Acta Bot. Neerl. 22(2), April 1973, p. 120–123.

OPEN PLASMODESMATA IN SIEVE PLATES OF LAMINARIA DIGITATA

J. L. VAN WENT, A. C. VAN AELST and P. M. L. TAMMES[1]

Afdeling Plantkunde, Landbouwhogeschool, Wageningen, ditto and
Centrum voor Plantenfysiologisch Onderzoek, Wageningen

SUMMARY

After cooling and killing by freezing in solid CO_2 and fixation at –2 °C, the white outer ring (probably callose) was found to be absent in electron micrographs of plasmodesmata. Some plasmodesmata were partly or entirely filled with a dark plug, but others were open with a lining of protoplasm. The observation of open plasmodesmata accords with the experimental fluid flow through them described in a previous paper (1972). The plugging mechanism seems similar to that of sieve-plate pores in higher plants.

1. INTRODUCTION

An experimental fluid flow through plasmodesmata in the sieve plates of the medullary filaments of *Laminaria* was observed by VAN WENT & TAMMES (1972). Electron micrographs given by ZIEGLER & RUCK (1967), however, indicate that the plasmodesmata are almost closed. A thick white ring of callose (probably) on the outside surrounds a darker ring, probably the plasmalemma, the central part is either open or plugged or with a central core.

In the pores of sieve plates of higher plants a mechanism for rapid closing exists whereby the pore is closed by callose and a fibrous plug in the centre. It could be that the same closing mechanism exists in plasmodesmata, which are the precursors in the evolution of sieve-plate pores. To test this, cauloids were cooled, killed by freezing, and fixed at low temperature.

2. MATERIAL AND METHODS

Whole *Laminaria* plants were stored in plastic bags at 2 °C. Entire cauloids were kept in melting sea-ice at –2 °C for a night and then deep-frozen in solid CO_2. Discs were sawn out from the middle part of the cauloid and thawed at –2 °C in 5% glutaraldehyde in sea water. Then small pieces of the medulla were cut out and postfixed in 2% $KMnO_4$ in sea water at –2 °C for 2 h. Or discs from the medulla were thawed in 2% $KMnO_4$ in sea water at –2 °C and then pieces cut from the medulla for embedding. Controls were fixed at normal temperature and postfixed with OsO_4. All material was dehydrated in ethanol and embedded in Epon 812.

[1] Guestworker: Nederlands Instituut voor Onderzoek der Zee, Texel

3. RESULTS

In electron micrographs it was observed that the white outer ring was absent from all plasmodesmata. Many of them were partly or entirely plugged by protoplasm but a considerable number were open or had a lining of protoplasm (*plate 1A* and *B*).

The controls show the usual plugging with a white ring and the concentric dark ring or plug (*plate 1C*) as was already found by ZIEGLER & RUCK (1967).

4. DISCUSSION

Sieve-plate pores of higher plants are probably developed from plasmodesmata during evolution. In such pores a mechanism for rapid closing occurs with a callose ring and a fibrous plug in the middle. In electron micrographs, pores are usually seen to be closed. Only with certain manipulations were open or partly closed pores observed (ESAU & CHEADLE 1961, ESCHRICH 1965. IE et al. 1966, ANDERSON and CRONSHAW 1970).

It could therefore be expected that in plasmodesmata the same closing mechanism exists. In electron micrographs of *Laminaria* a thick white ring enclosing a darker ring, probably the plasmalemma, is found in the plasmodesmata.

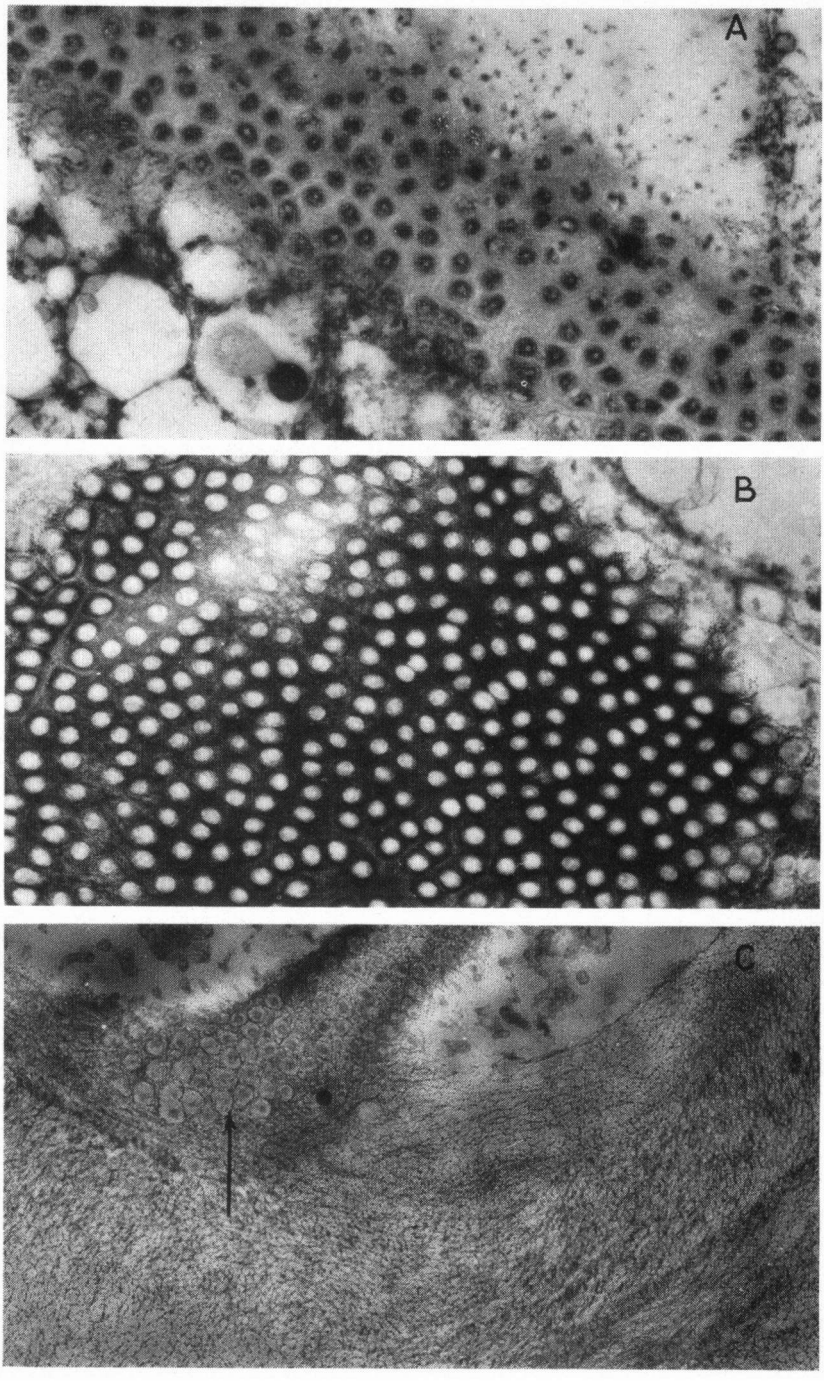
ZIEGLER & RUCK (1967) assayed positively for callose in sieve plates of *Laminaria*. (In the sieve-plate pores of higher plants the callose is found between the plasmalemma and the wall.) The central part is white or plugged or with a central core. The average diameter was 0.06 μm (ZIEGLER & RUCK 1967; see also *plate 1C*. ESCHRICH (1965) supposed that callose formation in plasmodesmata could occur in a fraction of a second and that the whole symplast is able to form callose in all its narrow passages. Callose formation in pits with plasmodesmata in lateral walls of phloem and in parenchyma has been observed after heat treatment in cotton by WEBSTER & CURRIER (1968).

In a previous paper, VAN WENT & TAMMES (1972), an experimental fluid flow through the plasmodesmata of *Laminaria* was described. We therefore tried to find whether open plasmodesmata could be observed by electron microscopy. For this purpose plants were first stored at 2°C, then brought to –2°C and finally frozen in solid CO_2. Discs of the medulla were cut out and thawed in the fixative at –2°C. The aim was to prevent biological activity by low temperature.

The electron micrographs showed an absence of the white ring in all the plasmodesmata. Many were partly or entirely plugged by protoplasm, but also several were open, lined with protoplasm. In other sections the plasmodesmata seem entirely open though some of them have light protoplasmic structures in them (*plate 1A* and *B*). *Plate 1C* shows the control for comparison.

The plugging mechanism seems similar to that of the pores in sieve plates of higher plants.

The existence of open plasmodesmata in vivo has also been supposed by


A
B
C

other authors, e.g. CLARKSON et al. (1971) for the passage of water and ions through the endodermis of barley roots. TYREE (1970) based on theoretical considerations, and SPANSWICK (1972) after studying electric coupling between cells of *Elodea*. It must be mentioned that MÜNCH (1930) already supposed pressure filtration through plasmodesmata in the symplasm.

ACKNOWLEDGEMENT

The authors are indebted to Mr J. C. Rigg for correcting the English.

REFERENCES

ANDERSON, R. & J. CRONSHAW (1970): Sieve plate pores in tobacco and bean. *Planta* **91**: 173–180.

CLARKSON, D. T, H. W. ROBARDS & S. SANDERSON (1971): The tertiary endodermis in barley roots: Fine structure in relation to radial transport of ions and water. *Planta* **96**: 292–305.

ESAU, K. & V. J. CHEADLE (1961): An evaluation of studies on ultra structure of sieve plates. *Proc. Nat. Acad. Sci. U.S.A.* **47**: 1716–1726.

ESCHRICH, W. (1965): Physiologie der Siebröhrencallose. *Planta* **65**: 280–300.

IE, T. S., P. M. L. TAMMES & J. VAN DIE (1966): Studies on phloem exudation from Yucca flaccida Haw. V. Electron microscopy of sieve plate pores. *Proc. Kon. Ned. Akad. Wetensch.* **C69**: 660–663.

MÜNCH, E. (1930): *Die Stoffbewegungen in der Pflanze*. G. Fischer, Jena.

SPANSWICK, R. M. (1972): Electrical coupling between cells of higher plants. A direct demonstration of intercellular communication. *Planta* **102**: 215–224.

TYREE, M. T. (1970): The symplast concept. A general theory of symplasmatic transport according to thermodynamics of irreversible processes. *J. Theor. Biol.* **26**: 181–214.

WEBSTER, D. N. & H. B. CURRIER (1968): Heat-induced callose and lateral movement of assimilates from phloem. *Can. J. Bot.* **46**: 1215–1220.

WENT, J. L. VAN & P. M. L. TAMMES (1972): Experimental fluid flow through plasmodesmata of Laminaria digitata. *Acta Bot. Neerl.* **21**: 321–326.

ZIEGLER, H. & I. RUCK (1967): Untersuchungen über die Feinstruktur des Phloems III. Die "Trompetenzellen" von Laminaria-Arten. *Planta* **73**: 62–73.

Plate 1. A. Plasmodesmata in a sieve plate with absence of the white outer ring (probably callose) and plasma lining of the wall, some are entirely plugged, × 30,000. B. The same but with more open structures and faint structures of protoplasm, plasmalemma not clearly visible due to the contrasting walls, × 30,000. C. Control with white outer ring and plugged centre, × 40,000. A and B. $KMnO_4$ fixation after killing by freezing. C. Osmium fixation without freezing.