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Short survey

Towards reconciliation of structure with function in plasmodesmata— who is the gatekeeper?

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Abstract

Whilst the structure of higher plant plasmodesmata was first described by Robards (1963. Desmotubule—a plasmodesmatal substructure. Nature 218, 784), and despite many subsequent intensive investigations, there is still much that remains unclear relating to their ultrastructure and functioning in higher plants. We have examined chemically fixed plant material, and suggest that the conformational changes seen in plasmodesmatal substructure, particularly the deposition of electron-dense extra-plasmodesmal material, is linked to either manipulation of the hormonal balance (as in Avocado fruit), or of osmotic potential in leaf blade material. These changes result in the deposition of ß 1,3-glucan (callose) at the neck region of these plasmodesmata. This electron-dense material is deposited at the neck region of plasmodesmata, and forms a collar-like structure. The formation of a collar is shown to be coupled with loss of lucence within the cytoplasmic sleeve. The formation of a collar at the plasmodesmatal orifice thus results in encapsulation and closure of the plasmodesmatal orifice. Closure of the orifice coincides with a loss of electron-lucence and a lack of resolution of the desmotubule. These ultrastructural changes are potentially significant and could contribute to, result in, or assist in the down-regulation of cell to cell trafficking via plasmodesmata.

Keywords: Desmotubule; Callose deposition; Neck constriction; Cell-to-cell communication; Cell-to-cell transport regulation; Plasmodesmata

1. Introduction

Thirty-six years have elapsed since the publication by Robards (1963) of the results of what we consider to be the first definitive ultrastructural study on plasmodesmata. Subsequently, many workers have added to the body of knowledge concerning the functioning of whole plasmodesmata, with respect to the characteristics of their component parts (see Botha, 1992; Lucas, 1995; Overall and Blackman, 1996, and literature cited by these authors). In addition to the widening of our understanding of this organelle through intellectual debate, major technological improvements, particularly in the resolving power of microscopes and the development of new imaging techniques and technologies, and in physiological techniques, have been applied to facilitate a better understanding of the fine structure of plasmodesmata. This is particularly so, with respect to their function, especially in regulating the cell cycle and in cell-to-cell transport in plants.

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2. Materials and methods

Fixation procedures for the tissues described here were standardized, with the exception that mesocarp tissues of Avocado were subjected to prolonged cold fixation (4°C, 5% glutaraldehyde in 0.05 M Na-cacodylate buffer, changed every hour during the course of the day and left overnight in a refrigerator). Fixative was changed several times the next day during re-trimming of blocks to approximately 2x2x2mm. Mesocarp tissues were washed in several changes of cold Na-cacodylate buffer, post-fixed in osmium tetroxide and dehydrated, infiltrated and embedded as described below.

Leaf blade tissue was taken from the mid-third of fully expanded leaf blades of the fifth or sixth visible leaf, counting from the apex downwards. Small (approximately 2x3 mm segments) of mature leaf blade material was fixed in three changes of 3.0% glutaraldehyde in cold 0.05 M Na-cacodylate buffer and then overnight in a refrigerator. Plant material used was taken from mature leaf blades of *Saccharum officinarum* var. Nco 376, *Bromus unioloides* L., *Sporobolis africanus* (Poir) Robyns & Tournay, and mesocarp tissue from the Avocado fruit, *Persea americana* Mill. Var *gautemalensis*.

Tannic acid (0.2% (w/v)) was added to some of the leaf tissue in the first change of glutaraldehyde. Leaf blade material was diced further into approximately 1x 2 mm pieces, before being washed in cold 0.05 M Na-cacodylate buffer, dehydrated in a cold graded alcohol series followed by two changes of cold propylene oxide (1 h total). Embedment was in Spurr's (1969) hard resin. Ultra-thin sections were cut with a diamond knife on an LKB Ultrotome III or Ultra-tome V ultramicrotome, and collected on 300-mesh copper grids. Sections were stained in uranyl acetate and lead citrate and viewed in a JEOL (Tokyo, Japan) JEM 1210 transmission electron microscope at 80 or 100 kV.

Fluorescence microscopy was carried out as follows— leaf blade tissue from mature *S. africanus* was used for the demonstration of callose-associated plasmodesmal pit fields. Leaf tissue was gently scraped with a new single-edged razor blade, and the scraped leaf segment was immediately immersed in a 0.5% (Gurr) Aniline blue solution in tapwater. Sections were examined using a Zeiss (Carl Zeiss, Oberkochen, Germany) epifluorescence microscope fitted with a 50 W high-pressure mercury lamp, under blue light. Colour slides were taken with Fujichrome 400 Provia Professional film, and digitized using a Nikon Coolscan II (Nikon Corporation, Japan) 35mm slide scanner.

3. Principal of construction

We recognize the significance of the work reported by Semenova and Tageeva (1972) who, after examining plasmodesmata in orange peel, wild radish, and red cabbage material, suggested that the 'principle of construction' of these different plasmodesmata was virtually uniform, and as a result, unity of organization occurs in these structures, due to their common function. Whilst this general observation remains largely undisputed, the interpretation of fine structure remains a major area where consensus has yet to be reached and where discussion on, and interpretation of, the electron micrographs continues. For example, Semenova and Tageeva (1972) were of the opinion that the axial structure of the plasmodesma is composed of two membranes, which are fused together, and not a central rod as argued by Robards (1968). Many models of the structure of plasmodesmata have been proposed over the years, but as noted by Ding et al. (1992) and subsequently by Botha et al. (1993) much of our current knowledge is based on results from material prepared for electron microscopy by chemical fixation. Ding et al. (1992) argued that observation of chemically fixed plasmodesmata is problematic, as fine structural details would be lost during to the relatively slow processes involved in chemical fixation procedures. However, Botha et al. (1993) presented evidence that their images of plasmodesmata in *Themeda triandra*, although prepared by chemical fixation, were properly representative of their real structure, and that direct comparison of the ultrastructural details visible in normal as well as digitally enhanced images, were directly comparable with those where rapid, high-pressure freezing techniques had been employed.

4. Regulation of intercellular transport

Regulation of intercellular symplasmic transport has been shown to be controlled by the plant, and thus transport capacity through plasmodesmata may be regulated during the course of morphogenesis (Heslop-Harrison, 1964a,b,c; Whelan, 1974; Kwiatkowska and Maszewski, 1985). Kwiatkowska and Maszewski (1985) also suggested that changes in plasmodesma structure led to changes in the nature of transport through plasmodesmata. These authors were amongst the first to state that the presence of 'open' plasmodesmata (i.e. those which contained electron-transparent cytoplasmic material) was necessary during synchronized cell division. More recently, this observation has received positive support from the elegant experiments conducted by Ehlers and Kollmann (l996), who have demonstrated that synchronous and asynchronous differentiation is linked to open or closed plasmodesmata between concomitant cells of *Solanum nigrum* protoplasts. In addition, there is strong evidence that plugging of plasmodesmata by osmiophilic electron-dense material precedes desynchronization of mitotic cycles (Kwiatkowska and Maszewski, 1976). Early studies have identified callose as a substance that is involved in the plugging of plasmodesmata after wounding (Whelan, 1974). There is evidence for the localization of callose antibodies attached only to the regions between collars and not to that structure itself [see Turner et al. (1994), cited in Overall and Blackman (1996)]. Clearly, this indicates that there has to be a role for callose, either in the wounding response, or in regulation of cell-to-cell transport. An interesting and exciting possibility revolves around a role for actin-like proteins (White et al., 1994) which have been noted in close association with plasmodesmata.

5. The nature, function and regulation of the axial components of plasmodesmata

The nature and function of the desmotubule has been the subject of much discussion ever since the report by Lo´pez-Sa´ez et al. (1966) that there was continuity between the endoplasmic reticulum and the desmotubule (Thomson, 1969; Fisher and Evert, 1982; Overall et al., 1982, and literature cited). Indeed, plasmolysis in up to 1.0 M sucrose apparently fails to rupture the connection between the desmotubule and the associated 'cytoplasmic strand' (Semenova and Tageeva, 1972). These authors claim this to be strong evidence of intercellular cytoplasmic unity between adjacent cells. In recent years there has been increasing attention focused on the association between the plasmalemma and the axial components of plasmodesmata, especially at the neck region where collars or sphincters are reported (see Robards, 1968; Evert et al., 1977; Olesen, 1979; Kwiatkowska and Maszewski, 1985; Beebe and Turgeon, 1991; Botha et al., 1993; Overall and Blackman, 1996; Cook et al., 1997; van Bel and van Kesteren, 1999 and literature cited in these papers). Clearly, the neck region plays a vital role in regulation of micro-and macromolecular trafficking, and determination of the structural organization and controlling or regulating mechan-ism(s) becomes central to any thesis on function. Many variations in method have been used to try to elucidate the substructure of the neck regions of plasmodesmata. For example, Hepler (1982) used an embedding routine that involved primary fixation by glutaraldehyde, followed by a mixture of osmium tetroxide and potassium ferricyanide (OsFeCN) that selectively stained the ER associated with plasmodesmata, and asymmetrically contrasted the outer and inner leaflets of the plasmamembrane. Numerous substructures associated with the outer plasmalemma leaflet were visualized with this technique, as was globular particulate material associated with the outer leaflet of the desmotubule. Importantly, the annulus was shown to be free of inclusions. Its electron-lucence was interpreted as evidence for the cytoplasmic sleeve being the major symplastic plasmodesmal transport pathway. Unfortunately, though, Hepler's paper shows no plasmodesmata in longitudinal view.

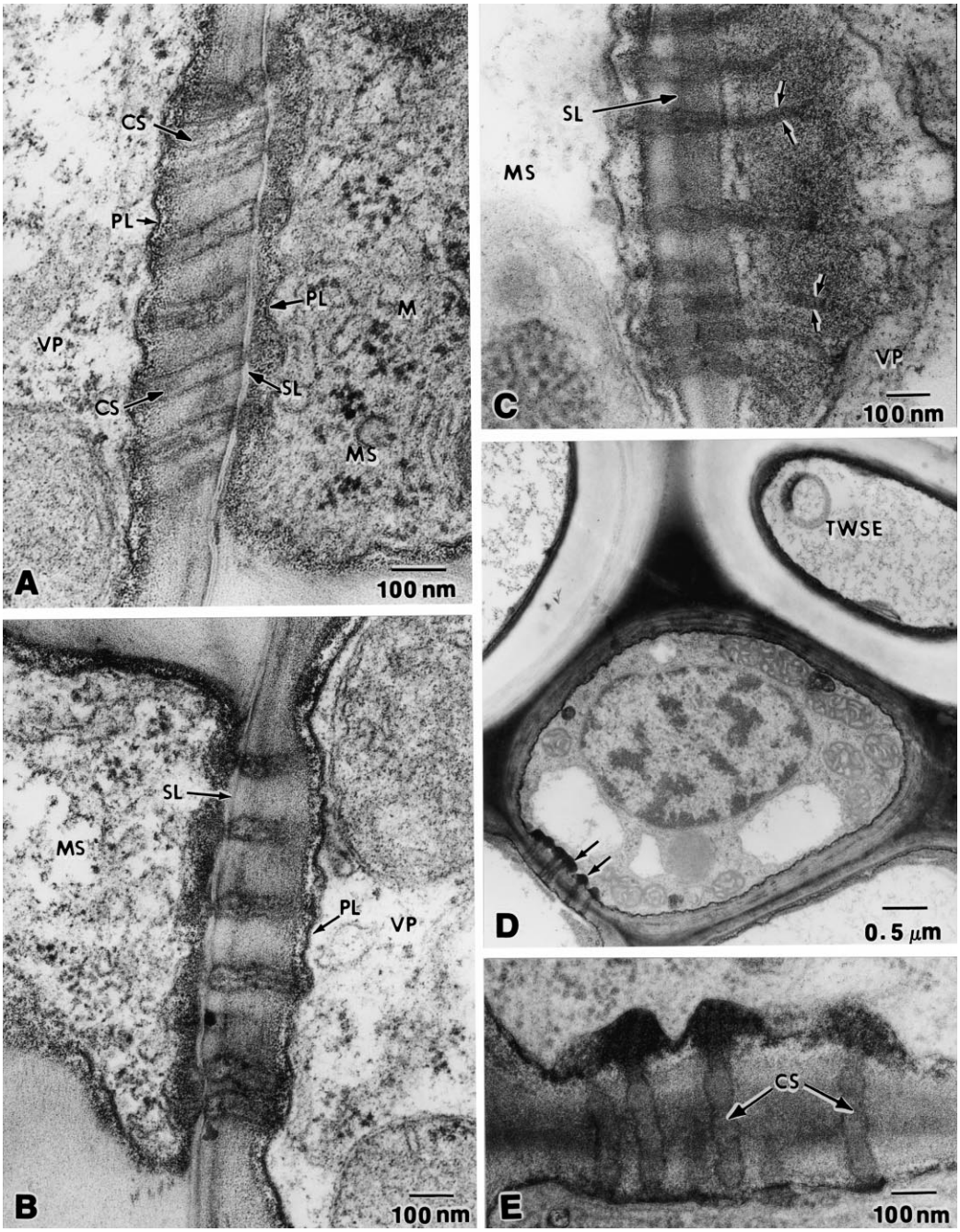

Fig. 1(A)–(E). Transections of plasmodesmata in leaf blade vascular bundles of the grass, *B. unioloides*. Plasmodesmal fields in inner tangential walls at the mesotome sheath–vascular parenchyma (MS–VP) interface. Suberin Lamella (SL) is visible in (A) and (B) but is not distinct in (C). Fine, granular electron-dense material is visible on the MS and VP side of the plasmodesmata in (A), but deposition seems more pronounced on the MS-side. Plasmalemma (PL) remains closely appressed to the plasmodesmata. Plasmodesmal tubules (including the cytoplasmic annulus and the desmotubule) are greatly extended beyond the normal wall structure, which is associated with a large granular, electron-dense deposit on the inner wall face, beneath the plasmalemma. Plasmodesmata contain desmotubules. (C) shows a plasmodesmal field between a MS cell (left) and a phloem parenchyma cell (right). As in MS–VP, the granular electron-dense deposits are more pronounced on the MS side, than on the phloem parenchyma cell side. Plasmodesmata contain clear cytoplasmic sleeves, and central to this, clearly resolved desmotubules. Plasmodesmata are constricted where they pass through the SL. (D) and at higher magnification, (E), shows transections of plasmodesmata traversing the VP–phloem parenchyma cell interface, adjacent to a late-formed, thick-walled sieve element (TWSE). Note deposition of a fine granular electron-dense matrix over these plasmodesmata, apparently occluding the plasmodesmal orifices. Desmotubules are not evident in (E). MS, mestome sheath; VP, vascular parenchyma; SL, suberin lamella; TWSE, thick-walled sieve element.

We have demonstrated that plasmodesmal function is related directly to the ratio of endogenous abscisic acid (ABA), (Moore-Gordon et al., 1998). Avocado fruit pretreated with ABA showed a significant (50%) reduction of ^{14}C-labelled sucrose in the seed coat, and a twofold increase in ^{14}C in the seed. Pretreatment of the fruit with ABA abolished the electrical potential (E_m). In addition, iontophoretic dye-coupling studies, revealed little cell-to-cell transport of Lucifer yellow in ABA-pretreated fruit (Moore-Gordon et al., 1998). In addition, application of ABA or 6-(γ(γ-dimethylallylamino) purine (iP) show branched plasmodesmata in control and iP-injected fruit, whereas plasmodesmal branching was reduced in ABA-injected fruit. A second effect is that electron-dense, particulate material is deposited at the neck region of plasmodesmata in the mesocarp and seed coat tissue. This material was not associated with plasmodesmata in mesocarp or seed coat tissue in either control or iP-injected fruit. Iontophoretic dye-coupling experiments confirmed little, if any, cell-to-cell transport in ABA-treated mesocarp or seed coat tissue, whilst control and iP-injected tissues showed positive intercellular Lucifer yellow transport. Clearly, this study supports the argument for hormonal control of plasmodesmal function through alteration of plasmodesmal morphology and ultrastructure.

Mutant plants, such as the sucrose transport deficient (SXD-1) maize mutant, have proved extremely useful in studies of sucrose export from mutant, compared with non-mutant leaves. Whilst the terminal two thirds of the mutant leaf cannot export sucrose, the basal third of the mutant, like the whole non-mutant leaf, is capable of sucrose export. Russin et al. (1996) reported that the lack of export capacity was due to a symplastic interruption of the transport pathway between the bundle sheath–vascular parenchyma interface, caused through aberrant plasmodesmal formation, and clearly this mediates assimilate traffic across this vital interface. The paper by Russin et al. (1996) therefore suggests that the mutant's lack of transport capacity is possibly due to a genetic malfunction of the plasmodesmal development sequence.

Clearly, the foregoing supports our contention that there is sufficient evidence in the literature for plasmodesmata not to be considered static structures, but structures that may respond to a number of stimuli—be these hormonally regulated, osmotically regulated, or under nuclear control during their development. There is also strong evidence for a role for callose in the regulation process.

Our principal aim is thus to review the ultrastructure of plasmodesma in important transport interfaces in the leaf blades of grasses, and in strong sink tissues such as Avocado.

6. Grass leaf blade plasmodesmata

The C_3 grass, *B. unioloides* (Fig. 1(a)–(e)) contains plasmodesmata at the mestome sheath–vascular parenchyma cell interface (MS–VP) that have unoccluded cytoplasmic sleeves (Fig. 1(a) and (b)). Plasmodesmata crossing the MS–VP interface are usually constricted where they traverse the suberin lamella (SL, Fig. 1(a)–(c)). Fig. 1(c) shows a large, electron-dense deposit on the VP cell side. Plasmodesmal canals have been formed through the dense material (paired arrowheads) suggesting some degree of functionality. Plasmodesmata are associated with variably granular but electron-dense to loosely fibrillar deposits, which appear first to be associated with the neck regions of these plasmodesmata (Fig. 1(a) and (b)) and concentrated over and around the plasmodesmal orifices. Note that deposits are more noticeable on the MS side of the interface in these electron micrographs. The electron-dense embellishments become more noticeable and pronounced in the MS–VP interfaces opposite phloem parenchyma (Fig. 1(d)). Here both orifices are associated with electron-dense material in interfaces between VP cells and even more prominent at the interfaces between phloem parenchyma cells (Fig. 1(d)). Of interest to us, was that the deposits were always more enhanced on the MS cell side at junctions between the MS and VP cells, and on the inner face of VP to phloem parenchyma cell interfaces. In all cases where enhanced electron-dense extraplasmodesmal deposits occur, resolution of the desmotubule within the plasmodesma becomes impossible (Fig. 1(e)), suggesting that they have either become altered and masked, or that the deposition of fine electron-dense material within the cytoplasmic sleeve masks the desmotubule, rendering it irresolvable.

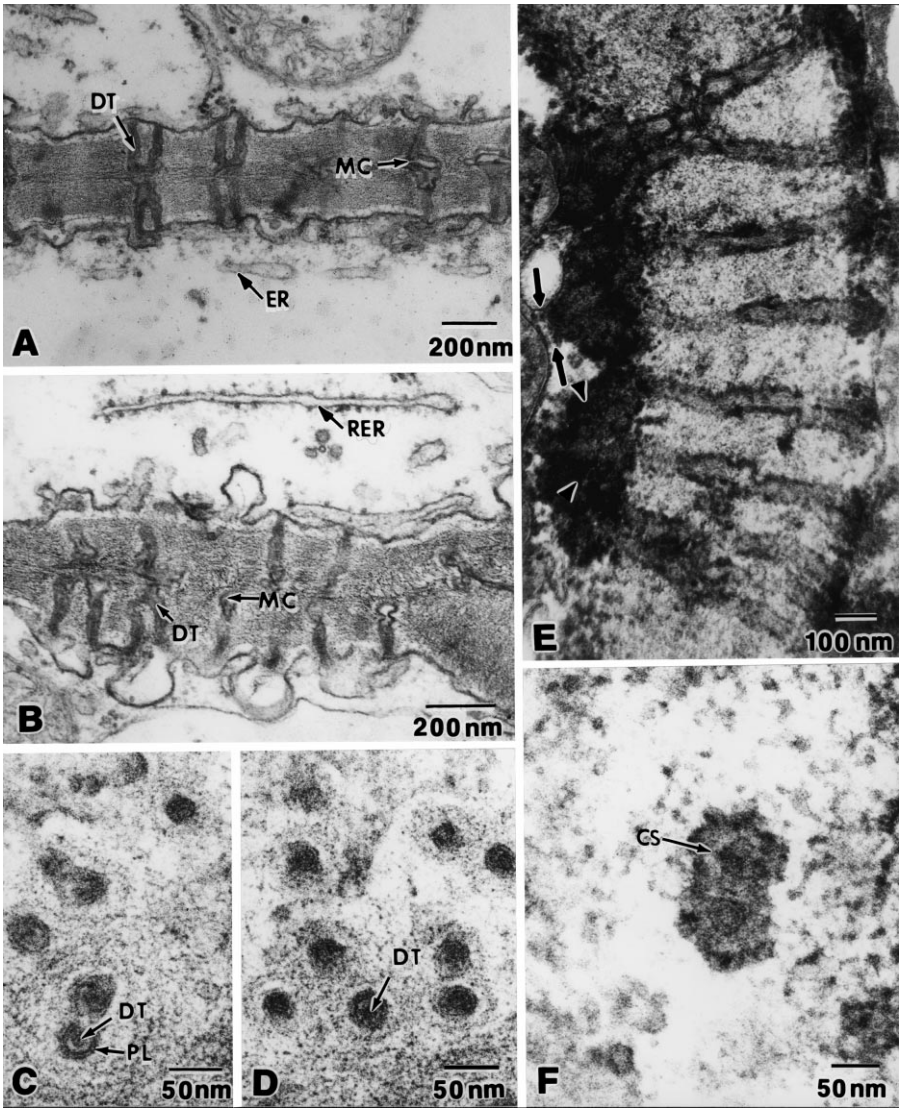

Fig. 2(A)–(F). Near-mature (226d) and young 40d post fruit set *Avocado* mesocarp tissue showing plasmodesmata in near-mature control ((A)–(D)) and ABA-injected (E) and young (F) tissue. Note the absence of electron-dense material at the plasmodesmal orifices in control, but marked asymmetric deposition of material at the neck region of plasmodesmata (darts point to granular deposits, (E)). Whilst plasmodesmata in control tissues are associated with clearly defined desmotubules (darts point to desmotubules) (C) and (D) are transections from the same plasmodesmal field, from control tissue. We recognize the outer and inner plasmalemma leaflets, surrounding the cytoplasmic sleeve, and, internal to this, the desmotubule with a central rod. Plasmodesmata in ABA-treated (F) 40-day-old tissues reveal no sign of a desmotubule. ER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; darts, neck region of plasmo
desmata covered by electron-dense material; MC, median cavity.

7. The effect of hormone treatment on plasmodesmatal structure

Near-mature (226-day-old) Avocado fruit has been examined in our laboratory, to determine the influence of exogenously applied ABA and iP, on the formation and ultrastructure of plasmodesmata in the mesocarp, as well as in the mesocarp–seed coat interface. Fig. 2(a)–(e) shows various aspects of the effect of unaltered ABA and IP concentration in control fruit on the formation of the plasmodesma. Fig. 2(a), (b) and (e) show plasmodesmata in longitudinal view and Fig. 2(c) and (d) in transverse view from 226-day-old mesocarp in control tissue. These plasmodesmata all apparently contain desmotubules, and a cytoplasmic sleeve is visible in most. Rough and smooth ER is closely associated with these plasmodesmata (ER & RER, Fig. 2(a) and (b)). All plasmodesmata are branched at the common middle lamella region between cells, and thus form median cavities (MC, Fig. 2(a)) at this junction.

ABA-injection 210d after full bloom (i.e. 16 days before fixation and sectioning) effected startling conformational changes in the mesocarp plasmodesmata. Fig. 2(e), (and at be differentially structured—less obvious to the right higher magnification in transverse section in the 40d tissue, (arrowheads) than to the left, where the electron-dense Fig. 2(f)) show examples of plasmodesmata where the neck matrix protrudes more deeply into the cell to the right. regions are associated with a protruding, electron-dense and Plasmodesmata are in close association with ER (arrows granular matrix, which we interpret as the plasmodesmal point to a junction of the neck region of a plasmodesma collar. In the mature tissue (Fig. 2(e)) the collar appears to with the ER). In the 40-day-old ABA-treated tissue (Fig. 2(f)) plasmodesmata are associated with electron-dense collar-like structures. Digital enhancement (Fig. 4(a) and (b)) of the plasmodesmata shown in Fig. 2(d) and (f), reveal more useful information of the substructure of the plasmodesmata themselves. The collar region (Fig. 4(a) and (b)) is for the most part, electron-lucent, and difficult to make out in normally stained material. The outer plasma-membrane leaflet (OPL) is granular, and contains very little electron-lucent area between itself and the inner plasmalemma leaflet (IPL), which is in turn attached to the outer wall of the desmotubule by fine spoke-like structures, (unlabelled arrows point to these) reminiscent of those seen in *T. triandra* plasmodesmata (Botha et al., 1993). Exogenously applied ABA thus has a marked effect on the structure of plasmodesma between differentiating mesocarp cells. The extra-plasmodesmal collar and neck regions are very electron-dense and particulate in transections of Avocado mesocarp (Fig. 2(e)). Whilst the inner and outer plasmalemma leaflets are visible here, there is no evidence of a central desmotubule in any of the plasmodesma in this transection. Digital enhancement (Fig. 4(b)) of the outer neck region clearly shows the granular, electron-dense and apparently six-sided extra-plasmodesmal collar complex, surrounding the outer (OPL) and inner (IPL) plasmamembrane complex. The cytoplasmic sleeve (CS) is visible internal to the plasmamembrane complex. Again, there is no desmotubule-like structure in this, or other transections of plasmodesmata cut in similar planes, either before or after digital enhancement. Interestingly, the collar appears attached to the plasmodesma with fine spoke-like structures, reminiscent of the plasmodesma shown in Fig. 1(c) in the work of Overall and Blackman (1996).

8. Resolution of the desmotubule

Examination of plasmodesmata in the pith parenchyma cell wall interfaces in *S. officinarum* L. reveals some interesting details. These high-resolution micrographs show that where no collar is evident (as in Fig. 3(a)) the desmotubule may be clearly resolved, central to the electron-lucent cytoplasmic sleeve. Even though slight (30 nm) plasmolysis has caused some extrusion (ectodesmal) formation, this does not seem to have affected or effected any conformational changes in the exterior regions, including the neck, and also not in the internal substructure of these plasmodesmata. The plasmodesmata in question thus appear to be ‘normal’ and could, we argue, like all others that contain therecognized substructures, be classified as potentially functional. In contrast, once evidence of electron-dense deposition around the collar is evident (Fig. 3(b)) the desmotubule in *Saccharum*, like other species examined in this review, seems either to be lost or to have undergone conformational changes leading to total electron-lucence. The loss of the desmotubule from such plasmodesmata is demonstrated in the glancing section through a pit field (Fig. 3(c), and at higher magnification in Fig. 3(d)), which contains more than 55 plasmodesmata. Note that the plasmodesmata at the edges of the field are associated with a granular electron-dense matrix (short arrows point to the granular matrix), clearly deposited on the outside of the plasmalemma leaflets, which conforms to the collar region.

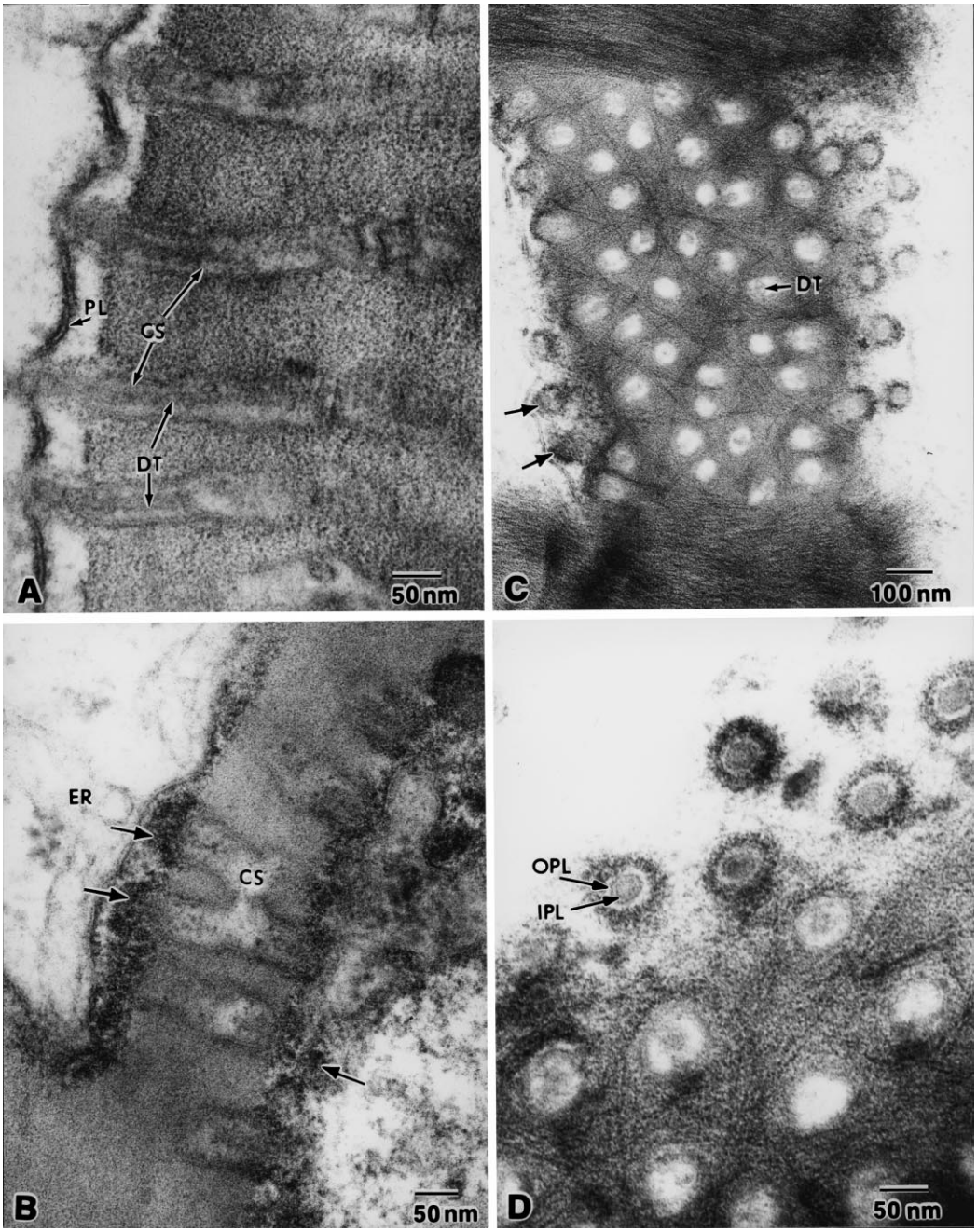

Fig. 3(A)–(D). Longitudinal ((A) and (B)) and transverse ((C) and (D)) of plasmodesmata within stalk storage parenchyma cells in mature internodal tissue in sugarcane (*S. officinarum* L.). Although there is a slight degree of plasmolysis evident in (A), plasmodesmata appear normal, and contain well-resolved plasmamembranes, desmotubules (DT) and unoccluded cytoplasmic sleeves (CS). (B) shows plasmodesmata from a similar field, but evidences the deposition of extra-plasmodesmal, electron-dense granular material, outside the neck region of these plasmodesmata. Desmotubules are either lacking or are entirely electron-lucent. (C) shows a glancing section through part of a large plasmodesmal field, between concomitant storage parenchyma cells. Central region of this field shows numerous plasmodesmata, but only a few evidence desmotubules. Those at the extreme left-and right-hand edges of this field, show granular deposits (arrows), which are clearly extra-plasmodesmal. The granular nature of the extra-plasmodesmal collar is evident at higher magnification (arrows, (D)). Note absence of the desmotubule in these collared plasmodesmata.

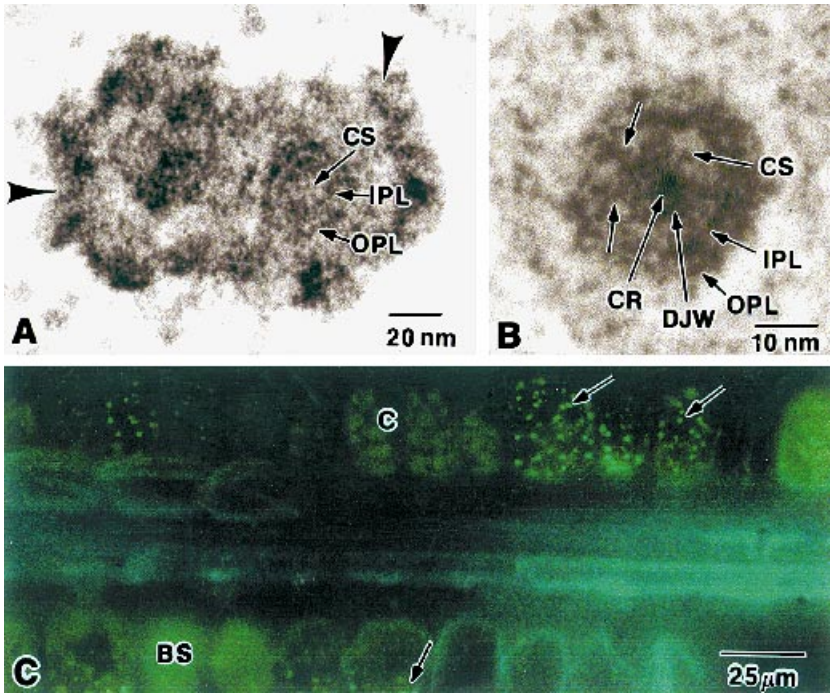

Fig. 4(A)–(C). Digitally enhanced images of plasmodesmata from 226-(Fig. 3(A)) and 40-day-old *Avocado* mesocarp (Fig. 3(B)). (A) shows that the collar surrounding these plasmodesmata (arrows) is partly electron-lucent and difficult to resolve, but spoke-like structures are visible, connecting it to the outer leaflet of the plasmamembrane (OPL), which seems to be composed of granular subunits. The inner plasmalemma leaflet is attached, in turn, to the outer wall of the desmotubule with fine, thread-like spokes. Digital enhancement of the plasmodesma shown in Fig. 2(D) (labelled with 'DT') is shown in (B). Note the electron-dense, globular collar, which is connected to the plasmodesma, via spokes (arrows). Internally, we recognize the outer (OPL) and inner (IPL) plasmalemma leaflets, a cytoplasmic sleeve (CS), and a desmotubule (DTW) with what appears to be a central rod (CR) at its centre. (C) is a digital fluorescence micrograph showing a longitudinal section through a small vein in the leaf blade of *S. africanus*. Note the large bundle-sheath cells (BS) surrounding this small vein in which chloroplasts (C) are clearly seen. Callose-encrusted plasmodesmal pits (arrows) appear as small dots.

9. Collar deposition

Clearly, the presence of electron-dense deposits in close association with the collar and neck region of plasmodesmata in grass leaf blade bundles, as well as between the mesocarp cells in Avocado fruit, suggests the existence of some kind of control mechanism at this point. This idea becomes more appealing when note is taken of the fact that the deposition always seems to be initiated in the plasmalemma, over and adjacent to the plasmodesmal neck regions. We have previously demonstrated that callose deposition is associated with plasmodesmata within the leaf blade vascular bundles of a number of grasses including *B. unioloides*, as well as at the mesocarp to mesocarp cell wall interfaces in young as well as mature Avocado fruit, when subjected to increased ABA concentration. The electron micrographs show unequivocally that this deposition is extra-plasmodesmal, and that it is associated with the collar-ER plasmamembrane complex at this junction. Complete closure by this extra-plasmodesmal material, suggests that either there is a loss of functionality at this stage, or that the deposition of the extra-plasmodesmal material is in some way linked to the loss of the desmotubule, and that this may be part of that process through which the transport pathway is either down-regulated or terminated. Hughes and Gunning (1980) suggested that callose deposition could result in closure of plasmodesmata— deposition of such substances clearly may prove to have a key role in controlling the physical aperture under normal circumstances. Fig. 4(c) is an example of callose-deposition associated with plasmodesmal fields in the radial walls of bundle-sheath cells within the loading veins in leaf blade bundles of *S. africanus*. Here callose-specific reactions are associated with a large number of plasmodesmal fields in this C_4 grass. Whilst the concept of a 'removable plug' (such as the beautiful example shown in Fig. 1(d), composed for the most part of ß-1,3-

glucan units, may not be a new one, it is exciting to speculate about the triggering mechanisms involved. We have shown that internal conformational changes in plasmodesmal ultrastructure (particularly in the electron-lucence or absence of desmotubules), the associated changes within the neck region and, external to this in the collar, are all co-incidental events, triggered in part by osmotic forces and in part by changes in the iP-ABA ratio. We believe that the collar is central to the regulation of cell to cell trafficking through plasmodesma, in the plants discussed in this paper.

The question that remains, is what is the function of the desmotubule? Clearly, the electron micrographs of unoccluded plasmodesmata show that this structure indeed exists and confirms Semenova and Tageeva's (1972) statement concerning the 'principle of construction'—this again seems to be true for the plasmodesmata investigated here. However, occlusion of the neck region by electron-dense material seems somehow to be related to either the loss of the desmotubule, or perhaps, through chemical alteration of its substructure, rendering it 'invisible' (electron-lucent) in conventionally fixed and stained tissues.

Whilst it is generally accepted that molecules of up to 1 kDa may traverse normal plasmodesmata with relative ease, large molecules have also been shown to achieve this. Clearly, the nature of plasmodesmata with respect to their role as a 'molecular sieve' is not yet precisely understood. Perhaps callose deposition holds the key? Regulation can thus be achieved through a change in osmotic potential, or due to an alteration of the endogenous hormone balance. Rapid pressure losses evidenced during wounding processes, result in the deposition of callose within 10–15 min of wounding (personal observation, this study). This collar-related material is interpreted as being composed of a mixture of proteinaceous and carbohydrate material, which we commonly interpret as callose. The callose deposition process therefore may hold the key to conformational substructural changes, and plasmodesmal gating ability. Plasmodesmal gating is most likely a complex interaction of events, in this case seemingly mediated by pressure (osmotic) changes as well as through subtle alteration of the iP-ABA ratios. What is remarkable is that we have seen that deposition of collar-related material effects substructural changes in all plasmodesmata studied in our laboratory thus far. Collar formation is strongly associated with lack of lucence of the cytoplasmic sleeve. It is therefore exciting to speculate and argue that the apparent dissolution or loss of the desmotubule must have significance—whilst the collar clearly encapsulates plasmodesmata at the plasmodesmal orifice, perhaps the appearance of the non-lucent cytoplasmic sleeve and the apparent loss of the desmotubule, must be significant, and, in some way, these must contribute to, result in, or assist in the down-regulation of cell to cell trafficking?

The question still remains intriguing—who is the gatekeeper?

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