FLAGELLAR ROOTS, MATING STRUCTURE AND GAMETIC FUSION IN THE GREEN ALGA ULVA LACTUCA (ULVALES)

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SUMMARY
The slightly anisogamous gametes of Ulva lactuca exhibit a cruciate flagellar root system consisting of 4 microtubular roots (4-2-4-2-system) and an elaborate system of fibrous roots associated with the 2-stranded microtubular roots. Two fibres (32-nm striation periodicity; system I fibres) closely underlie each of the 2-stranded roots, while different fibres (150-nm striation periodicity; system II fibres) run parallel to the root microtubules, and are 150-200 nm more internally located. Female gametes have 4 system II fibres, 3 of which are combined into a compound fibre associated with one microtubular root, while the fourth fibre is associated with the opposite root. In male gametes only 2 system II fibres are present, each underlying one of the two 2-stranded roots.

A special region of the plasmalemma of both gamete types about 0.5 μm away from the basal bodies and located between 2 adjacent microtubular roots is structurally specialized and acts as a mating structure in gametic fusion. The region is oval-shaped and up to 1.1 μm long with a maximum diameter of 0.7 μm. A continuous electron-dense boundary layer underlies the plasmalemma at the edges of the mating structure. In both gamete types the mating structure consists of a fuzzy layer of material underlying the plasmalemma and special granules (60 nm diameter) are associated with this layer on its cytoplasmic side. In addition diffuse material overlies the mating structure, especially in male gametes. The mating structure is connected to 3 different kinds of flagellar roots: the boundary layer is linked to a 2-stranded microtubular root and its associated system I fibre; the fuzzy layer of the mating structure is connected with a system II fibre; and in female gametes this is the compound system II fibre.

The ultrastructural changes which occur after mixing the 2 gamete types have been followed. Mating structure activation involves contraction of system II fibres (change of striation periodicity to 100 nm), detachment of special granules from the fuzzy layer of the mating structure and their replacement by electron-transparent vesicles at the prospective cell fusion site. Furthermore, release of electron-dense contents from Golgi-derived vesicles in the anterior part of both gamete types precedes cell fusion. Cell fusion is exclusively initiated in a region delimited by the 2 mating structures. After partial dissolution the 2 plasma membranes unite within the mating structure regions.

The ultrastructure of gametic fusion in Ulva lactuca is compared to that of other green algae and the significance of flagellar roots in the mating process of green algae is discussed.

INTRODUCTION
The ultrastructure of fertilization in green algae has been studied in only a limited number of genera. The most detailed studies have been made with different species of the biflagellate Chlamydomonas (e.g. Friedmann, Colwin & Colwin, 1968; Cavalier-Smith, 1975; Triemer & Brown, 1975a; Goodenough & Weiss, 1975). Following
flagellar agglutination the complex sequence of events that leads to cell fusion in *Chlamydomonas reinhardii* involves (a) digestion of gamete cell walls, (b) activation of mating structures, and (c) fusion between activated mating structures of opposite gamete types (for review see Goodenough, 1977). In the marine multicellular green alga *Ulva* previous light-microscopical studies (Levring, 1955) and electron microscopy (Braten, 1971) have indicated that the general mating behaviour of the gametes is similar to that of *C. reinhardii* gametes. The differences include absence of cell walls from *Ulva* gametes and the rapidity of the whole mating process. Within 10–20 s after initial mixing of the 2 gamete types cell fusion is initiated in most cells (Braten, 1971). Despite the precision of the mating process in *Ulva*, in a thin-section study of fertilization in *Ulva mutabilis* special mating structures were not found and the plasma membranes of the copulating gametes were said simply to fuse at the point of contact (Braten, 1971). It has therefore been concluded that *Ulva* gametes lack special mating structures (Pickett-Heaps, 1975). Recent experimental work with *Ulva mutabilis* gametes has, however, shown that flagellar agglutination triggers a signal that activates particular areas of the plasma membrane for fusion (Lovlie & Bryhni, 1976).

Zoospores of *Ulva lactuca* contain a complex set of cruciate flagellar roots including 2 different types of striated fibres (Micalef & Gayral, 1972; Melkonian, 1979). One fibre type is composed of a bundle of 6–8-nm filaments and therefore resembles fibres ('rhizoplasts', for review see Melkonian, 1980) that have been shown to be contractile and possibly involved in initiation and coordination of flagellar beat in the green flagellate *Tetraselmis* (Salisbury & Floyd, 1978; Robenek & Melkonian, 1979). Recently the presence of similar fibres was also described in the genus *Chlamydomonas* (Katz & McLean, 1979).

In the present study the occurrence of elaborate mating structures in both gamete types of *Ulva lactuca* is demonstrated. From comparison of the detailed structure of flagellar roots before and after gamete mixing it is concluded that a fibrous root is actively engaged in the mating process of *Ulva*.

**MATERIALS AND METHODS**

Fertile thalli of *Ulva lactuca* L. were collected on the rocky flats at the west cliff of the island of Heligoland during spring tides in April 1977. The thalli were individually placed in plastic Petri dishes in a moist but not wet condition. After return to the laboratory the Petri dishes were stored overnight in a cool chamber (10 °C). The next morning the thalli were flooded with fresh seawater and exposed to a northern window light. In less than a minute motile cells were discharged from the fertile regions of the thalli. Most thalli were gametophytes and the released gametes showed positive photoaccumulation and concentrated at the meniscus near the light source. Female gametes appeared as a dark-green mass, while male gametes, due to their prominent eyespot, exhibited a more yellow-green colour. Gametes were fixed for electron microscopy 5 min after their release. In gametic fusion experiments concentrated suspensions of male and female gametes were mixed in a Petri dish and exposed to a northern window light. A few seconds after mixing gamete pairs were swimming away from the light source, while the gamete type outnumbering the other sex showed positive photoaccumulation. Only the backward swimming gamete pairs were fixed for electron microscopy. The time interval between initial mixing and fixation of gamete pairs was exactly 20 s.
Gametic fusion in Ulva

Fixation was performed by mixing equal amounts of 2% glutaraldehyde, made up in 0.25 M sucrose without buffering but with final pH adjustment (pH 8.2), with the cell suspension, giving a final glutaraldehyde concentration of 1%. The cells were fixed for 30 min at 20 °C. After centrifugation the cells were washed 3 times with seawater and postfixed in 1% OsO₄ (prepared as a 2% solution in 0.25 M sucrose and mixed with equal amounts of seawater). Postfixation was carried out for 10 min at 4 °C. The fixed cells were again washed with seawater at 4 °C 3 times and stored for no longer than 2 days before further processing as previously described (Melkonian, 1975). Sections were cut either with glass knives or with a diamond knife (Dupont) and examined with a Siemens Elmiskop 102.

RESULTS

General

Gametophytes of Ulva lactuca produce anisogametes, the female gamete being slightly larger (mean length 8 μm as compared to about 6 μm for the male gamete). Gamete ultrastructure is similar to that of zoospores of the same alga (Micalef & Gayral, 1972; Melkonian, 1979) and to that of gametes of Ulva mutabilis (Bråten, 1971) and need not be described in detail. A longitudinal section through a female gamete showing organelle distribution is sufficient for present purposes (Fig. 2). Female and male gametes do not differ in their general ultrastructure, although differences exist in the detailed structure of the flagellar apparatus (see below). Special mention should be made of Golgi-derived electron-dense vesicles in the anterior part of the cells (e.g. Fig. 2). These vesicles measure about 200 nm in diameter.

Flagellar roots

The 2 basal bodies of a gamete form an angle of 180° with each other and at their proximal end they overlap for about 200 nm. The flagellar root system is cruciate, consisting of a microtubular system (4-2-4-2-system) and a complex system of striated fibres. Each basal body is connected to 2 microtubular roots (a 4-stranded and a 2-stranded root) and the disposition of root microtubules is shown in the diagram of Fig. 1. In the anterior part of the cell the 4 microtubular roots run inside 4 projecting ribs (e.g. Figs. 5, 6); a typical cruciform papilla, as in the quadriflagellate zoospores (Melkonian, 1979), is, however, not present in the gametes (Fig. 3). The termination of root microtubules was not observed, but in cross-sections at the level of the nucleus they were still seen. In addition to root microtubules a small number (about 10–20 per gamete) of secondary cytoskeletal microtubules was found (e.g. Fig. 11).

The striated fibres are of 2 types. One type (system I fibres according to Micalef & Gayral, 1972, and Melkonian, 1980) is closely associated with the two-stranded roots (see Fig. 1). Longitudinal sections through this fibre reveal a striation pattern of 32 nm. This fibre is about 1–2 μm long, 30 nm wide and 70–80 nm thick. It tapers distally and finally splits into 2 parts, each being associated with one of the two microtubules (see diagram Fig. 9 and Fig. 10). System I fibres are absent from 4-stranded roots.

The second type of striated fibres (system II fibres) is also associated with only the
Fig. 1. Schematic 3-dimensional reconstruction of the flagellar apparatus of female gametes of Ulva lactuca. Abbreviations: b, basal body; ms, mating structure; f₁, system I fibre; f₂, system II fibre; r₁, 2-stranded microtubular root; r₂, 4-stranded microtubular root; cf, connecting fibre.

Figs. 2–15. Ultrastructural aspects of the female gamete of Ulva lactuca.

Fig. 2. Longitudinal section through the female gamete revealing organelle distribution. n, nucleus; p, pyrenoid; s, starch grain; cl, chloroplast; arrow depicts Golgi-derived electron-dense vesicle. × 14,000.

Fig. 3. Cross-section through the anterior part of the cell. Only one root type shows associated electron-dense material (arrows). × 33,000.

Figs. 4, 5. Proximal (Fig. 4) and distal (Fig. 5) cross-sections through the 2-stranded microtubular root, curved arrow, system II fibre; straight arrow, microtubular root. × 64,000.

Fig. 6. Cross-section through the distal parts of the 4-stranded microtubular root with 3 over 1 configuration of root tubules (arrow). Note absence of system II fibre. × 60,000.

Fig. 7. Cross-section through distal parts of the 2-stranded microtubular root. Curved arrow, compound system II fibre; straight arrow, boundary layer of the mating structure in oblique view. × 77,000.

Fig. 8. Longitudinal section through the cell in a plane perpendicular to that of Fig. 2; arrows indicate limits of the mating structure. × 37,000.
Gametic fusion in Ulva
Fig. 9. Schematic 3-dimensional reconstruction of the mating structure in female gametes. Abbreviations: gr, granules arranged in rows and interconnected by microfilaments; fl, fuzzy layer of material; mz, 'membrane zone', bl, boundary layer. For explanation of other abbreviations see legend to Fig. 1.

Fig. 10. Cross-section through the mating structure of female gametes. Curved arrows, 3 profiles of the compound system II fibre; straight arrows, cross-sections through the boundary layer. × 78,000.

Fig. 11. Section similar to that of Fig. 10. Curved arrow, compound system II fibre with only one profile; small arrow, 2-stranded microtubular root associated with the edge of the boundary layer. × 78,000.

Fig. 12. Cross-section through the mating structure at higher magnification. Large arrows, special granules; short arrow, fuzzy layer of material underlying the plasmalemma in the mating structure region. Boundary layers, 2 profiles of system II fibres and the microtubular root attached to the boundary layer can also be seen. × 125,000.

Fig. 13. Oblique section through the mating structure. Curved arrows, 2 individual fibres of the compound system II fibre; short arrows, limits of the mating structure. × 59,000.

Fig. 14. Tangential section through the mating structure. Special granules are arranged in rows; arrow, granules are interconnected by fine filaments, $f_t$, system I fibre. × 67,000.
Gametic fusion in Ulva
Gametic fusion in Ulva

2-stranded roots. These fibres run parallel to the 2-stranded roots being about 200 nm more internally located (e.g. Figs. 4, 5, 7). System II fibres consist of a bundle of filaments (diameter 6–8 nm) and in longitudinal sections show cross-striations with a 150-nm repeat (Figs. 13, 15). The pattern is apparently constant within single fibres and also between fibres of different cells. The female gametes have 4 fibres, 3 fibres underlying one of the microtubular roots and the fourth fibre underlying the opposite root (Fig. 1). The 3 fibres over most of their length are united into a compound fibre (Fig. 15) and are twisted around each other. Distally they separate and oblique or tangential sections in this area establish the presence of at least two separate fibres (Fig. 13). The length of the compound fibre is about 2 μm. The fibres originate at the proximal end of a basal body embedded in electron-dense material. Regarding the 3 individual fibres of the compound fibre, 2 fibres originate at one basal body, while the third originates at the inner side of the other basal body (see Fig. 1). The significance of these peculiar fibre arrangements is related to the presence of a single mating structure (diagram Fig. 9).

In male gametes there are only 2 system II fibres. The length of the 2 fibres is approximately 1.2 μm and they are 50–60 nm wide. Each fibre originates at the outer side of the respective basal body (Fig. 18). The inner side of the proximal end of the 2 basal bodies also carries electron-dense material, but this does not give rise to additional striated fibres in the male gametes (e.g. Fig. 27).

Mating structure

Part of the plasmalemma region in both gamete types near the flagellar apparatus is specialized for gametic fusion and represents an elaborate mating structure. The mating structure lies perpendicular to the plane of beat of the 2 flagella, its anterior end being approximately 0.6 μm away from the proximal part of one of the basal bodies in female gametes (Figs. 1, 8, 15) and about 0.4–0.5 μm away from the respective proximal part of one of the basal bodies in male gametes (Figs. 18, 27).

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Fig. 15. Longitudinal section of compound system II fibre (fII); b,b cross-sections through both basal bodies; ms, mating structure; short arrow, proximal part of the opposite system II fibre. × 64,000.

Figs. 16–18. Ultrastructural aspects of the mating structure of male gametes.

Fig. 16. Longitudinal section through mating structure. Large arrow, proximal part of system II fibre; small arrow, fuzzy material overlying the mating structure. × 50,000.

Fig. 17. Cross-section through the mating structure at its anterior end. Curved arrow, system II fibre; long arrow, boundary layer; short arrow, 2-stranded microtubular root. × 75,000.

Fig. 18. Longitudinal section through the mating structure. b, 2 basal bodies in cross-section; short arrows, 2 ‘synthesizing complexes’ of system II fibres. gv, Golgi-derived vesicle. × 50,000.

Figs. 19–23. Activated gametes 20 s after mixing the 2 gamete types.

Fig. 19. Longitudinal section through the mating structure region of activated gamete in the same region as in Fig. 18. Notice changed structure of system II fibre and Golgi-derived vesicle (gv). Slight protrusion of mating structure (long arrow), short arrow, attachment point of system II fibre with the plasmalemma. × 50,000.
Gametic fusion in Ulva

basal body in male gametes (Figs. 15, 17). The mating structure is oval with an average length of 1-1 μm and a short diameter between 0-6 and 0-8 μm in the female gametes. In male gametes it is slightly smaller, being 0-9 μm long and 0-5 μm wide. Details of the mating structure and its connexion with flagellar roots are shown diagrammatically in Fig. 9. Below the plasmalemma an electron-dense boundary layer runs around the edge of the mating structure as a continuous line (Figs. 1, 7, 10). In a few favourable cross-sections the boundary layer could be seen to consist of 2 electron-dense lines, but often this substructure was obscured. Above the boundary layer a thin electron-dense 'membrane zone' is closely appressed to the inner leaflet of the plasmalemma (Figs. 10, 21). Electron-dense links occur between the membrane zone and the boundary layer at fairly regular intervals of about 10 nm (e.g. Fig. 28) as seen in longitudinal sections through the boundary layer. A fuzzy layer of material is associated with the inner leaflet of the plasmalemma in the region which is enclosed by the boundary layer (e.g. Figs. 10–12). In male gametes this layer appears to be more condensed (compare Fig. 15 with Fig. 16). At its cytoplasmic side the fuzzy layer is connected to special small granules (Figs. 11, 12, 16). The disposition of these granules form rows arranged at an angle of about 40° with respect to the short axis of the mating structure (Fig. 9). Individual granules measure 50–60 nm and appear to be interconnected by filaments 6–7 nm in diameter. The total number of granules per mating structure was calculated to be about 80 in female gametes and 50 in male gametes. A significant specialization of the mating structure in male gametes is the presence of prominent fuzzy material outside the cell adhering to the plasmalemma of the mating structure (Figs. 16, 18). This material is much less conspicuous on the plasmalemma of female mating structures (Figs. 12, 15) and was not observed elsewhere on the plasmalemma of either gamete type.

Fig. 20. Cross-section through activated mating structure. Short arrow, special granules still attached to the fuzzy layer of material. Membrane material accumulates below the granules. × 77000.

Fig. 21. Cross-section through activated mating structure at a later stage as in Fig. 20. Arrows, special granules associated with an electron-transparent vesicle (v). Notice that the fuzzy layer stains less intensely as in Fig. 20. × 77000.

Fig. 22. Longitudinal section through activated mating structure in the region of the boundary layer (long arrow), gv, Golgi-derived vesicle with partially retracted electron-dense contents; small arrows, links between cross-bars of a system II fibre and the boundary layer. × 75000.

Fig. 23. Tangential section through activated mating structure. Notice disposition of electron-transparent vesicles (v) and special granules (short arrows). Long arrow, Golgi-derived vesicle; b, basal body. × 60000.

Figs. 24–29. Different aspects of gamete pairs after cell fusion.

Fig. 24. Early fusion stage in cross-section. v, electron-transparent vesicle; short arrows, special granules; long arrows, cross-section through boundary layer of one gamete type. × 77000.

Fig. 25. Cross-section through the cell fusion site at a later stage as in Fig. 24. v, large electron-transparent vesicle; long arrows, cross-sections of boundary layers of both gametes; short arrow, region of the plasmalemma where recombination of membranes had taken place. × 90000.
Association of mating structure with flagellar roots

The mating structure in both types of gametes is closely associated with 3 different flagellar roots (Fig. 9). One edge of the mating structure is always linked to one microtubule of the two-stranded microtubular root (Figs. 9-12, 17) and this gives the mating structure a definite position with respect to the flagellar apparatus. Special links connect the root microtubule with the outer edge of the boundary layer over much of the length of the mating structure (e.g. Figs. 10, 11). The system I fibre, which underlies the 2-stranded root, is connected to the boundary layer only in a limited region in which the boundary layer can be seen to arch around into the cytoplasm to associate with the system I fibre (Fig. 12). The third kind of flagellar root associated with the mating structure is a system II fibre. In female gametes this is the compound system II fibre (Figs. 1, 9). In cross-sections and tangential sections through the mating structure it is seen that the compound fibre attaches to several of the special granules (Figs. 11, 13). A special connexion of the system II fibre with the boundary layer (Fig. 9) was clearly seen only during mating structure activation (see below). In male gametes the system II fibre seems to terminate together with the mating structure (Figs. 18, 19) while in female gametes at least one part of the compound fibre extends beyond the mating structure (Figs. 13, 15).

Mating structure activation

Gamete pairs of *Ulva lactuca* were fixed for electron microscopy 20 s after mixing the 2 gamete types. As seen in the EM more than 90% of the cells have established cell fusion (see below). A few cells, however, were found with an apparently intact plasmalemma in the mating structure region as judged from serial sections. These cells showed some remarkable differences in detailed structure when compared to unmated gametes.

The cross-banding pattern of the system II fibres as measured in 8 different cells

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Fig. 26. Longitudinal section through the cell fusion site. Both gametes in parallel arrangement. *fl*, flagellum; *b*, basal body; *flb*, system II fibre; short arrows, special granules attached to both sides of fusing electron-transparent vesicles (*e*); long arrow, recombination region of plasma-membranes; small arrows, cross-sections through boundary layers of the 2 gametes. ×77,000.

Fig. 27. Longitudinal section through the cell fusion site. Both gametes in antiparallel arrangement. *b*, basal bodies of the female gamete; *flb*, system I fibre of male gamete; arrows, oblique sections through 3 different system II fibres; *gv*, Golgi-derived vesicle near one system II fibre; *e*, electron-transparent vesicles at the cell fusion site. ×66,000.

Fig. 28. Oblique section through the boundary layers of 2 fusing gametes. Notice fuzzy material in the extracellular space overlying the boundary layers. ×105,000.

Fig. 29. Cross-section through the anterior part of a young zygote at a later stage of fusion. All 4 flagellar bases and the proximal parts of all 8 flagellar roots can be traced. Long arrows, bases of 4-stranded flagellar roots; short arrows, bases of 2-stranded flagellar roots. Note that the mating structures obviously fused in a trans-configuration. ×42,000.
was only 100 nm, compared to 150 nm in the unmated gametes. These fibres also appeared more electron-dense and thicker (compare Fig. 18 with Fig. 19). In the proximal part of such a fibre (near the basal body) the cross-banding pattern was slightly larger (about 115 nm) than in the parts closer to the mating structure (e.g. Figs. 19, 23). In longitudinal sections through the mating structure distinct links between the cross-bars of the fibre and the boundary layer were seen (Fig. 22). The contraction of the system II fibre led to a slight protrusion of the mating structure (Fig. 19).

The mating structure also showed some peculiarities in ‘activated’ cells. The granules seem to dissociate from the fuzzy layer (compare Figs. 20 and 21). Electron-transparent vesicles replace the granules (Fig. 21). The fuzzy layer appears less electron-dense and some of its material possibly coats the electron-transparent vesicles (Figs. 20, 21, 32). The granules are attached to these vesicles exclusively at their cytoplasmic side (Figs. 21, 23).

In the anterior part of the activated cells the Golgi-derived electron-dense vesicles lose their contents. The contents retract partially from the vesicle membrane, leaving a clear space (compare Fig. 18 with Fig. 19).

Due to the infrequent occurrence of activated cells in the gamete-pair suspension, it was not possible to determine whether male or female gametes differed with respect to activation.

Gametic fusion

Cell fusion is exclusively initiated in the region of the 2 mating structures enclosed by the 2 boundary layers (see Figs. 24–28). The initial fusion event seems to be a very rapid process since this stage was never observed, although activated but not yet fused gametes were occasionally found (see above). Probably only a single fusion site at both mating structures is involved in the process (Fig. 24). The fate of the 2 plasmalemmata at the fusion site is unknown, but measurements of plasmalemma length have indicated that part of the plasmalemma either dissolves during fusion or is incorporated into cytoplasmic vesicles. Large electron-transparent vesicles are prominent structures at the cell fusion site (Figs. 24–27). These probably do not represent incomplete fusion areas, but possibly arise from fusion of those electron-transparent vesicles which are already present in the activated gametes (see above). The vesicles are coated on 2 sides with the special granules, a further indication of their origin by fusion of vesicles from both gametes (Figs. 25, 26). The open plasmalemmata at the edges of the fused area recombine well within the two boundary layers. This is concluded from slight overlap in the fuzzy layer of the 2 plasmalemmata in this region in cross-sections of the fusion site (Fig. 26). The region in which the plasmalemmata recombine is approximately an equal distance away from both boundary layers (Figs. 25, 26). Therefore both boundary layers lie opposite each other in cross and longitudinal sections through the cell fusion site (Figs. 25–28).

Arrangement of gamete cell bodies during fusion followed 2 clearly distinguishable patterns. About half of the gamete pairs lie side by side with their longitudinal axes
Gametic fusion in Ulva

parallel to one another (Fig. 26), while another 50% are oriented antiparallel to each other (inverse gamete pair; Fig. 27).

Later stages of cell fusion were only rarely found 20 s after mixing both gamete types (e.g. Fig. 29).

DISCUSSION

Flagellar roots

Gametes of Ulva lactuca exhibit a cruciate microtubular root system which is of widespread occurrence in the Chlorophyceae (review by Moestrup, 1978). The presence of an additional system of 2 types of fibrous roots (system I and system II according to Micalef & Gayral, 1972) seems to be limited, however, to members of the Ulvales (summary by Melkonian 1979). In zoospores of Ulva lactuca (Melkonian, 1979) and Enteromorpha linza (Melkonian, 1980) all 4 microtubular roots are accompanied by a system II fibre, while in the gametes of Ulva lactuca only the 2-stranded microtubular roots are associated with system II fibres. This appears to be the first report on differences in flagellar root system structure between gametes and zoospores in a single green algal species, since previous studies have indicated that the flagellar root system in gametes and zoospores within a species is the same (e.g. Moestrup, 1978). Both gamete types of Ulva lactuca further differ with respect to the numbers of system II fibres associated with the 2-stranded microtubular roots. The smaller male gametes seem to be more specialized, in that two of the 4 'synthesizing complexes' located at the basal bodies do not give rise to fibres. This sex-specific difference in the flagellar root system might be related to different mating behaviour of the 2 gamete types (see below). Since anisogametes of other green algae have not been comparatively studied with respect to their flagellar apparatus, it is at present not possible to deduce that anisogametes would generally differ in the detailed structure of their flagellar apparatus, although this appears likely. Recently Hoffman (1979) found differences in the structure of the flagellar apparatus between the male gametes of anisogamous and oogamous species in the Sphaeropleaceae and observations on anisogametes of Bryopsis lyngbyei also indicate that the flagellar apparatus of male and female gametes is different (Melkonian, in preparation).

Mating structure

The term mating structure was introduced by Goodenough and coworkers (Martin & Goodenough, 1975; Goodenough & Weiss, 1975) for an organelle associated with the plasmalemma of gametes of Chlamydomonas reinhardii, which is engaged in the gametic fusion process. This term is more universally applicable than 'choanoid body' (Friedmann et al. 1968; Triemer & Brown, 1975a) or 'gamosome' (Cavalier-Smith, 1975). A mating structure can be defined as an organelle associated with the plasmalemma of one or both gamete types, which is activated following contact between different gamete types and is involved in establishing cell fusion between them. Within the green algae only in Chlamydomonas reinhardii (Friedmann et al. 1968; Goodenough & Weiss, 1975) and gametes of Hydrodictyon reticulatum (Marchant &
Pickett-Heaps, 1972) has the presence of mating structures been proven by electron microscopy. Katz & McLean (1979) have recently mentioned the presence of a 'choanoid body', which may be a mating structure in the plasma papilla area of *Chlamydomonas moewusii* gametes. Previously no mating structures have been detected in *C. moewusii* gametes (e.g. Triemer & Brown, 1975b). The occurrence of cytoplasmic protruberances facilitating gametic fusion in a greater number of green algae was described by light microscopy (summary by Wiese, 1969), but no ultrastructural data are available on these organisms. The absence of mating structures was reported in the isogamous *Polytoma papillatum* (Gaffal & Schneider, 1978), but no freeze-fracture observations were made to determine whether membrane specializations were present in the apical plasma papilla of *Polytoma* gametes. It is suggested that even if in thin-section studies a mating structure is not discernible, freeze-fracture might show membrane specializations at the future sites of cell fusion (e.g. Weiss, Goodenough & Goodenough, 1977).

When comparing the mating structure of *Chlamydomonas reinhardii* (e.g. Triemer & Brown, 1975a; Goodenough & Weiss, 1975) with that of *Ulva lactuca* some similarities and some differences are encountered. The boundary layer obviously corresponds to the 'doublet zone' in mt+ gametes of *C. reinhardii*, the small hole that is present in the doublet zone of *C. reinhardii* is enlarged in *Ulva* gametes and accounts for most of the mating structure area, i.e. the region enclosed by the boundary layer. Both gamete types in *Ulva* have a boundary layer, while in *C. reinhardii* a doublet zone was reported to be absent from the mt- gamete (Goodenough & Weiss, 1975). With an improved fixation schedule it was, however, later shown that an electron-dense boundary layer similar to that in *Ulva* is present in mt- gametes of *C. reinhardii* (Weiss et al. 1977). This may either be a reduced or a slightly developed doublet zone. A continuous 'membrane zone' as reported for mating structures of both gamete types in *C. reinhardii* (Goodenough & Weiss, 1975) is not found in *Ulva* gametes. In *Ulva* gametes a membrane zone is present only in the space between plasmalemma and the boundary layer. The fuzzy layer of material that underlies most of the plasmalemma area in the mating structure region of *Ulva* bears similarities to the 'bud zone' of activated mt+ gametes of *C. reinhardii* (Goodenough & Weiss, 1975), the whole appearance of the mating structures in *Ulva* is therefore more reminiscent of the activated mating structures of *C. reinhardii* than of the unactivated mating structures. The special granules underlying the fuzzy layer in both mating structures of *Ulva* have not been seen in *Chlamydomonas* or any other green alga.

**Mating structure activation and the role of flagellar roots**

Since the mating process in *Ulva* is very rapid it is difficult to find cells which have performed flagellar agglutination but have not yet commenced cell fusion. The interpretation that the few cells with intact plasma membranes in the mating structure region as revealed by serial sections correspond to the above-mentioned stages of the mating process is based on the fact that only gamete pairs swimming away from the light source were fixed for electron microscopy, and that a time of 15 s was allowed for backward swimming after initial flagellar agglutination. A confusion with the more
numerous gamete type (usually the male gamete) is highly unlikely, because during the experimental period this gamete type accumulated at the meniscus near the light source and furthermore the ultrastructure of these cells was not different from that of gametes before mixing the 2 gamete types (unpublished observations). Since gamete pairs were also seen in different stages of cell fusion 20 s after mixing the gamete types (compare early fusion stages (Fig. 24) with later stages like that in Fig. 29) the proposed interpretation seems most reasonable. It appears, therefore, that flagellar agglutination and not cell fusion signals phototactic reversal and that agglutinated gamete pairs are presumably able to swim in a coordinate manner. In *Chlamydomonas reinhardtii* flagellar agglutination signals a complex series of events, which include cell wall degradation and mating structure activation (Claes, 1971; Goodenough & Weiss, 1975; Solter & Gibor, 1977; Forest, Goodenough & Goodenough, 1978; Matsuda, Tamaki & Tsubo, 1978; Goodenough & Jurvich, 1978). In gametes of *Ulva mutabilis* it was shown that flagellar agglutination signals the activation of particular membrane areas for fusion for a limited period of time (Lovlie & Bryhni, 1976). The ultrastructural changes following flagellar agglutination in *Ulva lactuca* gametes observed during this study include mating structure activation, contraction of system II fibres and release of electron-dense contents from Golgi-derived vesicles. Mating structure activation in *Ulva* is different from that in *C. reinhardtii* gametes because it does not involve formation of a fertilization tubule in one gamete type (e.g. Friedmann *et al.* 1968). At present one can only speculate about the sequence of events involved in mating structure activation in *Ulva lactuca*. One important step in this process seems to be the detachment of the special granules from the fuzzy layer of material. Since the granules are interconnected by microfilaments, an involvement of microfilaments in the detachment process appears possible. In *C. reinhardtii* microfilaments originating from the doublet zone are involved in fertilization tubule elongation (Goodenough & Weiss, 1975). It is suggested that the detachment of granules from the fuzzy layer together with a concomitant partial dissolution of the fuzzy layer makes the mating structure in *Ulva* ready for cell fusion.

The function of flagellar root systems in green algae is still virtually unknown (some functional aspects are discussed by Melkonian, 1978, 1980). This study has given evidence that at least 3 different flagellar roots are associated with the mating structure of *Ulva lactuca* and it was further shown that a fibrous root (system II) contracts after flagellar agglutination. The finding that 2-stranded microtubular roots link to the edges of mating structures in *Ulva* gametes is in accordance with similar observations regarding *C. reinhardtii*, in which contact is mediated by a system I fibre overlying the 2-stranded root (Goodenough & Weiss, 1978). In female gametes of *Bryopsis lyngbyei* the elaborate mating structure is also associated with a 2-stranded microtubular root (Melkonian, in preparation). If this association is of more general occurrence in the green algae, then the function of cruciate microtubular root systems would be better understood. The exact position of the mating structure with respect to the flagellar apparatus, which is a prerequisite for coordinate cell fusion, would be attained by specific association with one root type, while the function of the other
root type can in similar way be explained by the need for exact positioning of the eyespot in relation to the flagellar apparatus (summary by Moestrup, 1978; and Melkonian, 1978). If this hypothesis is correct it could also explain why 2 root types have evolved in most of the green algae. Goodenough & Weiss (1978) have speculated that the system I fibre that associates with the mating structure in *C. reinhardii* may play an active role in signal transduction between the agglutinated flagellar tips and the mating structures. Contractility of this fibre type has until now not been proved (Melkonian, 1980) and there is at present no experimental evidence for the proposed function of this fibre.

The observations that a system II fibre contracts after flagellar agglutination but before cell fusion in *Ulva lactuca* gametes indicates that the signal which is created by the agglutinated flagella causes fibre contraction. Fibres of similar structure have been shown to be contractile in the green flagellate *Tetraselmis* (Salisbury & Floyd, 1978; Robenek & Melkonian, 1979). Cytologically, fibre contraction is detected by a change in the cross-banding pattern (e.g. Salisbury & Floyd, 1978). This change in *Tetraselmis* shows polarity, because the proximal regions of the fibre near the basal bodies are more contracted than the distal regions (Robenek & Melkonian, 1979). A polarity in cross-banding pattern of the fibre in activated *Ulva* gametes was also observed, the fibre was less contracted near the basal bodies and more contracted near the mating structure. In *Tetraselmis* calcium ions appear to trigger the contraction process (Salisbury & Floyd, 1978) and the polarity of contraction possibly reflects the polarity of an internal calcium gradient in the cell. Although involvement of calcium in the mating process of *Ulva* has not been shown yet, this appears likely since calcium plays a role in phototactic reorientation in green flagellates (e.g. Schmidt & Eckert, 1976; Litvin, Sineshchekov & Sineshchekov, 1978; Nultsch, 1979) and also stimulates cell wall lysis in gametes of *C. reinhardii* (Claes, 1980). The observed polarity in contraction of system II fibres of *Ulva* indicates that these fibres are probably not involved in the primary signal transduction from the agglutinated flagella to the cell proper. It appears more likely that they transduce a reciprocal signal either to cause agglutinated flagella to lose their sexual adhesiveness (see also Snell & Moore, 1980) or more probably to create the motive force by which the 2 gametes and their activated mating structures are brought close together to establish cell fusion. The involvement of fibrous roots in fertilization of *Oedogonium cardiacum* has been previously suggested, although no cytological evidence for it was presented (Hoffman, 1973). With respect to *Ulva* it is assumed that the 2 gamete types contribute to a different extent to the initiation of cell fusion. The female gametes with their prominent compound system II fibre might be more active in the directional turning movement. An evaluation of the 2 modes of mating behaviour in *Ulva* suggests that a turning movement initiated by the female gamete would ensure proper cell fusion if mating structures during flagellar agglutination are located on the same side along the longitudinal axis of the gametes. If, however, mating structures are located on opposite sides both gametes would slide along each other and the antiparallel mating configuration results. Similar modes of mating are known for *C. reinhardii* (Friedmann *et al.* 1968).
Gametic fusion in Ulva

The release of electron-dense contents from Golgi-derived vesicles has been observed in settling zoospores of Enteromorpha intestinalis (Evans & Christie, 1970; Christie, Evans & Shaw, 1970; Callow & Evans, 1974) and during the attachment of Ulva mutabilis zygotes (Braten, 1975). These vesicles possibly contain a glycoprotein which acts as an adhesive substance (Callow & Evans, 1974). Braten (1975) has unfortunately not studied the dynamics of changes in vesicle content during the cell fusion process. The results obtained in this study indicate that changes occur in vesicle content in response to flagellar agglutination, even before cell fusion has started. It might therefore be that vesicle discharge resembles the well known cortical granule exocytosis occurring in sea-urchin eggs after sperm attachment (e.g. Chambers & Hinkley, 1979).

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Gametic fusion in Ulva


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