Acta Bot. Neerl. 36(1), February 1987, p. 3–11

SEPTUM FORMATION IN THE GREEN ALGA ULOTHRIX PALUSALSA (CHLOROPHYTA, ULVOPHYCEAE)

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SUMMARY

The ultrastructure of cell division, with the emphasis on septum formation, is described in the ulvophycean green alga *Ulothrix palusalsa* Lokhorst. Septum formation involves a cleavage furrow which is initiated before the onset of mitosis. The development of the cleavage furrow is most pronounced at the final mitotic stages when it shows a serial arrangement of alternating thickened and flattened parts. Especially in the chloroplast region, a hoop of microtubules is sometimes associated with the leading edge of the cleavage furrow.

1. INTRODUCTION

In spermatophytes, cytokinesis is affected by centrifugal growth of a septum, which develops from fusing Golgi-vesicles in association with a microtubular system, the phragmoplast (see review of GUNNING 1982). A similar cell plate/phragmoplast system is also found in mosses, ferns, and in some charophycean green algae (for examples, see PICKETT-HEAPS 1975). In chaetophoralean green algae a Golgi-derived cell plate is associated with a system of transversally aligned microtubules (MTs), the phycoplast (PICKETT-HEAPS 1972). Many other green algae, however, do not produce a centrifugally developing cell plate, but instead a centripetally developing invagination of the plasma membrane (cleavage furrow). Recently, another cytokinetic system was suggested for chlorococcalean and 'pseudo-filamentous' green algae (SLUIMAN 1984, 1985), in which the endoplasmic reticulum would contribute directly to the formation of the plasma membrane, thus 'bypassing' the Golgi apparatus.

In the green algal class Ulvophyceae (*sensu* O'KELLY & FLOYD 1984) cytokinesis seems to be accomplished by a cleavage furrow which is not associated with a microtubular system. However, the findings in *Ulothrix zonata* and *U. tenuissima* (SLUIMAN et al. 1983) and probably in *U. implexa* (LOKHORST 1985) have given rise to doubts whether the cytokinetic mechanism in some ulvophycean species can always be conceived as a 'simple' cleavage furrow. Moreover, in several ulvophycean algae MTs have been observed in the cleavage plane although they mostly do not occur in large numbers (e.g., PICKETT-HEAPS & MCDONALD 1975, LOKHORST & STAR 1983, LOKHORST 1985).

In this study, we report on the cytokinetic mechanism of *Ulothrix palusalsa* which represents a variation of the ulvophycean pattern: The developing furrow in this alga is locally differentiated and shows association with MTs.

2. MATERIAL AND METHODS

The clone of *Ulothrix palusalsa* which was used for this study, and the fixation procedure for transmission electron microscopy (TEM) were the same as described previously (SEGAAR & LOKHORST 1987).

3. RESULTS

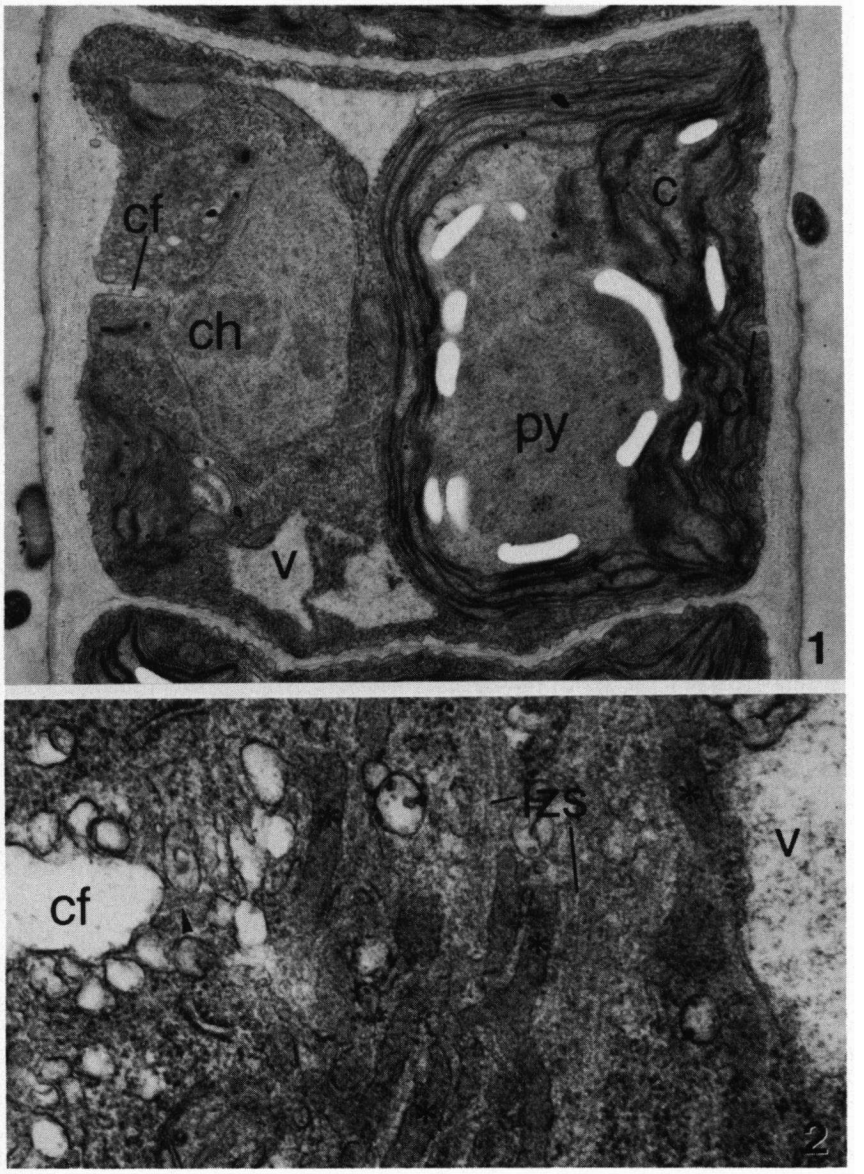
3.1. Mitosis

The ultrastructure of the mitotic pattern in *U. palusalsa* generally corresponds to the ulvophycean type. The replicated centrioles move in pairs to a position lateral to the spindle poles at prophase. During their migration they are associated with a clear zone which contains tubulin (see *figs. 15–17* in SEGAAR & LOKHORST 1987). The (early) anaphase migration of the chromosomes is simultaneous (*fig. 1*). The centriole pairs remain situated near the spindle poles until mid-anaphase. Thereafter they move back towards the cleavage plane where, at late telophase, they lie opposite to each other facing the advanced cleavage furrow (see *fig. 31* in SEGAAR & LOKHORST 1987). Throughout mitosis, the nuclear envelope (NE) is semi-closed. After restoration of the NE spindle MTs remain observable in the interzone (*fig. 3*). During mitosis numerous microbody-like profiles occur in the cleavage plane near the NE. These profiles are associated with the interzonal spindle when it strongly elongates (*fig. 2*).

3.2. Cytokinesis

The formation of a new septum is initiated at late interphase (before mitosis), after the nucleus has occupied a central position facing on one side the chloroplast and, on the other side, the longitudinal cell wall. The first sign of this precocious furrow is seen at the latter side where the centrioles are situated (see *fig. 9* in SEGAAR & LOKHORST 1987). At prophase, the cleavage furrow becomes also apparent at the side of the chloroplast, where it remains relatively small until telophase (*fig. 1*). Until telophase the cleavage furrow does not develop further significantly. At anaphase, conspicuous dictyosomes seemingly produce large amounts of vesicles and multivesicular body (MVB)-like profiles appear in the cytoplasm surrounding the nuclei (*fig. 3*).

At early telophase, the cleavage furrow at the centriolar side approaches the interzonal spindle but it is never seen to make contact with the spindle MTs. After spindle disintegration, the shape of the cleavage furrow changes drastically. Locally thickened ('inflated') parts of the septum may alternate abruptly with thin, flattened ('membraneous') septum regions (*figs. 4, 7*). This alternating pattern can be found along the whole length of the ingrowing septum, at the chloroplast side (*fig. 4*) as well as at the centriolar side. The leading edge of the cleavage furrow is mostly flattened (*figs. 4, 6, 7*). The diameter of thickened septum parts is relatively variable (250 $\pm$ 80 nm) while the flattened septum parts enclose an interspace of 15 $\pm$ 6 nm, and are relatively constant in diameter (25 $\pm$ 7 nm) (n = 35). MTs are sporadically present along the cleavage furrow



Figs. 1, 2. *Ulothrix palusalsa*. Fig. 1. Early anaphase. c = chloroplast; cf = cleavage furrow; ch = chromosomes; py = pyrenoid; v = vacuole. × 9000. Fig. 2. Late anaphase. Detail of interzonal spindle (izs) region. Microbody-like profiles (asterisks) are associated with this zone. A microtubule (arrowhead) is visible preceding the leading edge of the cleavage furrow. × 46200.

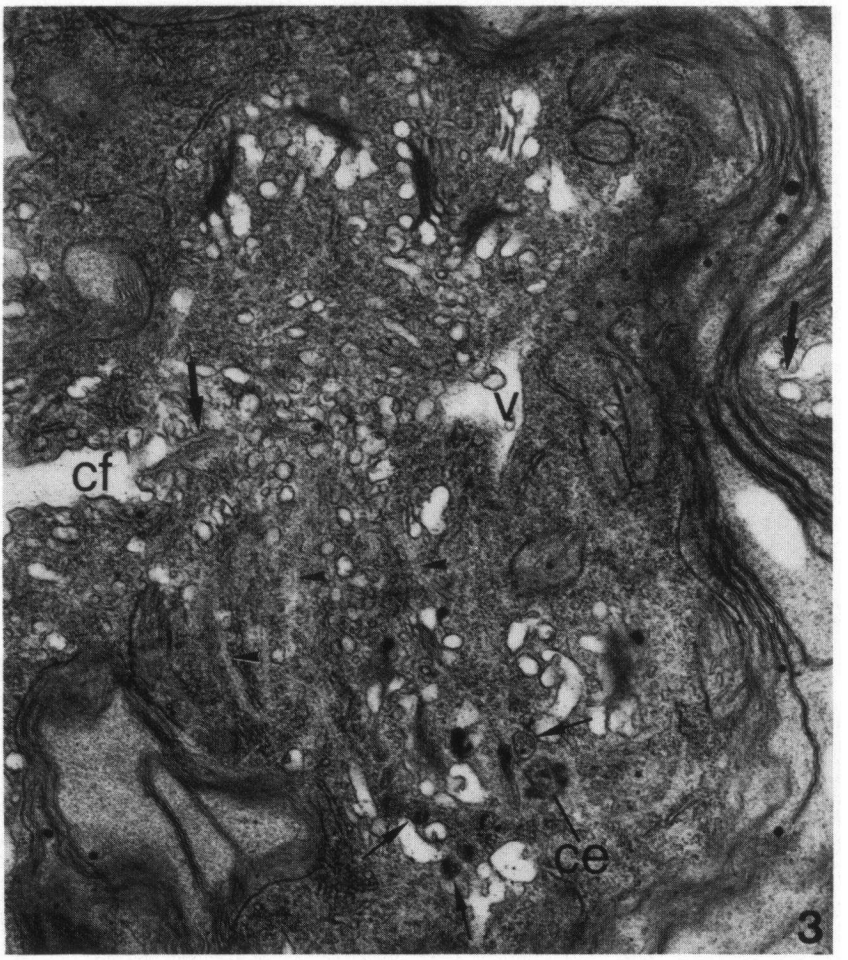
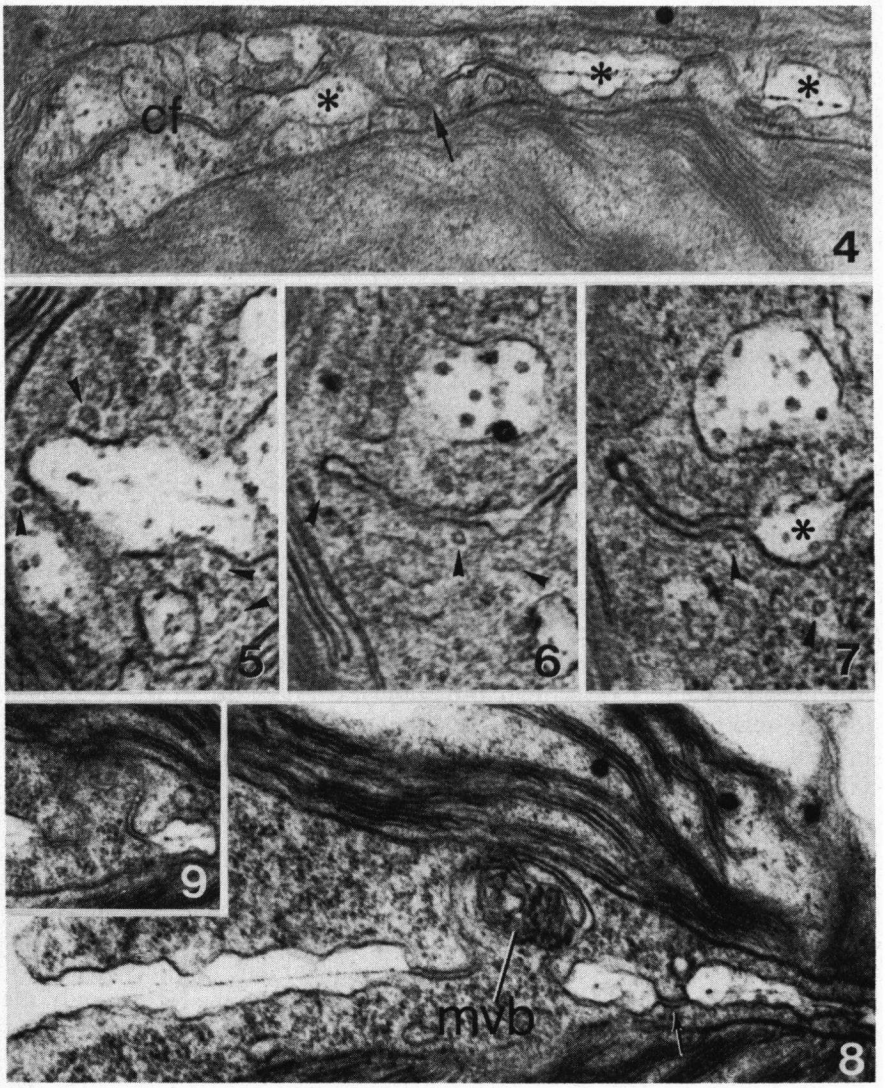


Fig. 3. *Ulothrix palusalsa*. Late anaphase. The perinuclear cytoplasm including one centriole (ce) is grazed. Seemingly active dictyosomes are mainly restricted to regions surrounding the nuclear poles. Numerous vesicles are visible in the interzonal spindle region including spindle microtubules (arrowheads). Multivesicular body-like profiles occur in the lower 'dictyosomal' region (arrows). The leading edge of the cleavage furrow (cf) is flattened (large arrows). v = vacuole. × 20400.

(*figs. 2, 4*). However, they are sometimes distinct in the chloroplast region (*figs. 5–7*) where they form a hoop which is intimately associated with the leading edge of the furrow.

The chloroplast (including the pyrenoid) becomes completely cleaved after completion of mitosis. During this process, seemingly autonomous (presumable Golgi-derived) vesicles are concentrated in the developing cytoplasmic strand between the dividing chloroplast halves. Some of these vesicles seem to fuse with the flattened leading edge of the ingrowing cleavage furrow (see *fig. 3* in



Figs. 4–9. *Ulothrix palusalsa*. Septum formation (chloroplast region). Fig. 4. Cleavage furrow (cf) consisting of alternating thickened (asterisk) and flattened parts. Arrow indicates apparent hiatus, where a vesicle has been grazed. × 35700. Figs. 5–7. Three consecutive sections of the leading edge of the cleavage furrow. Arrowheads indicate transversely cut microtubules surrounding the leading edge. A vesicle (asterisk) seems to have fused with a flattened part of the cleavage furrow. × 77000. Figs. 8, 9. Two consecutive sections showing a folded flattened part of a just closed septum. A multi-vesicular body-like organelle (mvb) is intimately associated with this septum part (*fig. 8*). Fig. 8 × 46600, fig. 9 × 39900.

LOKHORST 1985). Endoplasmic reticulum is concentrated near the parental cell wall on both sides of the cleavage furrow.

In longitudinal sections, advanced and just completed septa are often visualized as strongly waving strands, apparently caused by flattened septum parts that have assumed a folded appearance (*fig. 10*). In transverse sections, these folded parts prove to consist of vesicular structures having a double membrane (arrows in *fig. 11*). As a result of the strongly sinusoid appearance of the septum, thickened as well as flattened parts of the septum may be cut longitudinally as well as transversally in transverse sections (*fig. 11*). MVB-like profiles are often associated with the folded parts of the septum (*figs. 8, 9*). On the other hand, in some preparations the completed septum has also been seen to show a regular serial arrangement of alternating flattened and thickened parts (*fig. 12*).

During septum formation small, dark spots (*figs. 6, 7, 10, 12*) and a fibrillar layer (*figs. 4, 8, 10, 12*) are present inside the thickened parts of the cleavage furrow.

4. DISCUSSION

The ultrastructure of the cell division pattern in *Ulothrix palusalsa* basically conforms to the standard type of the Ulvophyceae (SLUIMAN et al. 1983), particularly to that of the Ulotrichales (LOKHORST 1984). Three main conclusions are drawn from the present study: (a) septum formation in *U. palusalsa* occurs via disposition of Golgi-derived vesicles into the annular ingrowth of the plasmalemma; (b) the developing septum is locally differentiated and (c) microtubules are associated with septum formation.

That Golgi-derived vesicles contribute to the development of the cleavage furrow in *U. palusalsa* is concluded from a seemingly increased activity of dictyosomes in producing vesicles at septum formation and, secondly, from fusion of these vesicles with the ingrowing septum as suggested by micrographs. Recently, SLUIMAN (1984, 1985) reported that endoplasmic reticulum (ER) can also contribute to the formation of the new plasma membrane in some chlorophycean green algae. Although ER is always associated with the formation of the septum in *U. palusalsa,* an intimate association of ER with septum formation is very unlikely in this alga since ER is not seen to produce vesicles. In other ulvophycean green algae vesicles contributing to the cleavage furrow are also considered to be Golgi-derived (SLUIMAN et al. 1983, LOKHORST 1986).

In *U. palusalsa,* a distinct morphological differentiation of the developing septum, including a regular serial arrangement of alternating thickened and flattened parts is present. This phenomenon has not been described earlier, although published micrographs of septum formation in the green algae *Scenedesmus* (NILSHAMMER & WALLES 1974), *Closterium* (PICKETT-HEAPS & FOWKE 1970) and the chrysophycean alga *Hydrurus* (VESK et al. 1984) might be interpreted this way. Despite the observed association of MVB-like profiles with the folding flattened parts of the forming septum in *U. palusalsa* it is not clear whether

Figs. 10–12. *Ulothrix palusalsa*. Final stages in septum formation. Fig. 10. A closed strongly waving septum showing folding of flattened parts (arrows). Flattened septum parts without folding are indicated by arrowheads and thickened parts by asterisks. ce = centrioles. × 22200. Fig. 11. Transverse section of a just closed septum. Vesicular structures with double membranes (arrows) are associated with the septum. A longitudinally cut flattened part of the septum is indicated by an arrowhead, a grazed thickened part of the septum is marked by an asterisk. The two circled areas possibly represent transversally grazed flattened parts of the septum. × 34800. Fig. 12. A closed septum showing a regular pattern of alternating flattened (arrows) and thickened parts. × 26200.

these organelles are the source of the membraneous flattened parts. The function of the flattened parts is unknown.

In *U. palusalsa*, microtubules are associated with septum formation. Although not present in large numbers, they are consistently present, especially around the leading edge of the cleavage furrow in the chloroplast region (*figs. 5–7*). Cytokinetic MTs have now been reported in several ulvophycean green algae (HUDSON & WAALAND 1974, PICKETT-HEAPS & MCDONALD 1975, MCDONALD & PICKETT-HEAPS 1976, HORI & ENOMOTO 1978, LOKHORST & STAR 1983, LOKHORST 1984, 1986, SEGAAR & LOKHORST 1987, this study). Their possible function in cytokinesis in ulvophycean algae has been discussed by LOKHORST & STAR (1983). The presence of cytokinetic MTs in ulvophycean algae may devaluate the use of the 'phycoplast' (PICKETT-HEAPS 1972) as an important taxonomic marker in the macro-classification of the green algae, as was discussed by SLUIMAN (1985).

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. C. Kalkman and Dr. H. J. Sluiman for critical reading of the manuscript. We thank the Department of Cell Biology and Genetics and the Department of Plant Molecular Biology for their hospitality and for the electron microscopy facilities provided.

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