A PATHWAY OF PLASMA MEMBRANE BIOGENESIS BYPASSING THE GOLGI APPARATUS DURING CELL DIVISION IN THE GREEN ALGA CYLINDROCAPSA GEMINELLA

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SUMMARY
Cell division in Cylindrocapsa geminella, in particular the mode of septum membrane biogenesis, has been studied with the transmission electron microscope. Septum formation takes place in a narrow layer of cytoplasm separating post-mitotic nuclei. First, each daughter nucleus develops a wide cytoplasmic pocket (invagination) containing numerous strands of rough endoplasmic reticulum (ER). Next, a proliferation of rough ER is observed in the equatorial zone of cytoplasm, which invariably contains a small number of widely scattered microtubules. The equatorially aligned cisternae of rough ER produce smooth-membraned vesicles, interpreted as smooth ER, which subsequently coalesce to form the membranous transverse septum. Thus, primary septum formation does not follow any of the two previously known basic cytokinetic patterns in green plants (i.e. plasma membrane furrowing and cell-plate formation), but instead represents a novel type of membrane flow, which effectively bypasses the Golgi apparatus. This pathway of membrane flow has remained largely ignored in current concepts of endomembrane structure and function in eukaryotes. However, it appears to be more widespread than has previously been recognized, especially in autospore-producing green algae and in red algae during the formation of tetraspores. It may represent an evolutionary intermediate type of cell division between the supposedly primitive method of plasma membrane furrowing and the more advanced cell-plate system.

INTRODUCTION
Ultrastructural knowledge of both the cytokinetic apparatus and the flagellar apparatus of green plants (Viridiplantae sensu Cavalier-Smith, 1981) obtained during the last two decades has significantly improved our understanding of the fundamental patterns of evolution of this group. It has led to major revisions of long-standing traditional taxonomic concepts of the higher categories, which are largely based on morphological manifestations at the supracellular level. Flagellar features have been found to be useful for identification of major lines of evolution of green plants, while cytokinetic data may help characterize subordinate groups (Mattox & Stewart, 1984; O’Kelly & Floyd, 1984).

Up to six basic patterns of mitosis and cytokinesis have been found so far in green plants (Van den Hoek, 1981; Mattox & Stewart, 1984), while the mechanism of septum membrane biogenesis in postmitotic cells is known to involve either

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centripetal invagination (furrowing) of the parental plasma membrane (PM), or centrifugal coalescence of dictyosome-derived cytoplasmic vesicles in a cell plate. Because invagination is so widespread amongst eukaryotes, it is generally considered more primitive than the formation of cell plates. Since both invagination and cell plates occur in single, presumably monophyletic lineages like the classes Chlorophyceae and Charophyceae (sensu Mattox & Stewart, 1984) one must assume that cell plates somehow evolved from an ancestral, invagination-type of septum formation. However, the very question of the evolutionary origin of the cell plate, which is fundamental to our understanding of the phylogeny of green plants, has surprisingly enough received little attention so far.

A recent electron-microscope study of cell division in the filamentous green alga *Cylindrocapsa geminella* has shed some new light upon the possible evolution of the cell plate. Some of the observations, i.e. those on septum formation, will be reported here. It will be demonstrated that, contrary to current dogma, a third, hitherto unknown pathway of septum biogenesis occurs in green plants (apart from plasma membrane furrowing and cell-plate formation), which appears to be widespread, especially amongst autospore-producing green algae, and which may be regarded as ancestral to the cell-plate system.

**MATERIALS AND METHODS**

*Cylindrocapsa geminella* Wolle was isolated from a ditch in the lake district in the NW part of the Dutch province of Overijssel. Unialgal cultures were grown in a modified Woods Hole MBL medium (see Francke & Ten Cate, 1980) at 20°C and a 16 h/8 h, light/dark photoregime. Samples of the culture were fixed at room temperature in 2-5 % glutaraldehyde made up in medium (pH 7.2), washed three times in medium, and postfixed in 1 % aqueous OsO₄. After a thorough wash in deionized water, the samples were embedded in 1-5 % agar, dehydrated in acetone, and embedded in Spurr's resin. Sections were stained with uranyl acetate and lead citrate and viewed in a Philips EM300.

**RESULTS**

*C. geminella* is a uniseriate filamentous green alga whose roughly cylindrical cells possess conspicuously layered walls, and an irregularly shaped, parietal chloroplast with a prominent pyrenoid protruding towards the cell centre (Fig. 1).

In postmitotic cells, daughter nuclei reapproach one another closely, leaving a narrow layer of equatorial cytoplasm in between, in which the transverse septum is to be formed (Fig. 1). Each nucleus is associated with a set of four centrioles, which have migrated from the former poles of the mitotic spindle to the centre of the equatorial cytoplasm (not shown).

The initiation of septum formation is marked by two phenomena. First, each daughter nucleus develops a wide cytoplasmic pocket facing the equatorial zone, invariably containing numerous strands of rough endoplasmic reticulum (ER) (Fig. 2). The nuclear envelope-associated set of centrioles is usually situated at or just below

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Fig. 1. Longitudinal section of a filament of *C. geminella*. Note the prominent pyrenoids (*p*), layered cell wall, and a postmitotic cell with appressed daughter nuclei (arrows). ×3700.
Plasma membrane biogenesis in Cylindrocapsa

Fig. 1
Fig. 2
Plasma membrane biogenesis in Cylindrocapsa the entrance to the pocket (Fig. 2). Second, a centripetal proliferation of rough ER is observed in the plane of cytokinesis, resulting in a more or less continuous series of ER cisternae across most of the equatorial zone (Figs 2, 3). A few widely scattered microtubules are consistently seen in the vicinity of the ER cisternae, both in the plane of division and inside the nuclear pockets.

It is the equatorial and "endonuclear" ER that has been found to play a key role in the construction of the new membranous septum. First, small smooth-membraned vesicles are seen in the immediate vicinity of the rough ER cisternae (Figs 2, 3), from which they apparently originate. This process can be interpreted as follows. Small, localized areas of the rough ER dissociate themselves from ribosomes and undergo dilation (Figs 2, 3). These areas are then separated from the rest of the ER, thereby giving rise to smooth-membraned vesicular elements, which may be referred to most appropriately as smooth ER vesicles. The latter appear to be the basic ultrastructurally identifiable elements involved in early septum ontogenesis, since all available evidence strongly suggests a process of coalescence of smooth ER cisternae in the plane of division whereby smooth membranes are continuously being added to the growing septum (Fig. 4). Theoretically, the alternative possibility of direct participation of the 'parental' rough ER itself should not be ruled out entirely, since localized dilation of its cisternae in concert with ribosome dissociation may account for the formation of at least portions of the new septum. At any rate, the ER is clearly the exclusive ultrastructurally identifiable source of membrane needed for primary septum formation. The central portion of a just-completed septum is shown in Fig. 5.

There is a sharp distinction between this first, ER-dominated stage of cytokinesis whereby a membranous septum is formed, and the next stage in which a cell wall is secreted all around each daughter protoplast by exocytosis of Golgi-derived vesicles containing wall material (not shown).

**DISCUSSION**

The cellular mechanism underlying cytokinesis in *C. geminella*, or, more specifically, the formation of transverse septa, involves a direct flow of ER membrane towards
Figs 3 and 4. For legend see p. 93.
Plasma membrane biogenesis in Cylindrocapsa

Fig. 5. For legend see p. 93.
the plasma membrane (PM). This has not been reported before in any green alga, and its occurrence in other eukaryotic systems is most uncertain. For this reason, the possibility of direct ER → PM membrane flow has received little, if any, attention in various reviews dealing with the structural and functional aspects of the endomembrane system since the introduction of the 'Endomembrane Concept' by Morré, Mollenhauer & Bracker (1971) (see, e.g., Morré & Mollenhauer, 1974; Morré, Kartenbeck & Franke, 1979; Mollenhauer & Morré, 1980; Morré, 1980).

In green plants (Viridiplantae sensu Cavalier-Smith, 1981) it is generally accepted that cell division involves either centripetal invagination (furrowing) by the parental PM (as, e.g., in volvocalean and other green flagellates), or coalescence of Golgi-derived vesicles in a cell plate (e.g. in numerous filamentous green algae, and in all archegoniate land plants) (Pickett-Heaps, 1975; Stewart & Mattox, 1975; Van den Hoek, 1981; Mattox & Stewart, 1984). However, the method of septum formation observed in C. geminella is difficult to reconcile with any of the existing models of cytokinesis. It is evident that PM furrowing does not occur. On the other hand, the cell-plate model is equally unfit to account for the cytokinetic phenomena observed in this alga since: (1) although several Golgi bodies are known to be present at the time of septum ontogenesis, they are consistently located far away from the plane of division, i.e. at the side of each daughter nucleus facing away from the equatorial plane; (2) there is no evidence of a selective flow of Golgi-derived vesicles towards the plane of septum ontogenesis; (3) dictyosomes do not appear particularly active during early septum formation. Rather, the observed pattern of membrane flow effectively bypasses the Golgi apparatus. It is therefore concluded that primary septum formation in C. geminella represents a cytokinetic mechanism previously unknown in green plants.

How unique is this type of PM biogenesis? In the literature the possibility of direct ER → PM membrane traffic has occasionally been raised. Schnepf (1969), for example, suggested that ER membrane could be transformed into PM when cell walls are secreted and when cell plates are formed. Conclusive evidence in support of this assumption, however, is entirely lacking. There seems to be no exception to the rule that wall materials are transported to the cell surface via dictyosome-derived vesicles, while in the case of cell plates there is increasing evidence that "the vesicles of the plate are derived solely from the dictyosomes" (Hepler, 1982), and that the structural role of the ER is limited to the formation of plasmodesmata (see Gunning, 1982, for a recent review).

The only available convincing example of ER acting as a source of membrane for PM is contained in a report on cytokinetic events leading to the formation of tetraspores in the red alga *Harveyella* (Kugrens & Koslowski, 1981). There are indications, however, that ER-mediated septum formation amongst red algae is more widespread than is presently recognized: according to published electron micrographs of tetrasporogenesis in *Erythrocystis* (Santisi & De Masi, 1981), Golgi activity is very low at the time of septum formation, while smooth membranous elements, probably smooth ER vesicles, aggregate in the plane of division. In another red alga, *Palmaria*, it has been established that ER-derived 'mucilage sacs' contribute to the formation of new septa during tetrasporogenesis (Pueschel, 1979).
The coincidence of constructive involvement of the ER in septum ontogenesis during cytokinesis in *C. geminella* and during tetrasporogenetic cleavage may be significant: in both instances daughter protoplasts are formed that secrete an extracellular coat over their entire surface, inside and separate from the pre-existing parental cell wall. This development can be interpreted as the coordinated but temporally and functionally discrete action of two different endomembrane components: the ER is the ultrastructurally identifiable source of membrane needed for primary septum formation, while the Golgi apparatus is responsible for subsequent wall deposition (cf. Whaley & Dauwalder, 1979; Farquhar & Palade, 1981; Robinson & Kristen, 1982).

Although the reasons for the coincidence of active ER participation in primary septum formation and circumferential wall deposition are not understood, it may help to cue other systems in which PM biogenesis during cytokinesis involves a flow of membranes bypassing the Golgi apparatus. For example, in autospore-producing green algae (Chlorococcales), daughter cells form an entire new wall inside and separate from the parental wall (as does, incidentally, *C. geminella*, which is therefore considered chlorococcalean by certain authors). Critical review of published information on membrane flow patterns in Chlorococcales has led to the conclusion that generalized statements that in these algae cytokinesis is effected by PM furrowing (Stewart & Mattox, 1975; Pickett-Heaps, 1975; Van den Hoek, 1981; Mattox & Stewart, 1984) are oversimplifications and thus misleading. Instead, the present knowledge of cytokinetic development in *C. geminella* necessitates a reinterpretation of numerous previous observations on septum ontogenesis in chlorococcalean algae, since available electron micrographs of *Kirchneriella* (figs 11, 15c, 19, 20a, 20b, 30, 31a, 31b of Pickett-Heaps, 1970), *Tetraedron* (plates 5D, 6A of Pickett-Heaps, 1972a), *Ankistrodesmus* (fig. 4 of Pickett-Heaps, 1972b), *Chlorella* (fig. 3 of Atkinson, Gunning & John, 1972; figs 2, 3, 5, 11 of Wilson, Wanka & Linskens, 1973), *Scenedesmus* (figs 8.3, 8.4 of Dodge, 1973; figs 6, 9 of Nilshammar & Walles, 1974; fig. 8 of Pickett-Heaps & Staehelin, 1975) and possibly *Sorastrum* (fig. 11 of Marchant, 1974), *Coelastrium* (fig. 13 of Chan & Wong, 1975) and *Eremosphaera* (figs 3–5 of Robinson, Sachs & Mayer, 1976) are more readily understood by reference to the *C. geminella* cytokinetic model instead of the PM cleavage model. Interestingly enough, it is noted that in some of these reports (Wilson *et al.* 1973; Nilshammar & Walles, 1974) smooth ER cisternae are apparently mistaken for Golgi vesicles since consistent reference is being made to centrifugally expanding 'cell plates'. The unexpected use of this term is a further indication that division in these algae cannot be explained in simple terms of PM furrowing. Finally, in species of *Tetraselmis*, a genus of thecate green flagellates, daughter protoplasts produce new individual thecae within the parental theca by exocytosing Golgi-derived vesicles (Manton & Parke, 1965; Domozych, Stewart & Mattox, 1981). Hence, it may not be entirely unexpected that cytokinesis in *T. striata* has been reported to involve "fusion of membranous vesicles" (Ricketts & Davey, 1980). A detailed reinvestigation of septum initiation should confirm their origin from the ER. The latter possibility is not precluded by a published electron micrograph on cell division in another species, *T. subcordiformis* (fig. 16 of Stewart, Mattox & Chandler, 1974).
In order to avoid confusion, it seems appropriate at this point to emphasize that the alga referred to as 'Cylindrocapsa involuta' (strain LB653 from the UTEX culture collection), whose cell division has been studied with the electron microscope by Pickett-Heaps & McDonald (1975), and C. geminella, the subject of the present investigation, are taxonomically unrelated (see Hoffman & Hofmann, 1975; Hoffman, 1976). It is therefore not surprising to find that in these organisms the patterns of septum biogenesis are entirely different: strain LB653 uses the PM furrowing mechanism like, e.g., Ulva, Ulothrix, Pseudendoclonium and Trichosarcina (Sluiman, Roberts, Stewart & Mattox, 1983), whereas in C. geminella fusion of ER-derived cisternae takes place (present paper). To complicate matters further, in the much quoted book by Pickett-Heaps (1975, p. 536) strain LB653, elsewhere known as 'Cylindrocapsa involuta' (Pickett-Heaps & McDonald, 1975), is referred to by the taxonomically non-existent name of 'Cylindrocapsa brebissonii'.

Of the two cytokinetic categories that were traditionally known to occur in green plants PM furrowing is generally regarded as the more primitive (Pickett-Heaps, 1975; Van den Hoek, 1981). However, the evolutionary origin of the presumably advanced cell-plate system is poorly understood. Clearly, it has the advantage (in terms of efficiency) that both new PM and extracellular material are deposited at the same time, due to the outward flow of Golgi-derived vesicles, and that wall precursors are initially laid down in specific areas only, i.e. at the new septum, instead of being secreted 'wastefully' all around each daughter protoplast. Since the Golgi apparatus is part of the structural, ontogenetic and functional continuum known as the endomembrane system (cf. Morré & Mollenhauer, 1974; Morré et al. 1979), and in view of the overall pattern of vectorial migration and interconversion of endomembranes in eukaryote cells, one can easily envisage a primitive cell-plate mechanism in which endomembrane components other than the Golgi apparatus account for the supply and insertion of new PM. The present study suggests that the ER-dominated cytokinetic apparatus of C. geminella and certain other algae may exemplify such an ancestral cell-plate system.

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REFERENCES.
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