

Chapter 5

CELL ATTACHMENT MECHANISMS IN THE FLAGELLATE, COLACIUM (EUGLENOPHYCEAE)

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5.1 INTRODUCTION

Fouling is the opportunistic colonization by sessile organisms in aquatic systems of a man-made or man-provided substrate with subsequent succession into a community which may affect man's economic interests. The character of the resulting community will be determined by the nature of the substrate, the availability and diversity of colonists, the efficiency of their attachment to the substrate, and the biotic/abiotic events (often man-directed) occurring during and after colonist attachment. If we can set aside the anthropocentric focus involved in the study of fouling, we will see that the process and problems involved are not unlike those experienced by a substrate organism in relation to an epibiont. The host provides a substrate surface and the epibiont is the fouling colonist. The consequences of epibiosis to small, motile substrate organisms, such as aquatic arthropods, can be severe in terms of predator avoidance, maintenance of position in the water column (for zooplankton), and feeding and respiratory movements - namely their economic interests. From the point of view of the epibiont, the substrate organism may represent just another surface, or it may represent, an important source of nutrients and the association may coevolve into a highly integrated system (e.g., mutualism or parasitism). In epibiosis, the resulting community is simpler than that of a ship bottom or a wharf piling. In fact, it may be represented by a single species. However, we can assume that even this reduced community of one will be controlled by the same parameters of colonist dispersal, attachment mechanisms, maintenance, reproduction and extinction, as pertains to fouling communities.

Euglenoid flagellates of the genus Colacium Ehrenberg are typically epibionts on freshwater arthropods. We have become interested in the attachment process as a determinant of attachment success as well as host selectivity. Cells of Colacium libellae Rosowski & Willey, normally found in the hindguts of odonate larvae, readily respond, in culture, to artificial substrate surfaces. Laboratory observations have determined that they have efficient adaptations for establishing, maintaining, and breaking contact with their substrate organisms

(Killen et al., 1984). We present the behavioral sequence of cell attachment in Colacium and an histochemical analysis of the several bridging polymers involved. Studies of Colacium should contribute significantly to the understanding of the attachment mechanisms of flagellated organisms, some of which are found as early stages in the development of fouling communities.

5.2 MATERIALS AND METHODS

5.2.1 Culture of algae

Monoalgal cultures of Colacium libellae (Rosowski and Willey, 1975), Warren clone number 15 (W-15) and Peshtigo clone number 1 (P-1), were maintained in glass Petri dishes containing soil-water-pea extract (Starr, 1964) with 10% Alga-Gro (Carolina Biological Supply, Burlington, North Carolina, U.S.A.). Cultures were maintained at room temperature (approximately 25°C) under a 1000 lux Gro-light on a 14:10 hr (day:night) cycle. Specimens used in this study were originally isolated in 1972 from the hindgut of damselfly larvae obtained from ponds at the Warren Dunes State Park, Warren, Michigan, and in 1983 from a damselfly larva obtained from a sand quarry on the southern edge of Peshtigo, Wisconsin, U.S.A..

5.2.2 Light microscope preparations

Whole mount preparations were made by placing a drop of cell culture on a clean coverslip for approximately 30 minutes to ensure the presence of all attachment stages (see Killen et al., 1984). The cultures used were 6 to 7 days old (near the end of log phase growth) and the preparations were timed so that the cells would be observed or fixed at approximately 1400 hours (0800 CT). Fixatives used were: 4% glutaraldehyde (GA) in 0.1M cacodylate buffer, pH 6.5 and 7.2; or a mixture of GA plus 1% alcian blue 8GX (AB) (Polysciences, Warrington, Pennsylvania, U.S.A.) after Edgar and Pickett-Heaps (1982). Preparations were stained in various combinations with the periodic acid-Schiff procedure (PAS) (Willey et al., 1977) and 1% AB in 3% acetic acid (Pearse, 1968). Acetic-aniline and sodium borohydride were used initially as blocking agents for the PAS reaction to mask aldehydes resulting from fixation in GA. However, since non-specific staining turned out to be negligible, the use of these blocking agents was discontinued. Control slides for the PAS reaction substituted distilled water for the periodate oxidation. Dehydration in ethanol and mounting in resin caused a severe reduction in AB staining. Therefore, preparations were routinely mounted directly after washing in distilled water with Dow-Corning Silicone Oil #710, a nonorganic medium with a refractive index of 1.533 (Zugibe and Fink, 1966). Cells were prepared both at room temperature and after being chilled in an ice bath.

5.2.3 Enzyme extraction

Protein content of the attachment structures was assessed by reduction in stainability and loss of structure after treatment, up to 36 hours, with trypsin (Sigma, St. Louis, Missouri, U.S.A.) (0.1 mg/ml, 1.0 mg/ml and 10 mg/ml) in 0.1M tris-maleate buffer with 1 mM CaCl_2 at pH 8.0 and with pronase (Sigma, St. Louis, Missouri, U.S.A.) (10 mg/ml) in the same buffer at pH 7.0, at room temperature and at 37°C. Coverslips were sputter-coated with approximately 60 Å gold/palladium and scored in a grid pattern so individual cells could be located repeatedly. Hanging drop preparations were made, cell number, arrangement, and general condition were recorded and individual attachment positions were noted. The culture medium was drained off, replaced first with buffer solution and then with the requisite enzyme-buffer solution. The cells were photographed at 15 min, 30 min, and 1, 24, and 36 hr intervals.

5.2.4 Scanning electron microscope (SEM) preparations

Drops of cells were placed on coverslips coated with 60 Å gold/palladium. After 5, 10, 15 and 30 min, most of the coverslips were flooded with the 1% AB in 4% GA in cacodylate buffer at pH 6.5 at room temperature or at 4°C, and then immediately flooded with 1% OsO_4 in the same buffer for 30 min in the dark (Edgar and Pickett-Heaps, 1982). Some coverslip preparations were fixed in a mixture of 2% GA and 0.5% osmium tetroxide in 0.05M cacodylate buffer at pH 7.2 (Willey, 1984). After dehydration in ethanol, the coverslips were critical point-dried (Bomar SPC-50/LX) and sputter coated with 12 nm gold (Technics Hummer Jr.). Individual cells were examined with an ISI DS-130 SEM at an accelerating voltage of 10-20 kV.

5.2.5 Transmission electron microscope (TEM) preparations

Cells for TEM examination were fixed in either of the two fixatives for SEM, pelletized by centrifugation, embedded in BSA, and processed according to Willey (1984).

5.3 RESULTS

Cells of Colacium libellae follow a basic attachment process involving: (1) a long range translocation (migration into the immediate vicinity of the substrate organism), (2) a subsequent short range translocation (swimming close to and subsequent orientation to the host surface), (3) initial surface contact, and (4) a permanent (more than 24 hrs) attachment mediated by a flexible stalk (Fig. 5.1). Our observations presented here focus on the last two parts of the process which are specifically associated with bridging polymer formation.

Cells undergoing attachment, when close to the substrate, abruptly change their swimming patterns so that they spiral rapidly in place with the anterior end very close to the surface. The flagellum appears to be coiled about the cell and generates a spinning movement which may last several seconds. Initial contact occurs when the spiral maneuver abruptly ceases. The flagellum becomes

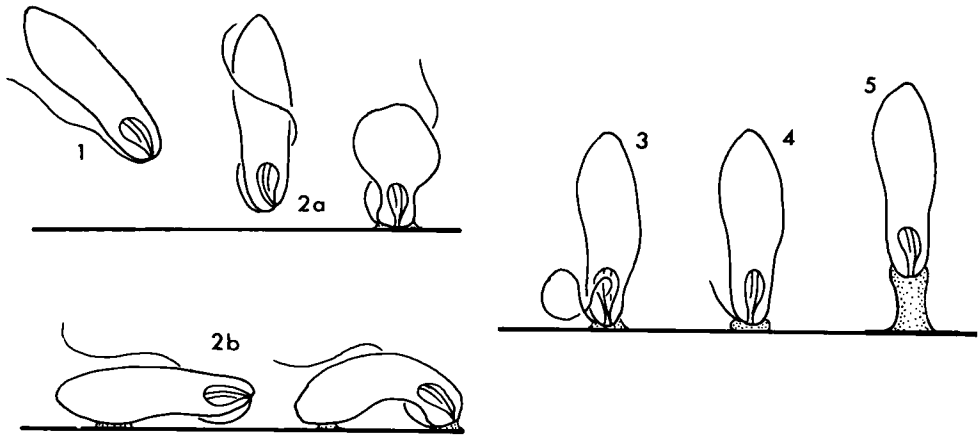


Fig. 5.1. Diagrammatic representation of the basic attachment process of *Colacium* involving: (1) short range translocation, (2) orientation and initial contact with the formation of the AD, (3) orientation of the anterior pole of the attached cells towards the substrate accompanied by uncoordinated movements of the flagellum, (4) resorption of the flagellum, and (5) formation and elongation of the stalk. During initial contact, cells may orient normal to the surface with a characteristic, spiralling swimming movement just before initial contact (2a) or may swim in at an angle to the surface and make initial contact along the side of the cell with the lateral AD (2b). In the latter case, the cell must establish a second contact by its anterior pole with a terminal AD before the subsequent flagellar resorption (3,4) can occur.

uncoordinated and, over the succeeding 20 to 30 min, is resorbed into the cell. Further details of this process have been published by Killen et al. (1984).

Early studies with the SEM had difficulty demonstrating the extent of polymer involvement (see Killen et al., 1984). However, the incorporation of the phthalocyanin dye, alcian blue 8GX, with the fixative (after Edgar and Pickett-Heaps, 1982) provided excellent SEM images of, first, an adhesion disc (AD) bridging the space between the cell and substrate and, next, a stalk which may branch and subsequently support many cells (Fig. 5.2).

The AD appears to be formed by small strands of polymer extruded from the anterior pole of the cell. The points of extrusion coincide with pores between the pellicular strips. These pores are the secretion channels for biphasic granules (Fig. 5.3) which are specialized mucocysts. In alcian blue preparations of chilled cells for the light microscope, all stages of the extrusion process are clearly delineated from the appearance of blue product in focal spots (at the pores) to the fully formed AD (Fig. 5.4).

The above described process of cell attachment (Fig. 5.1; steps 1, 2a, 3-5) is typical of all populations of *C. libellae* observed. However, in clone P-1, a variation of the normal procedure was commonly observed. The cells frequently

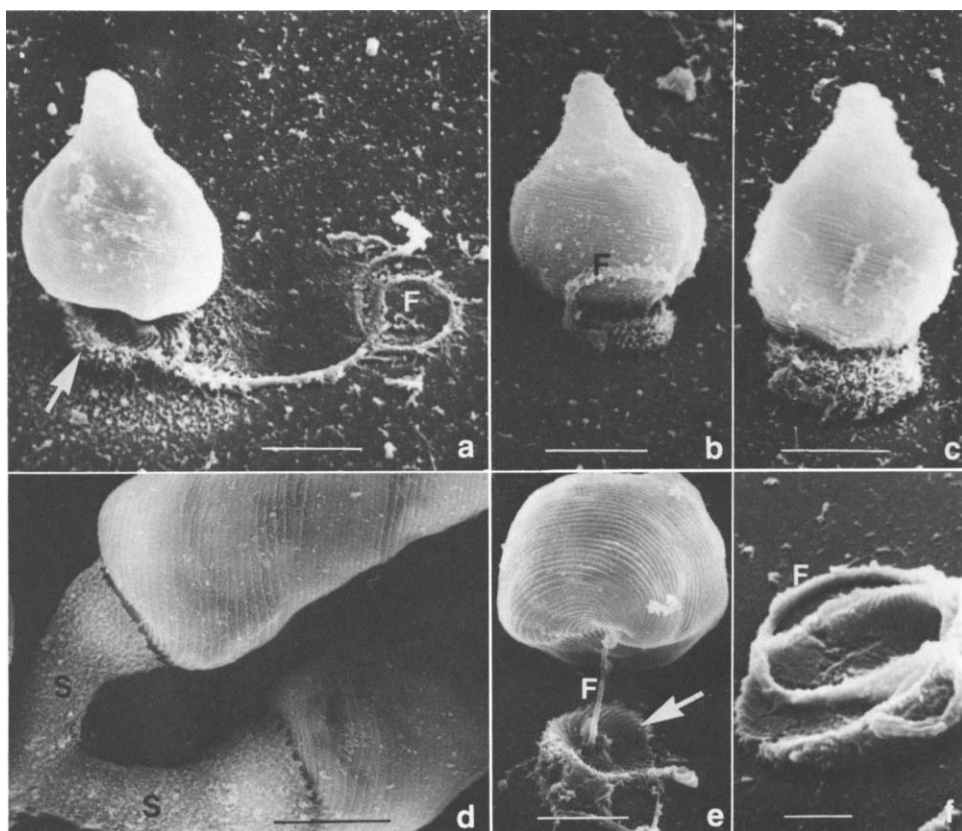


Fig. 5.2. Scanning electron micrographs of selected stages in the attachment process of *C. libellae*. (a) Initial contact is accompanied by the extrusion of an AD (arrow) which mediates contact between the anterior pole of the cell and the substrate. Flagellum (F). Scale = 5 μm . (b) The flagellum (F) is resorbed after initial contact. Scale = 5 μm . (c) At the end of the resorption step, the emergent flagellum has been shortened so that it occurs only in the reservoir. Scale = 5 μm . (d) After flagellar resorption, a stalk (S) is extruded from the anterior end of the cell. When the cell divides, each daughter cell continues to secrete stalk material creating a colony held together by a branched stalk system. Scale = 5 μm . (e) The AD (arrow) exhibits strong cohesion and adhesion to the substrate. Adhesive failure occurs relatively easily at the cell-adhesive interface. The flagellum (F), in this preparation, maintains contact of the cell with the AD. Scale = 5 μm . (f) During processing, cells frequently break free from the AD leaving flagellum (F) and AD attached to the substrate. Scale = 2 μm .

swim straight toward the substrate surface at a low angle without utilizing the spiral maneuver (Fig. 5.1; steps 1, 2b, 3-5). They make initial attachment by the side, rather than the anterior end, of the cell - a "pancake" landing. Observations with the SEM revealed pores located over the pellicular surface in

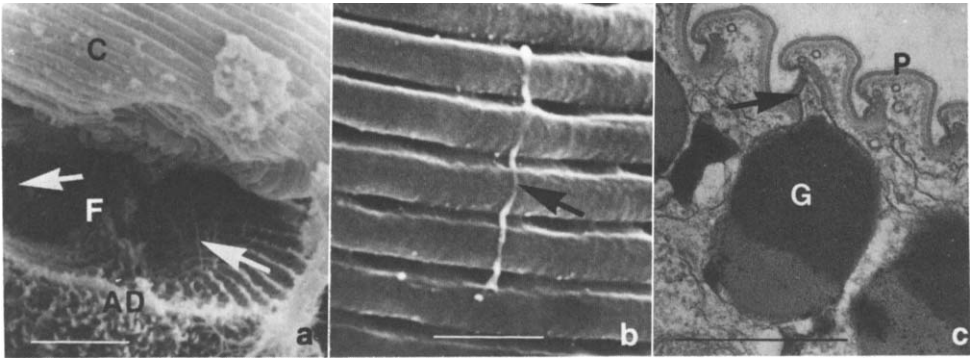


Fig. 5.3. The AD is extruded from the anterior end of the cell. (a) Strands of polymer (arrows) extend from the pellicular surface to the AD in this preparation in which the cell is only slightly disturbed by processing. Cell (C), flagellum (F). Scale = 2 μm . (b) Pores in the pellicle of the anterior end of the cell appear to secrete polymer (arrow) which will form the AD. Scale = 0.5 μm . (c) Biphasic granules (G) lie directly under the pellicle (P) at the anterior pole of the cell. Tubular extensions from the granules coincide with the pellicular pores (arrow). Scale = 0.5 μm .

ADHESION DISC FORMING

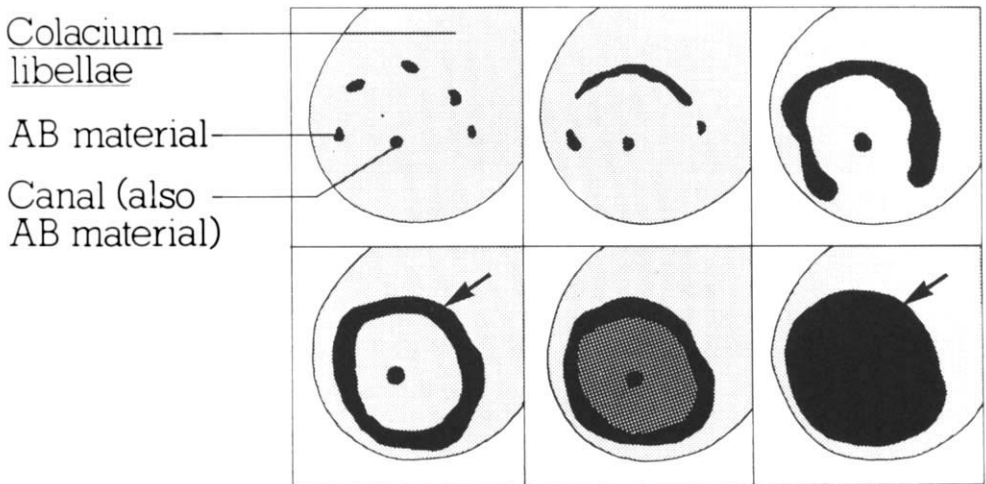


Fig. 5.4. The AD (arrows) is formed by alcian blue-staining material extruded from the anterior pellicular pores.

addition to the anterior end. Subpellicular mucocysts associated with these pores are somewhat different ultrastructurally from the biphasic granules in the same cell (Fig. 5.5). The product within the granule has a distinctly different pattern and characteristically is much more osmiophilic. In clones

TABLE 5.1

Qualitative results of histochemical reactions (+ = positive reaction; - = negative reaction).

Bridging Polymer	Source	Stain Reaction	
		AB	PAS
AD (anterior)	biphasic granules	+++	-
AD (lateral)	pellicular mucocysts	+++	- (?)
stalk - core	reservoir	+	+++
- periphery	biphasic granules	+++	+

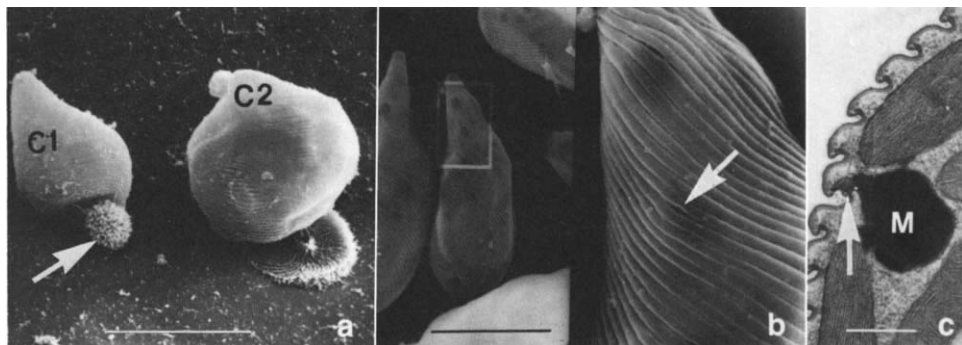


Fig. 5.5. Cells in clone P-1 of *C. libellae* can make the initial contact by the side of the cell. (a) The cell on the left (C1) has attached by a lateral AD. The spherical mass of anterior AD material (arrow) may have been extruded in response to the fixative. Each cell must, however, establish contact by the anterior AD before the attachment process can proceed (C2). Scale = 10 μ m. (b) Cells which are capable of lateral initial contact exhibit pellicular pores (arrow) along the side as well as the anterior end of the cell. The image on the right is a 5X magnified view of the area boxed in the cell image on the left. Scale = 10 μ m. (c) Mucocyst-like granules (M) lie under the lateral pellicular pores (arrow) and, like the biphasic granules, are attached by tubular extensions (arrow). Scale = 1 μ m.

in which the cells primarily utilize the spiral maneuver, the pellicular mucocysts are rarely observed. Regardless of the type of approach (2a or b), the cells have to, ultimately, establish contact by the anterior pole of the cell and its AD (step 3 in Fig. 5.1). Only then do the cells initiate flagellar resorption and complete the attachment process.

The AD and the stalk exhibited different reactions with the histochemical stains (Table 5.1). The adhesion discs, from both types of mucocyst, stain dark blue with alcian blue if it is incorporated in the fixative (Fig. 5.6). Fixation without alcian blue did not retain sufficient polymer to react with subsequent stain procedures. Alcian blue also reacts with the stalk, but only gives a strong reaction with the peripheral part (the "cortex" of Ward and

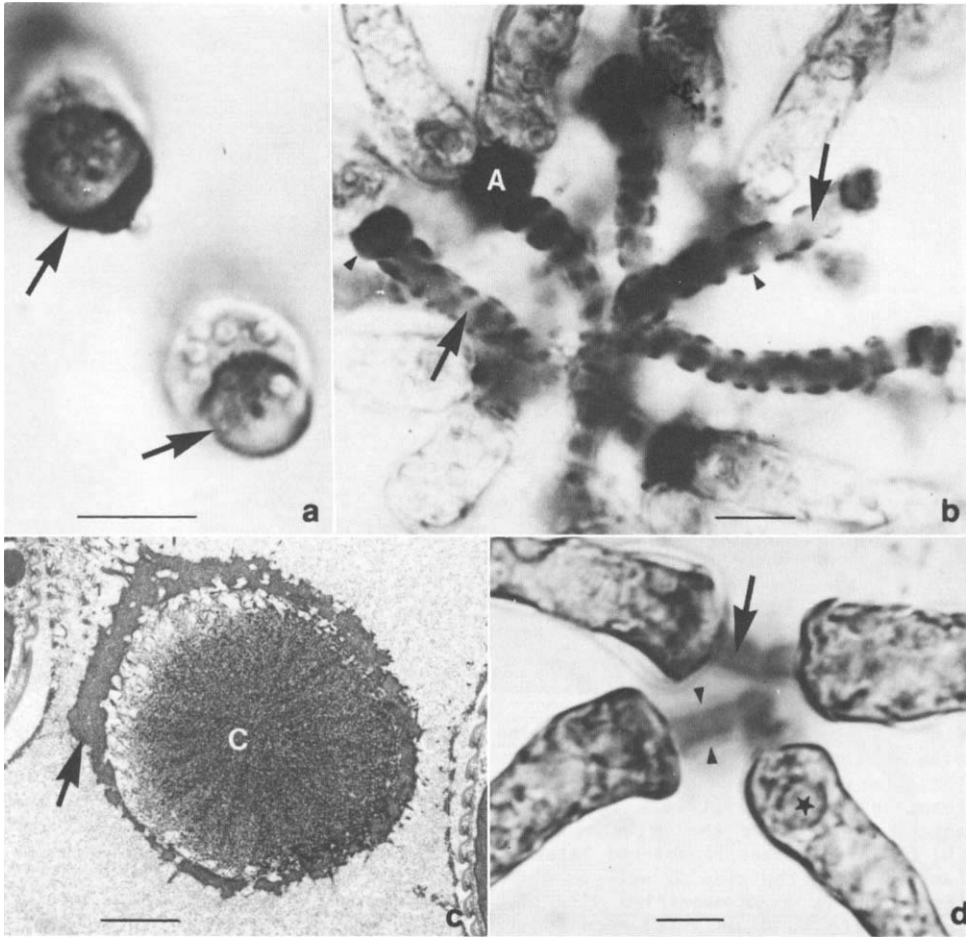


Fig. 5.6. Attachment by *C. libellae* involves two different polymers. (a) The adhesion discs (arrows) stain intensely with alcian blue. These cells were exposed for 36 hrs to the tris-maleate buffer used as a control for the trypsin extraction. Scale = 10 μ m. (b) The stalk reacts strongly with alcian blue on its periphery (arrowheads) and with PAS in its central core (arrows). The alcianophilic material (A) around the cell canal probably represents AD material which continues to be secreted during stalk formation. Note that the AB-staining patches of the stalk periphery are largest closest to the cell and are smaller (older) farther away from the cell. The AB-staining material appears to dissolve more rapidly than that of the stalk core. Scale = 10 μ m. (c) Cross-section of a stalk fixed with 1% AB in GA followed by OsO_4 delineates the peripheral alcianophilia (arrow) and the core part which reacts with PAS (C). Note the radiating pattern of dense fibrillar material in the core which corresponds to the pattern of strips forming the pellicle around the canal. Scale = 1 μ m. (d) The stalk stained with PAS reacts most intensely in the core (arrow) whereas the peripheral portion (arrowhead) reacts only slightly. The reservoir (*) also contains PAS-reacting material. Scale = 10 μ m.

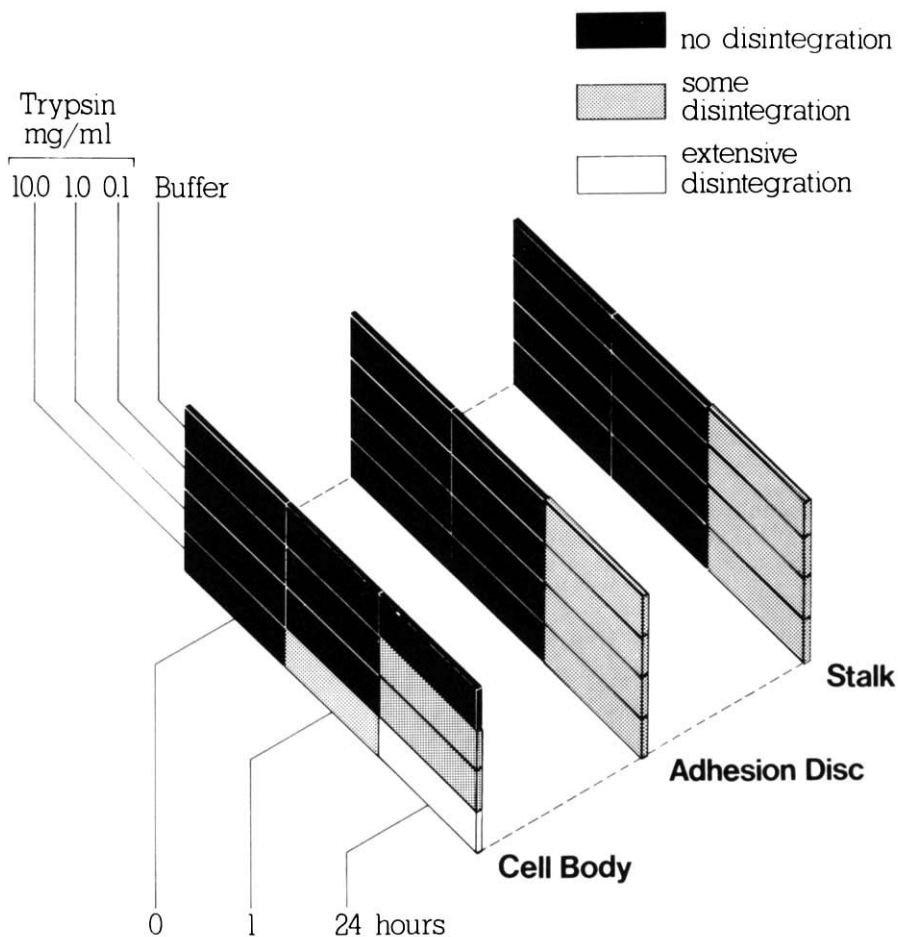


Fig. 5.7. Graphic representation of the response of cell body, AD, and stalk to enzyme degradation with trypsin. Pronase gives similar results.

Willey, 1981) and with freshly formed stalk directly around the canal opening. The peripheral part is structurally distinct from the rest of the stalk. The central portion of the stalk characteristically reacts with PAS (Fig. 5.6). We observed no reaction of the AD with PAS, but we frequently observed red staining material in the reservoir.

Extraction of whole mount preparations with trypsin is summarized in Fig. 5.7. The results with pronase are essentially the same. The AD and stalk are resistant to these proteases under conditions in which the whole cell is digested away (Fig. 5.8).

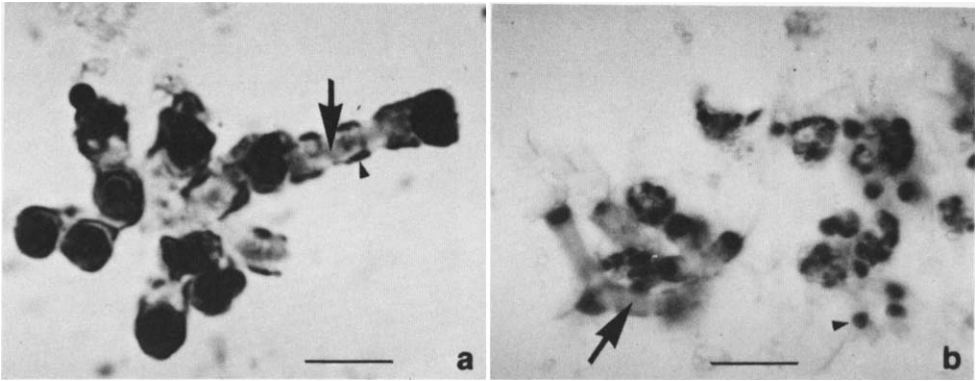


Fig. 5.8. The stalk and AD are resistant to protease digestion. (a) After 36 hr exposure to 10 mg/ml trypsin, the cells have disintegrated leaving behind only the stalk (stained with AB) with its periphery (arrowhead) and core (arrow) structurally intact. Scale = 10 μ m. (b) After 33 hr exposure to 10 mg/ml pronase, the AD (arrowhead) and stalk (arrow) are structurally intact. Control cells are illustrated in Fig. 5.6. Scale = 20 μ m.

5.4 DISCUSSION

The general pattern of attachment by cells of Colacium is not unique. The same general steps have been outlined for the association-specific behavior patterns of parasites (Whitfield, 1979) as well as, in part, for zoospore attachment by Characium and the ship-fouling alga, Enteromorpha (Evans and Christie, 1970; Lee and Bold, 1974). Perhaps the most complete investigations of attachment have focussed on bacteria (see Marshall, 1984) and most of the theoretical work has been carried out on this latter system.

Organisms as small as Colacium have a very small Reynolds number (Gittelsohn, 1974). They will be affected by hydrodynamic and electrostatic forces at the substrate's boundary layer. Relatively little is known of the effect of these forces on eukaryotic flagellates (see Charters et al., 1973). Presumably the spiralling behavior and/or the pancake landings of Colacium are both in response to a turbulent boundary layer on the surface of an active substrate organism. Why some clones should exhibit two methods of penetrating this layer and other clones have only one cannot be explained with the existing data.

The cells of Colacium make initial contact with the pellicular surface at the anterior pole of the cell even though the emergent flagellum is anteriorly located. This behavior differs from flagellated cells which initiate contact with the flagellum itself. These latter cells may then be drawn to the substrate surface during flagellar resorption (e.g., zoospores of Chorda tomentosa; Toth, 1976), remain attached by the flagellum itself (e.g.,

Trypanosoma vivax; Vickerman, 1973), or glide along the surface mediated by a flagellar surface glycoprotein (e.g., Chlamydomonas; Bloodgood and Workman, 1984). However, in species of Characium, the zoospores, like Colacium, attach directly by their anterior poles. The zoospores of Pseudocharacium americanum, on the other hand, attach by their flagellar tips (Lee and Bold, 1974). It is not clear how the fouling alga, Enteromorpha, makes initial attachment. However, since spiral swimming which is oriented perpendicular to the surface is typical of Characium, and not of Pseudocharacium, it appears likely that the zoospores of Enteromorpha, which also undergo the spiralling approach (see Evans and Christie, 1970), also attach directly by the anterior cell surface.

The maintenance of cell-substrate contact by one or more types of bridging polymers is well known in microbial systems (Sutherland, 1983). Two basic types of polymers appear to mediate the attachment process - an initial, low viscosity (Stefan) adhesive and a subsequent, more permanent adhesive (Crisp, 1972). In algal cells, the adhesive polymers have been shown generally to be polysaccharide-protein complexes (Chamberlain, 1976). Differences in physical character of the polymer at different stages in the attachment process have been attributed to the chemical transformation of the externalized polymer over time (e.g., Enteromorpha zoospores; Christie et al., 1970), or the secretion of two separate polymers (e.g., Ulva zygotes; Bråten, 1975). The cells of Colacium appear to have the ability to externalize different compounds separately. The AD secreted by the biphasic granules is histochemically distinct from the core of the stalk which appears to be extruded through the canal from the reservoir (see Fig. 5.6).

The lateral AD associated with the pellicular mucocysts of clone P-1 reacts strongly with AB just as does the anterior AD. The granules are ultrastructurally and positionally distinct and, therefore, we cannot ignore the possibility that they also may prove to extrude a third, biochemically distinct, polymer. Euglenoids, in general, also secrete a mucilage which is often associated with slime trails and euglenoid movement from specialized channels of the ER - the muciferous bodies (Leedale, 1967). Muciferous body product does not yet appear to have a role in attachment of Colacium. However, its possible role in attachment, particularly in relation to gliding behavior, cannot be totally disregarded either. Therefore, the cells of Colacium have the potential of producing four separate compounds through four different organellar routes. Such biosynthetic diversity may have contributed to the success of these cells in attaching to active, moving substrate organisms such as freshwater arthropods.

The reactions of the AD and stalk with AB and PAS strongly indicate a major polysaccharide component (Pearse, 1968). However, the AB was buffered at pH 6.5 because it had to be incorporated in the fixative. Polyanions reacting

with AB at this pH could be sulfate or carboxylate forms of acid polysaccharide or could be the carboxyl groups of aminoacids (Behnke and Zelander, 1970). Therefore, enzymatic extraction of the bridging polymers with trypsin and pronase was important in determining not only the general presence of protein but also the polysaccharide or proteinaceous identity of the AB-reacting polymers. Both trypsin and pronase had little, if any, effect on the structural integrity or stain reactivity of the stalk or the AD (see Figs. 5.7, 5.8). We, therefore, presume that these two polymers, at least, are primarily polysaccharide in composition. We have no comparable observations on the lateral AD or the muciferous body product.

We propose that the polyanionic AD (either anterior or lateral) represents a rapidly extruded polymer of relatively high solubility (a Stefan adhesive) which "tacks" the cell to its substrate during the initial step in attachment. The major stalk material is primarily a neutral polysaccharide (PAS-reactive) of relatively high flexibility, tensile strength and low solubility. It effectively maintains adhesion of the cell to actively moving substrate organisms. The cortical stalk material which reacts strongly with AB probably represents AD material which continues to be secreted by the biphasic granules during stalk elongation. Whether these polyanions contribute in any way to stalk characteristics is unknown.

Most algal adhesives studied have been shown to be polysaccharide-protein complexes of considerable variability with some part of their biosynthesis channeled through the Golgi apparatus (Christie et al., 1970; Chamberlain, 1976). In C. calvum, the biphasic granules, also, have been attributed a Golgi origin (Willey, 1984). The lateral mucocysts may prove to be formed, in part, by Golgi vesicles (unpublished observations). The Golgi of Colacium cells, therefore, exhibits considerable synthetic versatility. Components of at least two types of polymer appear to be produced at different times or by separate Golgi organelles. A similar versatility has been suggested for Golgi function in Ectocarpus (Baker and Evans, 1973).

The basic steps of attachment of diverse flagellated cells are probably quite similar - approach, orientation, with initial and permanent attachment utilizing a bridging polymer. However, the mechanisms for each of these steps are relatively diverse - even within the same cell system. The attachment mechanisms of the flagellated cells of Colacium reflects this versatility in that there appear to be: (1) two different approach mechanisms, (2) two different initial adhesive systems, and (3) two different types of adhesive for initial and permanent contact. The cells of Colacium must attach, and remain attached, to the active, moving surfaces of their substrate organisms. The diversity of approaches and polymers may reflect the adaptations to the rigorous circumstances under which attachment must be achieved. The basic

behavior pattern appears to be predictable, however, and we have some evidence that it can be manipulated. Therefore, we are exploring the various factors (biotic and abiotic) which contribute to attachment success of cells of Colacium libellae by determining what factors alter their behavior. We hope that some of our findings will contribute to the understanding of fouling systems.

REFERENCES

- Baker, J.R.J. and Evans, L.V., 1973. The ship fouling alga Ectocarpus. I. Ultrastructure and cytochemistry of plurilocular reproductive stages. Protoplasma, 77: 1-13.
- Behnke, O. and Zelander, O., 1970. Preservation of intercellular substances by the cationic dye alcian blue in preparative procedures for electron microscopy. J. Ultrastr. Res., 31: 424-438.
- Bloodgood, R.A. and Workman, L.J., 1984. A flagellar surface glycoprotein mediating cell-substrate interaction in Chlamydomonas. Cell Motility, 4: 77-87.
- Bråten, T., 1975. Observations on mechanisms of attachment in the green alga Ulva mutabilis Foyn. Protoplasma, 84: 161-173.
- Chamberlain, A.H.L., 1976. Algal settlement and secretion of adhesive materials. In: J.M. Sharpley and A.M. Kaplan (Editors), Proc. Third International Biodegradation Symposium. Applied Science Publishers Ltd., London, pp. 417-432.
- Charters, A.C., Neushul, M. and Coon, D., 1973. The effect of water motion on algal spore adhesion. Limnol. Oceanogr., 18: 844-896.
- Christie, A.O., Evans, L.V. and Shaw, M., 1970. Studies on the ship-fouling alga Enteromorpha. II. The effect of certain enzymes on the adhesion of zoospores. Ann. Bot., 34: 467-482.
- Crisp, D.J., 1972. Mechanisms of adhesion of fouling organisms. Proc. 3rd International Congress of Marine Corrosion and Fouling. National Bureau of Standards, Gaithersburg, MD, U.S.A.: 691-699.
- Edgar, L.A. and Pickett-Heaps, J.D., 1982. Ultrastructural localization of polysaccharides in the motile diatom Navicula cuspidata. Protoplasma, 113: 10-22.
- Evans, L.V. and Christie, A.O., 1970. Studies on the ship-fouling alga Enteromorpha. I. Aspects of the fine-structure and biochemistry of swimming and newly settled zoospores. Ann. Bot., 34: 451-466.
- Gittelsohn, S.M., 1974. Flagellar activity and Reynolds number. Trans. Am. Microsc. Soc., 93: 272-276.
- Killen, R.P., Willey, R.L. and Durum, F.A., 1984. Docking behavior of Colacium libellae (Euglenophyceae): Cell-substrate adhesion and flagellar resorption. Trans. Am. Microsc. Soc., 103: 67-73.
- Lee, K.W. and Bold, H.C., 1974. Phycological studies. XII. Characium and some Characium-like algae. Univ. of Texas Publication No. 7403, Austin, Texas.
- Leedale, G.F., 1967. Euglenoid Flagellates. Prentice-Hall, Inc., Englewood Cliffs, NJ, 242 pp.
- Marshall, K.C. (Editor), 1984. Microbial Adhesion and Aggregation. Dahlem Workshop Life Sciences Research Report, 31. Springer-Verlag, Berlin/Heidelberg/New York/Tokyo, 423 pp.
- Pearse, A.G.E., 1968. Histochemistry: Theoretical and Applied. Williams and Wilkins, Baltimore, 759 pp.
- Rosowski, J.R. and Willey, R.L., 1975. Colacium libellae sp. nov. (Euglenophyceae), a photosynthetic inhabitant of the larval damselfly rectum. J. Phycol., 11: 310-315.
- Starr, R.C., 1964. The culture collection of algae at Indiana University. Amer. J. Bot., 51: 1013-1044.

- Sutherland, I.W., 1983. Microbial exopolysaccharides - their role in microbial adhesion in aqueous systems. *CRC Critical Reviews in Microbiology*, 10: 173-201.
- Toth, R., 1976. The release, settlement and germination of zoospores in Chorda tomentosa (Phaeophyceae, Laminariales). *J. Phycol.*, 12: 222-233.
- Vickerman, K., 1973. The mode of attachment of Trypanosoma vivax in the proboscis of the tsetse fly Glossina fuscipes: an ultrastructural study of the epimastigote stage of the trypanosomes. *J. Protozool.*, 20: 394-404.
- Ward, K.A. and Willey, R.L., 1981. The development of a cell-substrate attachment system in a euglenoid flagellate. *J. Ultrastr. Res.*, 74: 165-174.
- Whitfield, P.J., 1979. *The Biology of Parasitism: An Introduction to the Study of Associating Organisms*. University Park Press, Baltimore, 277 pp.
- Willey, R.L., 1984. Fine structure of the mucocysts of Colacium calvum (Euglenophyceae). *J. Phycol.*, 20: 426-430.
- Willey, R.L., Ward, K., Russin, W. and Wibel, R., 1977. Histochemical studies of the extracellular carbohydrate of Colacium mucronatum (Euglenophyceae). *J. Phycol.*, 13: 349-353.
- Zugibe, F.T. and Fink, M.L., 1966. A new ion association fractionation technique for demonstrating polyanions in tissue sections. *J. Histochem. Cytochem.*, 14: 147-152, 153-158.