An Electron Microscope Study of Dinoflagellate Flagella

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SUMMARY

The two flagella of the dinoflagellates examined possess the normal axoneme structure with $9+2$ filaments, the nine outer doublets forming triplets in the basal region. Unlike other flagella which possess one basal disc, the dinoflagellates have two basal discs and two diaphragms in the transition region. The two flagella differ from each other in their external morphology and extra-axonemal structures. The longitudinal flagellum contains much packing material in its proximal two-thirds, whereas the distal third contains only the axoneme. This flagellum bears short fine hairs. The transverse flagellum differs from any other type which has been described. It has a helical ribbon-like form due to the presence of a striated strand, which is shorter than the axoneme, and an expanded sheath. It also bears long fine hairs in a unilateral array. The distal end of this flagellum is attached to the cell in the region of the flagellar bases. The structure of the two flagella appears to be compatible with their respective forms of movement.

INTRODUCTION

Dinoflagellates have long been known to possess two flagella which differ from one another in appearance and position (Fig. 1). One, the longitudinal flagellum, beats in a posterior plane in organisms belonging to the order Peridiniales and anteriorly in the order Prorocentrales. The other, the transverse flagellum, beats around the girdle in the Peridiniales and in an anterior position in the Prorocentrales. The structure of the transverse flagellum has been the subject of much speculation and it was at one stage thought to consist of a ring of cilia. Kofoed & Swezy (1921) however found that 'the longitudinal flagellum was thread-like and more rigid than the transverse flagellum which was frequently ribbon-like in form consisting of a deeply staining thread or stout fibril bordered on one side by a comparatively wide fin-like sheet of transparent protoplasm or membrane, somewhat greater in length than itself and thrown into ripples or folds of wider amplitude than the fibril'. These were very accurate observations considering that they were made with an ordinary light microscope. Later, Deflandre (1934) used a negative staining technique and described the transverse flagellum of *Glenodinium uliginosum* as having a single row of hairs. Thus he called it a 'stichonématé' flagellum. He also noted that this flagellum had a distinct outline on one side but not on the other and that the longitudinal flagellum was cylindrical with a long pointed tip.

Dragesco (1952) was the first to look at dinoflagellate flagella with the electron microscope. He reported that the two flagella of *Oxyrrhis marina* appeared to be identical, and were surrounded by a flattened sheath containing two marginal fibres. Pitelka & Schooley (1955) looked at the flagella of a *Gyrodinium* sp. They found the

longitudinal flagellum to be surrounded by 'apparently tubular sheath with no visible structure' which was continuous to the tapered end of the flagellum where it surrounded a single thick fibre from the axoneme. The transverse flagellum consisted of a dense axoneme and a sheath of low electron density which was expanded unilaterally, being about two or three times as wide as the axoneme. A unilateral array of fine mastigonemes (hairs) was found on one side of the flagellum. Pitelka & Schooley did not mention the presence of an accessory fibril although this is visible in their micrograph (Pitelka & Schooley, 1955, pl. 23*a*). This structure we have recently termed the striated strand (Leadbeater & Dodge, 1966).

Although the internal structure of many cilia and flagella is now well known, the dinoflagellates have been neglected in this respect. This is surprising since these organisms have obviously a unique flagellar system. The present paper shows that the dinoflagellates have the usual basic flagellar structure but that this is modified in several ways some of which are probably related to the peculiar mode of movement of the organisms.

METHODS

A wide range of dinoflagellates was examined during the present work but most of the observations were made on three species: *Woloszynskia micra*, *Gymnodinium vitiligo* and *Gymnodinium veneficum*. All organisms were maintained as uni-algal cultures under standard conditions described previously (Dodge, 1963). The cultures originated from the collection of Dr M. Parke at the Laboratory of the Marine Biological Association, Plymouth.

For electron microscopy, whole organisms were fixed with osmic acid vapour or by adding a few drops of 1% (w/v) osmic acid solution to a small volume (about 5 ml.) of culture. Preparations were shadowed at 20° with a mixture of gold + palladium. For sectioned material two fixation methods were used: (a) 1% (w/v) osmic acid in acetate veronal buffered balanced salt solution (Gibbs, 1962) at pH 8.2; (b) 6% (v/v) glutaraldehyde in phosphate buffer and balanced salt solution at pH 8.0, followed after washing, by 1% (w/v) osmic acid in the same medium. Dehydration was carried out in an ethanol/water series and after transfer to propylene oxide small pellets of organisms were embedded in Araldite. Sections were cut on a Porter Blum M.T.1 ultramicrotome and after staining in uranyl acetate in 50% (v/v) ethanol/water followed by lead citrate (Reynolds, 1963), they were examined in a Zeiss EM 9 electron microscope.

RESULTS

External morphology from shadowed organisms

The difference in the appearance of the two flagella is clearly seen (Pl. 1, fig. 1; Fig. 1). When dried (for shadowing) the longitudinal flagellum becomes more or less straight and rigid. The basal two-thirds of the flagellum is wide and contains other material in addition to the axoneme (the 9 + 2 filaments). The distal third, or less, is narrow, with the sheath fitting firmly around the axoneme, it terminates in a rounded point into which extend some of the axoneme filaments, probably the central two. Fine hairs (about 0.5 μ long) clothe the entire flagellum, probably in bilateral arrangement. The hairs are particularly abundant on the distal portion (Pl. 1, fig. 3).

The transverse flagellum is about twice as long as the longitudinal flagellum. When fixed it is quite distinct since it retains a series of waves (Pl. 1, figs. 1, 4) which are often

remarkably even in height and length. The axoneme is surrounded by a thin expanded sheath which also surrounds an accessory fibre, the striated strand (Pl. 1, figs. 1, 4). This strand describes a shorter path than the axoneme and is partly responsible for maintaining the expanded ribbon-like form of the sheath (Fig. 5). On one side of the flagellum is borne a single row of long fine hairs about 2μ long (Pl. 1, figs. 2, 4). They appear to arise in groups of two or three from short stiff usually curved bases. The hairs appear to be attached to the sheath since they remain with the sheath when this is detached from the axoneme. The distal region of the transverse flagellum (Pl. 1, fig. 2) is tightly covered by its sheath, the final micron of its length is devoid of hairs and the flagellum ends in a smooth rounded tip. The striated strand appears to terminate at the point where the hairs cease.

Fig. 1. Diagram of *Gymnodinium* to show the position of the longitudinal and transverse flagella. A, Ventral view of the organism; B, dorsal view of the organism.

The distal end of the transverse flagellum in some species of the Peridiniales is lightly attached to the organism in the region of the flagellar bases but readily becomes detached on fixation. As detached flagella show an entire distal end (Pl. 1, fig. 2) it would seem likely that there is little structural contact with the organism but that the tip reposes in a special infolding of the theca. Some evidence for this has been obtained from sectioned organisms.

Internal structure of the flagella

For convenience of description the flagella can be divided into three regions: the part outside the organism, the external morphology of which has already been described; the transition region where the flagellum enters the organism; the basal region

(kinetosome) which anchors the flagellum in the organism. The transition and basal regions are identical in the two flagella and so in the description of those regions no distinction is made between the two flagella.

The longitudinal flagellum outside the organism. In transverse section (Pl. 2, fig. 6) this part of the flagellum consists of the axoneme, occupying rather less than half the volume surrounded by the flagellar sheath, and a large amount of packing material. The axoneme is of the usual structure (Pl. 2, fig. 6; Fig. 2A) with nine outer pairs of

Fig. 2. Diagrammatic reconstruction of a flagellum and basal body. A-F show transverse sections at levels indicated on the median longitudinal section. The root fibres are omitted from the diagram.

tubular filaments and two inner filaments joined by delicate membranes. Between each peripheral doublet and the inner filaments is a secondary fibre. The secondary fibres appear to be connected by fine fibrils to the doublets (Fig. 2A). The centre two filaments are inclined at an angle of about 45° to a line drawn through the broad axis of the flagellum (Pl. 2, figs. 5, 6).

In longitudinal section (Pl. 3, fig. 16) the packing material is composed of regularly organized longitudinal rows approx. 360 Å apart. This material is absent from the narrow distal tip of the flagellum.

The transverse flagellum outside the organism. The axoneme is exactly as described for the longitudinal flagellum and is of the standard 9 + 2 type (Pl. 2, fig. 5). The unique feature of this flagellum is the striated strand which runs from just above the base of the flagellum (the exact point has not been determined) to near the distal end of the flagellum. In length it is shorter than the axoneme. In section the striated strand consists of a bundle of fibrils, or possibly tubules, generally situated at the opposite side of the flagellar sheath to the axoneme. In longitudinal section (Pl. 3, figs. 13–15; Fig. 3) it exhibits a complex transverse banding. The mean overall periodicity is 660 Å with a basic unit of banding about 110 Å long. Four light bands (440 Å) are followed by two dark bands (220 Å) in regular sequence. However, there is considerable variation in the measurements obtained, ranging from 400 to 800 Å for the overall periodicity. This suggests that the striated strand may alter in length. Regularly arranged packing material separates the striated strand from the axoneme and fills the remaining space within the flagellar sheath (Pl. 3, fig. 15). It is similar in structure to that described for the longitudinal flagellum.

Fig. 3. Diagram showing the detailed pattern of cross-banding of the striated strand.

The transition region where the flagellum enters the organism. The outer extremity of the transition region is marked by the basal disc at which the two central filaments of the axoneme terminate (Pl. 3, fig. 13; Fig. 2B). A single thin-walled central filament runs from below the second basal disc to the base of the flagellum. The second diaphragm marks the proximal end of the transition region. The basal disc has a diameter equivalent to that of the secondary fibres. A short distance below is a second basal disc (Pl. 3, fig. 13; Pl. 4, fig. 17; Fig. 2) which is similar in appearance to the first disc. Connected to the second disc and beneath it is the first of a pair of diaphragms which project into the lumen of the axoneme but are pierced in the centre by an aperture of the same diameter as the two basal discs (Pl. 2, fig. 8, Pl. 3, fig. 13; Fig. 2). The second diaphragm is a short distance beneath the first but is clearly distinguishable from it in transverse section since the outer doublets of the axoneme have there increased to triplets (Fig. 2D). The addition of one filament to each doublet occurs mid-way between the two diaphragms. Also at this level the flagellar pore invagination of the outer cell membranes join the membrane of the flagellar sheath; and associated with each of the newly formed triplets is a centrifugally radiating spoke or fibre terminating in a densely stained circular body (Pl. 2, fig. 9; Fig. 2D).

The basal region (kinetosome) and roots anchoring the flagellum. The beginning of the basal region is marked by the centrifugally arranged accessory 'spokes' or fibres attached to the triplets (Pl. 2, fig. 9). The structure of the basal region is almost uniform throughout its length and consists of a single central tubule and nine peripheral triplets. Fine connexions or septa join the triplets to the central filament (Pl. 2, fig. 11;

Fig. 4. Diagrammatic reconstruction of the root system associated with the flagellar bases (FB): flagellum, F; root, R; theca, T.

Fig. 2E). Towards the base of this region the angle which the triplets make to the radius to the centre of the flagellum decreases from about 85° to 50° (Pl. 2, figs. 9–12). At the base of this region the inner filament of each triplet is joined by a septum to the outermost filament of the next triplet in a clockwise direction (Pl. 2, fig. 12; Fig. 2F).

Both flagellar bases are joined by branches of a common striated root system (Pl. 4, fig. 19; Fig. 4). In transverse section the flagellar base is seen to be closely associated with a row of 15–20 fine filaments. These filaments join to form a single root which runs below the cell theca. In longitudinal section the root appears to be striated,

with a periodicity of about 360 Å. The filaments of the root appear to join the subthecal filament system (Leadbeater & Dodge, 1966). Smaller minor roots are also associated with the flagellar bases, one joining a triplet (Pl. 4, fig. 18) in the mid-basal region of the flagellum to another minor root which passes from the base of the flagellum to the subthecal region of the flagellar pore.

Fig. 5. Diagram of the transverse flagellum after its emergence from the flagellar pore, showing the suggested relationship between the axoneme (*A*), the striated strand (*S*) and the flagellar sheath. The fine hairs are not shown on this drawing.

DISCUSSION

The work reported in the present paper shows that the structure of the axoneme of dinoflagellate flagella conforms to the 9+2 (+secondary fibrils) pattern found in almost all cilia and flagella. In the dinoflagellates there are, however, several unique features in the construction of the flagella which are undoubtedly associated with the unusual movement, particularly that of the transverse flagellum.

The longitudinal flagellum is relatively simple in structure and externally has the shape described by Deflandre (1934) and Pitelka & Schooley (1955). The fine hairs, which we have observed for the first time, appear roughly similar in size to the deciduous hairs which are found in the Prasinophyceae (Manton, Rayns & Ettl, 1965). However, in dinoflagellates they do not appear to be easily detached and the flagella do not have a scaly covering like those of the Prasinophyceae. Internally the dinoflagellate longitudinal flagellum contains a large volume of packing material in the thick proximal region. This material is arranged in a definite manner (Pl. 3, fig. 16) and bears some resemblance to the 'paraxial rod' of trypanosome flagella (Vickerman, 1962).

The movement of the longitudinal flagellum of the large dinoflagellate *Ceratium* was studied in detail by Jahn, Harmon & Landman (1963) and by Brokaw & Wright (1963). They found it to show a base to apex planar wave which approximated to a sine wave. Our observations on living material (with phase microscopy) would suggest that this is normally the case in *Gymnodinium*, but as the wave is never retained after fixation we have been unable to make a detailed check of the form that this takes. It appears that sometimes the longitudinal flagellum is held straight, perhaps functioning like a rudder.

The transverse flagella examined possess several unique features. Externally, the

single array of long fine hairs appears rather similar to that of *Euglena* (Leedale, Meuse & Pringsheim, 1965) but internally, and in its movement, there is little similarity with the euglenoid flagellum. One distinctive dinoflagellate feature is the regularly banded striated strand which is probably constructed of fibrous protein. If this strand has elastic properties these will be of great importance in the structure of the flagellum which we describe below. The ribbon-like sperm tails of the toad; *Bufo arenarum* (Burgos & Fawcett, 1956) contain an accessory body (in addition to the axoneme), but unlike the striated strand, this body does not show banding and the tails move with a planar wave.

Observations of living organisms by high-speed photography (Jahn *et al.* 1963) suggest that the transverse flagellum moves in a base to apex circular or elliptical helical wave. From our sections it is clear that the flagellum is ribbon-like with the axoneme at one side of the hollow ribbon and the striated strand at the other (Pl. 2, fig. 5). Between these two structures is found packing material similar to that described from the longitudinal flagellum. From shadowed preparations it is clear that the striated strand, which normally runs in a more or less straight course, is much shorter than the axoneme. This latter is always thrown into a series of waves, even after fixation, and normally passes under and over the striated strand. These facts can be explained if the flagellum is visualized as having the form of a drawn out helix rather like a steep spiral staircase in which the axoneme forms the outer boundary of the spiral, and the striated strand the inner boundary. The packing material forms as it were the steps between the striated strand and the axoneme. This interpretation is illustrated in Fig. 5. From models which we have constructed it would appear that the tightness of the striated strand is to a considerable extent responsible for the helical form of the flagellum and therefore also for its unique helical movement. It is necessary, for this model to work, to have each end of the striated strand firmly attached to the axoneme. Our sections appear to show that it tapers off adjacent to the axoneme but no connexion has so far been observed.

Occasional micrographs have been obtained in which the striated strand and the axoneme do not appear to be intertwined but lie one over the other. In these cases it is possible that the relationship between the strand and axoneme was disturbed when the distal end of the flagellum became detached from the organism. Presumably if detachment takes place before fixation some rearrangement is likely to occur in a tensioned system like this. The model described above appears to be compatible with the observed movement of the flagellum. The broad spiral would appear to present a much greater surface area to the water than would a simple cylindrical structure. Whether this is also responsible for the forward movement of the organism, which Jahn *et al.* (1963) believe the transverse flagellum to provide, will only become clear after detailed hydrodynamic study.

The internal structure of the transition region differs somewhat from that described for certain other flagellates (e.g. Manton, 1963). Instead of the normal single basal disc two are found and, beneath these, two diaphragms. The pairs of discs and diaphragms may provide a useful taxonomic character for dinoflagellates. The size of the basal discs and the width of aperture of the diaphragms is of interest in that it corresponds with the diameter of the circle enclosed by the secondary fibrils of the axoneme. These fibrils appear to be continuous through the length of the flagellum, eventually joining the radial septae at the bottom of the flagellar base (Fig. 2).

As in *Pseudotriconympha* (Gibbons & Grimstone, 1960) the peripheral doublets of the axoneme of the dinoflagellate flagella become triplets, but here this takes place not at the basal disc but between the two diaphragms. At this same point the outwardly radiating spokes make the structure appear like that of a centriole. The lower half of the basal region, the connexion between the triplets, their change in angle, and finally the central hub with radiating spokes, conform very closely with what has been found in other flagella. Similarly, the striated roots are like those of ciliates, but there appears to be no connexion with the nucleus of the type found in *Prasinocladus* (Parke & Manton, 1965). In fact the root in *Gymnodinium* does not penetrate very deeply into the organism but probably only joins the subthecal fibrillar system.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Shadowed whole mount of *Gymnodinium vitiligo* showing longitudinal flagellum and transverse flagellum displaced above the organism. Shadowed gold+palladium. $\times 3500$.

Fig. 2. Detached distal tip of transverse flagellum of *Gymnodinium vitiligo* showing unilateral array of long hairs with curved bases. The striated strand (S) terminates at the same level as the hairs. Shadowed gold+palladium. $\times 14,400$.

Fig. 3. Distal portion of the longitudinal flagellum of *Woloszynskia micra* showing the firm sheath around the axoneme and the short fine hairs. One filament protrudes into the tip, the other filaments terminate short of this. Shadowed gold+palladium. $\times 17,500$.

Fig. 4. Small portion of the transverse flagellum of *Gymnodinium veneficum* showing the undulating axoneme enclosed in an expanded sheath and bearing a unilateral array of long hairs. The striated strand runs more or less straight through the sheath. Shadowed gold+palladium. $\times 10,500$.

PLATE 2

Transverse sections through the flagella of *Wolszynskia micra*.

Fig. 5. External portion of transverse flagellum. The expanded sheath contains the axoneme of nine outer doublets, two central filaments and the secondary fibres, and is expanded laterally to contain the fibrils of the striated strand (s). $\times 78,400$.

Fig. 6. Proximal portion of longitudinal flagellum. The axoneme is surrounded by the sheath and a large amount of packing material. $\times 58,800$.

Fig. 7. Distal transition region, the two central filaments are surrounded by the dense basal disc (B) of similar diameter to the secondary fibres. $\times 58,800$.

Fig. 8. Proximal transition zone. A diaphragm (D) projects into the lumen of the axoneme, from the periphery of the triplets. The diaphragm is penetrated by an aperture of similar diameter to the basal disc, and in the centre is a single filament. $\times 108,000$.

Fig. 9. Distal basal region, the triplets are attached to centrifugally radiating fibres terminating in dense staining areas. The central filament is just visible. $\times 108,000$.

Fig. 10. Mid-basal region, the triplets are orientated at approximately 30° to the circumference. $\times 71,000$.

Fig. 11. Distal basal region, showing central filament. $\times 83,600$.

Fig. 12. Proximal basal region. Triplets orientated at 35° to the circumference and joined to the central filament by fine fibres or septa. The inner filament of each triplet is joined to the outer filament of the adjacent triplet by a fine fibre (F). $\times 108,000$.

PLATE 3

Longitudinal sections through the flagella of *Woloszynskia micra*.

Fig. 13. Transverse flagellum and basal body showing median and tangential longitudinal sections of axoneme and the striated strand. The two central filaments of the axoneme terminate in the first basal disc (B); immediately below this is the second basal disc and the two diaphragms (D). Attached to the base of the flagellum is part of the root extending to the subthecal region of the flagellar pore. The flagellum has recently divided and the duplicate base is seen above. $\times 37,500$.

Fig. 14. External portion of the transverse flagellum showing fibrous connexions between the two central filaments of the axoneme and the complex cross banding of the striated strand (S). $\times 58,800$.

Fig. 15. Transverse flagellum cut obliquely and showing the striated strand (S), some of the axoneme filaments and packing material. $\times 58,800$.

Fig. 16. Longitudinal flagellum showing peripheral filaments of axoneme and regularly arranged packing material. $\times 84,800$.

PLATE 4

Fig. 17. Longitudinal section of two flagellar bases of *Woloszynskia micra*. Showing the lower basal disc, the diaphragms and cross-striations in the basal region. (see Fig. 2). $\times 54,000$.

Figs. 18, 19. Roof structure of *Woloszynskia micra*. Fig. 18. Transverse section of the basal region of two flagella showing the connection between one triplet and the root (r). $\times 71,000$. Fig. 19. Longitudinal section of flagellar bases (k) with associated root structure (r). Below the lower base a narrow band of striated fibres joins the main root. $\times 63,000$.

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