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Chara tomentosa antheridial plasmodesmata at various stages of spermatogenesis

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Abstract

Chara tomentosa antheridial plasmodesmata are described during proliferation and spermiogenesis. In antheridial filament cells which are cycling completely synchronously, unplugged plasmodesmata are filled with light cytoplasm. The same plasmodesmata are observed after cessation of mitotic division followed by the onset of synchronous spermiogenesis. Walls separating cells at different cell cycle stages and dividing antheridial filaments into asynchronous domains are plugged with a dense osmophilic substance. Similarly plugged plasmodesmata are present between antheridial cells of different types, *e.g.*, capitular cells and antheridial filaments. In mid spermiogenesis when abundant endoplasmic reticulum (ER) appears temporarily it penetrates into plasmodesmata enabling cell-to-cell transport via ER cisternae. In late spermiogenesis there are no cisternae in plasmodesmata.

Additional key words: endoplasmic reticulum, plugged plasmodesmata, unplugged plasmodesmata.

Introduction

Plasmodesmata penetrating a cell wall, being canals lined with plasmalemma *via* which plant cells are in contact, are of various structure. They can contain modified ER in the form of desmotubules, unmodified ER or no ER at all. Plasmodesmata form straight or branched canals. They can also be blocked – filled with an osmophilic substance forming a plug or there can be various constrictions limiting their lumen (Lucas 1999, Pickard and Beachy 1999, Ehlers and Kollmann 2001). All these structural modifications undoubtedly influence plasmodesmata functions.

Higher plant plasmodesmata are the main subject of research but there are various plasmodesmata in lower plants as well. Our studies focus on plasmodesmata of complex structure present in antheridia of higher algae the genus *Chara*. Previously we described them in *Chara vulgaris* (Kwiatkowska and Maszewski 1976, 1985, 1986, Kwiatkowska 1988, 1991, 1999). Now we present them in *Chara tomentosa* – a dioecious species forming very big antheridia with similar structural pattern as *C. vulgaris*. In *C. tomentosa* antheridia there are about 20 times more spermatozoids than in *C. vulgaris*. This is due to a greater number of capitular cells producing very numerous antheridial filaments. Spermatogenesis, however, is very similar to that in *C. vulgaris* and other *Chara* species (Maszewski 1991).

The present observations have shown that the structure and position of plasmodesmata (plasmodesmogram) are very similar in *C. tomentosa* and *C. vulgaris* antheridia.

Materials and methods

Thalli of *Chara tomentosa* L. coming from lake Powidz near Konin in Poland were used. For electron microscopy the apical parts of the thallus carrying antheridia were fixed for 2 h at room temperature in 3 % gluteraldehyde in 0.0125 M phosphate buffer, pH 7.2, with 0.007 M $CaCl_2$ added. The antheridia were then isolated and individual rosettes of the filaments were gently squeezed out onto slides and embedded in 2 % agar in phosphate buffer.

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Abbreviations: ABA - abscisic acid, GAs - gibberellins, ER - endoplasmic reticulum.
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Subsequently the material was postfixed in veronal-buffered (pH 7.2) 1 % OsO_4 solution for 1 h, dehydrated in an ethanol series, stained overnight in 2 % uranyl acetate, and embedded in Spurr's medium. Ultra-thin sections were double stained with uranyl acetate and lead citrate. The sections were examined and photographed in a *JEOL JEM-1010* (Japan) transmission electron microscope.

Results and discussion

Plugged and unplugged plasmodesmata: The structure of plasmodesmata joining *Chara tomentosa* antheridial filament cells shows that there is a direct relation between an ability of neighbouring sister cells to develop fully synchronously and the presence of specific unplugged plasmodesmata forming about 67 nm wide canals filled with light cytoplasm (Figs. 1, 2). Appearance of plugs made of unidentified osmophilic substance leads to asynchronous development of cells: plugged plasmodesmata are invariably connected with asynchronous development of neighbouring antheridial filament cells (Figs. 1, 3, 4). Research on *C. tomentosa* supports these observations made on *C. vulgaris* (Kwiatkowska and Maszewski 1976, 1985, 1986).

Quantitative analysis of *C. vulgaris* antheridial filaments at consecutive stages of proliferation seems to point out that the appearance of plugs is a primary cause of desynchronisation of cell development, as in all cases of asynchronous development there were plugs in plasmodesmata while in very few walls between fully synchronously developing cells (*e.g.* in metaphase) small plugs *in statu nascendi* were observed (Kwiatkowska and Maszewski 1976).

Recent studies on a scutellar callus of the grass *Molinia caerulea* (Ehlers *et al.* 1999) and on a micro-callus of *Solanum nigrum* (Ehlers and Kollmann 2000) prove that plugging of plasmodesmata is a common phenomenon during morphogenesis. Blockage of plasmodesmata by appearing plugs leads to a separation of domains with different rhythms of development while unplugged plasmodesmata enable synchronous mitoses in cells connected by them.

Analysis of antheridia development has shown that plugging of plasmodesmata is a reversible process. While antheridial filament cells pass from proliferation to differentiation, *i.e.* spermiogenesis, plugs disappear from plasmodesmata of filaments which earlier did not develop synchronously (Fig. 1*A,B*). Plugs also disappear from plasmodesmata connecting cells of neighbouring antheridial filaments emerging from the same capitular cells (Figs. 1*B*, 5). These plasmodesmata must have been blocked during proliferation because each of the filaments has a different developmental rhythm (Fig. 1*A,B*). When synchronisation spreads from one filament onto a whole filament complex these plasmodesmata become unplugged (Fig. 1*B*). The same process was observed in *C. vulgaris* (Kwiatkowska and Maszewski 1985). These unplugged plasmodesmata connect cells till late stages of spermiogenesis (Fig. 5). Neighbouring plasmodesmata between a capitular cell and a spermatid cell are plugged with a thick, osmophilic substance because in antheridia of *C. tomentosa* and *C. vulgaris* (Kwiatkowska and Maszewski 1985, 1986) plugged plasmodesmata are present always between cells of different type (Figs. 1*A,B*, 5).

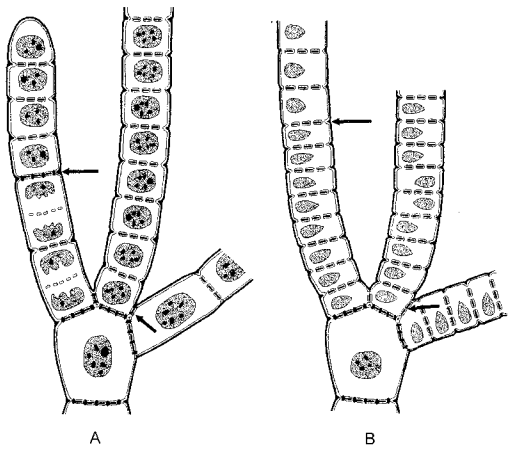
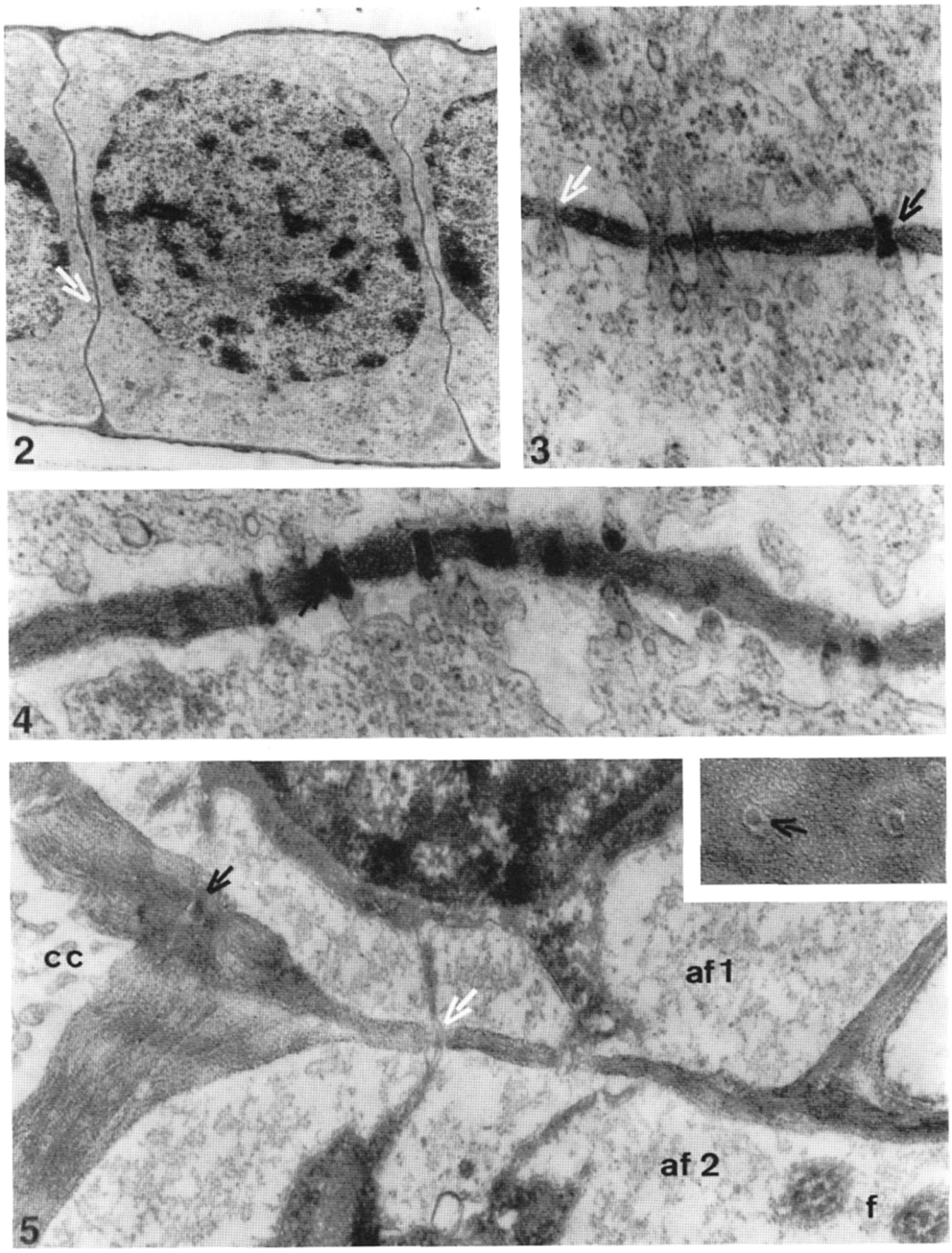


Fig. 1. Schematic illustration of plasmodesmal connections in *Chara tomentosa* within a capitular cell – antheridial filament complex: *A* - during mitotic divisions in antheridial filaments (proliferative phase), *B* - during spermiogenesis. *Arrows* indicate walls where plugged plasmodesmata become unplugged.

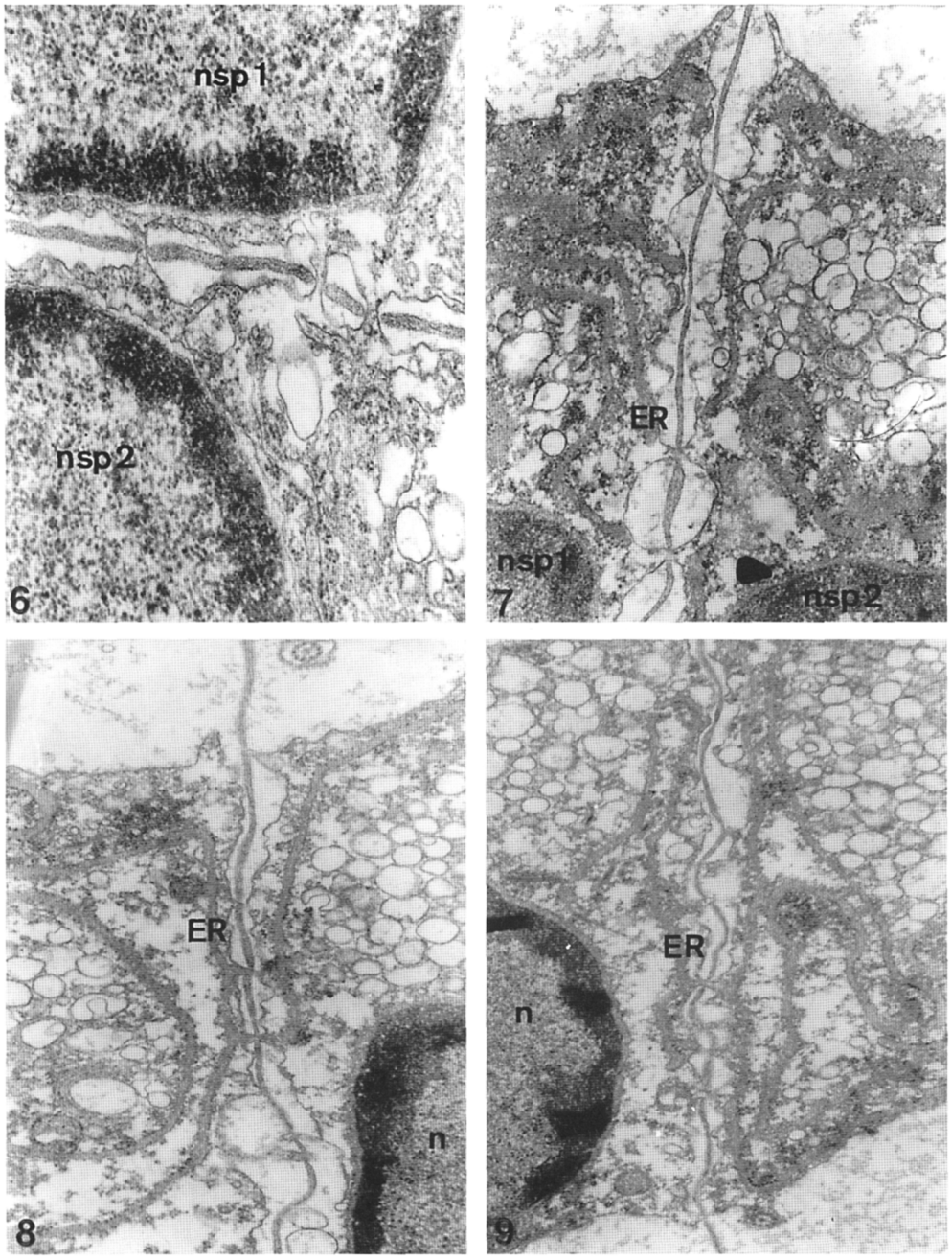
Experiments of Maszewski and van Bel (1996) with Lucifer Yellow have shown that this fluorochrome may penetrate from one cell to another via plugged straight plasmodesmata of young antheridia of *C. vulgaris* joining shield cells with manubria and manubria with capitular cells. The fact that plugged plasmodesmata join antheridial filament fragments which are asynchronous with each other, indicate indirectly that regulatory factors responsible for a synchronous cell cycle development do not pass through them. Thus plugs are a type of "molecular sieve" limiting a symplasmic transport.

Moore-Gordon *et al.* (1998) have shown that a reversible process of plasmodesmata plugging during the development of *Avocado* fruit limiting sucrose transport is controlled by hormones, *i.e.* it is regulated by a definite cytokinin/ABA ratio. It seems probable that in *Chara* antheridia hormones may influence the structure of plasmodesmata as well. This may be suggested by the fact that disappearance of plugs coincides with the decrease in gibberellin levels in the antheridium (Kaźmierczak *et al.* 1999) caused by a symplasmic

isolation of antheridia from thallus resulting from a breakage of plasmodesmata between a basal cell and the antheridium (Kwiatkowska 1988) that probably decreases the import of GAs from thallus to antheridia (Kwiatkowska 1991, Kwiatkowska and Malinowski 1995).



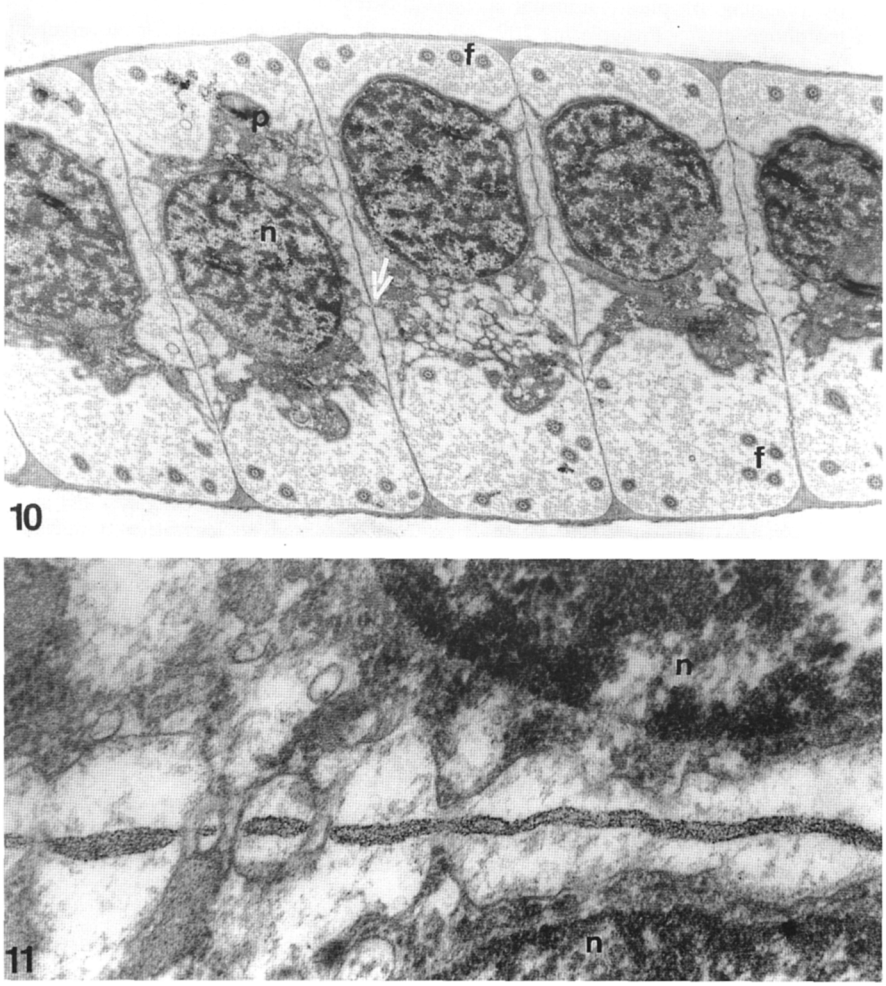
Figs. 2, 3, 4. Plasmodesmata in *C. tomentosa* at proliferative phase of spermatogenesis:
2 - Interphase cells from 16-celled antheridial filament with completely synchronous development joined by unplugged plasmodesmata (*white arrow*), ×10 200.
3 - Three unplugged plasmodesmata (*white arrow*) and one plugged (*black arrow*) – the beginning of plasmodesmata plugging? In unplugged ones ribosomes are visible, ×66 000.
4 - A fragment of a cell wall dividing two asynchronous domains of an antheridial filament with plugged plasmodesmata (*black arrow*), ×55 000.
Fig. 5. Fragments of a capitular cell (cc) and 2 antheridial filaments (af1, af2) with completely synchronous spermiogenesis connected with an unplugged plasmodesmata (*white arrow*). Plugged plasmodesmata (*black arrow*) between a capitular cell and spermatid cells in longitudinal- and cross-sections, f - flagellum, ×46 500.



Figs. 6, 7, 8, 9. Plasmodesmata of *C. tomentosa* at spermiogenesis stage.
6 - Early spermiogenesis. Unplugged plasmodesmata (*white arrow*) without ER, ×37 500.
7, 8, 9 - Mid spermiogenesis.
7 - ER cisternae approaching plasmodesmata, nsp1 - nucleus of spermatid 1, nsp2 - nucleus of spermatid 2, ×22 500.
8, 9 - ER cisternae penetrating plasmodesmata, n - nucleus, 8 - ×22 500, 9 - ×18 600.

Plasmodesmata and ER: The formation of a cell plate in *Chara* during cytokinesis is not correlated with an appearance of numerous ER cisternae on the plane of division (Kwiatkowska and Maszewski 1979, Kwiatkowska 1997, 1999) as is the case, *e.g.* in higher plants (Hepler 1982, Ehlers and Kollmann 2001). In newly formed plasmodesmata and in plasmodesmata in fully formed walls the canal of unplugged plasmodesmata is filled with light cytoplasm in which there are ribosomes (Fig. 3) and sometimes small vesicles, and they are quite rarely penetrated by ER (Kwiatkowska and Maszewski 1976, 1986). During early spermiogenesis

plasmodesmata look the same (Fig. 6). In mid spermiogenesis, however, in spermatids numerous ER cisternae filled with a dark substance the same as in intermembranous spaces of a nuclear envelope appear (Kwiatkowska 1996, Kwiatkowska and Popłońska 2002). Cytochemical studies show that this process occurs in the period of an exchange of histones into protamine-type nuclear proteins. This was proved by cytochemical analysis (Popłońska 2002) the results of which are in agreement with the data obtained from capillary electrophoresis. With the latter method during *C. tomentosa* spermiogenesis a replacement of histones with proteins with electrophoretic mobility similar to that in salmon protamines was observed (Kaźmierczak 2000). At this specific stage, which lasts very short, as can be supposed on the basis of a rare appearance of this phase (Popłońska unpubl. results), ER cisternae penetrating plasmodesmata right through or in an intimate contact with plasmodesmata at both wall sides can be seen (Figs. 7, 8, 9). This means that ER penetrates into plasmo-desmata in most of which ER cisternae were not present before. The present observations have revealed a similar regularity in *C. tomentosa* as in *C. vulgaris* (Kwiatkowska 1996) concerning both mass appearance of ER cisternae in mid spermiogenesis and their relationship with plasmodesmata. In *C. tomentosa* similarly as in *C. vulgaris* (Kwiatkowska 1996) these ER cisternae do not become apressed and do not form desmotubules. Observations of many *C. tomentosa* cells suggest that at this stage ER cisternae are both resilient and rigid. Often they are arched or straight. Therefore, it seems, they can penetrate relatively large plasmodesmata or even form continuous ER cisternae. Thus, they enable intercellular exchange and transport of substances from these cisternae being an additional factor synchronizing spermatid development at this relatively short, but very probably decisive, stage of morphogenesis.



Figs. 10, 11. Plasmodesmata of *C. tomentosa* at spermiogenesis stage. Late spermiogenesis. Unplugged plasmodesmata (*white arrow*) without ER.
10 - f - flagellum, n - nucleus, p - plastid, ×11 000.
11 - n - nucleus, ×77 500.

The possibility of transport via ER penetrating plasmodesmata was suggested by experiments on higher plants (Cantrill *et al.* 1999).

At later spermiogenesis phases (Figs. 10, 11) in plasmodesmata joining spermatids again no ER cisternae are observed. Thus ER penetration into plasmodesmata joining spermatids is confined only to a certain period of time. Unplugged, open plasmodesmata persist till late spermiogenesis phase and disappear only at the end of differentiation before spermatozoid liberation from a cell when a cross wall between spermatids becomes gelatinized (Kwiatkowska 1996).

The results of *C. tomentosa* examinations fully support those obtained from *C. vulgaris* which proves that the observed phenomena are of general character.

The above observations prove that plasmodesmata ultrastructure in *Chara* antheridia dynamically changes in relation to specific morphogenetic situations. These proceses are supposed to enable adjustment of plasmodesmata functioning in cell-to-cell communication to these situations.

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