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THE ULTRASTRUCTURE OF PROKARYOTIC–EUKARYOTIC CELL JUNCTIONS

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SUMMARY

Freeze-fracture and thin-section electron microscopy were used to describe the sites of attachment of 2 kinds of ectosymbiotic bacteria to a devescovinid flagellate from termites. In each case, surface specializations in both partners occur at the junctional complexes. Rod bacteria lie in pockets of the eukaryotic membrane which are coated by dense material and contain high densities of intramembrane particles. A double row of closely spaced particles circumscribes the edges of the pockets on the P face. The surface of the rod bacteria which is exposed to the external medium bears a thick glycocalyx and flagella. Fusiform bacteria are attached along ridges of the protozoon surface. Dense material underlies the ridges, and particles are aggregated on both P and E faces of the ridge membrane. The outer layer of the fusiform bacteria is grooved to match the host ridge. The bacterial–devescovinid junctions are considered to serve mainly as attachment sites, and the membrane specializations at the sites of contact are discussed in this respect. Control of junction assembly by the cortical bacteria is suggested by the parallel patterns of junctional replication and bacterial reproduction.

INTRODUCTION

Associations of eukaryotic cells in multicellular organisms are characterized by specialized regions of contact between the membranes of adjacent cells. These intercellular junctions are of different types, and serve to integrate and coordinate the various kinds of cellular interactions (Gilula, 1974; McNutt, 1977; Staehelin, 1974).

Associations also occur between prokaryotic and eukaryotic cells. These relationships are extremely diverse, but are generally called symbiotic (Ball, 1969; Henry, 1966; Kirby, 1941*a*; Starr, 1975). This report deals with ectosymbiotic associations, in which the prokaryotes live permanently attached to the surface of the eukaryotic cell.

The junctions formed between ectosymbiotic prokaryotes and eukaryotic cells are not well understood. Conventional electron-microscopic descriptions of prokaryotic–eukaryotic cell junctions have revealed various kinds of structural specializations at the site of contact between prokaryote and eukaryote (Bloodgood & Fitzharris, 1976; Cleveland & Grimstone, 1964; Hollande & Carruette-Valentin, 1970, 1971, 1972;

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Hollande & Valentin, 1969; Lavette, 1969; Smith & Arnott, 1974; Smith, Buhse & Stamler, 1975; Tamm & Tamm, 1974, 1976; Wagner & Barrnett, 1974). Freeze-fracture replication has greatly clarified the nature of intercellular junctions, but until recently this method has not been applied to the study of prokaryotic–eukaryotic cell junctions.

The hindgut of the wood-eating roach, *Cryptocercus*, and lower termites is a good place to investigate prokaryotic–eukaryotic cell junctions. This environment harbours numerous kinds of associations between bacteria and flagellate protozoa. In particular, these symbioses reach their highest level of development in devescovinid flagellates and related polymastigotes (Ball, 1969; Cleveland & Grimstone, 1964; Kirby, 1941*a*, *b*, 1942*a*, *b*, 1945, 1949). The best-known example is the regular attachment of spirochaetes and rod bacteria to specialized surface structures of *Mixotricha paradoxa*, a flagellate from *Mastotermes darwiniensis*; the coordinated undulations of the spirochaetes propel the protozoon (Cleveland & Grimstone, 1964).

We previously described the association of 2 kinds of ectosymbiotic bacteria with the surface of a remarkable devescovinid flagellate from a Florida termite (Bloodgood & Tamm, 1977; Tamm, 1976, 1978*a*, *b*, *c*, 1979; Tamm & Tamm, 1974, 1976). The anterior part of this protozoon continually rotates in the same direction relative to the rest of the cell. Rotational movements are caused by a rod-like axostyle complex running through the cell body (Tamm, 1978*a*). We were interested in the adherent bacteria because they provided useful markers for following movements of the cell's plasma membrane. Recently, we discovered that these bacteria are also useful to the protozoon: one type is flagellated, and provides locomotion for the host (Tamm, 1978*c*, manuscript in preparation).

In this paper we present a correlated thin-section and freeze-fracture description of the junctional complexes formed between the 2 kinds of bacteria and the devescovinid. The results, reported previously in preliminary form (Bloodgood & Tamm, 1977; Tamm, 1978*b*, 1979), illustrate several new features of the ultrastructure of prokaryotic–eukaryotic cell junctions.

MATERIALS AND METHODS

The organism studied here is the same (unnamed) devescovinid flagellate from *Cryptotermes cavifrons* used in previous work (Tamm, 1976, 1978*a-c*, 1979; Tamm & Tamm, 1974). The methods for thin-section electron microscopy and freeze-fracture of this protozoon have been reported previously (Tamm & Tamm, 1974, 1976; Tamm, 1979).

RESULTS

General features of the bacterial–devescovinid associations

Rod-shaped and fusiform-like bacteria are found in intimate association with the surface of the protozoon (Figs. 1, 2). The rod bacteria are ~ 2–3 μm long, $\sim 0{\cdot}6$ μm in diameter, and are flagellated on one side (Fig. 3B; Tamm, 1978*c*). The fusiform bacteria are longer (~ 5–6 μm) and more slender ($\sim 0{\cdot}2$ μm in diameter), and do not

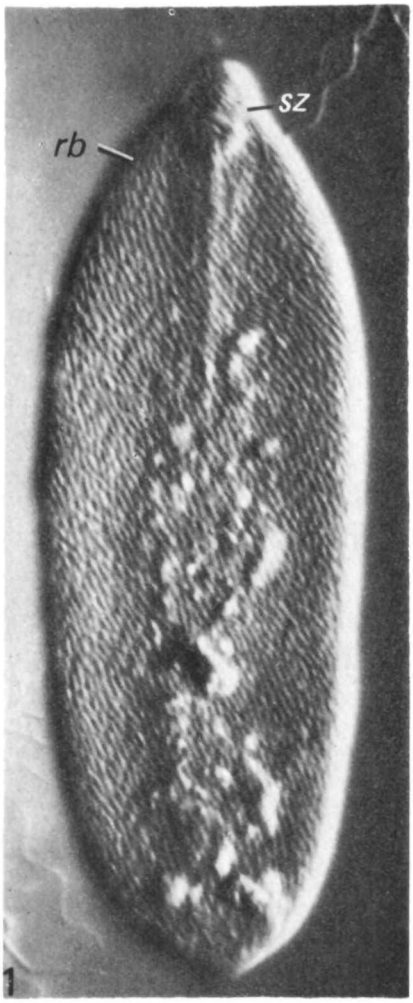


Fig. 1. Pattern of ectosymbiotic rod bacteria (*rb*) on the surface of the protozoon. The adherent fusiform bacteria are not visible at this magnification. The anterior end of the cell is continually turned in a clockwise direction by the rod-like axostyle complex running through the cell body. The bacteria-free zone of membrane between the head and the body (*sz*) undergoes continual shear. Zeiss Nomarski optics. × 1100.

have flagella. As in other devescovinid flagellates (Kirby, 1941*b*, 1942*a*, *b*, 1945, 1949), the bacteria are attached in a characteristic and specific pattern to the surface of the host. The rod bacteria are arranged end to end in parallel rows which follow a helical path on the devescovinid's body, but run more transversely on the anterior end, or head (Fig. 1). The fusiform bacteria live only on the body surface, and lie end to end in single rows or several parallel rows between the rows of rod bacteria (Fig. 2).

The cell envelopes of the bacteria have the multilayered structure typical of Gram-negative bacteria (Sleytr, 1978). The surface layers include a plasma membrane, a dense peptidoglycan layer (not clearly visible in the fusiform bacteria), and an outer membrane (Figs. 3–5). Extending from the outer membrane is a fuzzy filamentous

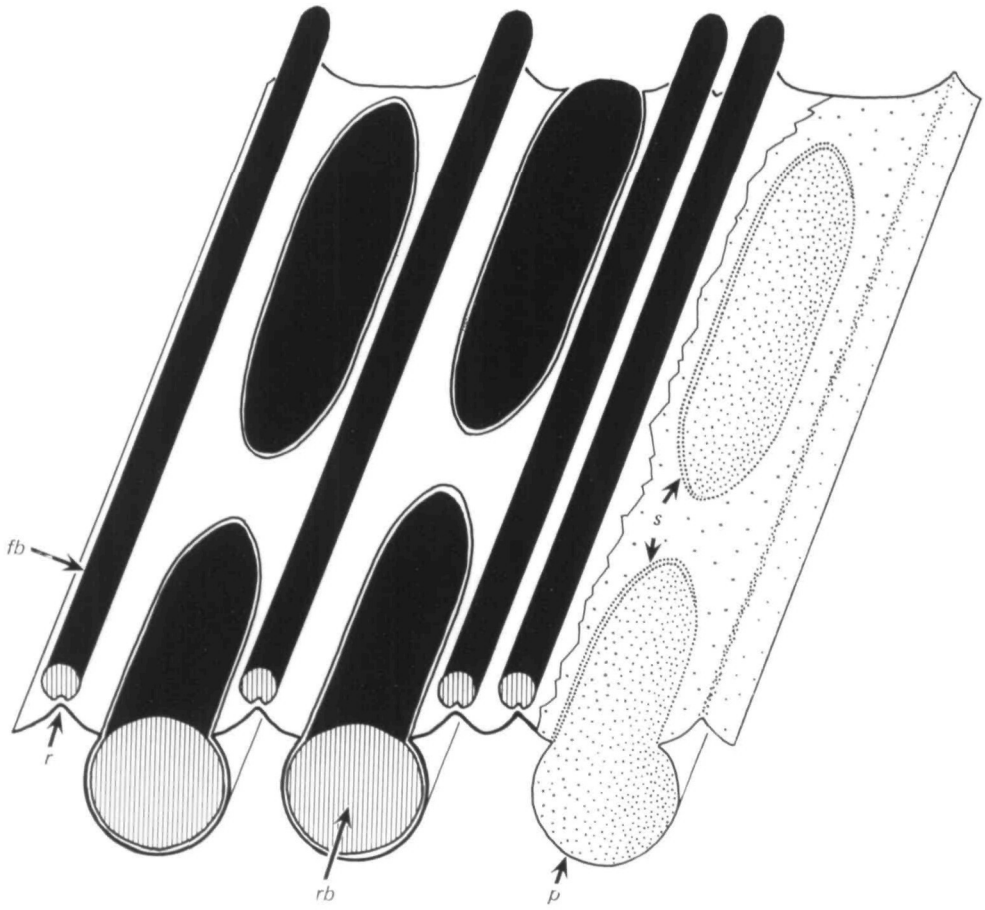
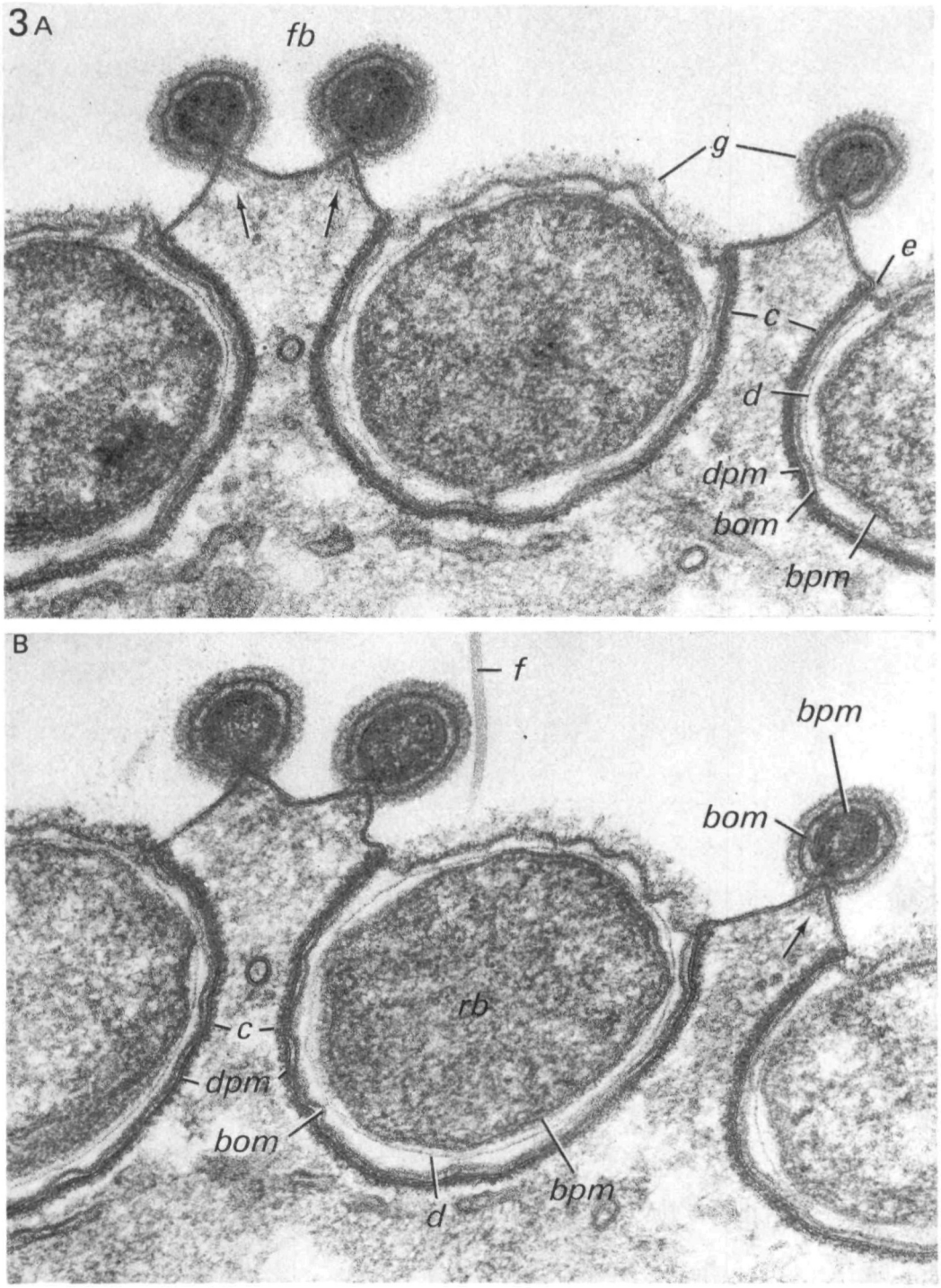


Fig. 2. Reconstruction of a small area of the body surface to show the arrangement of the ectosymbiotic bacteria, and the intramembrane particle arrays associated with the attachment sites. Rod bacteria (*rb*) lie in deep pockets (*p*) of the protozoon plasma membrane. Fusiform bacteria (*fb*) are attached along ridges (*r*) of the host surface, and are grooved along the site of contact. The P face of the eukaryotic membrane (exposed at right) exhibits aggregations of intramembrane particles (dots) along the ridges and in the pockets. In addition, a double row of particles circumscribes the opening of the pocket ('seam', *s*).

Fig. 3A, B. Adjacent thin sections cut transversely through the rows of rod bacteria (*rb*) and fusiform bacteria (*fb*) on the surface of the protozoon. The pockets of the devescovinid membrane (*dpm*) surrounding the rod bacteria (*rb*) are coated by an electron-dense layer (*c*) on the cytoplasmic side. Less-dense material (arrows) underlies the ridges of the devescovinid membrane to which the fusiform bacteria (*fb*) attach. Favourable transverse sections through the edge (*e*, in A) of the pocket membrane show no opacities corresponding to the particle seam in freeze-fracture replicas (cf. Figs.



7, 8). The bacterial cell envelopes consist of a plasma membrane (*bpm*), a dense peptidoglycan layer (*d*) (not visible in the fusiform bacteria), and an outer membrane (*bom*) and glycocalyx (*g*). The rod bacteria bear a thick glycocalyx and flagella (*f*) only on the exposed part of their surface at the pocket opening. Note the groove in the glycocalyx and outer membrane of the fusiform bacteria where they contact the surface of the host. × 100000.

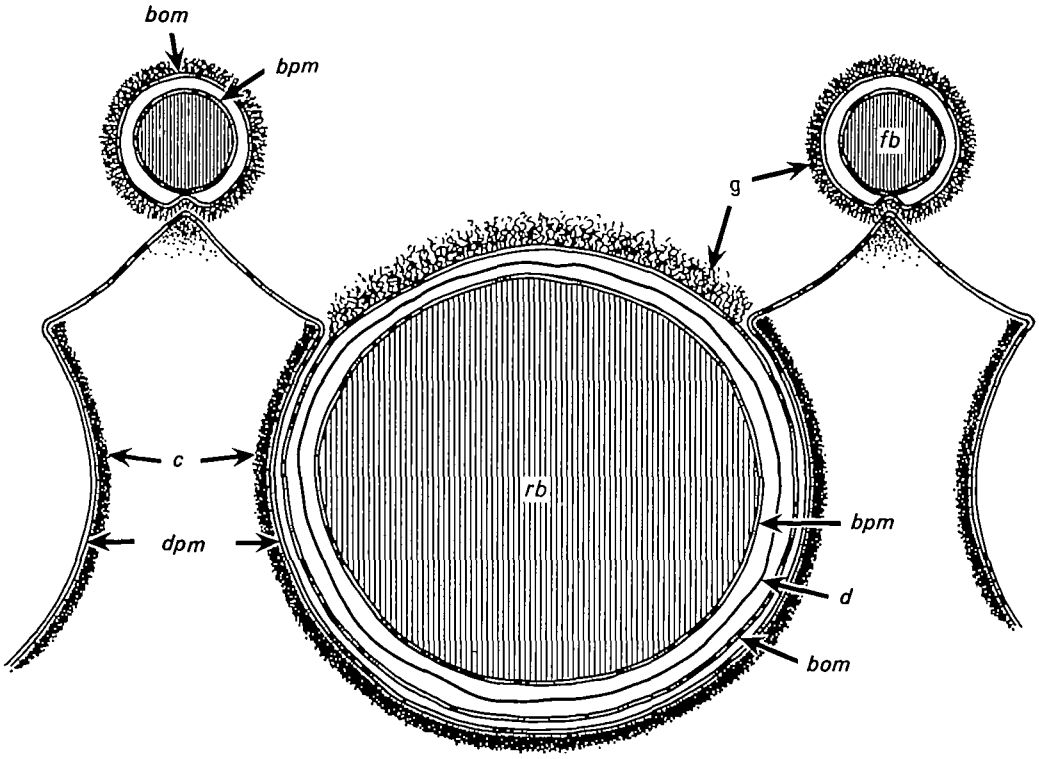


Fig. 4. Diagram of the junctional complexes with rod bacteria (*rb*) and fusiform bacteria (*fb*) as seen in transverse thin sections (cf. Figs. 3 and 5). The flagella on the exposed surface of the rod bacteria are omitted. *dpm*, devescovinid pocket membrane; *c*, membrane coat; *bpm*, bacterial plasma membrane; *d*, dense peptidoglycan layer; *bom*, bacterial outer membrane; *g*, glycocalyx.

layer which is termed a glycocalyx here, although its polysaccharide nature has not been demonstrated. In this report, only junctional specializations of the bacterial outer membrane and glycocalyx are described.

As will become apparent, the 2 types of bacteria form 2 structurally distinct kinds of junctions with the protozoon.

Rod bacterial junctions

The rod bacteria lie in deep invaginations of the protozoon's plasma membrane (Figs. 3–5). These pockets do not completely enclose the bacteria, but leave part of their surface exposed to the external medium.

A layer of dense fuzzy material is closely applied to the cytoplasmic side of the pocket membrane (Fig. 3). This dense coat ends abruptly at the edges of the pockets where the plasma membrane everts over the cell surface.

Freeze-fracture images reveal specialized distributions of intramembrane particles in the pocket membrane of the devescovinid (Figs. 6–9). The most striking specialization is a double row of closely spaced particles that circumscribes the edges of the pockets on the P face, giving the appearance of a distinct 'seam' at the pocket opening.

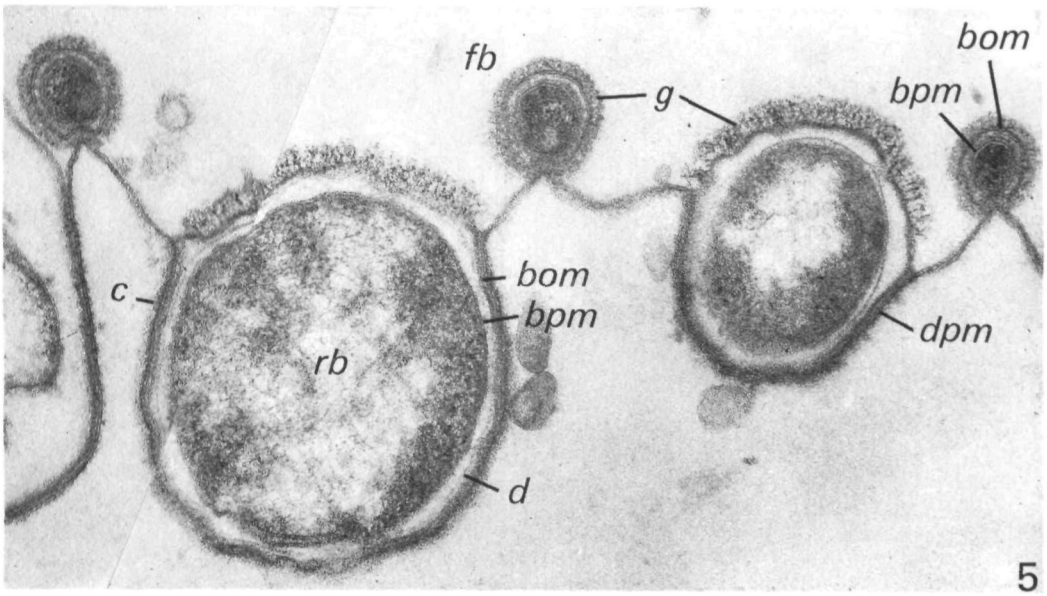


Fig. 5. Section through a fragment of plasma membrane from a glycerinated devescovinid, showing persistence of bacterial attachment. The characteristic structure of the junctional complexes is preserved – and even clarified – after disruption and extraction of the cell. *fb*, fusiform bacterium; *rb*, rod bacterium; *dpm*, devescovinid pocket membrane; *c*, membrane coat; *bpm*, bacterial plasma membrane; *d*, dense peptidoglycan layer; *bom*, bacterial outer membrane; *g*, glycocalyx. × 68 000.

This particle array is not evident on the E face of the pocket membrane, nor are complementary depressions found there (Fig. 9). In favourable thin sections through the edges of the pockets, no densities in the lipid bilayer corresponding to the particle seam are observed (Fig. 3A).

In addition, the particle density on both faces is approximately 2-fold greater in the pocket membrane than in surrounding regions of the cell membrane (Figs. 8, 9). For comparison, it should be noted that the particle distribution on the P face of non-junctional regions of the devescovinid membrane – i.e. *between* the sites of bacterial attachment – is more or less random, with a density of ~800 particles/μm^2 (Tamm, 1979). The size and shape of the intramembrane particles (approximately 7–11 nm in diameter) appear similar in both junctional and non-junctional regions of the protozoon membrane.

The rod bacteria also exhibit surface specializations at the junctions. The part of their surface which faces the external medium bears a prominent glycocalyx, as well as flagella (Figs. 3–5; Tamm, 1978*c*). The filamentous nature of the glycocalyx is particularly evident after glycerination (Fig. 5). In contrast, the glycocalyx is reduced or absent, and flagella are missing, on the bacterial surface that is enclosed by the host pocket. Consequently, only a narrow, somewhat irregular space separates the outer membrane of the bacterium from the pocket membrane of the devescovinid.

Division stages of the rod bacteria can be found almost anywhere on the surface of

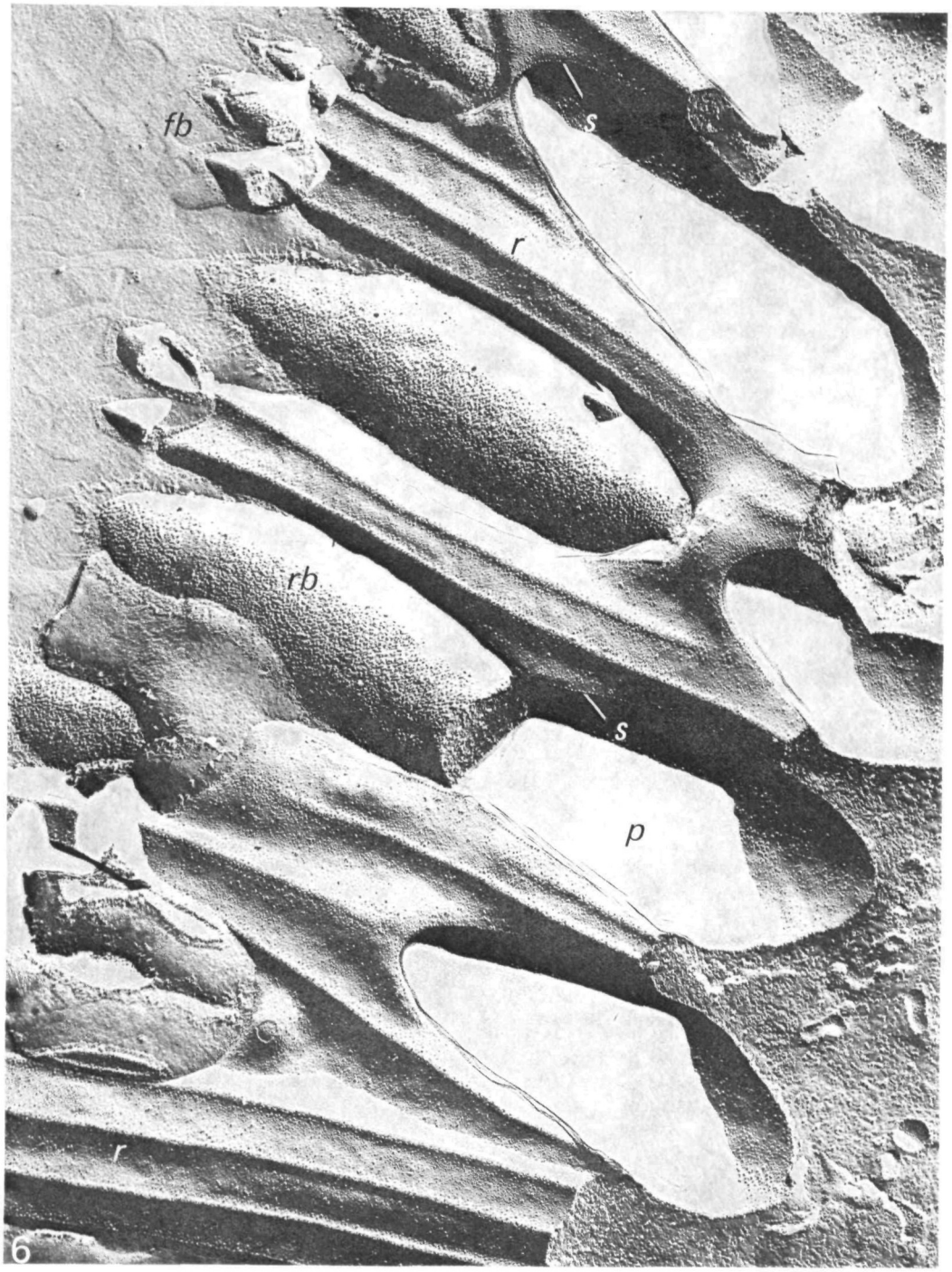


Fig. 6. P face of the body membrane. Particles are aggregated along the ridges (*r*) to which the fusiform bacteria (*fb*) adhere. Several rod bacteria (*rb*) are removed from the membrane pockets (*p*) by the fracturing process. Only 1 row of particles in the seam (*s*) around the edges of the pockets is visible here. In the centre, 1 member of a dividing rod bacterium is retained; the other daughter has been lost, revealing particle arrays typical of the junctional complex in the replicating pocket of the host. × 43 000.

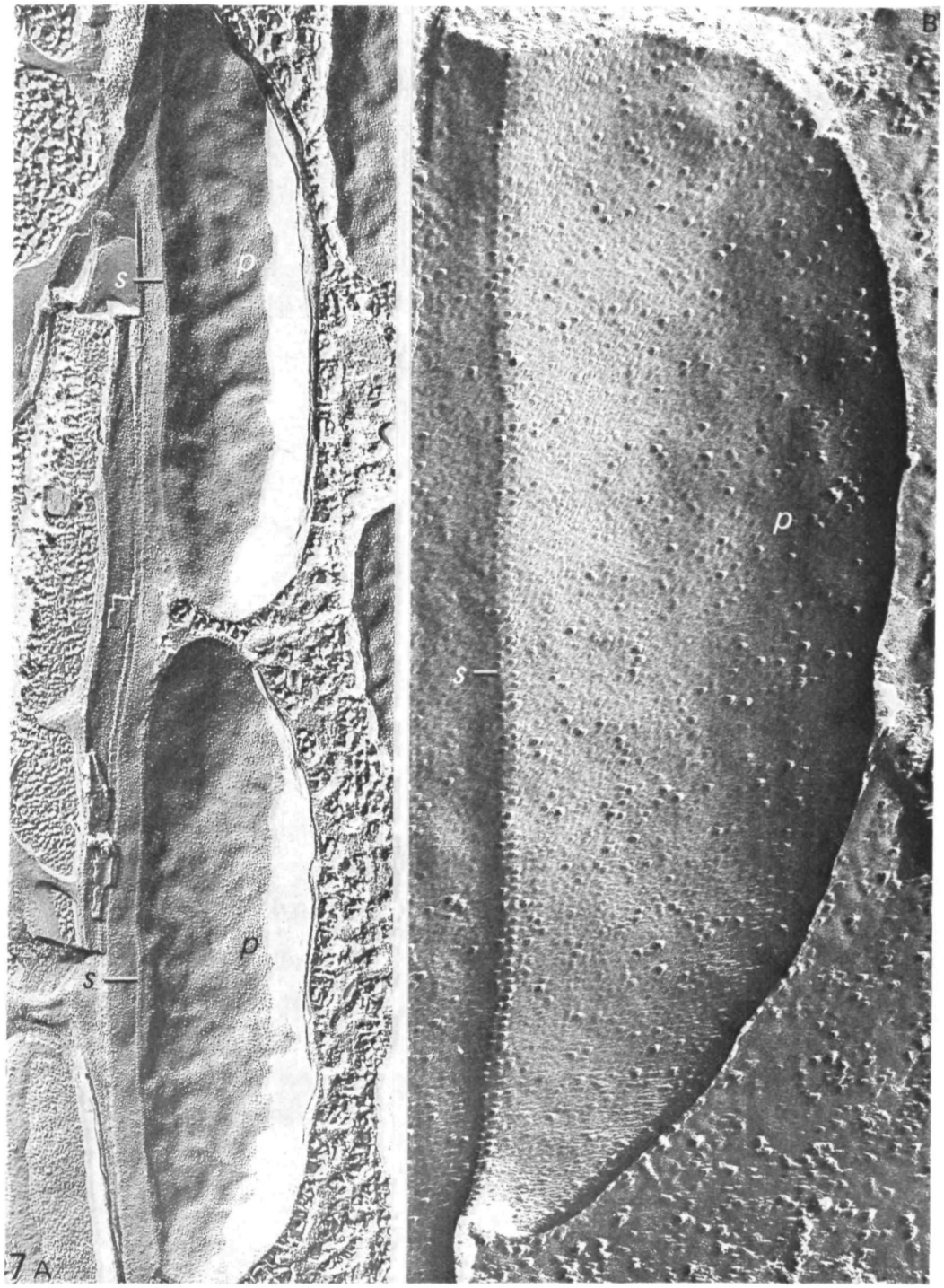


Fig. 7A, B. P face of membrane pockets (*p*), from which rod bacteria have been removed during fracturing. A double row of closely spaced particles forms a seam (*s*) around the edges of the pockets. A, × 40 000; B, × 110 000.

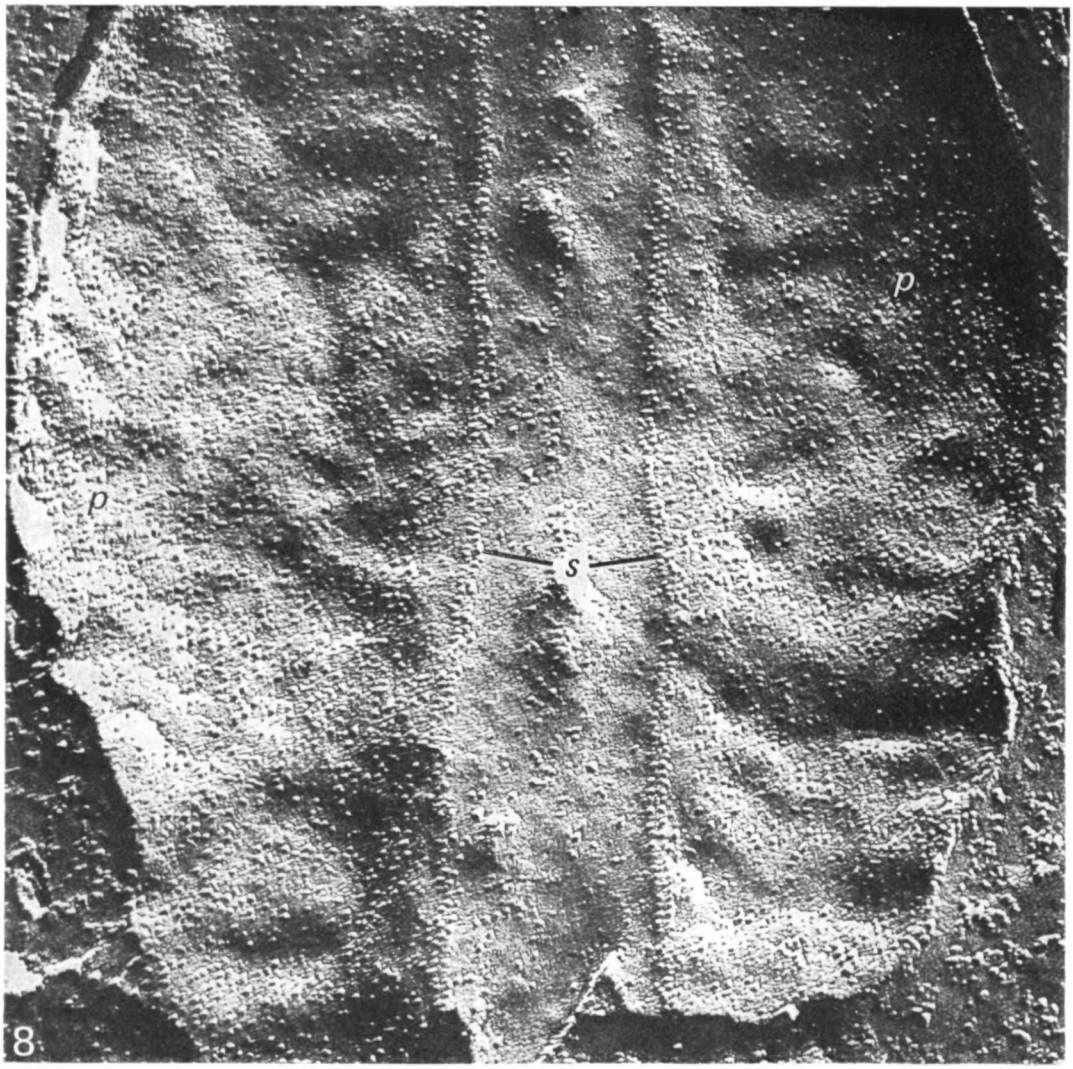


Fig. 8. P face of 2 adjacent membrane pockets (*p*). The particle seams (*s*) bordering 1 edge of each pocket are visible. The particle density in the pocket membrane is ~2 × that in non-junctional regions (centre strip between seams). × 96 000.

most devescovinids (Figs. 6, 9). Since the protozoa are not dividing, the bacteria evidently reproduce *in situ* throughout the cell cycle of the host. The various junctional specializations of the devescovinid membrane are reproduced in parallel with the division of the bacteria (Figs. 6, 9).

Fusiform bacterial junctions

The less-conspicuous fusiform bacteria are attached along their length to ridge-like elevations of the host surface (Fig. 2). The membrane ridges are underlain by electron-dense material which is less noticeable than the coating of the membrane pockets

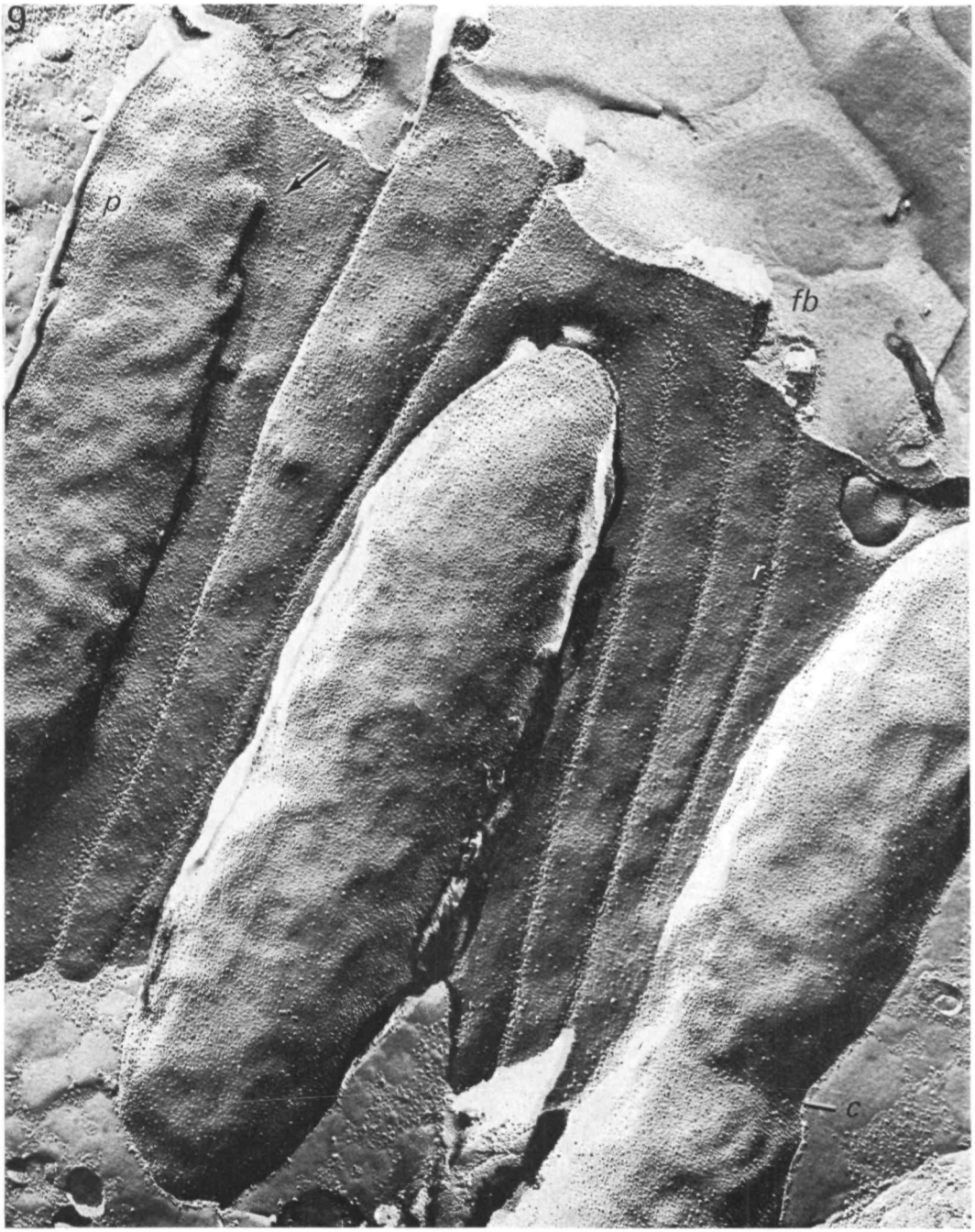


Fig. 9. E face of the body membrane. Particles are aggregated under the ridges (*r*) to which fusiform bacteria (*fb*) attach. Note the absence of depressions complementary to the particle seam at the edges of the membrane pockets (*p*) (arrow). The elongated pocket at the lower right has a constriction (*c*), indicating replication of the junctional complex in parallel with the division of the enclosed rod bacterium, as shown in Fig. 6. × 47000.

surrounding the rod bacteria (Figs. 3, 4). Freeze-fracture replicas reveal aggregations of 7–11 nm diameter particles along both P and E faces of these ridges (Figs. 6, 9).

Opposite to the surface ridge of the protozoon, the outer membrane and glycocalyx of the fusiform bacterium are indented to form a matching groove (Figs. 3–5). This complementary configuration resembles a tongue-and-groove joint, and is probably an

Table 1. *Surface specializations of protozoon and bacteria at junctional complexes*

Devescovinid ..... Rod bacteria		Devescovinid ..... Fusiform bacteria	
Membrane pocket	Glycocalyx and flagella absent	Membrane ridge	Groove in outer membrane and glycocalyx
Dense coat		Dense underlying material	
Particle aggregation		Particle aggregation	
Particle 'seam'			

adaptation for attachment (see Discussion). The fusiform bacteria are completely covered by a glycocalyx; this layer does not appear modified at the junction but lies in close contact with the surface ridge of the host. As a result, the grooved outer membrane of the bacteria is separated from the ridged membrane of the protozoon by a uniform distance that corresponds approximately to the thickness of the intervening glycocalyx.

Dividing fusiform bacteria have not been observed in freeze-fracture replicas, but their greater length compared to the rod bacteria would decrease the chances of finding fission stages by this method.

Table I summarizes the thin-section and freeze-fracture findings on the various surface specializations exhibited by the 2 types of bacteria and the devescovinid at the junctions.

Bacterial attachment to membrane fragments

Devescovinids were disrupted by glycerination or detergent treatment, and the resulting pieces of cell membrane examined by electron microscopy. Both rod and fusiform bacteria remained attached in their characteristic pattern to the membrane fragments (Fig. 5).

The structural specializations at the sites of contact between prokaryotes and protozoon therefore provide firm mechanical anchorage for the bacteria.

DISCUSSION

In comparison to intercellular junctions, the nature of the junctions formed between prokaryotic and eukaryotic cells is not well understood. We therefore used a correlated thin-section and freeze-fracture approach to describe the attachment sites of 2 kinds of bacteria to the surface of a devescovinid flagellate from termites. The 2 prokaryotes were found to form structurally distinctive junctions with the eukaryote, and in each case the surface elements of both partners were modified at the sites of contact (Table 1).

The most obvious structural contribution of the protozoon to the junctions is its elaborate cortical sculpturing: deep invaginations surround each rod bacterium, while long ridges underlie the sites of attachment of the fusiform bacteria. The significance of the surface topography for attachment of the prokaryotes is discussed below.

Complex surface adaptations for accommodating bacteria are also found in *Mixotricha paradoxa*, a related flagellate from *Mastotermes darwiniensis*. Spirochaetes are attached to small depressions on the posterior side of regularly arranged projections (brackets) of the protozoon's surface, while rod bacteria lie in concavities on the opposite side of the brackets (Cleveland & Grimstone, 1964). Somewhat similar attachment sites for spirochaetes occur on the surface of *Pyrsonympha*, but in addition a striated rootlet – looking amazingly like a ciliary rootlet – extends from the point of attachment of the bacterium into the cytoplasm of the protozoon (Smith & Arnott, 1974; Smith *et al.* 1975).

Shallow pockets or cup-like depressions of the eukaryotic cell surface have been reported where bacteria attach to other insect gut flagellates (Ball, 1969; Cleveland & Grimstone, 1964; Hollande & Valentin, 1969; Kirby, 1941*a*), as well as to intestinal epithelial cells (Chase & Erlandsen, 1978; Neutra, 1979, 1980; Snellen & Savage, 1978; Wagner & Barrnett, 1974). In addition, ridge-like elevations of the host surface have been described at the sites of contact with adherent bacteria in other termite flagellates (Hollande & Carruette-Valentin, 1970, 1971; Lavette, 1969; Tamm & Tamm, 1976).

A common feature shared by most of these junctions and the bacterial-devescovinid junctions described here is the presence of dense, cytoplasmic material underlying the junctional membrane of the host. Membrane coats or dense plaques are also found at desmosomes and synaptic contacts in higher animals (Ellisman, Rash, Staehelin & Porter, 1976; Gilula, 1974; Heuser & Reese, 1977; McNutt, 1977; Staehelin, 1974).

Adhesive junctions, such as desmosomes, also possess a constant intercellular space, typically ~ 25–35 nm wide, containing dense filamentous material (Gilula, 1974; McNutt, 1977; Staehelin, 1974). Similar well-defined gaps, also spanned by filaments or dense material, are found at many prokaryotic–eukaryotic junctions, particularly those in which only one end of the bacterium is attached to the eukaryote (Cleveland & Grimstone, 1964; Hollande & Valentin, 1969; Smith & Arnott, 1974; Smith *et al.* 1975; Wagner & Barrnett, 1974). In several such end-on attachments, a 30–40-nm space, bridged periodically by filaments specific to the junction complex, separates the bacterial cell wall from the membrane of the eukaryotic cell (Hollande & Valentin,

1969; Wagner & Barrnett, 1974). In one case (Hollande & Valentin, 1969), this filamentous material exhibits a central dense stratum resembling that found in the interspace of spot desmosomes. Whether the extracellular material originates from the prokaryote or the eukaryote, or both partners, is not known. However, the marked resemblance of this feature of prokaryotic–eukaryotic junctions to desmosomes suggests a similar function.

In associations where the bacteria adhere along their length, the junctions often exhibit less obvious filamentous connexions between the prokaryote and eukaryote (Bloodgood & Fitzharris, 1976; Cleveland & Grimstone, 1964; Hollande & Carruette-Valentin, 1970, 1971; Lavette, 1969; Tamm & Tamm, 1976). In many of these cases, as in the fusiform bacterial-devescovinid association described here, the bacterial glycocalyx rests in direct contact with the junctional membrane of the host. Since no other structural connexion between prokaryote and eukaryote is evident, adhesion is evidently mediated by the (bacterial) glycocalyx alone.

The fusiform bacterial–devescovinid junctions described here have an additional feature which seems uniquely designed to promote the association. The outer layer of the bacterium is grooved to match the apposing surface ridge of the host. This tongue-and-groove configuration is probably a structural adaptation for adhesion. Similar ridge–groove joints have been reported between another devescovinid flagellate and adherent fusiform bacteria (Tamm & Tamm, 1976).

That the bacterial–devescovinid junctions serve mainly, if not exclusively, as attachment sites seems self-evident from the nature of the association. Further evidence that the junctions provide strong mechanical anchorage for the prokaryotes comes from the persistent adhesion of the bacteria to disrupted fragments of the host membrane. The junctional complexes are therefore considered to perform an adhesive function, analogous to the role played by desmosomes in higher organisms. Because of the morphological asymmetry of the prokaryotic–eukaryotic junctions, however, a better analogy may be to hemidesmosomes – i.e. the devescovinid would be comparable to the basal epithelial cell, and the outer layer of the bacteria to the extracellular lamina.

Freeze-fracture

Freeze-fracture replicas of the devescovinid membrane demonstrated specific arrays and/or distributions of intramembrane particles at the sites of contact with each type of bacterium. In this respect the prokaryotic–eukaryotic junctions resemble intercellular junctions in multicellular organisms.

The morphological similarities between desmosomes and prokaryotic–eukaryotic cell contacts, based on thin-section electron microscopy, were pointed out above. Our freeze-fracture images, however, reveal important differences between the bacterial–devescovinid junctions and desmosomes. The fracture faces of desmosomes either bear few particles (belt desmosomes), or, in the case of spot desmosomes, contain patches of irregularly shaped particles, often fibrillar in form and apparently unorganized (McNutt, 1977; Staehelin, 1974). In contrast, the particles seen in the

devescovinid membrane at the bacterial attachment sites are dimensionally uniform, and highly organized in the case of the particle seams.

These differences may be significant, since the fibrillar appearance of particles in fractured desmosomes is interpreted to represent transmembrane connexions which have been broken off and plastically deformed during the fracturing process (McNutt, 1977; Staehelin, 1974). Such differences do not necessarily rule out participation of the particles observed here in cell adhesion, but rather point up the uniqueness of the prokaryotic–eukaryotic cell contacts.

The double seam of closely spaced particles lining the edges of the membrane pockets may function in a novel way to promote attachment of the rod bacteria. The circumference of these surface invaginations remains constant, never closing completely over the bacteria, nor opening wider. The particle seam may stabilize this highly asymmetric configuration of the membrane by acting as a fixed aperture. By so doing, this particle array would help to retain the bacteria, as well as to ensure that the flagellated portion of their surface – which provides motility for the protozoon (see below) – is not covered over.

To date, the freeze-fracture method has been applied to only a few other prokaryotic–eukaryotic junctions. Chase & Erlandsen (1978) and Snellen & Savage (1978) found changes in intramembrane particle density in rat mucosal cell membranes at the attachment sites of filamentous bacteria. In contrast, a freeze-fracture study of the attachment sites of 2 kinds of bacteria to primate intestinal cells revealed no particle specializations in the host cell membrane, but particle arrays were found on the outer membranes of both types of bacteria (Neutra, 1979, 1980). In the present study, we did not determine whether specialized particle arrays also occurred in the outer membrane of the rod or fusiform bacteria at the junctions with the devescovinid.

An important corollary of this study of the bacterial–devescovinid junctions is that the intimate and highly specialized nature of the association provides strong justification for using the ectosymbiotic bacteria as visible markers to follow movements of the devescovinid plasma membrane (Tamm, 1976, 1979; Tamm & Tamm, 1974, 1976).

Relations between junctions and the type of symbiotic association

Bloodgood & Fitzharris (1976) considered it unlikely that junctional structures apparently specialized for promoting and maintaining prokaryote–eukaryote associations would evolve in either the protozoon or the bacteria if the association conveyed no selective advantage upon the partner elaborating the specialization. They argued that a partner which contributed a structural specialization to the junctional complex must therefore benefit from the association. These authors used this approach to classify the type of symbiotic relationships between bacteria and insect gut flagellates. Although examples were found in which one or the other partner provided structural specializations for attachment, Bloodgood & Fitzharris did not observe cases where both partners contributed to the formation of the junctional complexes.

Such associations, indicative of true mutualistic symbiosis, have been reported between adherent spirochaetes and *Pyrsonympha* (Smith & Arnott, 1974; Smith *et al.*

1975), and possibly in several other cases (Hollande & Valentin, 1969; Wagner & Barrnett, 1974). The junctional complexes described here are additional examples of participation between surface elements of both the bacteria and the eukaryote (Table 1), and thus may indicate a mutualistic symbiosis. The presumed advantage conferred upon the bacteria by this association is not understood. However, recent work using selective inhibitors of prokaryotic vs. eukaryotic motility has shown that the flagellated rod bacteria provide locomotion for the devescovinid (Tamm, 1978*c*, manuscript in preparation), analogous to the spirochaete motility-symbiosis in *Mixotricha* (Cleveland & Grimstone, 1964). It is also possible that the prokaryotes provide other functions for the host, such as cellulose digestion (cf. Bloodgood & Fitzharris, 1976) or nitrogen fixation (Breznak, 1975). The junctions may therefore serve other purposes besides adhesion, and a role in metabolic or physiological coupling between the partners cannot be ruled out by ultrastructural comparisons alone.

Control of junction assembly

The positional information that specifies the location and spatial organization of cell junctions is unknown. The bacterial–devescovinid association provides some insight into this problem. As noted above, the bacteria reproduce *in situ* on the surface of the host. The various junctional structures contributed by the protozoon, including the specialized arrays of intramembrane particles, are reproduced in parallel to the division of the bacteria (cf. Figs. 6, 9).

Of the 2 concurrent processes, bacterial reproduction and junctional replication, the former seems more likely to be the primary causative event. If so, the sites and arrangement of the junctional complexes would be determined by the reproductive pattern of the attached prokaryotes. The positional information for the assembly of the junctional membrane specializations of the protozoon may thus be provided by the bacteria themselves – much like the pattern of cortical inheritance in ciliate Protozoa (Sonneborn, 1970). This hypothesis could be tested by determining the effects of inhibiting bacterial growth on the formation of junctional specializations by the protozoon.

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