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Spine-Scale Reorientation in *Apedinella radians* (*Pedinellales, Chrysophyceae*): the Microarchitecture and Immunocytochemistry of the Associated Cytoskeleton

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Summary

Motile unicells of *Apedinella radians* have the extraordinary ability to instantaneously reorient six elongate spine-scales located on the cell surface. Extracellular striated fibrous connectors (termed microligaments) attach spine-scales to discrete regions of the plasma membrane underlain by intricate cytoplasmic plaques. A complex cytoskeleton is associated with the plaques and appears responsible for spine-scale movement. Three cytoskeletal proteins have thus far been identified by immunofluorescence using anti-tubulin, anti-actin, and anti-centrin. The three-dimensional configuration of the cytoskeleton has been established and consists of filamentous bundles of actin and centrin which form stellate systems interconnecting the plaques. Additionally, there is a network of microtubular triads which originate on the surface of the nuclear envelope and subtend the plasma membrane and also support several tentacular protrusions. It is proposed that contraction of the actin and/or centrin filamentous bundles is responsible for the reorientation of the spine-scales.

Keywords: Actin; *Apedinella*; Centrin; Cytoskeleton; Motility; Tubulin (Microtubules).

1. Introduction

The cytoskeleton of unicellular algae varies from simple ground matrices, which maintain basic cell shape, to highly complex three-dimensional structures. In many instances cytoskeletal components perform remarkable functions, and in some cases are the basis for the generation of motility (for review see GRAIN 1986).

Apedinella radians (Lohmann) Campbell [synonymous with *Apedinella spinifera* (Throndsen) Throndsen, THRONDSEN 1971] is a marine unicellular phytoflagellate of chrysophycean affinity assigned to the order *Pedinellales* (ZIMMERMANN *et al.* 1984). Swimming cells bear six elongate, cellulosic spine-scales (THRONDSEN 1971) which undergo a striking reorientation just prior to the cells changing direction. Although the general ultrastructure of the cells has already been established (THRONDSEN 1971), no detailed information on spine-scale movement or the mechanism responsible has been presented.

Observations of thin-sectioned cells as well as immunofluorescence studies using FITC-labelled antibodies have revealed the presence of three cytoskeletal components in *A. radians*; microtubules (tubulin), microfilaments (actin) and centrin filaments. The almost universal presence of tubulin and actin in eukaryotic cells has been extensively recorded in the literature, although the presence of centrin is a more recent discovery (SALISBURY *et al.* 1984, COLING and SALISBURY 1987). Centrin is an apparently ubiquitous 20 kD calcium-modulated contractile phosphoprotein usually associated with eukaryotic basal bodies (WRIGHT *et al.* 1985, SALISBURY *et al.* 1986, MCFADDEN *et al.* 1987, SCHULZE *et al.* 1987, MELKONIAN *et al.* 1988). As well as an amorphous region surrounding the basal bodies of *A. radians*, we have also found filaments forming a stellate network that are immunoreactive to the anti-centrin antibody and is independent of the basal bodies. Actin filaments form another stellate filamentous network in close proximity to the centrin network, and together they interconnect the spine-scale bearing plaques at the cell surface as well as the microtubular triads near the cell centre.

In this report we describe the three-dimensional con-

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figuration of the cytoskeleton and discuss its relationship to spine-scale movement.

2. Materials and Methods

2.1. Maintenance of Culture and Light Microscopy

A. radians was isolated by Peter Luke Beech from Hobsons Bay, Victoria, Australia. Cells were grown in f/2 medium (GUILLARD and RYTHER 1962) at 20 °C and subjected to a 12:12 hour light:dark cycle.

Light micrographs were taken on a Zeiss Photomicroscope III using Nomarski and phase contrast optics, and using Kodak Tech Pan Film (ASA 50).

2.2. Wholemounts

Wholemounts were prepared by fixing the cells in 1% Lugol's solution for 15 minutes. Following fixation, cells were brought into freshwater by sequential transfers and then placed onto formvar coated grids and allowed to settle for 30 minutes. Grids were then rinsed in filtered distilled water, allowed to dry, and shadow-cast with gold-palladium alloy, at an angle of 30°, using a Selby's evaporative coater. The grids were then examined in a Siemens 102 electron microscope in the Botany School, University of Melbourne.

2.3. Sectioned Material

Cells in a logarithmic growth phase were fixed semi-simultaneously. Four ml of concentrated cells were placed in a 10 ml disposable centrifuge tube to which was added 4 ml of 4% glutaraldehyde in f/2 medium at room temperature (final concentration of 2% glutaraldehyde). After 7 to 9 minutes, 1 ml of 2% OsO_4 in f/2 medium was added, at which time the centrifuge tubes were placed in ice. The cells were fixed for 40–50 minutes, pelleted and washed twice in f/2 medium. Cells were brought into fresh distilled water by sequential transfers, dehydrated in 10% gradations of ethanol at 10 minute intervals, and, when in 100% ethanol the centrifuge tubes were taken out of the ice. Ethanol was replaced by acetone (50% steps), and the cells were embedded in Spurr's resin (SPURR 1969). Thin and thick sections were taken on a Reichert ultramicrotome using a diamond or glass knife and were stained in uranyl acetate (10 mins) and lead citrate (3–5 mins). Thin sections were examined in a Siemens 102 electron microscope while 0.25 µm thick sections were examined in a JEOL high voltage electron microscope (operated at 750 kV) located at the University of Colorado, U.S.A.

2.4. Indirect Immunofluorescence

Three antibodies were used for immunolocalization of cytoskeletal components. An affinity purified IgG fraction of a mouse monoclonal antibody directed against tubulin (MAb 1–2.3; ASAI *et al.* 1982) was kindly provided by Dr. David Asai, Purdue University, Indiana, U.S.A. A second affinity purified IgG fraction of a mouse monoclonal antibody directed against actin was obtained from Amersham Int. U.K. (N. 350). A third affinity purified IgG fraction of a polyclonal rabbit antiserum, generated against a 20 kD polypeptide from the striated flagellar root of the green flagellate *Tetraselmis striata* (for details see SALISBURY *et al.* 1984), was the generous gift of Dr. J. L. Salisbury, Case Western Reserve University, Cleveland, U.S.A.

Cells were fixed in 0.5% glutaraldehyde in PBS-marine/1 mM EGTA (2.25 g Na_2HPO_4; 0.62 g NaH_2PO_4 2 H_2O; 28.6 g NaCl; 0.1 g NaOH; 0.38 g EGTA to 1,000 ml deionized water) for 30 minutes. Following fixation, cells were brought into PBS-normal/1 mM EGTA (as in PBS-marine/1 mM EGTA, differing in having only 8.6 g NaCl) by sequential transfers. Cells were then washed in 0.5 mg/ml sodium borohydride in PBS-normal/1 mM EGTA then rewashed in PBS-normal/1 mM EGTA and allowed to dry down onto polylysine coated coverslips (0.02% polylysine) overnight. Cells were rehydrated by washing the coverslips in PBS-normal/1 mM EGTA and then incubated in an aliquot of the primary antibody for 2 hours at 37 °C in a moist chamber. Cells were then washed and incubated in an aliquot of the secondary antibody (goat-anti-mouse or goat-anti-rabbit) conjugated to FITC (Miles Yeda) for 2 hours at 37 °C. Coverslips were washed extensively in PBS-normal/1 mM EGTA and mounted onto glass microscope slides using Mowiol mounting media (6 g glycerol; 2.4 g Mowiol 4-88; 6 ml H_2O; 12 ml of 0.2 M Tris buffer, pH 9–10; 2% n-propyl gallate). The FITC fluorescence was observed

Fig. 1. Light micrograph (phase contrast optics) showing a lateral view of a cell. A single flagellum emerges from the apical pit while the six spine-scales project posteriorly forming a cone behind the cell. Scale bar = 5 µm

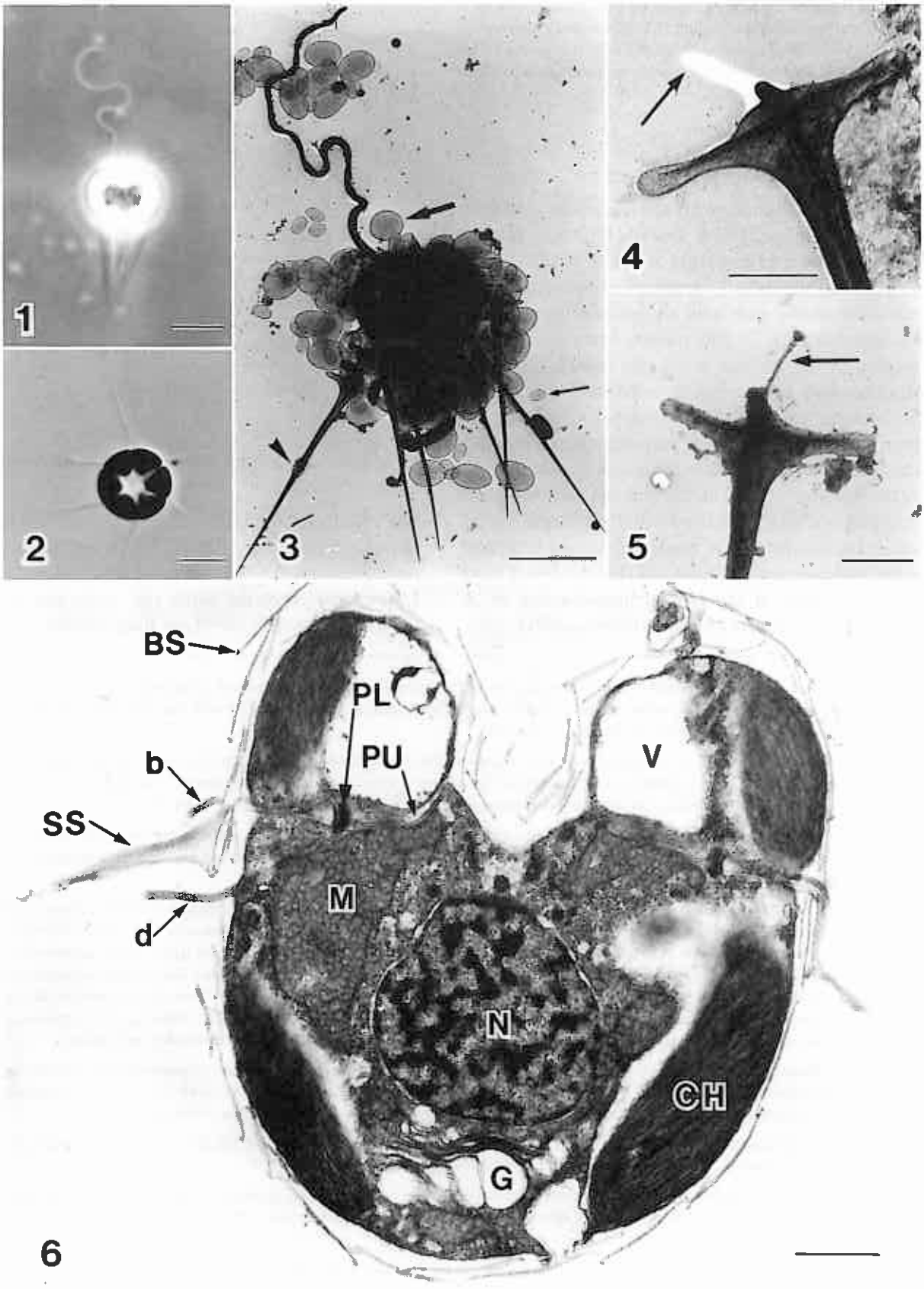
Fig. 2. Light micrograph (Nomarski optics) showing an apical view of a cell. Six spine-scales are located between the six peripheral chloroplasts. In this case the spine-scales have all reoriented laterally. Scale bar = 5 µm

Fig. 3. Shadow-cast wholemount of a whole cell. Two scale types are present; six long tapering spine-scales (arrowhead) and numerous ovoid body scales (small [small arrow] and large [large arrow]). Note that the mastigonemes have fallen off the flagellum during the preparation of the specimen. Scale bar = 4 µm

Fig. 4. Shadow-cast wholemount of the base of a spine-scale. The shadow (arrow) indicates the presence of a central evagination on the proximal surface causing the spine-scale to have a triangular base. Scale bar = 1 µm

Fig. 5. Shadow-cast wholemount of the base of a spine-scale. A microligament (arrow) is attached to the central evagination of the proximal surface. Scale bar = 1 µm

Fig. 6. High-voltage electron micrograph of a thick longitudinal section showing general morphology. Note the covering of body scales (*BS*), centrally placed nucleus (*N*), posterior Golgi (*G*), peripheral chloroplasts (*CH*), various mitochondrial profiles (M), anterior vacuoles (*V*) and the sac-like pusules (*PU*) just below the latter and closely associated with the filamentous network. The spine-scales (*SS*) are oriented laterally while the plaques (*PL*) are located a significant distance from the circumference of the cell (in deep invaginations). The tentacle supported by the type (*b*) microtubular triad is above the spine-scale while the tentacle supported by the type (*d*) microtubular triad (observed internally) is present below and follows the direction of the spine-scale. Note the anterior depression of the cell representing the apical pit. Scale bar = 1 µm



Figs. 1–6

on a Zeiss Photomicroscope III, equipped with epifluorescence for FITC excitation and a barrier filter (KP 560) to block chlorophyll autofluorescence. Fluorescent micrographs were taken using Ilford XP 1 film (ASA 400). Control experiments were performed by omission of the primary antibody.

3. Results

3.1. General Morphology

Cells of *Apedinella radians* are apple-shaped (7–10.5 μm wide, 6–9.5 μm long) with a single flagellum bearing mastigonemes emerging from an apical pit (Figs. 1, 3, and 6). Cells are radially symmetrical with six peripheral chloroplasts, each with a prominent pyrenoid on the innerside (Fig. 2). The nucleus occupies a central position in the cell and is roughly spherical (Fig. 6). Mitochondrial profiles run longitudinally through the cell between the chloroplasts (Fig. 34), coalescing anteriorly to form one large mitochondrial reticulum which encircles the apical pit (Figs. 6, 20, and 30 *a–c*). In the anterior region of the cell the mitochondrial reticulum is closely associated with the filamentous cytoskeleton (see below). A single dictyosome is located at the posterior region of the cell (Fig. 6). For a more detailed account of the general ultrastructure of *A. radians* (= *A. spinifera*) see THRONDSEN (1971).

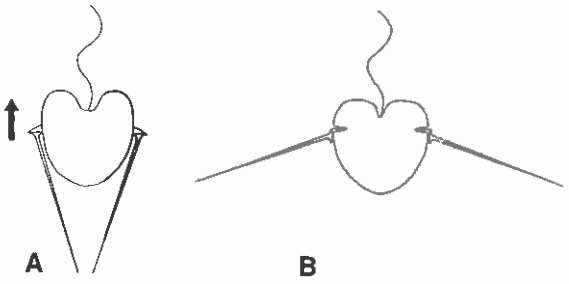


Fig. 7. Schematic representation of cells exhibiting the two different orientations of the spine-scales. Only two spine-scales are drawn for simplicity. *A* When swimming (in the direction of the arrow), the spine-scales are in the relaxed position and are oriented posteriorly to form a cone. *B* Immediately prior to stopping, the cell rapidly reorients the spine-scales through an angle of 90° to radiate laterally. After a short interval, the spine-scales return to position *A* and the cell resumes swimming in the forward mode

3.2. Scale Morphology and Spine-Scale Attachment Sites

The cells have two scale types: ovoid body scales and elongate spine-scales (Fig. 3). The ovoid scales are of two size classes (0.6–0.8 × 0.4–0.5 μm and 1.5–2 × 1.1–1.3 μm) and cover the entire cell. Cells also bear six slender spine-scales, (9–13 μm long) which, according

Fig. 8. High voltage electron micrograph of a thick oblique section. The spine-scale is attached to the cell at the plaque (*PL*) via an extracellular microligament (arrow). The tentacle supported by a type (*b*) microtubular triad is closely associated with the microligament. Note that the plaque is located between the chloroplasts. Scale bar = 500 nm

Fig. 9. Longitudinal thin section of the extracellular microligament. The extracellular microligament is attached to the plasma membrane and immediately proximal to this region is a plaque. Note the striated appearance of the microligament. The plaque is located on the circumference of the cell indicating that in this cell the spine-scales are oriented posteriorly. Scale bar = 200 nm

Fig. 10. Cross section of a cell, longitudinal section of an extracellular microligament. The plaque and microligament are located in a deep invagination between the chloroplasts indicating that in this cell the spine-scales are oriented laterally. Note the filaments emanating internally from the plaque (arrow). Scale bar = 500 nm

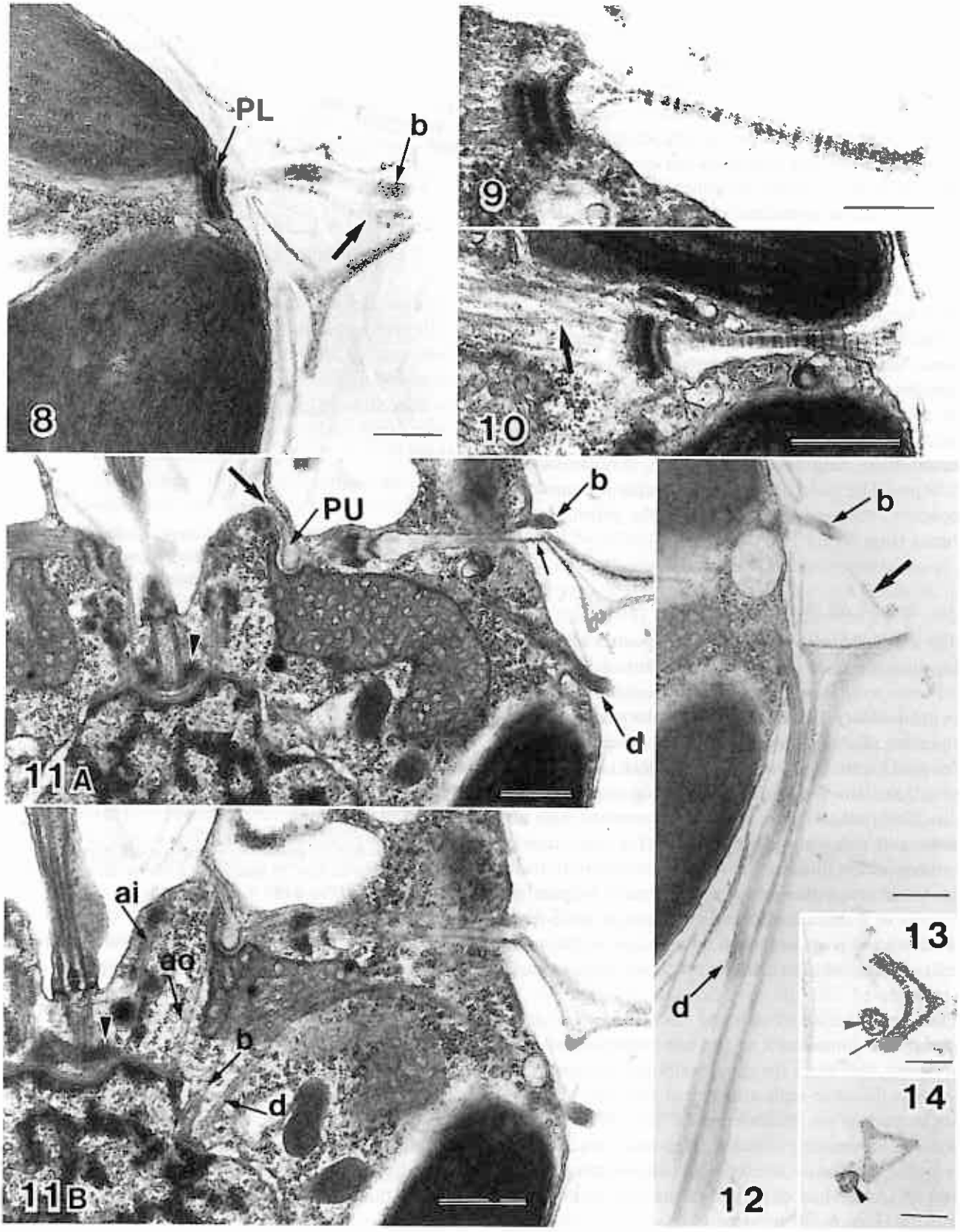
Fig. 11 AB. Two serial longitudinal sections of a cell with the spine-scale oriented laterally and the plaque located in a deep invagination. Note the sac-like pusule (*PU*) with the pore (large arrow) leading to the apical pit which is in the constricted state. Type (*a*) both inner (*ai*) and outer (*ao*) whorls, type (*b*) and type (*d*) microtubular triads, are present. The type (*d*) microtubular triad clearly supports the tentacle below the spine-scale. The type (*c*) microtubular triad is not visible in this cell. The nucleus is depressed anteriorly to accomodate the two basal bodies and anteriolaterally to accommodate (at least) the type (*b*) and (*d*) microtubular triads. Note the amorphous electron dense material at the base of the basal bodies just distal to the nuclear membrane (arrowhead). The extracellular microligament is present, attached to the spine-scale (small arrow), however it is impossible to detect the cross-striations. Scale bars = 500 nm for both figures

Fig. 12. High voltage electron micrograph of a longitudinal section of a cell with the spine-scales oriented posteriorly. The tentacle supported by the type (*d*) microtubular triad remains in a close association with the proximal surface of the spine-scale. Parts of the tentacle supported by a type (*b*) microtubular triad and microligament (arrow) are also present in this section. Scale bar = 500 nm

Figs. 13 and 14. High voltage electron micrographs showing cross sections of the spine-scales and the closely associated tentacle supported by the type d microtubular triad. Fig. 14 is towards the tapering end of the spine

Fig. 13. The tentacle supported by the type (*d*) microtubular triad is inserted into the concave proximal surface of the spine-scale and appears to be connected to it (arrow). Note that apart from the three microtubules the tentacle contains an additional tubular structure (arrowhead). Scale bar = 250 nm

Fig. 14. Towards the end of the tapering spine-scale the concave proximal surface is not present, however the tentacle is still associated with the spine-scale indicating that it remains with it along its length. Again another longitudinal member is present (arrowhead) in addition to the three triad microtubules. Scale bar = 250 nm



Figs. 8–14

Consider each plaque/plaque system, consisting of two alternate plaques which are associated with two members of the innermost whorl of cylindrical caps and one member of the outermost whorl of cylindrical caps, as an isolated unit (Fig. 29, inset). It consists of two discrete actin bundles which merge in the middle and are closely associated, if not attached, to the outermost cylindrical cap. At present, the polarities of these two actin filamentous bundles is unknown. Additionally, each actin filamentous bundle is branched such that the innermost branch by-passes the first (or closest), but attaches to the second member of the innermost whorl of cylindrical caps (Fig. 29, inset). These attachments are the likely cause for the rugose fluorescent appearance on the innerside of the "Star of David" (Fig. 26). Therefore two actin branches from two actin filamentous bundles in the same plaque/plaque filamentous connection overlap (Fig. 29, inset).

In effect, there are twelve discrete actin filamentous bundles (two emanating from each plaque), and part of each is associated with one member of the innermost whorl of cylindrical caps, while the other part merges with an actin filamentous bundle from the same plaque/plaque filamentous connection. Although each plaque/plaque filamentous connection has a unique member of the outermost whorl of cylindrical caps, they share the innermost cylindrical caps with another actin filamentous bundle from another plaque/plaque system. Consequently, each member of the innermost whorl of cylindrical caps anchors part of two actin filamentous bundles from different plaque/plaque filamentous connections. The immunofluorescence results also suggest that, in some cases, the actin filamentous bundles do not actually contact the plaques, but do remain directed towards these structures (Fig. 26).

3.4.2.2. Centrin Filamentous Bundles

Two centrin filamentous bundles emanate from each plaque, at two different angles in the same horizontal plane and attach to the two closest members of the innermost whorl of cylindrical caps forming a second, yet quite different, six pointed star (Fig. 27). These closest cylindrical caps are the cylindrical caps which the actin filamentous bundles by-pass (see Fig. 29, inset). Similarly, there are twelve discrete centrin filamentous bundles, however, bundles do not branch and each is associated with only one member of the innermost whorl of cylindrical caps. Consequently, each member of the innermost whorl of cylindrical caps anchors two centrin filamentous bundles that emanate from adjacent plaques.

An additional fluorescent ring is also observed using the anti-centrin antibody (Fig. 27) and is located pos-

Fig. 16. Oblique sections showing microtubular triads. Note the thin filaments (arrows) connecting the three microtubules forming the microtubular triad. Three microtubular triads are present representing types (*b*), (*c*) and (*d*). Scale bar = 250 nm

Fig. 17. Median longitudinal section in the anterior region of the cell. A type (*a*) microtubular triad (*MT*) emanates from the nucleus and terminates at a cylindrical cap (*CC*). Note the filamentous bundle attached to the cylindrical cap (small arrow). Also note the electron dense material surrounding the base of the basal body (arrowheads) and the close association of the mitochondrion (*M*) with the filamentous bundle. Note the wide diameter of the apical pit (large arrow) indicating that in this cell the spine-scales are oriented posteriorly. Scale bar = 500 nm

Fig. 18. Cross section of a cylindrical cap. The three microtubules of a microtubular triad are encircled by the cylindrical cap. Scale bar = 200 nm

Fig. 19. Cross section just anterior to the nucleus. The two basal bodies are surrounded by an amorphous electron dense material. Numerous type (*a*) microtubular triads are also present (arrowheads). Scale bar = 250 nm

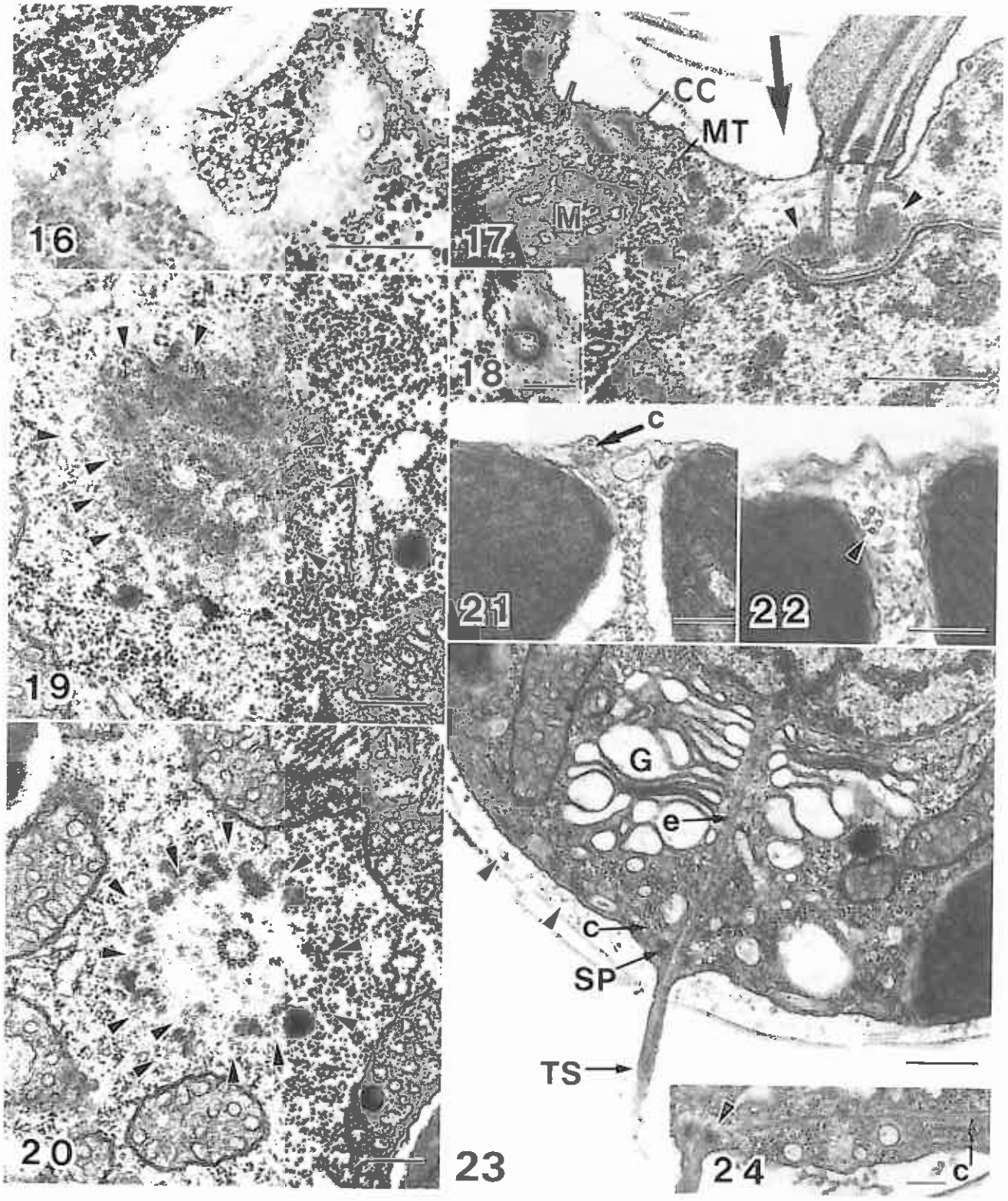
Fig. 20. Cross section just anterior to Fig. 19. Only the basal body leading to the emergent flagellum is present and is not surrounded by any material. However, there is some amorphous electron dense material between the microtubular triads. Again numerous type (*a*) microtubular triads are present (arrowheads). Scale bar = 250 nm

Fig. 21. Cross section between two chloroplasts. When deflected the type (*c*) microtubular triads continue posteriorly along the circumference of the cell, between the chloroplasts. Scale bar = 250 nm

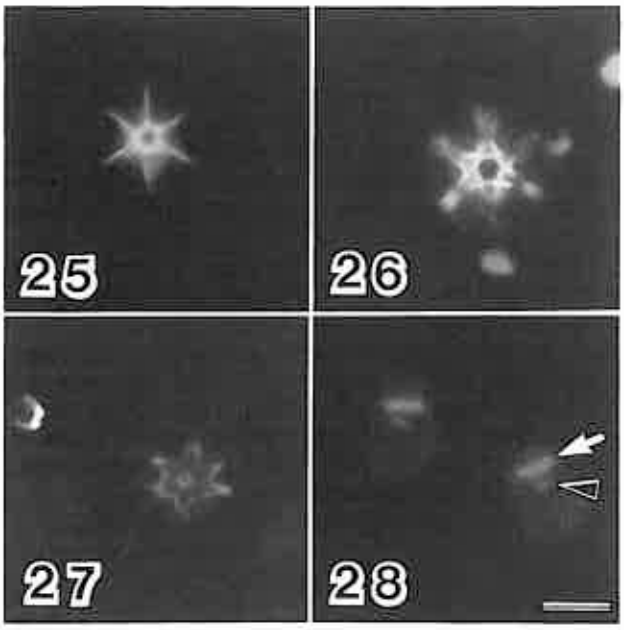
Fig. 22. Similar section to that in Fig. 21, however in this case the type (*c*) microtubular triad is associated with an additional microtubule (arrowhead). Scale bar = 250 nm

Fig. 23. Median longitudinal section in the posterior region of the cell. The type (*e*) microtubular triad emanates from the nucleus and projects posteriorly passing through the Golgi apparatus (*G*) and the sphincter (*SP*), to support the trailing stalk (*TS*). The type (*c*) microtubular triad, terminates at the sphincter. Note the fibrillar material which presumably is involved in attaching the body scales to the plasma membrane (arrowheads). Scale bar = 500 nm

Fig. 24. Median longitudinal section in the posterior region of the cell. The type (*c*) microtubular triad follows the circumference of the cell and terminates at the sphincter (arrowhead). Scale bar = 250 nm



Figs. 16–24



Figs. 25–28. Indirect immunofluorescent localization of cytoskeletal components using antibodies to three different cytoskeletal proteins. Scale bar = 5 μm

Fig. 25. Immunolocalization of tubulin in a fluorescent micrograph showing an apical view of a cell. The pallisade of anteriorly directed microtubular triads (type *a*) appear as dots which form a ring around the apical pit, while the anteriolaterally directed microtubular triads radiate laterally between the six chloroplasts

Fig. 26. Immunolocalization of actin in a fluorescent micrograph showing an apical view of a cell. Actin filamentous bundles form a very distinctive "Star of David" pattern. The six-pointed star consists of two discrete triangles. Note that the sides of the triangles, which are directed from one plaque to every alternate plaque, are not always continuous indicating that they consist of two separate bundles. In this cell it appears that the filamentous bundles do not reach the plaques. Also note the rugose fluorescence on the innerside of the star

Figs. 27 and 28. Immunolocalization of centrin in fluorescent micrographs showing an apical (Fig. 27) and lateral (Fig. 28) view of a cell

Fig. 27. Centrin filamentous bundles form a second, but quite different, six-pointed star not composed of two distinct triangles. Centrin also forms a ring in the middle of the cell which surrounds the basal bodies

Fig. 28. The centrin surrounding the basal bodies (arrowhead) is located posteriorly and is apparently not connected to the centrin filamentous bundles (arrow)

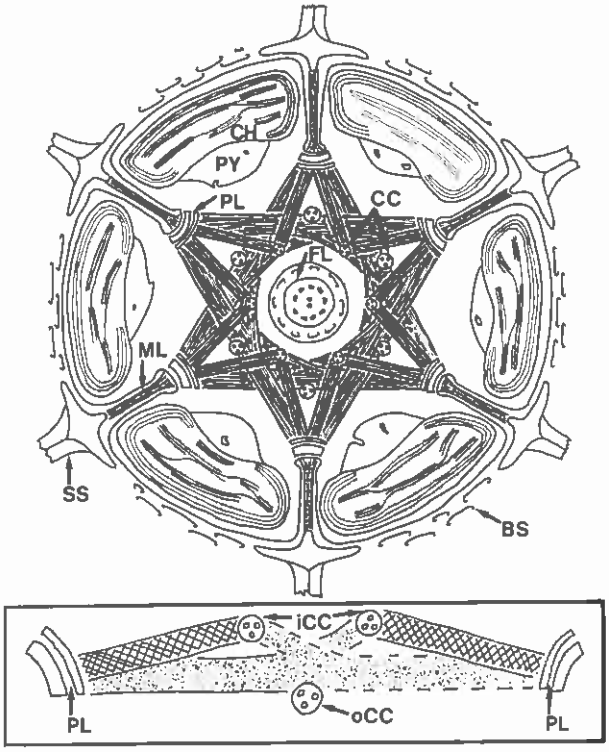


Fig. 29. Schematic representation of a cross section of a cell showing some cytoskeletal components viewed from the anterior end of the cell. Note the two whorls of cylindrical caps with the various filamentous bundles attached to them. The inset shows the general organization of the filamentous bundles. A plaque/plaque system consists of two alternate plaques (*PL*), two members of the inner whorl of cylindrical caps (*iCC*) and one member of the outer whorl of cylindrical caps (*oCC*) which are interconnected by both actin filamentous bundles (stippled bundles with continuous and broken lines) and centrin filamentous bundles (hatched bundles)
Abbreviations: *BS* body scale, *CC* cylindrical cap, *CH* chloroplast, *FL* flagellum, *ML* microligament, *PL* plaque, *PY* pyrenoid, *SS* spine-scale

terior to the centrin filamentous system adjacent to the apical pole of the nucleus (Fig. 28). It is believed to be associated with the two basal bodies as has been found in other protists (SALISBURY *et al.* 1986). In sectioned material it appears as an amorphous electron dense material surrounding the basal bodies (Figs. 17, 19, and 36). There does not appear to be a connection between the centrin surrounding the basal bodies and the filamentous centrin system.

Actin filamentous bundles therefore form both plaque/plaque connections and plaque/cylindrical cap connections while centrin filamentous bundles only form plaque/cylindrical cap connections.

3.5. Configuration Changes in the Cytoskeleton

As mentioned previously, when the spine-scales are oriented laterally, the plaques (spine-scale attachment sites) are drawn into deep invaginations between the chloroplasts (Figs. 6, 10, and 11 *a*, *b*). Consequently, the distance between opposite plaques is reduced to

approximately two thirds the length observed when the spine-scales are in the posterior configuration. Observations of living material indicates that the reorientation event is synchronous and sectioned material supports this finding as all the plaques, in any one cell, are pulled into invaginations (Figs. 30 *a–c*).

Some other structural changes accompany spine-scale reorientation. When the spine-scales have a posterior orientation the filamentous bundles emanate from the plaques in an anterior direction to reach the cylindrical caps (Figs. 31, 32, and 35). Conversely, when the spine-scales are oriented laterally the whole filamentous system is observed in a few transverse sections (ref. Figs. 30 *a–c*), indicating that the filamentous bundles are in the same horizontal plane as the cylindrical caps (Figs. 11 *a* and 30 *a–c*).

The three cylindrical cap (two form the inner whorl and one from the outer whorl) associated with each plaque/plaque system (see Fig. 29 inset) form three points of an "imaginary" triangle. The angle subtended at the outer cylindrical cap changes dramatically during this phenomenon. When the spine-scales are oriented posteriorly the angle is at least 90° (Fig. 32). Conversely, when the spine-scales are oriented laterally, the angle is approximately 60° (Fig. 30 *c*). This alteration in the relationship between the three cylindrical caps is brought about by the relocation of the two inner cylindrical caps such that they are in a closer proximity. Finally, when the spine-scales are oriented laterally the apical pit is closely appressed to the emergent flagellum (Figs. 11 *a, b*, and 36). Conversely, when the spine-scales are oriented posteriorly the apical pit is dilated (Figs. 17 and 35). Interestingly, the inner whorl of cylindrical caps is always closely associated with the plasma membrane of the apical pit (Figs. 11 *a*, 17, 30 *a–c*, 32, 35, and 36), irrespective of the orientation of the spine-scales. However, it is uncertain how this association is mediated.

3.6. Pusules, Mitochondrion and Polyribosomes

Pusule-like structures have been noted previously in *A. radians* (THRONDSEN 1971) and are located just posterior to the large anterior vacuoles (Figs. 6, 11 *a, b*, and 35). They are sac-like inclusions continuous with the plasma membrane with a narrow pore opening into the apical pit and appearing to be closely associated with the complex filamentous network (Figs. 6 and 11 *a, b*). The major portion of the mitochondrial reticulum is present in the anterior region of the cell where it too is closely associated with the complex filamentous network (Figs. 34 and 35). Polyribosomes are also abundant in the anterior region of the cell (Fig. 34).

4. Discussion

By combining traditional thin and thick section electron microscopy with immunofluorescent techniques using three antibodies to eukaryotic cytoskeletal proteins, it has been possible to make the first comprehensive, three-dimensional reconstruction of the cytoskeleton in a pedinellid flagellate. The cytoskeleton of *A. radians* is an intricate, radially symmetrical network comprising (at least) three main varieties of longitudinal elements (microtubules, actin microfilaments and centrin filaments) that interconnect via two varieties of anchoring sites (plaques and cylindrical caps). The intimate association of this elaborate cytoskeletal network with the moveable spine-scales strongly suggests that a primary function of the cytoskeleton is the reorientation of the spine-scales.

4.1. Microligament

The spine-scales of *A. radians* remain attached to the cells via an extracellular striated fibrous connector (microligament). The oar-like axopods of the planktonic protozoan, *Sticholonche* propel the organism through seawater and are connected to each other by extracellular filaments (CACHON *et al.* 1977). However, the authors did not assign any effective motive force to the filaments. The extracellular location of the microligament in *A. radians* also does not provide much scope for its exploitation as an effective force generating structure. Presently, it is considered only to connect the spine-scales to the cells.

4.2. Microtubular Triads and Associated Structures

This study demonstrates that thirty-one microtubular triads form a peripheral basket delineating the cell shape (type c) as well as a pallisade of microtubular triads encircling the apical pit (type a). Microtubular triads also support two sets of tentacular protrusions (types b and d) and a trailing stalk (type e).

The angle at which the inner whorl of type a microtubular triads in *A. radians* emanates from the nuclear envelope appears to alter when the cell changes the orientation of its spine-scales. As mentioned previously, the actin branches that are attached to the innermost whorl of cylindrical caps of a plaque/plaque system overlap. Therefore any interdigitation between these filamentous actin branches will form a filamentous connection between adjacent members of the in-

nermost whorl of cylindrical caps. Any contraction of these filamentous bundles would result in the lateral movement of these cylindrical caps and, as they are closely associated with the plasma membrane of the apical pit, the diameter of the latter would be reduced. At this stage, it is presumed that this movement marginally aids the filamentous network (see later) in relocating the plaques from the circumference of the cell.

The six tentacles supported by the type b microtubular triads project directly over the extracellular microligament, but no physical connection between the structures was observed. The location of the tentacles is suggestive of a protective function to shield the microligaments during swimming.

The function of the type c microtubular triads, which run longitudinally between the chloroplasts forming a peripheral basket, is presently unknown. It is also uncertain whether they are present in a normal interphase cell. They are ideally situated, between the chloroplasts, to play one of the classical cytoskeletal roles: cell-shape maintenance.

The location of the tentacles supported by type d microtubular triads suggest that they play a key role during spine-scale reorientation. These tentacles remain on the proximal surface of the spine-scales whether they are oriented posteriorly or laterally, by an evident physical connection. As the three microtubules of an individual microtubular triad are interconnected by thin filaments there is a possibility of intermicrotubular sliding causing these tentacles to flex. Although unlikely to be involved in the relocation of the spine-scales to their lateral orientation, these tentacles could well be responsible for restoring the spine-scales to their posterior configuration (see later). Presumably these tentacles are also responsible for keeping the seemingly unwieldy spine-scales in the same vertical plane during reorientation.

The trailing stalk, which is supported by the type e microtubular triad in *A. radians*, is very much reduced in comparison with that observed in other members of the *Pedinellales* and is not always visible by light microscopy. This is the first account of a trailing stalk in *A. radians;* however, the structure THRONDSEN (1969, 1971) called a cytoplasmic strand was probably the "reduced" trailing stalk. Although the vacuolar system associated with the trailing stalk in other pedinellids is absent in *A. radians*, the presence of a microtubular triad passing through a posterior sphincter indicates that the structure observed in *A. radians* is indeed a true trailing stalk. It has been observed that the long posterior trailing stalk in *Pseudopedinella elastica* suddenly contracts prior to the cells stopping and changing directions (ZIMMERMANN *et al.* 1984). Although uncertain, it appears that the spine-scales in *A. radians* could be performing a similar function. It seems reasonable therefore to suggest that cells of *A. radians* do not require such an elaborate trailing stalk as seen in other pedinellids. The function, if any, of the apparently homologous, but much reduced, structure in *A. radians* remains obscure.

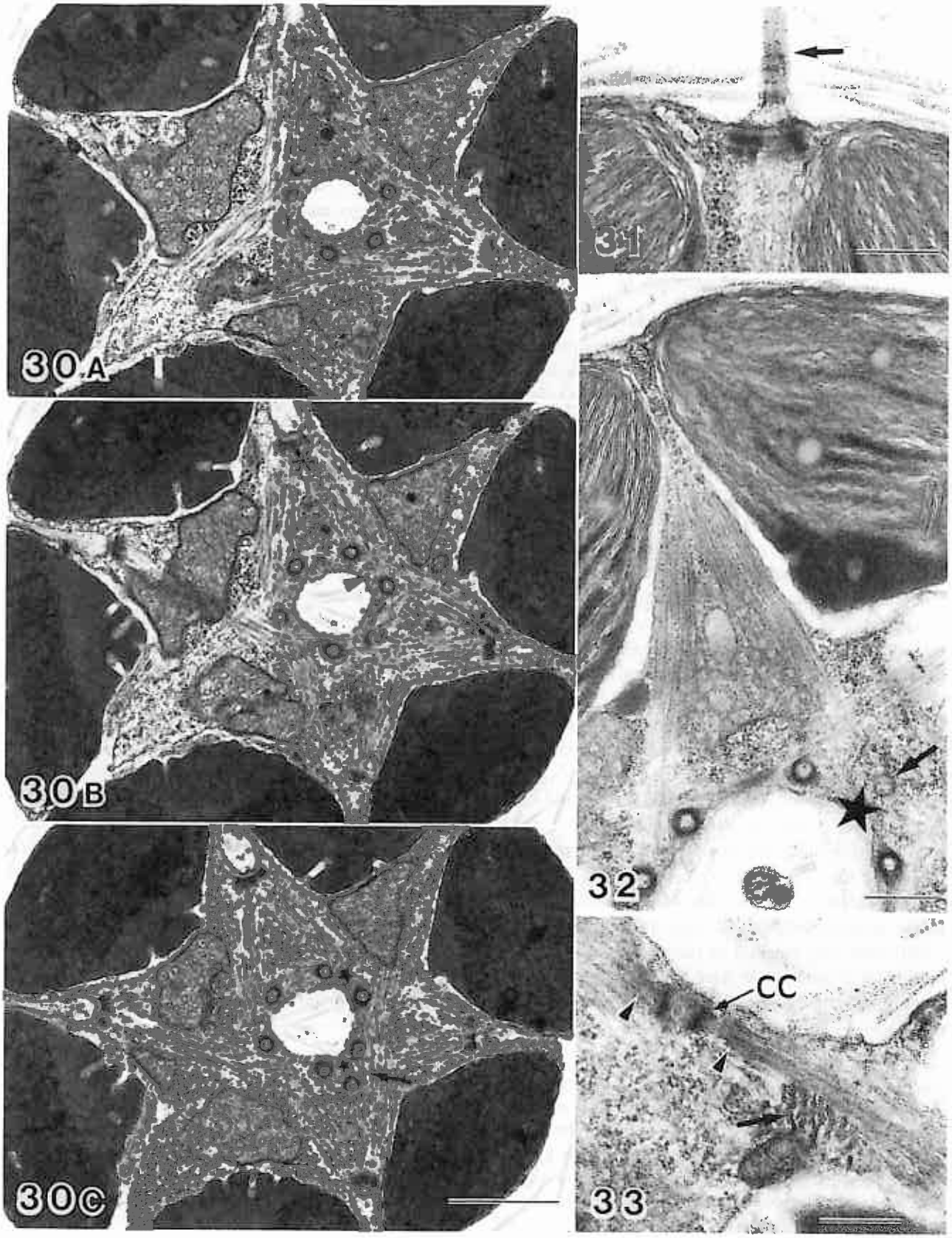
Fig. 30 *A–C*. Three transverse serial sections of the anterior region of the cell. The plaques are located in deep invaginations between the chloroplasts indicating that in this cell the spine-scales are oriented laterally. One triangle of the "Star of David" is present in *A*. The six members of the inner whorl of cylindrical caps are present in *B* and *C* while an additional two members of the outer whorl of microtubular triads come into the plane of the section in *C*. In *B* consider the plaque/plaque filamentous connection emanating from the plaques marked by an asterix. The two actin filamentous bundles merge in the middle while a branch of an actin filamentous bundle emanating from the uppermost plaque is attached to a member of the inner whorl of cylindrical caps (arrowhead). As the spine-scales are oriented laterally the "imaginary" triangles (two present in *C* surrounding the star shape) are in the characteristic equilateral triangular formation. A centrin filament (arrow) emanates from the lowermost plaque and is attached to the closest member of the inner whorl of cylindrical caps. Note that the plaques and cylindrical caps are in the same sections indicating that in this cell the filamentous network is in the same horizontal plane. Scale bar = 1 µm

Figs. 31 and 32. High voltage electron micrographs of the same cell. Note that in this cell the plaques and cylindrical caps are not in the same section. Fig. 31 is a more posterior section indicating that the filamentous bundles emanate from the plaques in an anterior direction to reach the cylindrical caps

Fig. 31. Cross section between two chloroplasts. The plaque is located on the circumference of the cell indicating that in this cell the spine-scales are oriented posteriorly. The extracellular microligament (arrow) is also present. Scale bar = 400 nm

Fig. 32. When the spine-scales are oriented posteriorly the "imaginary" triangles (surrounding the star shape) form approximate right angled triangles. The outer cylindrical cap is just visible in this section (arrow). Scale bar = 500 nm

Fig. 33. High voltage electron micrograph of an oblique section in the anterior region of the cell. Filamentous bundles cut longitudinally (arrowheads) are attached to a cylindrical cap (*CC*) while a filamentous bundle cut obliquely (arrow) gives a wavy appearance. Scale bar = 400 nm



Figs. 30–33

4.3. Filamentous Network and Its Role in Spine-Scale Reorientation

The extremely complex network of filamentous bundles present in the cytoskeleton of *A. radians* is unique among the eukaryotes and consists of at least two elements: actin microfilaments and centrin filaments. The related species *Actinomonas pusilla* and *Pteridomonas danica* also possess an ordered filamentous network (LARSEN 1985); however, they are much simpler structures and as yet no immunocytochemical analyses of the cytoskeletons and their functional properties have been made.

Thin and thick sectioned material observed under the electron microscope reveals that when the spine-scales are oriented posteriorly, the plaques are located on the cell margins. Conversely, when the spine-scales are oriented laterally, the plaques are situated in deep invaginations between the chloroplasts. The relocalization of the plaques into deep invaginations and the consequent lateral reorientation of spine-scales is apparently mediated by the shortening of the filamentous bundles. In order to exert a mechanical force, a contractile assembly must be anchored to other cellular components. Actin microfilaments form both plaque/plaque interconnections and plaque/cylindrical cap interconnections, while centrin filaments form only plaque/cylindrical cap interconnections. Contraction of either plaque/plaque or plaque/cylindrical cap interconnections could hypothetically lead to spine-scale reorientation. Therefore both filamentous systems are potential mechano-chemical motors.

In order to achieve lateral orientation, the spine scales must pivot at a fulcrum, near their bases on the proximal surface. Since two points of the triangular base of a spine-scale rest on the cell surface, the latter may act as a fulcrum. The location of tentacles supported by the type d microtubular triads also allows the possibility that they may act as fulcra. The central evagination of a spine-scale base is attached to the cell membrane via a microligament (as opposed to a direct connection) allowing such a pivoting action (see Fig. 37). If we consider the spine-scales as levers then their attachment near their fulcra results in a very short force arm. The force to reorient the spine-scales must therefore be great as they are located at mechanically unfavourable attachment sites. However, the relationship between the distance of the force arm and the angle a lever is displaced is inversely proportional. Therefore any shortening of the internal filamentous cytoskeleton would result in the greatest angle of spine-scale reorientation possible. This indicates that the spine-scales are located at the most advantageous attachment sites for this particular function.

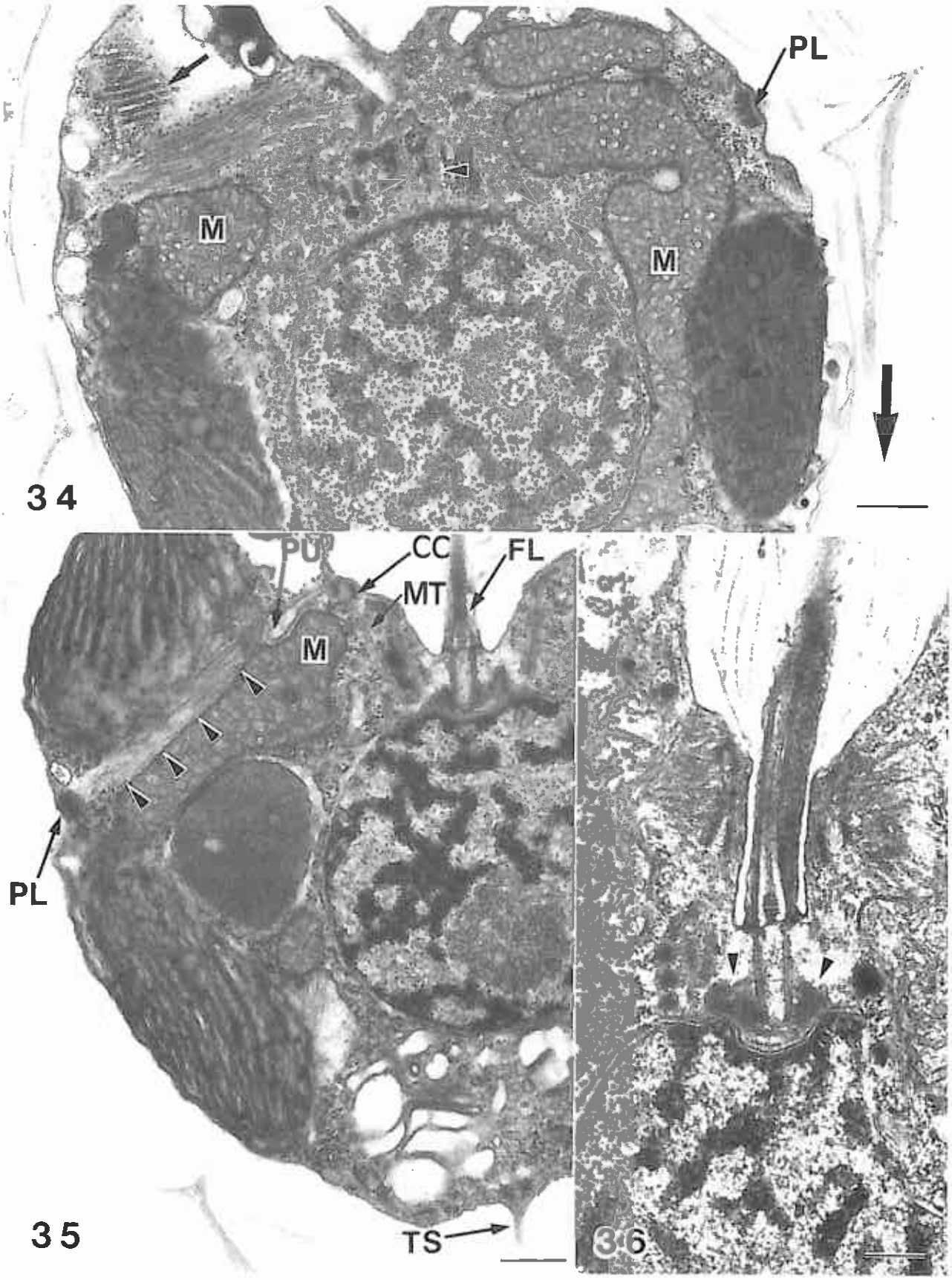
Preliminary immunocytochemical studies using an anti-myosin antibody have failed to demonstrate the presence of myosin in *A. radians*. Myosin, however, is the only known actin-associated protein that can generate mechanical force (FULTON 1984), and is always present when actin microfilaments form contractile bundles (ALBERTS *et al.* 1983). We are continuing efforts to establish whether or not myosin is present. Contraction of centrin in response to increased calcium concentrations has been well documented (SALISBURY *et al.* 1984, WRIGHT *et al.* 1985, MCFADDEN *et al.* 1987). It is therefore possible that filamentous bundles composed of centrin undergo a calcium induced contraction to reorient the spine-scales.

While actin microfilaments form a bundle that provides a structural backbone to which a motor for mechano-chemical energy transduction (myosin) is attached (ISENBERG and JOCKUSCH 1982), centrin undergoes supercoiling causing the whole bundle to contract (SALISBURY *et al.* 1984). Even though the mechanisms by which the two motility systems function are different, they both have a requirement for ATP and contraction is elicited by calcium (PERRY 1979, SALISBURY *et al.* 1984, MCFADDEN *et al.* 1987). ATP is provided by the mitochondrion and its close association in *A. radians* with the filamentous network is an example of the localization of this organelle near sites of potentially

Fig. 34. Oblique longitudinal section showing a mitochondrial profile (*M*) present in the anterior end of the cell closely associated with the filamentous network. The plaques (*PL*) are located on the circumference of the cell and the spine-scales are oriented posteriorly (direction of large arrow). Microtubules from microtubular triads can be seen emanating from the nucleus (arrowheads) and there is an abundance of polyribosomes (small arrow) in the anterior region of the cell. Scale bar = 500 nm

Fig. 35. Median longitudinal section through the flagellum (*FL*) and trailing stalk (*TS*). When the plaques (*PL*) are located on the circumference of the cell (spine-scales oriented posteriorly) the filamentous bundles (arrowheads) emanate upwards at a significant angle to reach the cylindrical caps (*CC*). Additionally the apical pit is considerably open. Note the pusule (*PU*), closely associated with the filamentous bundle and mitochondrion (*M*). A microtubular triad (*MT*) is present and terminates at a cylindrical cap (*CC*). Scale bar = 500 nm

Fig. 36. Median longitudinal section of the anterior region of the cell. The diameter of the apical pit is constricted indicating that in this cell the spine-scales are oriented laterally. Note the electron dense material at the proximal end of the basal body (arrowheads). Scale bar = 250 nm



Figs. 34–36

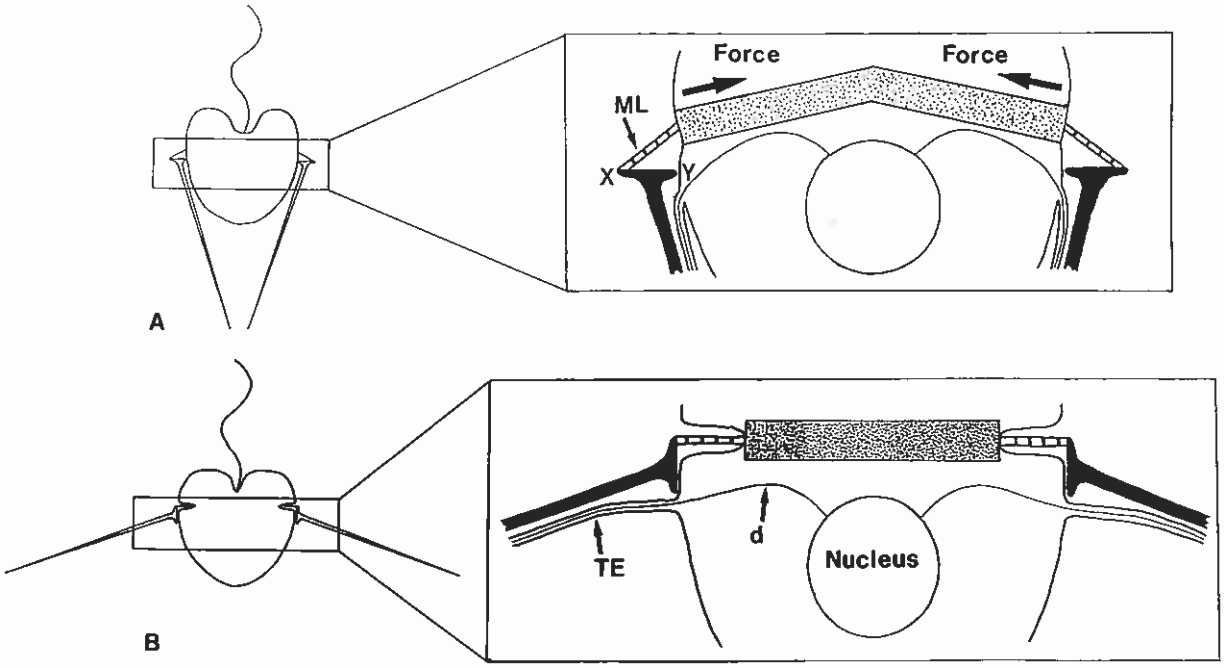


Fig. 37. Schematic representation of the elements presumed to be involved in spine-scale reorientation. For simplicity only one plaque/plaque filamentous connection and the two associated spine-scales are shown. One point of the triangular base of each spine-scale (darkened structures) is shown resting on the cell surface at or near the point Y (*A*). A force (apparently caused by a contraction of the internal filamentous cytoskeleton–stippled region) acts at the central evagination at the base of the spine-scales (*X*) via extracellular microligaments (*ML*) which link this region of the spine-scales to the cell membrane. The pivoting of the spine-scales at or near point *Y*, after the initiation of the force, results in the lateral orientation of the spine-scales (*B*). The short force arm (the distance between points *X* and *Y*) is mechanically advantageous for this particular function (see text). Note also that the tentacles (*TE*), supported by the type (*d*) microtubular triads, remain closely associated with the spine-scales and are possibly involved in their restoration to the posterior configuration (*A*)

high ATP usage. The position of the pusules just anterior to the filamentous network could also have a significant role in spine-scale reorientation, perhaps acting as calcium reservoirs.

The mechanism for restoring the spine-scales to their posterior orientation is unknown but does correlate with the relaxation of the filamentous cytoskeleton. Previous workers (SALISBURY and FLOYD 1978, SALISBURY *et al.* 1984, 1987), have shown the extension of centrin to be ATP-dependant, presumably priming the motor for contraction. Similarly, any extension of actin filamentous bundles would produce the same effect. Alternatively, intermicrotubular sliding causing bending in the tentacles associated with the spine-scales could reorient the spine-scales to their posterior configuration. Interestingly, these particular tentacles contain additional longitudinal, perhaps tubular, members. Finally, the drag force on the spine-scales when the cells resume swimming may cause them to passively return to their posterior orientation. At this stage it is perceivable that all of these mechanisms are involved, or at least supplement, the return of the spine-scales to their posterior configuration.

The intricate cytoskeletal network of *A. radians* has been shown to undergo conformational changes that suggest that it is the motor system responsible for the remarkable spine-scale reorientation process seen in this organism. Two potentially contractile elements, actin and centrin, have been localized immunocytochemically within the cytoskeleton and are the likely motors for this unusual form of cell motility.

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PROTOPLASMA

An Impregnated Suberized Wall Layer in Laticifers of the *Convolvulaceae*, and Its Resemblance to That in Walls of Oil Cells

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Summary

The cell wall in laticifers of the *Convolvulaceae, Calystegia silvatica, C. soldanella, C. tuguriorum, Convolvulus cneorum, C. verecundus, C. sabaticus* subsp. *mauritanicus*, and *Ipomoea indica*, contains an impregnated layer that surrounds the cells. The impregnated layer lies inside the primary wall of the laticifer, separated from the protoplast by a third (tertiary) layer of variable thickness. Histochemical and cytochemical staining give a positive reaction for suberin. The layer is often differentiated into dark and translucent regions, the latter frequently being composed of lamellae. The ultrastructure of this layer and its position within the cell wall of the laticifer is comparable to the condition found in oil cells where the walls contain a suberized layer. A suberized layer within the wall is unique for a laticifer system.

Keywords: Laticifers; *Convolvulaceae;* Suberized wall layer; Oil cell comparison.

1. Introduction

Anatomically, the *Convolvulaceae* are noted for the occurrence of internal phloem and other anomalous features (METCALFE and CHALK 1950). Secretory structures are also widespread in the stem and leaf, either solitary or in vertical rows. Laticifers are sometimes recognized and these are typically of the articulated non-anastomosing type (METCALFE and CHALK 1950, METCALFE 1967, 1983, ESAU 1965). Early studies on the laticifers were made by VOGL (1863, 1866-7), TRÉCUL (1865, 1866), ZACHARIAS (1879), CZAPEK (1894), MIRANDE (1898), and GRÉLOT (1903). Subsequent information has been mostly incidental to other aspects of the plant (*e.g.*, MAIRE 1913, ARTSCHWAGER 1924, PALIWAL and KAVALHEKAR 1971) or re-statements from earlier literature (*e.g.*, SOLEREDER 1908, HABERLANDT 1914, METCALFE and CHALK 1950, METCALFE 1967, 1983, ESAU 1953, 1965, FAHN 1979, 1982). The most recent work on laticifers in the *Convolvulaceae* is that of BRUNI *et al.* (1974) who examined the nuclei in *Calystegia soldanella*.

A survey of laticifers in New Zealand plants revealed an impregnated layer in walls of *Calystegia soldanella* and *C. silvatica* (CONDON 1985). A detailed investigation was initiated to determine the occurrence of such a layer in other laticifers of the *Convolvulaceae* and the results are reported in this paper. A comparison is also made with the suberized wall of oil cells in selected taxa.

2. Materials and Methods

Plants in the *Convolvulaceae* examined for laticifers included: *Calystegia silvatica* (Kit.) Griesb., *C. soldanella* (L.) R. Br., *C. tuguriorum* (Forst. f.) R. Br. ex. Hook. f., *Convolvulus cneorum* L., *C. sabaticus* Viv. subsp. *mauritanicus* (Boiss.) Murb., *C. verecundus* Allan, and *Ipomoea indica* (Burm.) Merr. Samples processed for microscopy included segments of rhizome, stem, petiole, lamina and pedicel of the flower. Secretory cells were also examined in stem and leaf tissues of *Dichondra repens* J. R. et G. Forst. According to CZAPEK (1894), *Dichondra* has laticifers consisting of tuberous vessels devoid of transverse septa originating by fusion of individual cells. The walls are also thick but non-suberized. In our material of *Dichondra* laticifers were not positively identified among the secretory cells examined. Oil glands examined for comparison with the laticifers were taken from leaves of *Hedycarya arborea* J. R. et G. Forst. (*Monimiaceae*), *Macropiper excelsum* (Forst F.) Miq. (*Piperaceae*), and *Pseudowintera colorata* (Raoul) Dandy (*Winteraceae*), all indigenous to the New Zealand flora and collected from the wild.

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