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EXPERIMENTAL FLUID FLOW THROUGH PLASMODESMATA OF LAMINARIA DIGITATA

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SUMMARY

Electron-microscopy (EM) revealed that the conducting filaments in the medulla of the cauloid are interconnected by numerous plasmodesmata in sieve plates. The filaments contain a vesicular ground plasm with a nucleus, mitochondria, and plastids. After a cauloid had been cut through, an exudate flowed from the medulla. Afterwards longitudinal sections showed an accumulation of cytoplasm on the plates near a wound, both basal and apical. On the side facing the wound cytoplasm was absent. EM confirmed this. The authors argue that, for several reasons, a fluid flow of exudate must pass the plasmodesmata. Cytoplasm moved with the exudate and was sieved out by the plates.

1. INTRODUCTION

After a cauloid (stipe) of *Laminaria* is cut through a little exudate appears on the medulla in the centre. Such an exudation is well-known from larger species, e.g. *Macrocystis* (CRAFTS 1939; PARKER 1966), where a more profuse exudation was observed.

Electron-microscopy (EM) revealed that sieve plates of *Laminaria* have many plasmodesmata and no other connections between the trumpet filaments (ZIEGLER & RÜCK 1967 for *Laminaria digitata*). The filaments are long cells with a vesicular cytoplasm, a nucleus, mitochondria, and plastids.

An experimental mass flow, probably through plasmodesmata, seemed of sufficient interest to justify investigation. MÜNCH (1930) has supposed a pressure filtration though plasmodesmata in parenchyma of higher plants. Though a translocation through them is obvious (ARISZ 1969), it is not known whether this is a flow or an exchange by diffusion of soluble substances, stimulated by cytoplasmatic streaming.

2. MATERIAL AND METHODS

Laminaria digitata plants were harvested from the sublittoral zone in summer. The rhizoid was detached from the stones. Whole plants were stored in plastic bags at 5°C where they keep well for a week.

a. Hand sections through living material were examined as follows:

1. In sea water or 3% NaCl, to which some eosine was added. Cytoplasm stains bright pink after previous rinsing in ethanol.

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2. After fixation in ethanol, staining in lactophenol cotton blue, rinsing in water and mounting in glycerol. The cytoplasm stains blue.

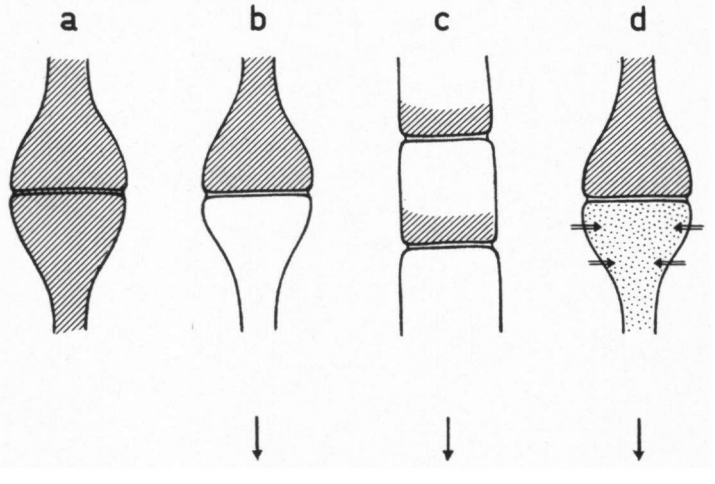
b. Material frozen at -20 °C. Treatment as a2 after thawing.

c. EM preparations, fixation in 5% glutaraldehyde in sea water for 12 h at 4 °C. Postfixation and staining in 1% OsO_4 in sea water for 24 h at 4 °C. Poststaining in 2% uranyl acetate in 70% ethanol for 1 h at 35 °C. Dehydration in ethanol, embedding in Epon 812 or ERL 4206.

3. OBSERVATIONS

3.1. In longitudinal sections through the medulla of a living cauloid, characteristic accumulations of cytoplasm on the sieve plates were observed. Near the wounds, both on apical and basal sides where the cauloid was cut transversely, cytoplasm had accumulated only at one side of the sieve plates. On the side facing the wound the lumen of each trumpet filament was empty. This phenomenon also occurred in the sieve filaments of the inner cortex (*Plate 1B, C, D; Scheme 1b, c*).

3.2. Light-microscopy (LM) observations with living material were confirmed by the EM observations. EM revealed that near the wounds, both apical and basal, on the sides of the sieve plates facing the wounds cytoplasm was completely absent, whereas on the opposite side cytoplasm had accumulated. The accumulated cytoplasm stained strongly. Organelles were hardly distinguishable and the cytoplasm seemed to be dislocated and disorganized.



Scheme 1. Single arrows indicate exudate flow to wound. a. Trumpet filament, frozen material with cytoplasm on both sides of sieve plate. b. Trumpet filament, section near wound, all cytoplasm below sieve plate flushed away. c. Sieve filament with accumulation of cytoplasm in successive cells. d. Trumpet filament, theoretical possibility of water attraction (double arrows) and dilution of contents.

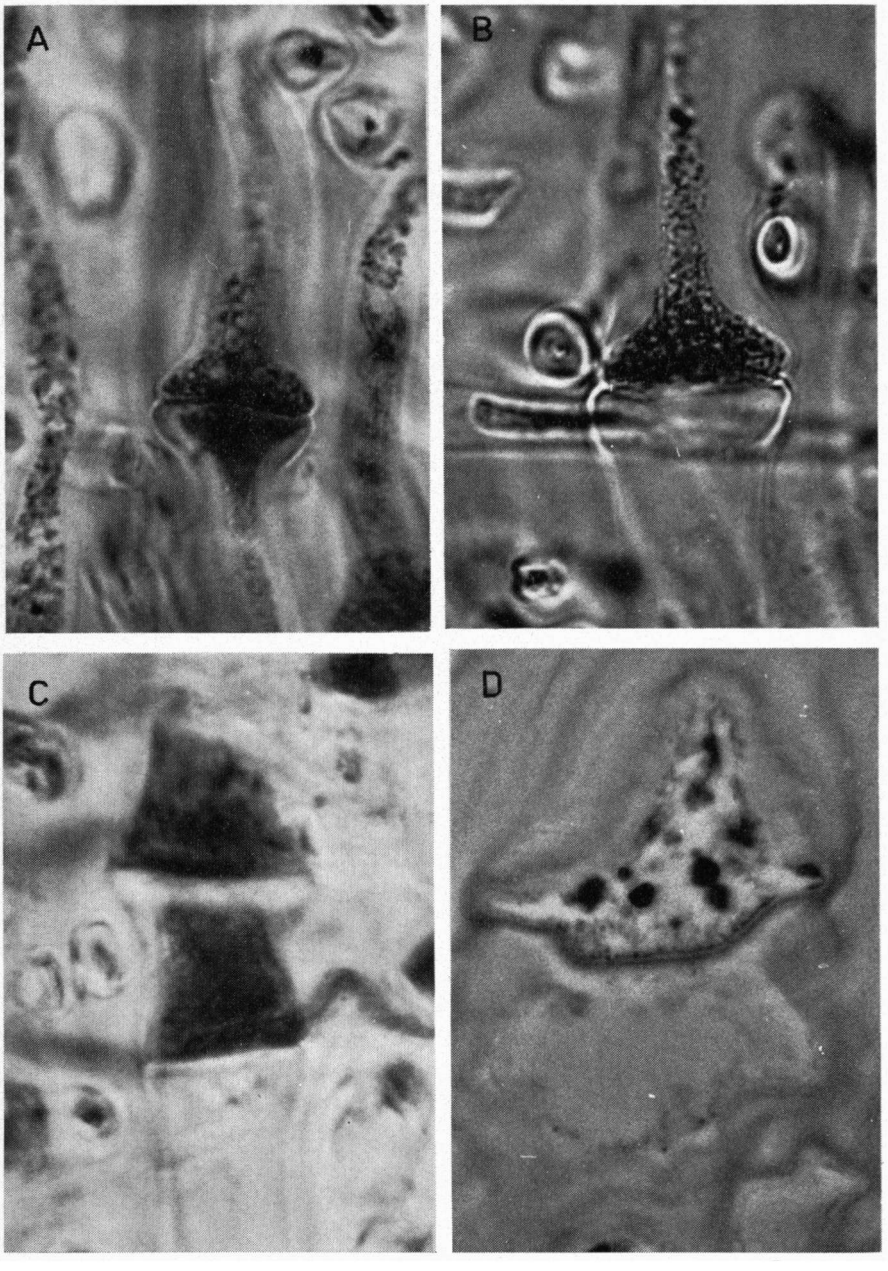


Plate 1. A.B. Trumpet filaments, and sieve filament C, hand-sections in cotton-blue-lactophenol ×800. A. frozen: cytoplasm on both sides of sieve plate. B. near a wound with empty half facing wound. C. sieve filament of inner cortex with cytoplasm accumulating in direction of flow. D. 2μm section EM method but under LM phase contrast ×1800. Note empty half trumpet facing wound.

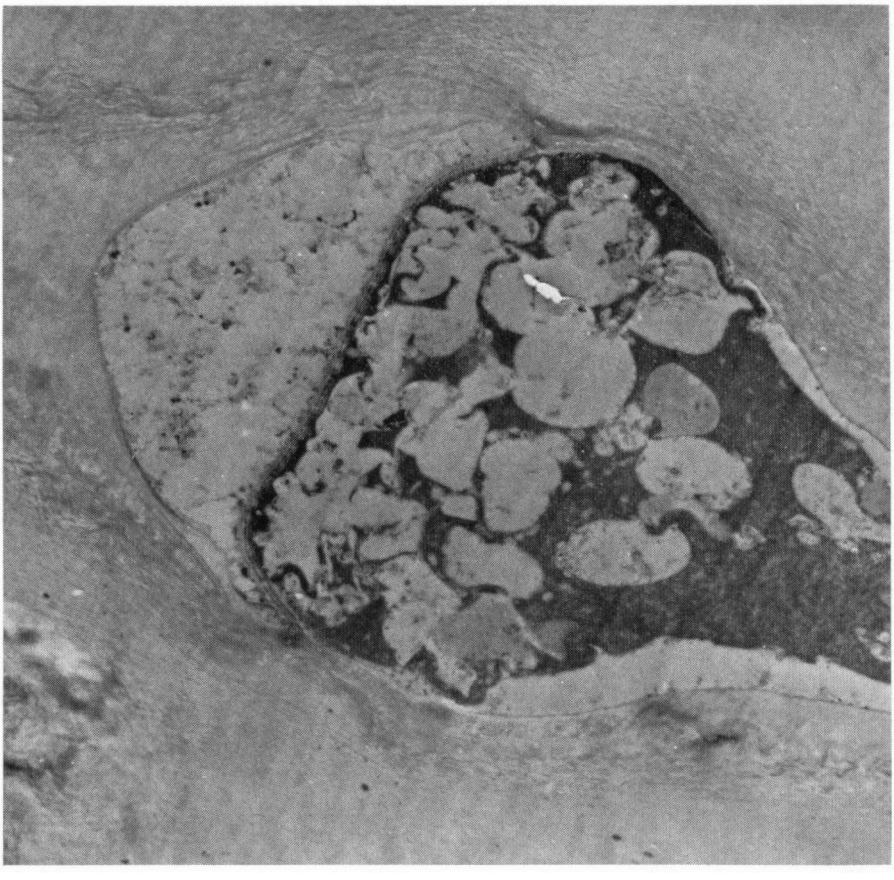


Plate 2. EM of trumpet filament with empty half towards wound and vesicular cytoplasm ×7200.

3.3. In longitudinal sections through the medulla of frozen material, cytoplasm was observed on both sides of the sieve plates: in the central part of the sections, as in sections through living material, as well as in the basal and apical parts near the wounds. The trumpet filaments that were cut through were not observed to empty. The presence of cytoplasm was much more obvious in sections from the frozen material.

4. DISCUSSION

Exudation from the medulla of the cauloid of Laminariaceae is well-known. It stops after a few moments but can be renewed by cutting off a thin slice (CRAFTS 1939 for *Macrocystis*). From results of repeated cutting of slices he calculated a velocity of 63.3 cm in 30 min. In an intact plant of *Macrocystis*, after labelling

the phylloids (fronds) with $^{14}CO_2$, PARKER (1965) found a translocation velocity in the cauloid of 65 to 78 cm h^{-1}, which is considerable. Exudation in *Laminaria*, though limited, is quite distinct. *Macrocystis* has large pores 2–3 μm in diam. in the sieve plates (ZIEGLER 1963). *Laminaria* has only plasmodesmata-fields with 20.000–30.000 in a single plate, average diameter 0.06 μm, occupying a sixth of the plate area (ZIEGLER & RÜCK 1967). For *Macrocystis* the occurrence of the pores seems an important step in the phylogeny of cellular translocation. Both have the advantage of long filaments interconnected at their ends by sieve plates. The number of sieve plates to be passed is thus reduced. Further the plates are very thin, 0.2 μm in *Laminaria.*

In EM the plasmodesmata of *Laminaria* have a concentric structure. From outside to inside first a light zone, probably callose, then a darker ring (the plasmalemma) followed by a lighter ring and often with a central core (ZIEGLER & RÜCK 1967).

The average diameter was 0.06 μm, range 0.05–0.1 μm, but they might be larger in vivo as dehydration before embedding would cause shrinking. Also their condition in vivo is unknown, e.g. the possible absence of callose, as a plugging mechanism as in sieve plate pores of higher plants caused by injury or fixation. The exudate flow stops after a few moments. Thick callose layers on the plates were observed in Laminariaceae by OLIVER (1887).

Translocation through the walls (free space) can be excluded. The walls of brown algae are in open connection with the sea water (TAMMES 1954) and substances present in the walls would diffuse into the surrounding medium and thus be lost. Also one would expect more sodium in the exudate if translocation were taking place through walls. PARKER (1966) found that the sodium concentration of *Macrocystis* exudate was only 8% of that of sea water, whereas that of potassium was 24 times as high as in sea water. His analysis showed a typical cell fluid with mannitol, amino acids and some protein. Walls and biomembranes would have such a high resistance that large-scale translocation through the free space of the walls would be prohibited. The biomembrane on the outside of cells is almost impermeable to many substances in the lumen. It is known that various substances once in the symplasm are kept there, though they can be translocated to the symplasm of other cells (ARISZ 1969).

SPANSWICK (1972), measuring the electrical coupling between adjacent cells (*Elodea*), found a communication with a resistance lower than could be expected if the plasmodesmata were closed.

As shown by our observations, exudate velocity through the filaments near the wound in *Laminaria* was so high that cytoplasm moved with the stream. When it reached a sieve-plate, it was sieved out. On the other side of the sieve plate facing the wound, the cytoplasm was flushed away, leaving an empty space. The phenomenon occurred both below and above a wound. In frozen material cytoplasm can be observed on both sides of sieve plates. In cut living material this is not so clear, perhaps because much cytoplasm drains sideways in longitudinal sections through the medulla. In frozen material all biomembranes are killed, probably preventing dislocation of protoplasm after injury.

Perhaps cut filaments loose part of their contents by osmotic attraction of water from the surrounding medium. But if so, one would expect dilution and not an emptying near the wounds; emptying is observed. EM confirms this.

The difference in pressure between cut cells and turgid cells behind them would be considerable. If this leads to a fluid flow through plasmodesmata, such flow could perhaps occur, also in intact plants, though more slowly.

The filaments of medulla have often been compared with sieve tubes of higher plants. But *Laminaria* differs in having a vesicular cytoplasm, nucleus, mitochondria, and other cell organelles; instead of pores it has plasmodesmata in the sieve plates. MÜNCH (1930) has proposed pressure filtration through plasmodesmata in the symplasm of parenchyma. Later authors thought that he might have underestimated the resistance of the plasmodesmata to fluid flow. Recently CLARKSON *et al.* (1971) calculated that water and ion passage through plasmodesmata in the endodermis was possible, so that flow must pass through plasmodesmata. TYREE (1970) also considers trans-symplasmatic streaming under certain conditions.

In *Laminaria digitata* an exudate flow through plasmodesmata can be induced by cutting through the cauloid.

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