AN ELECTRON-MICROSCOPICAL STUDY OF THE PLASMODESMATA IN THE ROOTS OF YOUNG BARLEY SEEDLINGS*

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SUMMARY

Many plasmodesmata can be found in the walls of the cells of the endodermis and the pericycle of the roots. A mutual symplasmatic contact is thus established between the cells of the pericycle, endodermis and the innermost layer of the cortex.

The radial walls of the endodermal cells are very thin and tend to become folded as a result of the fixation and embedding procedure. Virtually no plasmodesmata could be observed in these walls nor any striking feature which could be related to the presence of a Casparian strip.

The plasmodesmata are spindle-shaped and lined by a membrane, which is a continuation of the plasmalemma. They all possess a central osmophilic strand. The pores on either end of the plasmodesmata are closed by the contact between plasmalemma and this strand. The substructure of the strand and its relationship to the endoplasmatic reticulum is discussed.

Some rough calculations with respect to the role these structures may play in the process of intercellular transport are given and the significance of the plasmalemma for this type of transport is emphasized.

1. INTRODUCTION

Young barley roots have been used intensively in studies of active absorption and transfer of ions. Whereas the active absorption involves an active passage of the cell membrane, the transfer from cell to cell need not consist of a secretion by one cell and a subsequent absorption by the adjacent one. In fact, a large amount of evidence has been gathered with *Vallisneria* leaves in favour of the idea of a symplasmatic transport (ARISZ 1969, HELDER 1967). The same concept could be applied to the root tissue of young barley seedlings. Experiments on the simultaneous uptake of chloride and bromide ions suggested that there is only one discriminating step in the overall-process of uptake into the root tissue and subsequent transfer to the shoots (HELDER 1964).

The idea of symplasmatic transport assumes the presence of plasmatic connections between the cells of a tissue. An important peculiarity of plant roots is the endodermis with its modified wall areas known as Casparian strips, which deserve special notice with regard to absorption phenomena.

It was with these facts in mind that we embarked on a study of the fine structure of young barley roots. As it was the first study of this kind in our laboratory and as we did have neither the experience nor the facilities for this type of work, progress was bound to be slow. Moreover, our object, differentiated root tissue, has proved to be a difficult one from a technical point of view.

* Dedicated to Professor Dr. W. H. Arisz.

2. MATERIAL AND METHODS

Barley seeds were washed, desinfected, rinsed and allowed to germinate between sheets of moist filter paper for about two days. Germinated seeds were then raised on a half strength Hoagland solution under constant conditions of light and temperature for 7 days (HELDER 1964). At that time the longer roots were some 7 cm in length.

From these roots 1 mm segments, about 5 mm from the tip, were cut and fixed in 1% osmium tetroxide in veronal acetate buffer (pH 7.4) at 4°C for 3½ hours, rinsed, dehydrated by moving through 30%, 50% and 70% alcohol to 100% alcohol. The tissue was then treated in propylene oxide and embedded in Epon 5:5. Infiltration took place at 50°C for one hour. The mixture was renewed after half an hour. The resin was polymerized at 35°C (15 hrs), 45°C (7 hrs) and 60°C (48 hrs) respectively.

Sections were cut with glass knives on a L.K.B. Ultrotome I. The sections were mounted on formvar-coated 200 mesh grids and then double-stained with 1% uranyl acetate for 45 minutes followed by lead citrate for 5–20 minutes.

The sections were examined in a Philips EM 200 at 60 kV. Double condensor illumination with an aperture of 10^{-3} rad was used. The spot-size of the electron-beam amounted to 10 μ. The objective aperture was 0.025 rad. The microscope was equipped with an anti-contamination device cooled by liquid nitrogen. The magnification was calibrated with a grating.

3. OBSERVATIONS AND RESULTS

During the first part of our research into the fine structure of the young barley root we paid special attention to the radial walls of the endodermal cells. As can be seen from *fig. 1A* no thickening of these walls can be observed. On the contrary, these walls are of very delicate nature. This may be one reason for their folded appearance. If we assume that this folding is entirely due to a shrinkage during the fixation and embedding process the radial dimension of these cells *in vivo* would be about twice the one shown in the figure. It can be calculated that this would mean that the shrinkage of the outer tangential walls of the endodermal cells was of the order of about 10%. Although this contraction in a one-dimensional direction seems to be fairly high we still think it a reasonable one for these very young cell walls.

But, whether the radial walls in the normal living tissue are folded or not, they do not show any feature pointing to Casparian strips. All that could be found on cross section was that the median region of these walls appeared to be slightly more opaque than the other regions, which might indicate that this region may differ chemically from the rest.

This somewhat disappointing result was compensated by the discovery of the plasmodesmata in the tangential walls of the endodermal cells. In *fig. 1A* a group of 5 plasmodesmata can be seen in an intervening wall of endodermis and pericycle.

From a large number of sections we arrived at the conclusion that these

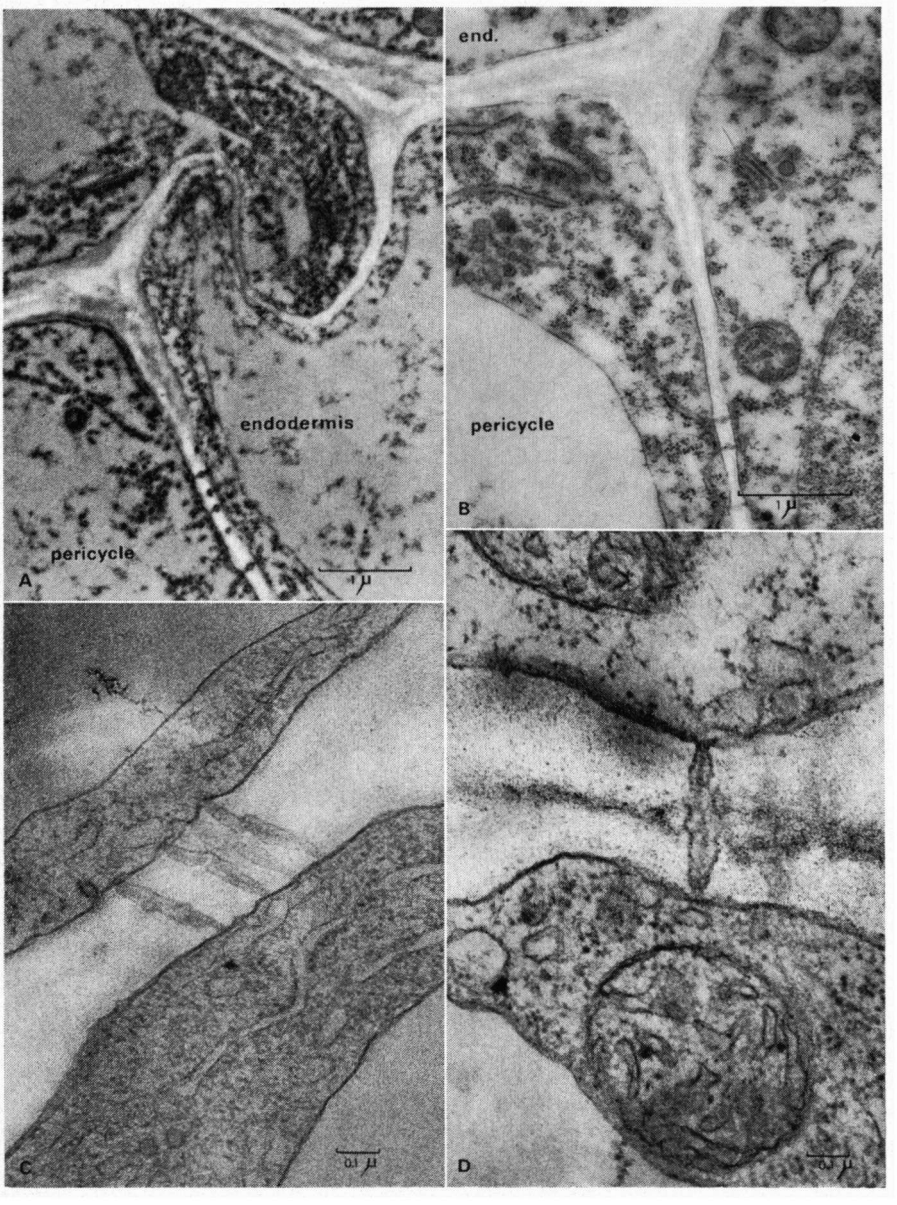

Fig. 1. Sections through the cells of endodermis and pericycle of young roots of barley seedlings.

A. A group of plasmodesmata is visible in the inner tangential wall of the endodermis. Similar groups can be found in the outer walls.

B. Plasmodesmata in a wall between two pericycle cells. Elements of the endoplasmatic reticulum can be seen to come up to these plasmodesmata.

C. A longitudinal section of a group of plasmodesmata in a pit of the inner tangential wall of an endodermal cell.

D. A single plasmodesma sectioned longitudinally. At the upper end of the plasmodesma the pore is cut through. The plasmalemma is continuous across the wall.

plasmodesmata are mainly present in both the inner and the outer tangential walls of the endodermis and in the radial walls of the pericycle *(fig. 1B)*. In this way pericycle, endodermis and the innermost layer of cortical cells are interconnected by plasmodesmata.

In the cortex itself the occurrence of a plasmodesma proved to be rare. This is, no doubt, related to the presence of the large intercellular spaces.

In only one case a single plasmodesma was demonstrated in a radial wall of the endodermis. It was situated very close to the inner tangential wall.

As was indicated above, the plasmodesmata are grouped together. On the average there were some five plasmodesmata in each group, but this number could vary from 1 up to 7–10 *(fig. 1C* and *D)*.

The same picture was arrived at when starting from observations on longitudinal sections. From this it could be inferred that the plasmodesmata must be present in the primary pit fields of the cell walls. The fact, that they always occur in the thinner parts of the walls *(fig. 1C* and *D)* is in accordance with this view.

Full corraboration was supplied by the grazing views that we obtained occasionally. Although we concentrated on sections in which the plasmodesmata were cut longitudinally we also obtained sections of cell walls with the plasmodesmata cut transversely at various levels *(fig. 2E and F)*.

The total number in a single field can be 40–60 plasmodesmata, which appear to be divided into sub-groups of 6–10 plasmodesmata. From these data a very rough estimate of the total number of plasmodesmata in an intervening wall of endodermis and pericycle was calculated as being 20000–30000.

In accordance with the findings with different objects by other authors, we found the plasmodesmata of the cells of the endodermis and pericycle of the younger parts of barley roots to have the larger diameter, ranging from 600–900 Å, in the region of the middle lamella, whereas the diameter of the pores on either end of the plasmodesmata was very small *i.e.* of the order of 300 Å.

Most plasmodesmata studied were simple, showing no branching. Some pictures suggested a somewhat more complicated structure, in particular with respect to the internal features of the central nodule, suggesting the existence of branched plasmodesmata (KRULL 1960, FALK & SITTE 1963, O'BRIEN & THIMANN 1967, SPANSWICK & COSTERTON 1967). As the latter produced vaguer images no further attention was paid to them till now.

The general shape of the plasmodesmata varied. Two main types were found: one type with a marked spherical central nodule *(figs. 1A, 2B, 2C)* and another more spindle-shaped one *(figs. 1C* and *D)*. As the first type was found during the former part of our research the suspicion arose that slight alterations of our fixation procedure might be the cause of these diverging results. Measurement of the total length of the plasmodesmata and the thickness of the walls, they are found in, reinforced this suspicion.

Whereas the length of the plasmodesma in *fig. 1 D* amounts to 0.35 μ, those in *figs. 2A* and *D* amount to 0.2 μ and in of *fig. 2B* to only 0.12–0.15 μ. Clearly, unless we assume a large variability of the thickness of the walls in the pits of these cells, we must conclude that shrinkage may be considerable and also

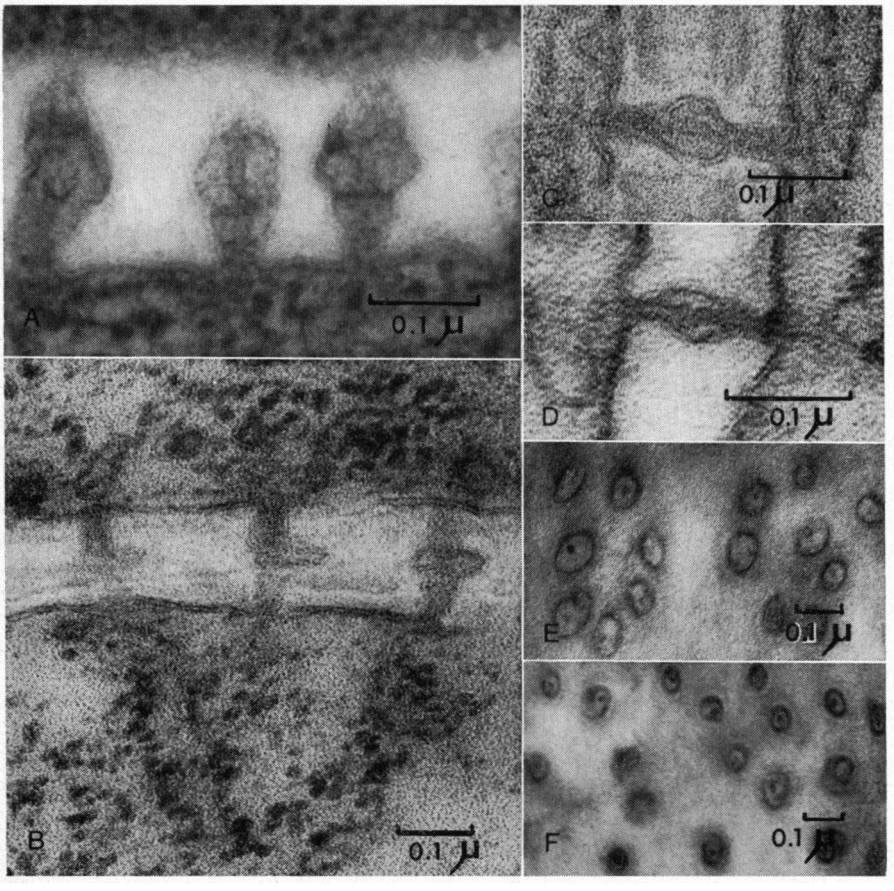

Fig. 2. Sections of plasmodesmata in the walls of the endodermis of the young root of barley seedlings.

A. Slightly contracted plasmodesmata. The surrounding membrane is continuous with the plasmalemma. Loops are to be seen in the central strand of electron dense material.

B. Severly contracted plasmodesmata. Cytoplasmatic elements are clearly situated with respect to the plasmodesmata.

C. An under-focused image of a plasmodesma.

D. A slightly under-focused image, not corrected for astigmatism.

E. and F. Under-focused and parafocal images of transverse sections of plasmodesmata.

variable according to the technique employed and that a considerable deformation of these tiny structures may be introduced.

The extent of the deformation is hard to determine. It will also depend on the kind of tissue studied. In an investigation into the fine structure of the leaf cells of the aquatic *Potamogeton* we made during the last year, shrinkage was such that we had to adopt Sitte's diffusion method (FALK & SITTE 1963). This method

proved to be so successful in this particular case that we adopted it for our barley roots, too. The first results were similar to those shown in *figs. 1C* and *D*.

Each plasmodesma is lined by a membrane of about 80 Å thickness. All investigators now agree that this membrane represents the plasmalemma which folds into the perforations of the intervening wall. Our results are in harmony with this concept, but it should be added that it is extremely difficult to collect pictures which show the plasmalemma to be continuous across the walls quite clearly and convincingly. In the majority of the sections the plasmalemma is seen to pass by the plasmodesmata *(fig. 1C)*. In some cases, however, the membrane could be traced from the cell into the pore at least at one end of the plasmodesma *(fig. 1D)*. That the same can be seen at either end of the plasmodesma is so exceptional that we have included the two examples we have obtained, in spite of their apparent technical shortcomings; under-focusing and astigmatism *(figs. 2C* and *D)*. It seems superfluous to state that this is caused by the smallness of the pores.

If a plasmodesma has been sectioned in the appropriate way a strand of opaque material will always be discernable. Its thickness is about 180 Å. In some cases the strand seems to consist of a tube of electron-dense material with a somewhat lighter content. The strand may show a few loops *(fig. 2A)* but one may wonder whether this reflects the shrinkage-phenomena mentioned above.

Of special importance is the connection between these strands and the endoplasmatic reticulum of the adjacent cells. There is no doubt that the organization of the cytoplasm and in particular that of the endoplasmatic reticulum is influenced by the presence of the plasmodesmata *(figs. 1B* and *2B)*. The crucial point is that the images of the region between plasmodesma and the endoplasmatic reticulum tends to remain faint for some unknown reason and hard to be interpreted.

All we could see were short extensions of the strand of the plasmodesma into the cytoplasm while, moreover, *fig. 2D* suggests that the strand is a part of the reticulum, which forms a loop just before entering into the plasmodesma.

3. DISCUSSION

In a recent article ROBARDS (1968) discusses the ultra-structure of the plasmodesma and in particular the substructure of the central strand, which is referred to as desmotubule. By applying the image reinforcement technique the conclusion is arrived at that the dark desmotubule-wall consists of 11 sub-units, and that the desmotubules may be nuclear spindle fibres.

Although it is admitted, that misinterpretation of structure may easily arise from this technique, the author feels that his result is not attributable to artifacts. Still, tilting experiments with these specimen in the electron-microscope may have yielded stronger evidence for the presence of discrete three-dimensional sub-units.

It seems appropriate to point to still another difficulty. It is the problem that arises from the combined presence of amplitude and phase patterns in defo-

cused images taken at high electron optical magnifications (RUSKA 1966). One should be most reserved in deducing specimen structure from defocused images, because the phase components in the final image do not allow a direct physical description of the object (VAN DORSTEN *c.s.* 1968).

The effect of enhanced overall-contrast as a result of under-focusing makes it understandable why many authors select defocused images. However, it is important to know whether the parafocal image of the object under discussion would have exhibited the same structural features as the underfocused one.

Another interesting aspect of Robards findings is that the outer membrane of the plasmodesma shows a similar substructure. Robards indicates that there are 33 sub-units. If this figure is combined with the diameter one can calculate that the inter-sub-unit spacing in this membrane is also 45–50 Å. Such a structure in the form of globular micelles would be more stable than a bimolecular leaflet with respect to the strain imposed on the structure as a result of the small diameter of the plasmodesmatal canal.

In this context it is only important to realize that, even if this membrane would prove to be particulate, no one would deduce that this membrane is not continuous with the normal cell membrane. In a similar way the particular nature of the wall of the desmotubule need not be considered sufficient proof that it does not represent a continuation of the reticulum.

Our own observations have not provided much evidence in favour of the idea that the osmophilic strands represent remnants of nuclear spindle fibers, apart from the fact that the difficulty to obtain clear images of the contact between these strands and the endoplasmatic reticulum may be considerd evidence for a different nature of these strands.

Apart from the problem whether the desmotubule may be a spindle-fiber or not one may consider whether these tiny structures, which connect the endoplasmatic reticuli of adjacent cells, can be of any significance for intercellular transport. It was calculated that some 20000 plasmodesmata may be present in the tangential wall of an endodermal cell.The total cross section of the desmotubules of such a number of plasmodesmata is about 5 μ^2, whereas the length of the tubule through the plasmodesmata is on the average 0.25 μ.

From absorption studies (HELDER 1964) performed with young barley plants similar to those used in this submicroscopical investigation we made a rough estimate of the transport of chloride ions through a single endodermal cell. It appeared to amount to 3.10^{-4} μeq Cl per day.

If it is assumed that this transport involves simple diffusion through the desmotubules, and that the diffusion constant is the same as for normal solutions, the concentration gradient from one end of the tubule to the other one can be calculated. We obtained a value of about 0.1 μeq per litre. This small difference reflects the well-known fact that diffusion in water is a very effective way of transport over a short distance.

This concentration gradient needed for the transport of ions as found in absorption studies must be increased according to the degree of reduction of the free diffusion in the desmotubules.

SPANSWICK & COSTERTON (1967) assumed from measurements of the specific electrical resistance that there is some restriction of ions in the plasmodesmata of internodal cells of *Nitella*. The presence of osmophilic substances in the tube indicates that such a reduction is very likely.

One may even feel that diffusion is almost impossible and that permeability may be as low as that for the cell membrane. If this is correct, the tubules would constitute a serious barrier for intercellular transport and substances taken up into the cisternae of the endoplasmatic reticulum would become sequestered. (O'BRIEN & THIMANN 1967). As a consequence the plasmalemma would remain as the only means of intercellular transport.

This conclusion is based on the evidence for "symplasmatic transport" (HELDER 1967, ARISZ 1969) and the preliminary assumption, that the pores of the plasmodesmata are sealed by the contact of plasmalemma and desmotubule.

It also involves that the plasmalemma would have a double task; active absorption across the membrane and translocation along its inner surface. Intercellular transport would therefore take place in a two-dimensional, though in many ways folded, space. Spreading and 'surface migration" are well-known notions which could be associated with a suchlike system, but admittedly, we still have little understanding of the factors, such as concentration-differences and membrane-potentials, which may influence such a transport. That such factors as well as metabolism must come into play is obvious in view of the polarity of some transport processes, which can not be explained by the submicroscopical details of the plasmodesmata, which show complete symmetry. In this last connection it is tempting to speculate about the potential significance of the valve-like structure of the plasmodesma.

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REFERENCES

ARISZ, W. H. (1969): Intercellular polar transport and the role of the plasmodesmata in coleoptiles and Vallisneria leaves. *Acta Bot. Neerl.* **18**: 14–38

BORRIES, B. VON & F. LENZ, (1957): Über die Entstehung des Kontrasten im elektronenmikroskopischen Bild. *Proc. Stockholm Conference for Electron Microscopy*, 60–64. Almqvist and Wiksell, Stockholm.

DORSTEN, A. C. VAN, J. E. MELLEMA, & H. F. PREMSELA, (1968): Possible procedures for dealing with the problem of simultaneous occurrence of amplitude and phase contrast in images of macromolecules. *Proc. IV European Conference for Electron Microscopy, Rome.*

FALK, H. & P. SITTE, (1963): Zellfeinbau bei Plasmolyse. *Protoplasma* **57**: 290–303.

HELDER, R. J. (1964): The absorption of labelled chloride and bromide ions by young intact barley plants. *Acta Bot. Neerl.* **13**: 488–506.

— (1967): Translocation in Vallisneria spiralis; in RUHLAND (Ed.): *Encyclopedia of plant physiology* XIII. Springer-Verlag, Berlin.

KOLLMANN, R. & W. SCHUMACHER, (1962): Über die Feinstruktur des Phloems von Metasequoia glyptostroboides und seine jahreszeitlichen Veränderungen. II. Mitt. Vergleichende Untersuchungen der plasmatischen Verbindungsbrücken in Phloemparenchymzellen und Siebzellen. *Planta* **48**: 366–386.

KRULL, R. (1960): Untersuchungen über den Bau und die Entwicklung der Plasmodesmen im Rindenparenchym von Viscum album. *Planta* **55**: 598–629.

LÓPEZ-SÁEZ, J. F., G. GIMÉNEZ-MARTIN & M. C. RISUEÑO (1966): Fine structure of the plasmodesm. *Protoplasma* **61**: 81–84.

MEEUSE, A. D. J. (1957): Plasmodesmata (Vegetable Kingdom). *Protoplasmatologia* Band II, A 1c. Springer-Verlag, Wien.

O'BRIEN, T. P. & K. V. THIMANN (1967): Observations on the fine structure of the oat coleoptile. II. The parenchyma cells of the apex. *Protoplasma* **63**: 417–442.

ROBARDS, A. W. (1968): A new interpretation of plasmodesmatal ultrastructure. *Planta* **82**: 200–210.

RUSKA, E. (1966): Past and present attempts to attain the resolution limit of the transmission electron microscope. *Advances in Optical and Electron Microscopy* Vol. I, Academic Press, London and New York.

SPANSWICK, R. M. & J. W. F. COSTERTON (1967): Plasmodesmata in Nitella translucens: structure and electrical resistence. *Journ. of Cell Science* **2** (3): 451–464.