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The Ultrastructure of Plasmodesmata

A. W. Robards

Department of Biology, University of York, Heslington, York, England

With 3 Figures

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Summary

It is suggested that the central strand which traverses plasmodesmata is in open continuity with the endoplasmic reticulum of adjacent cells, and that this strand (desmotubule) represents a modulation of a normal ER membrane so that it comprises solely spherical protein subunits. This concept is used to illustrate how plasmodesmata could form a median nodule or anastomosing central strands. The implications of this model in relation to current theories of symplasmic transport are discussed, and the possibility for further experimental work is outlined.

The concept of symplasmic transport between plant cells must take account of the possible role of plasmodesmata; this causes difficulties, because the size and situation of plasmodesmata within plant cell walls makes high resolution electron microscopical examination most difficult, and a generally acceptable description of plasmodesmatal ultrastructure has not been published. The commonest suggestion is that plasmodesmata comprise plasmalemma-lined canals through cell walls; each canal containing a tubule of endoplasmic reticulum (see, for example, literature in: Buvat 1969, Frey-Wyssling and Mühlethaler 1965, Kollmann and Dörr 1969, Northcote 1968. Also, see Clowes and Juniper, 1968, for a concise review of the subject of plasmodesmatal structure). It is the structure of the plasmodesmatal core which is of paramount importance. The suggestion that the central tubule is endoplasmic reticulum appears to stem from the finding that this membrane system (ER) most commonly approaches closely both ends of each plasmodesma. Although continuity at a late stage in cell plate formation is often seen (*e.g.*, Buvat 1969, p. 109), there is no published micrograph of a com-

pletely differentiated plasmodesma through which a strand of ER is unequivocally seen to pass. This is not surprising as the difficulties posed by limited resolution in observing sectioned material, low contrast in very thin sections, and in obtaining accurately longitudinal sections through the plasmodesmatal core all mitigate against the probability of obtaining a definitive image. Even in good micrographs the possibility of image misinterpretation due to the combined effects of amplitude and phase changes arising from the normal practice of recording slightly underfocused images at high electron optical magnification still needs to be considered. [This problem has been discussed by Haydon (1969), in relation to the molecular structures of ferritin and in connection with the current topic by Helder and Boerma (1969).] Despite limitations to high resolution study of plasmodesmata, it is useful to discuss their possible structure in relation to current electron microscopical evidence and theories concerning the molecular configuration of subcellular components.

Arisz (1969) and Tyree (1970) have given evidence for supposing that plasmodesmata must function in symplasmic transport. In common with the plasmodesmata which penetrate tertiary endodermal walls and other similarly impermeable walls, those which are found between the ray cells of secondary xylem (Robards 1968 b) are of special interest because they must, presumably, represent the only channels for intercellular transport. The studies on plasmodesmata between ray cells confirmed that the canal is lined by the plasmalemma which is continuous from cell to cell; it was also concluded that the central strand has a structure similar to that of a cytoplasmic microtubule, and the term "*desmotubule*" was coined to describe it. Largely for the reasons already described, it was not possible to resolve the nature of the junction between the desmotubule and the ER which undoubtedly closely approaches its two ends. A model was constructed (Fig. 1) which, while embodying the concept of the desmotubule, and illustrating the structures as seen, did little to interpret or clarify how plasmodesmata might function in symplasmic transport.

Initially, the idea of a desmotubule might appear incompatible with the possibility that ER is continuous between cells; the difficulties are augmented because the molecular configuration of cell membranes is itself a matter of current speculation. If the ER membranes are lipoprotein bilayers, then it is clear that the 20 nm diameter desmotubule is unlikely to be a direct continuation of such bilayers because a unit membrane would not be expected to assume a stable configuration when describing such a small circumference (Robertson 1964). The alternatives are either that the ER is not continuous with the desmotubule, or that there is continuity but through a considerably modified membrane structure. The first hypothesis is least attractive, if only because ER continuity is clearly evident during late stages of cell plate formation; further, lack of continuity leads to difficulty in explaining some of the

enzyme activities found associated with plasmodesmata (ROBARDS and KIDWAI 1969), as well as the presence of median nodules (KRULL 1960) and branching central strands (KOLLMANN and SCHUMACHER 1962, 1963, CLOWES and JUNIPER 1968). If the desmotubule is assumed to be a continuation of the ER, but with modified structure, the following features must be taken into account. The ER

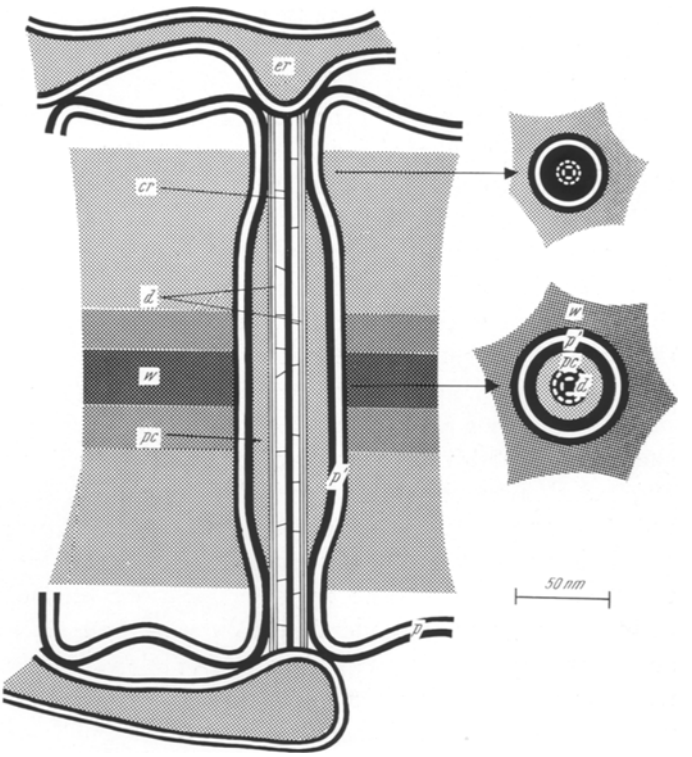

Fig. 1. Diagrammatic representation of longitudinal and transverse sections of a simple plasmodesma, as shown by ROBARDS (1968 b). *cr* = central rod, *d* = desmotubule, *er* = endoplasmic reticulum, *p* = plasmalemma, *p'* = plasmalemma through plasmodesmatal canal, *pc* = plasmodesmatal cavity, *w* = cell wall

membranes are about 8.0 nm thick, and the desmotubule wall is little more than half this. The ER membranes appear typically trilaminar after conventional preparation for electron microscopy while the wall of the desmotubule appears to comprise particulate subunits (ROBARDS 1968 b).

Current concepts of cell membrane structure commonly embody the possibility that expanded bimolecular leaflets alternate with areas of included lipidic and proteinaceous micellar subunits about 4.0 nm in diameter (see, for example, LUCY 1964, GLAUERT 1968). Such particles, if proteinaceous, would therefore be very similar in size, shape, and composition to those which are

the components of microtubules. This point has recently been given more weighty support by Mazia and Ruby (1968) who have shown that there are important similarities between proteins of as diverse origin as erythrocyte membranes, microtubules, and muscle actin; they have forwarded the name *tektins* for a genus of related proteins which, they suggest, are employed in the assembly of a variety of cell structures. It is not difficult to envisage that there is a basic particulate, proteinaceous, subunit common to the ER membrane and desmotubule, so that modulation from the former to the latter may occur merely by an increase in the particulate component with a concomitant reduction in non-particulate protein and probable complete elimination of lipidic material.

Consequently, continuity of ER from cell to cell via plasmodesmata *is* thought possible, but the traversing strand (desmotubule) would comprise a particulate, tubular structure, while cytoplasmic ER membranes are in some way different from this. If this hypothesis is true, then the question of whether the strand through the plasmodesmatal canal is endoplasmic reticulum or some form of microtubule becomes a purely semantic one.

Such close association between membranes and microtubules is not without precedent. It has been suggested that the ER may itself act as a nucleating site for polymerization of microtubular subunits (Burgess and Northcote 1968); and microtubules can certainly be attached to the plasmalemma in plant cells (Robards 1968 a). Wooding (1968) has recently criticized the concept of the desmotubule on the grounds that "... there is no precedent for microtubules to branch or connect with endoplasmic reticulum ...". Apart from the dubious use of "precedent" in following the logical course of a scientific argument, the scheme described here both allows the desmotubule to connect with the endoplasmic reticulum, and also offers an explanation of how the median nodule and branching plasmodesmata might exist (Fig. 3). Further, although there does not appear to be any good evidence for microtubules connecting with the endoplasmic reticulum in plant cells, they have been reported to join both the inner (Falk, Wunderlich, and Franke 1968) and outer (Anteunis, Fautrez-Firlefyn, and Fautrez 1967) nuclear membrane in animal cells. These facts suggest that it is unlikely that any real difficulty exists in microtubules connecting with cell membranes. Continuity of ER from cell to cell, although in modified form, is both probable and necessary for the reasons given, and it is suggested that the term desmotubule is retained as a completely unambiguous word to describe the central core.

In the original model (Fig. 1) there is no intercellular continuity, and an electron-opaque central rod is shown within the desmotubule. If the cavity of the ER is in open continuity between adjacent cells, then the central rod would act as a barrier to transport. It is known that microtubules in sectioned material may sometimes be demonstrated to advantage by a form of negative staining brought about during processing (Ledbetter and Porter

1964); in some cases an electron opaque core has even been demonstrated within cytoplasmic microtubules (in JENSEN and PARK 1967). If the central rod is some form of artifact, then it is most likely to have arisen from one of two causes: it may represent a true negative staining phenomenon; or it could possibly be an accumulation of osmiophilic material extracted during

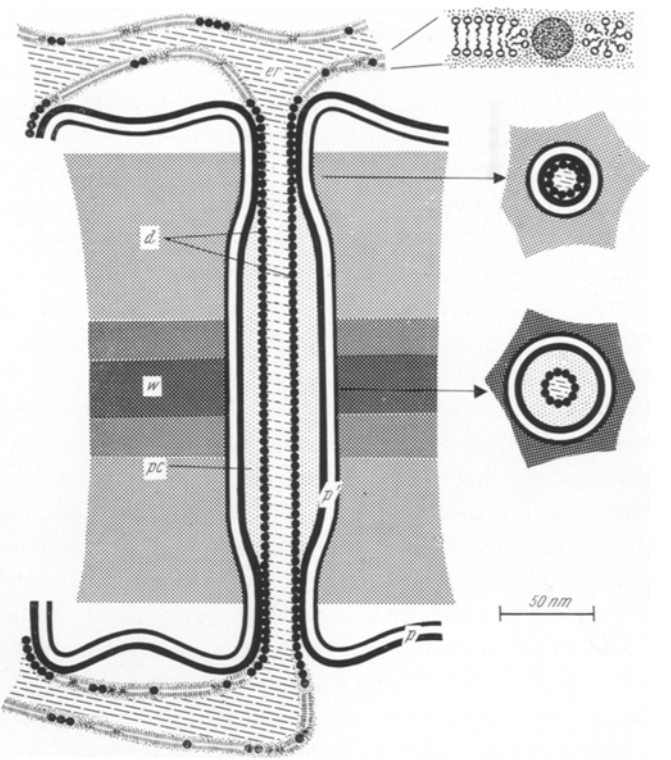

Fig. 2. An interpretation of plasmodesmatal ultrastructure. The endoplasmic reticulum is shown to be continuous with the desmotubule but in modified form. The structure of the endoplasmic reticulum membranes (detail shown top right) is based upon an alternating expanded bilayer and micellar inclusion model as, for example, illustrated by GLAUERT (1968). Abbreviations are as in Fig. 1

processing. If the latter, it is conceivable that the lipid had become stripped from a lipoprotein system as has been shown to occur in other systems (for example, see ASHWORTH, LEONARD, EIGENBRODT, and WRIGHTSMAN 1966, ONGUN, THOMSON, and MUDD 1968). This is regarded as unlikely, as the same limitations to molecular dimensions of the central strand as have already been considered would apply. In fact, taking account of the known phenomenon of negative staining in fixed and embedded tissues, it is very probable that the central rod (Fig. 1) *is* such an artifact. Using this assumption, in

addition to the one previously made, that the desmotubule provides open continuity between the cisternae of the ER in adjacent cells, it is possible to construct a further model for plasmodesmatal ultrastructure (Fig. 2).

This new model suggests areas for further research on plasmodesmata: the model can be assessed in relation to observed plasmodesmatal structure and variability; it can be incorporated into schemes for intercellular transport in plants; and the structure of the desmotubule indicates that experimental methods which are used to study cytoplasmic microtubules might also be usefully employed in research on plasmodesmata.

The model for plasmodesmatal ultrastructure described here implies that the structural change from ER membrane to desmotubule is brought about directly or indirectly by forces exerted when the ER becomes trapped in the cell plate. At early stages clear continuity of ER from cell to cell will thus be seen to exist (Fig. 3 *a*; also see BUVAT 1969). Where plasmodesmata have a median nodule, the actual canal through the wall is usually wider in the central region; the same restriction upon the size of the desmotubule would not exist and the tubule could remain in the form of ER in normal configuration. This is compatible with the evidence for enzyme activity associated with the median nodule. Similarly, where the plasmodesmatal canal is less constricting than as illustrated in Fig. 2 (as often found in potential sieve elements, for example), it would be expected that the central strand would not be caused to adopt the more compact desmotubule configuration. However, such examples are exceptions rather than the rule and, of the plasmodesmata that I have examined in roots, stems, leaves, and xylem, the majority conform to the model suggested here. In some cases (for example, DOLZMANN 1965, as well as my own results) the desmotubule appears to be connected to the plasmalemma which lines the canal through the wall; further, the desmotubule has sometimes been shown with apparent openings into the plasmodesmatal cavity (again, for example, DOLZMANN 1965). The significance of these attachments and openings is obscure and must await further studies on the mode of action of transport through plasmodesmata before further speculation can take place.

In view of the evidence presented here, that the desmotubule probably represents a locally modified tubule of ER, the possibility that microtubules of the phragmoplast become trapped in the cell plate and so form plasmodesmata seems unlikely. Quite apart from functional considerations, it has been pointed out by NEWCOMB (1969) that microtubules of one daughter cell are rarely continuous through the cell plate with those of the other. If, occasionally, phragmoplast microtubules do become so trapped, then it would be difficult to make a distinction from "normal" desmotubules during electron microscopic examination. However, it is possible that the occasional, solitary plasmodesmata which are found isolated from the more usual groups could have arisen in this way.

So far as the functional role of the plasmodesmata in symplasmic transport is concerned, it is clear that much further experimentation is required before any valid scheme can be proposed. However, the evidence that plasmodesmata *do* act as channels for intercellular communication (ARISZ 1969), together with the general opinion that it is the ER which is continuous from cell to cell, makes it surprising that the implication of this—that the ER is synonymous with the symplasm (as proposed by O'BRIEN and THIMANN

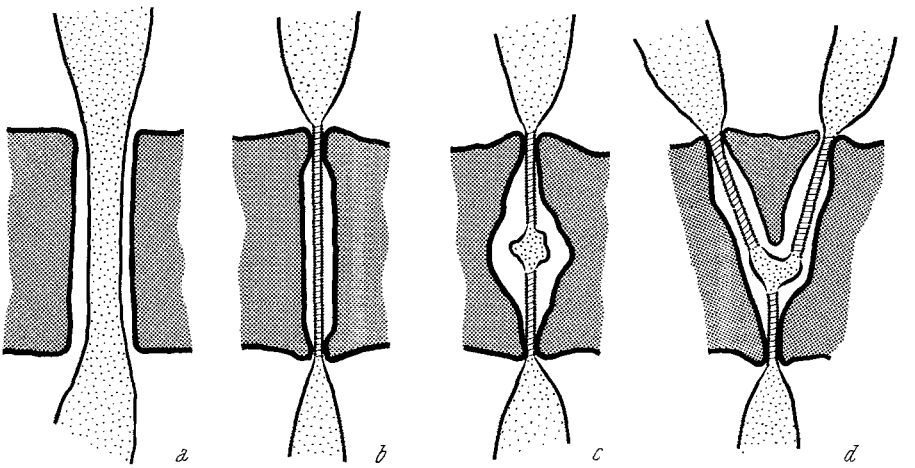

Fig. 3. Diagrammatic representation of some of the types of plasmodesmatal structure found which can be explained using the model proposed here. *a* Continuity of endoplasmic reticulum from cell to cell during cell plate formation. *b* A simple, mature plasmodesma as shown in detail in Fig. 2 and possessing as desmotubule. *c* A plasmodesma possessing a median nodule which could exist in the form of normal endoplasmic reticulum membrane configuration owing to absence of the compressive effect of the cell wall in the central region. *d* Anastomosing plasmodesmata showing how desmotubules could be joined by normal ER membranes where space permits

1967)—has not been more fully discussed. Recent calculations of fluxes through plasmodesmata in the inner tangential wall of the endodermis of barley roots indicate that neither water nor phosphate crosses a lipid membrane during passage through the plasmodesmatal pore (CLARKSON, ROBARDS, and SANDERSON 1971). If the endoplasmic reticulum were continuous with the desmotubule as suggested here, this would imply that these materials move within its cisternal cavity. However, while in higher plants intercellular translocation may be mediated by the endoplasmic reticulum, it is relevant to comment upon the work of FRASER and GUNNING (1969) who have clearly demonstrated the existence of a fundamentally different type of plasmodesma in the filamentous green alga, *Bulbochaete hiloensis.* In this alga there appears to be no question of ER participation in, or continuity with, the plasmodesmatal complex. *Prima facie,* therefore, it appears that it may be necessary

to construct more than one model for symplasmic transport in plants. This will only be resolved by physiological experiments in conjunction with examination of the structure of plasmodesmata in a variety of plant groups and plant organs.

Finally, the often-quoted evidence for the passage of virus particles through plasmodesmata (see, for example, BEHNKE 1966, ESAU 1967, ESAU, CRONSHAW, and HOEFERT 1967, and DAVISON 1969) is probably misleading in the current context as the size of the particles involved is usually large compared with the diameter of the desmotubule. The micrographs of DAVISON (1969) suggest that the desmotubule may have the capacity to distend, and so accommodate the virus particles, while ESAU's (1967) illustrations do not reveal a desmotubule-like structure at all. It is possible that the presence of the virus may itself alter the plasmodesmatal structure, but evidence obtained from infected cells should be regarded with suspicion when related to general theories of symplasmic transport until more is known about the normal condition.

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Author's address: Dr. A. W. ROBARDS, Department of Biology, University of York, Heslington, York, Y01 5DD, England.