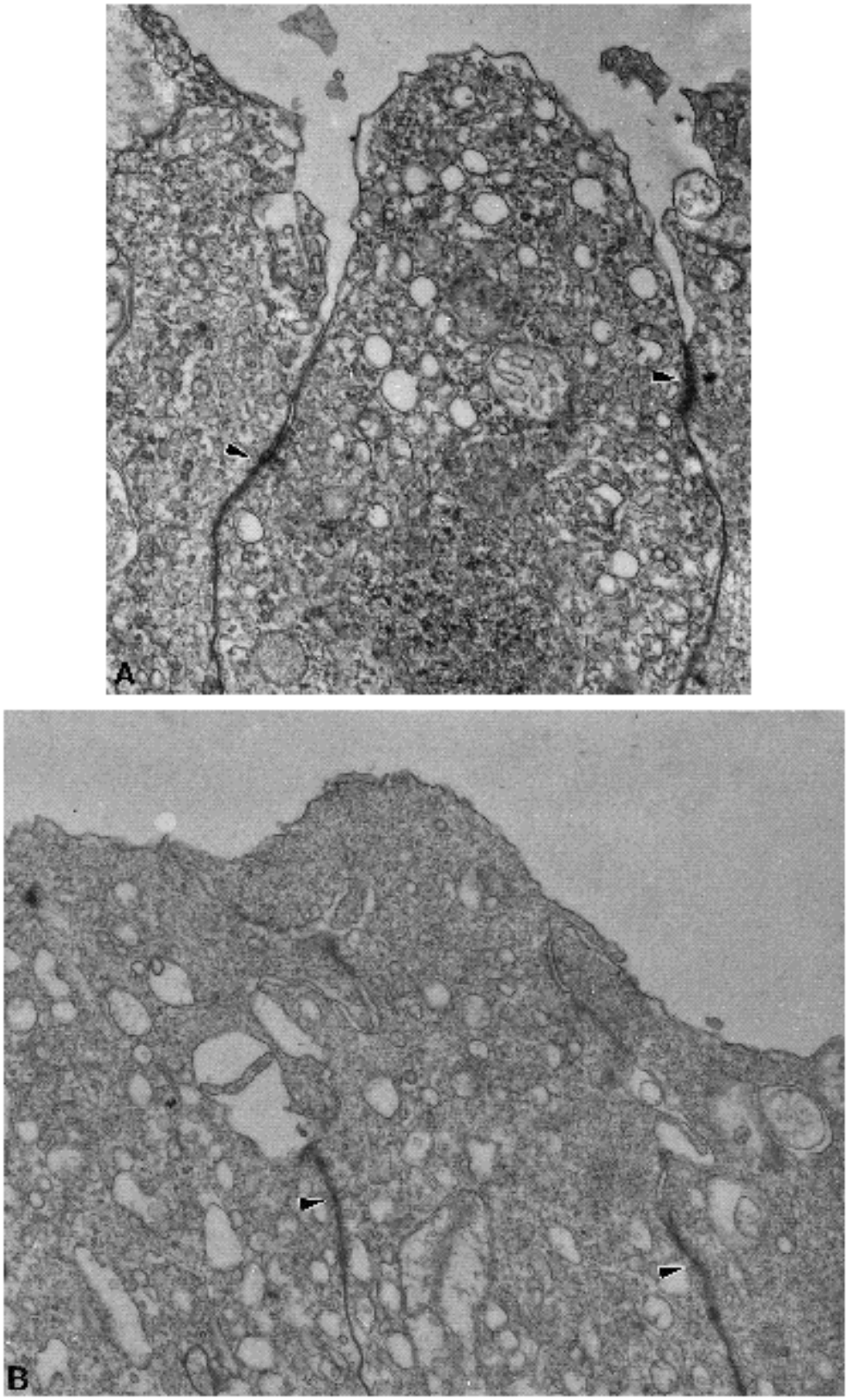


Fig. 8. Transverse thin sections through the apical ends of adhesive cells in two food-opened mouths. The plasma membranes are intact and lack filamentous cytoplasmic coats. The cell surfaces are rounded and bulge above the encircling apical belt junctions (arrowheads). (A) ×18,900. (B) ×23,500.

the adhesive cell junctions, the submembranous actin cytoskeleton and the folded topography of the apical plasma membranes disappear completely. Loss of the cortical actin cytoskeleton is expected to weaken or collapse the surface interdigitations of the adherent cells. Withdrawal of mechanical support for the junctions should facilitate their separation when the lips are peeled apart by muscular activity. Alternatively (or concomitantly), if cadherin molecules are involved in homophilic cell adhesion between the paired epithelial strips, then depolymerization of membrane-associated actin should reduce or eliminate their cell binding activity (Takeichi, 1990). Cell adhesion mediated by cadherins depends on their association, via linkage of their cytoplasmic domains to catenins, with the actin-based cytoskeleton; cadherin function may thus be regulated by the state of the actin cytoskeleton (Nagafuchi and Takeichi, 1989; Takeichi, 1990). However, we have no evidence so far that cadherins play any role in lip adhesion in *Beroë*. To the contrary, we found in preliminary studies that both pulled-apart and food-opened lips can re-adhere normally in Ca-free artificial sea water, indicating that epithelial adhesion is not Ca-dependent. However, the high concentration of Mg (approx. 100 mM) used in these experiments probably would have substituted for Ca in possible cadherin function, rendering these results inconclusive for deciding on mechanisms. Repeating these experiments in Ca-Mg-free sea water should be instructive; in addition, we plan to use broad spectrum pan-cadherin antibodies (Geiger et al., 1990) to probe for possible *Beroë* cadherins by immunofluorescence, and to test the effects of blockade of N-cadherin adhesion by monoclonal antibodies and synthetic peptides (Mege et al. 1992).

The role of the vacuolar intercellular spaces between the adherent surfaces of the epithelial cells is not understood. The plasma membranes of these widely spaced regions are not lined by dense filamentous coats, so these regions probably do not contribute to adhesion. These intercellular spaces may serve to increase the surface area of the paired adhesive strips without unduly increasing their adhesive strength. Wider strips would be more likely to overlap and re-establish contact after the mouth closes over ingested prey. In fact, sections through fixed closed mouths often show a slight offset or mismatch between the paired adherent strips (Tamm and Tamm, 1991b). If the junctional areas were continuous without these intervening spaces, the strips themselves would be much narrower, and perhaps more likely to miss one another upon closure of the stretched stomach walls after feeding.

The rapid disappearance of the membrane appositions and associated actin filaments in food-opened adhesive strips cannot be due simply to the mechanical stresses of lip separation. Peeling apart lips in the absence of food does not lead to similar changes. Instead the still-adherent junctions are ripped off the cells. Pulled-apart lips can re-adhere in about 15 min, however (Tamm and Tamm, 1991b), showing the well-developed wound healing and regenerative powers of ctenophores (Coonfield, 1936).

Disappearance of the adhesive cell junctions and membrane-associated actin filaments must therefore be signaled by the animal itself. Mouth opening is triggered by contact of prey with mechano- and chemoreceptors on the lips. The presumed receptors are cells bearing actin pegs and onion-root cilia (Tamm and Tamm, 1991a). These cells, also found along the adhesive strip epithelium, make synaptic contacts with the nerve net (Hernandez-Nicaise, 1974; Tamm and Tamm, 1991a), which in turn synapses onto lip muscles and cells of the adhesive strips. Food stimuli may thus signal not only muscular contractions that peel apart the lips, but also disassembly of the actin-supported appositions between the adhesive cells, thereby diminishing their binding and facilitating lip separation.

After engulfing prey, the mouth closes and the lips reseal. We have not yet examined re-adhered epithelial strips, but presumably the cortical actin cytoskeleton and interdigitating membrane appositions readily reform. The assembly and disassembly of the adhesive cell junctions and associated actin cytoskeleton is therefore a reversible process that is regulated by the ctenophore.

It will be interesting to follow the process of re-adhesion, particularly whether the same regions of the plasma membranes that were associated with actin filaments and formed appositions before mouth opening do so again when the epithelial strips rejoin. Formation of the elaborately sculptured interlocking surfaces of the adherent cells may be driven by polymerization of membrane-associated actin filaments. Actin polymerization provides the motive force for rapid elongation of the acrosomal process of *Thyone* sperm (Tilney and Inoué, 1982); actin polymerization is also reported to drive lamellipod extension in chemotactically stimulated neutrophils (Wymann et al., 1990). Actin polymerization induces morphological changes in actin-containing lipid vesicles, demonstrating directly that actin filament assembly can cause biological membranes to change shape (Cortege et al., 1989; Miyata and Hotani, 1992).

Although the adhesive properties of the epithelial strips are presumably regulated by nerves, the ionic or electrical responses of the adhesive cells to nervous signals are unknown. The intracellular messengers and processes that mediate the rapid changes in junctional morphology and actin cytoskeleton are also unknown. For example, we do not know whether the disappearance of cortical actin is due to depolymerization or redistribution of actin filaments. A variety of actin filament-severing, depolymerizing, nucleating, capping and cross-linking proteins, whose activities may be regulated by Ca, phosphoinositides or other messengers have been described (Hartwig and Kwiatkowski, 1991; Hartwig et al., 1992; Pollard and Cooper, 1986). These actin-binding proteins are thought to play roles in dynamic changes in the actin cytoskeleton and cell shape in response to various stimuli (Hartwig and Kwiatkowski, 1991; Pollard and Cooper, 1986). For example, the activity of the actin cross-linking protein, MARCKS, and its attachment to the plasma membrane is regulated by protein kinase C and calcium-calmodulin, suggesting that MARCKS provides a regulated cross-bridge between the actin cytoskeleton and the membrane (Hartwig et al., 1992). In addition, tyrosine phosphorylation of actin in *Dictyostelium* in response to growth medium is correlated with alterations in the actin cytoskeleton, cell shape and adhesion to the substratum (Howard et al. 1993).

Although described as ‘dynamic’ and ‘rapid’, most of the

previously described changes in the actin cytoskeleton take place over a period of 5-10 min or longer. One exception is the oscillations of actin polymerization and depolymerization that drive lamellipod extension and retraction in stimulated neutrophils: the periodicity of the changes in F-actin content is about 10 s (Wymann et al., 1990). Another example is the reduction of F-actin in toad bladder epithelial cells as early as 1 min after stimulation with vasopressin (Ding et al., 1991). In contrast, the disappearance of the cell junctions and associated actin coat in food-opened epithelial strips of *Beroë* is probably much faster: the mouth opens in 0.2-0.3 s, and at our earliest fixation time after opening (20 s) the actin/junctional complex is already gone. The disassembly of the junctions and actin cytoskeleton probably occurs before, or during, the muscular separation of the lips, i.e., in less than 0.3 s. *Beroë* adhesive strips show one of the fastest changes in actin-based cell junctions yet discovered and provide unique experimental advantages for studying the dynamic control of reversible cell adhesions and associated actin cytoskeleton in cells.

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