Minireview

Regulation within the supracellular highway — plasmodesma are the key

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Plasmodesmatal connections are unique, highly dynamic intercellular structures that are lined by the plasmamembrane. They are believed to be a vital intercellular communication channel between living cells, linking numbers of living cells into interconnected, highly specialised cellular domains, thus enabling the plant to act as an integrated organism. Their evolution in the higher plant was inevitable. It is accepted that cell heterogeneity rather than cell divergence pressurised developing plant systems along a route that led to the formation of intercellular passages and connections. With time, these connections have evolved to allow some degree of regulation and traffic control. This paper explores some of the structure/function relationships in plasmodesmata. Attention is focused on the potential role of the neck region of these remarkable structures and discusses models which may explain the processes involved in regulating the movement of substances from cell to cell.

Introduction

Plasmodesma are unique intercellular cytoplasmic communication channels that traverse the walls between living plant cells. Within the limiting tubule formed by the plasmamembrane, which traverses the common wall between living cells, the endoplasmic reticulum joins the adjacent cells through the plasmodesma in a highly-modified ER structure, called the desmotubule. The desmotubule is separated from the inner leaflet of the plasmalemma by a space that is termed the cytoplasmic sleeve. It is the cytoplasmic sleeve which is believed to be involved in the direct cell-to-cell trafficking process. Plasmodesma have diverse functions and it seems that they are capable of trafficking a large variety of molecules and signalling structures, of widely differing molecular mass.

The movement of substances through the transport systems in plants has occupied the attention of plant scientists for nearly 150 years. Improvements in technologies such as microscopy and microinjection have allowed more refined observations on cell-to-cell communication processes, and have allowed us to focus on regulation and control mechanisms within the processes involved.

Given the strong interrelationship between plant structure and function, it is not surprising that many researchers remain focussed on the leaf and, in particular, the processes that govern uptake and transport of assimilates and related materials. These interests remain concentrated at the microstructural and physiological levels. Exciting advances made in the past decade in light and confocal microscope-based microinjection techniques have allowed these techniques to be applied in a number of study programmes (Schulz, 1999 and references cited) including real-time observation of injection of fluorescent probes into living cells. This new dimension has provided answers to some important, yet fundamental questions relating to intercellular trafficking (see Botha and Cross 1997, Botha and Cross 2000, Botha et al. 2000). Table 1 summarises some of the concepts associated with the structure and function of plasmodesma.

Trafficking and transport processes

We recognise that substances are able to move through adjacent cells, following a pathway that is entirely contained by living interconnected protoplasts, collectively termed the symplasm. In contrast, the apoplasm, defined as that region of the plant not bounded by the plasmamembrane, also is involved with energy-free short- and long-distance transport in plants. The existence of two transport systems means that division of labour is possible, but, at the same time, complicates the transport processes within large supracellular organisms. It cannot be disputed that the symplasmic pathway (in this context, the phloem) is highly dependent upon the apoplasmic pathway (the xylem) for a steady supply of water, without which little if any symplasmic transport through the complex higher plant supracellular organism would be possible. How are these two contrasting, but com-
plementary pathways and specifically, their contents kept separate from each other? Are there control points and if so how does the plant maintain a normal functional physiological state? Clearly, the interactivities between these systems will result in a balanced and finely tuned transport system that can be regulated at several points.

Cell to cell transport is a complexity of processes — each interacting or acting in solitude to produce a potential cascade controlling mechanism. Water loss and carbohydrate transfer must be interlinked within the whole plant; otherwise, an unorchestrated discordant system will exist. The loading of assimilates must therefore be complex, involving a series of cell-to-cell short distance transfer processes via plasmodesma. These are coupled with a freer apoplastic water transfer from the xylem to the cell walls and intercellular spaces of the mesophyll, as well as into the cytoplasm of the mesophyll and surrounding tissues. In grasses, part of the overall phloem loading controlling mechanism may be found at the junction between the bundle sheath (BS) and the mesophyll (MS). It is at the MS–BS interface where many species regulate outward water loss and inward carbohydrate uptake. Regulation of the outward loss of water can be undertaken by the suberin lamella (Botha and Evert 1986 and literature cited), which may force adoption of a more regulated symplasmic pathway through plasmodesma at this interface. Internal to the bundle sheath, three alternative phloem-loading pathways exist — along entirely symplasmic or apoplastic pathways, or via a mixed mode symplasmic-apoplastic pathway. Clearly, there are many plants where phloem loading occurs via a mixed pathway (references cited in Botha and Cross 1997).

Undoubtedly these important, yet diverse transport superhighways remain the subject of significant and directed research, and there are now obviously defined focus areas which are being actively pursued in a number of laboratories worldwide. The re-focussing of ideas has come about as a result of the aforementioned application of advances in light and electron microscopy, particularly those using fluorophores, and coupled with this, the rapid improvements in confocal microscopy which have contributed a great deal to the rekindling of ‘old’ interests and, significantly, in some new discoveries (Knoblauch and van Bel 1996).

Many of the new ideas associated with intercellular trafficking in plants are encompassed in the work published recently by van Bel, Günther and van Kesteren (1999), who state that there is widespread opinion that the evolution of the multicellular plant body implicitly requires that corridors of communication have to become established. The authors argue convincingly that this is necessary in order to regulate and, in part, possibly control intercellular trafficking of metabolites, as well as to co-ordinate and integrate the activities of the many hundreds of thousands of cells making up a typical average plant. It is now accepted dogma that cell heterogeneity rather than cell divergence would have pressurised the developing plant systems along a route that led to the formation of passages and connections. With time, these connections enabled some degree of regulation and traffic control. Cell division is recognised as being an early pressure for the formation of the sub-microscopic intercellular communication channels, termed plasmodesma, which are at the heart of cell to cell traffic control and regulation (Marchant 1976, Lucas et al. 1993, Bergmans et al. 1997, Jane and Chiang 1993, Eleftheriou 1993, Franceschi et al. 1994, Vasil’ev 1997). Plasmodesmal permeability is an active business (Pickard and Beachy 1999) in which messengers such as IP3 and Ca++ participate in the control of permeability.

Plasmodesma. meristems, domains and field boundaries

Convergent evolution amongst divergent taxa may well tell us something about plasmodesmal evolution. Cook and Graham (1999) suggest that there is a clear correlation between the ability to form plasmodesma and the ability to form meristematic tissue (van der Schoot and Rinne 1999a). Previously, Jian et al. (1997) conducted a series of experiments which demonstrated that there were changes in the ultrastructure, as well as in the subcellular localisation of Ca++ in poplar (Populus deltoides Bartr. ex Marsh) apical bud cells during the induction of dormancy by short-day (SD) photoperiods. These authors reported an increase in the number of starch granules, as well as a significant accumulation of vacuolar storage proteins in the apical bud cells. Coupled with this, they observed constriction and blockage of the plasmodesma. These results suggest that under the influence of SD photoperiods, there are alterations in subcellular Ca-2+ localisation, and changes in ultrastructure of apical bud cells during the development of dormancy. The constriction and blockage of plasmodesma it seems, may cause the cessation of symplastic transport, limit cellular communication and signal transduction between adjacent cells. This in turn, may lead to events associated with growth cessation and dormancy development in buds.

Thus, complex cellular interactions, which require the exchange of morphogenetic signals, underlie the process of morphogenesis within shoot apical meristems (Rinne and van der Schoot 1998, 1999b). It has been suggested that all apical meristem cells are interconnected by plasmodesma and thus could be subject to regulation, especially during the cell division cycle (Ehlers and Kollmann 1998). Rinne and van der Schoot (1998) were able to demonstrate that two concentric fields exist in the apical meristem of Betula pubescence, which effectively restricts the symplastic diffusion of small morphogens to the cells within the boundaries of their specific fields. These authors determined that transient connections could arise between the fields, thus allowing transfer of potential signalling molecules between the fields under these conditions, and potentiating the radial exchange of symplastically diffusible signalling molecules. These exciting results suggest that the apical meristem is a compartmentalised structure and that the presence of intercellular communication partitions could involve boundary interactions that can be demonstrated electrophysiologically.

Interestingly, tannic acid staining and immunolocalisation revealed that cell isolation was due to the activation of glucan synthase complexes (the enzyme responsible for the synthesis of callose) near or at plasmodesmal orifices. Callose plugs formed as a result, and these are associated
Plasmodesmal origins, classification and transport properties

| Origins and definitions |
|-------------------------|
| **Origins:** Primary plasmodesma are formed during cytokinesis and cell plate formation, from the fusing of Golgi-derived vesicles — thought to be a direct product of the endoplasmic reticulum. These plasmodesma are formed in transverse cell walls in pit fields or singly. Plasmodesmal frequencies within longitudinal walls decline as cell wall expansion is continued, due to sharing between daughter cells.

Plasmodesmsa that undergo modification after formation, or those which are formed at the scion during grafting, are called secondary plasmodesmsa. There is evidence that plant viruses may alter the structure of the primary plasmodesmsa, thus forming secondary plasmodesmsa. If they are formed de novo then they are true secondary plasmodesmsa.

Plasmodesmsa may become modified with deposition of additional cell wall material (associated with the primary wall). These plasmodesmsa are modified primary plasmodesmsa.

**Ultrastructural & molecular considerations**

Diameter across outer plasmalemma membrane leaflet varies from 70-120nm.

Plasmalemma leaflets have been demonstrated to form a helix of electron-dense and electron-lucent structures. These are interspersed with and connected to the central desmotubule (confusingly referred to as 'appressed ER' in some literature) by fine spoke-like structures. The 'space' available within the cytoplasmic annulus is from 2-14nm, sufficient to accommodate small molecules. Molecular size exclusion limits from 0.7 to 4.4kDa (latter by Kempers et al. 1993) for pore plasmodesmal units (PPU's).

Desmotubule concomitant with ER.

The neck region has been demonstrated to be able to be restricted or constricted under certain circumstances. Dilatation can be achieved by 2-deoxy-D-glucose, callose synthesis has been shown to be enhanced in the presence of 10-40mM Ca++, and to be retarded by the sequestration of Ca++ via EDTA.

Transport through the desmotubule would require conformational changes.

A gating structure would narrow down the plasmodesmal orifice. This would form a bottleneck and could act as a rate-controlling step (Schulz 1999). Neck diameters when throttled back usually 20-40nm (Overall et al. 1982, Botha et al. 1993, Schulz 1995).

Cytosolic gateway would be advantageous for symplasmic solutes, as no membrane translocation step will be involved.

with morphogenetic deactivation. Clearly, domain or symplasmic field formation, and the isolation of these fields or domains within the corpus, must lead to specific cellular differentiation steps. The involvement of callose in the activity of sphincters in a controlled deposition of callose supports the evidence presented recently by Botha and Cross (2000a), in which these authors concern themselves with the role of callose in regulating cell to cell communication and transport in plants. It follows that the mechanism controlling plasmodesmal narrowing or closure at the field boundaries could be set up as a gradient of effect in the initial cells (Rinne and van der Schoot 1998).

**Plasmodesma – regulated entry to the functional intercellular transport pathway?**

Plasmodesmsa have been described as highly specialised gatable trans-wall channels that interconnect contiguous cells and function in direct cytoplasm-to-cytoplasm intercellular transport (Epel 1994). Analysis of computer-enhanced

**Table 1:**

| Transport, functionality and regulation |
|----------------------------------------|
| Current flow. Many authors have shown that electrical potentials may be transferred from cell to cell via plasmodesmsa. Transfer from cell to cell is assumed to be via the cytoplasmic sleeve or via the desmotubule. |
| **Trafficking** — Small molecules (< 1kDa) have been shown to traffic easily along diffusional gradients. These molecules are assumed to be confined to the intrinsic spaces within the cytoplasmic sleeve. Transport is diffusional, or may involve pressure flow. |
| Macromolecules have been shown to be able to be trafficked under certain circumstances. Viral proteins and viral nucleic acids, small proteins and mRNA may traffic. |
| **Molecular size exclusion limits** |
| Evidence for plasmodesmal size exclusion limit (SEL) with raffinose series sugars - the 'polymer trap' model (Turgeon and Gowen 1990, Turgeon 1991, 1996). Groups of plants transport raffinose series of oligosaccharides. Low molecular mass sucrose and galactinol pass into intermediary cells and are synthesised into raffinose, maintaining the diffusion gradient. |
| **Physiological Control** |
| The neck region has been implicated in plasmodesmal gating (see Botha et al. 1999, and literature cited) which has been suggested to be Ca++ enhanced (Botha et al. 2000, and literature cited). Neck region and exterior of plasmodesma have been associated with fimbrin, and/or actin or myosin filaments. These filaments are suggested to play a role in plasmodesmal modulation (Overall 1999). |
| Regulation through chelating agents that may form soluble complexes with the divalent cation. Calcium has been implicated as a messenger for a number of plant responses. It may bind to calmodulin in the cytosol (Hepler and Wayne 1985). Phentolamines and calmidozolium can inhibit, by attaching to the Ca++ binding proteins. |
| Plasmodesmal permeability has been demonstrated to be an active process (Pickard and Beachy 1999, Tucker 1988). Control is thought to be via Ca++ and IP3 messengers. |
| Gating can effect non synchronous cell division in apical cells, and separation of cell domains or fields. |
digital electron micrographs has provided new information on plasmodesmal fine structure (/Botha et al. 1993 and literature cited). It is now becoming clear that plasmodesmas are dynamic quasi-organelles whose conductivity can be regulated by environmental and developmental signals. New findings suggest that signalling and controlling mechanisms exist which allow the plasmodesmal pore to dilate to allow macromolecular transport. Schulin (1999) states that it is possible to envisage a control mechanism, which involves the dilation of the plasmodesma. Many authors have studied the regulation, control and functional state of plasmodesmas (Botha and Cross 2000, Botha et al. 2000 and references cited) and others (Holdaway-Clarke 1996, 2000, and references cited) have been concerned with the controlling mechanisms. Transport through these microchannels and, particularly, the regulation of pore aperture size, must influence the transport capacity on a per plasmodesma, per cell and whole organ basis. Schulin (1999) envisages this gating process to be similar to that of a lock in which, through a selective state of 'openness' or 'closedness', the passage of variously sized molecules will either be allowed or denied. This gating model envisages plasmodesmas as the regulator of intercellular molecular size exclusion (SEL) limits. This is an important concept, as many researchers believe that conformational changes (such as those that virosp have to make) are necessary to allow unhindered passage of large proteins and virosp with or without pilot molecules or chaperones.

Olesen (1980) suggested that there was a structure akin to a sphencter associated with the neck region of plasmodesmas. This region has been linked to putative glucan synthase complexes at the neck region (Olesen 1980, Olesen and Robards 1990, Rinnie and van der Schoot 1998), which were thought to act as a crude form of sphencter. Clearly, plasmodesma play an important role in intercellular traffic control.

Wounding responses — the role of calcium and other messengers?

In 1995 Apitius and Lehmann showed that cells of the liverwort Rie1ia could be separated from the thallus in two different ways; firstly, by killing the surrounding cells very carefully with a glass needle, without touching the isolated cells, and secondly, by touching the cells with a scalpel while simultaneous killing the neighbouring cells. In the first case, the only wound stimulation observed was the destruction of plasmodesmas and a change in cell turgor. A thin layer of new wall material was seen to become attached to the old wall within 24 hours as a wound reaction, which, within six days of wounding produced a thicker new cellulosic wall. When the thallus was more severely damaged, callose was formed in the cell wall adjacent to the dead cells within 10 minutes of wounding. Most of the damaged cells on the other side of the callose plugs died within hours of the appearance of the relatively large callose deposits. Significantly Apitius and Lehmann (1995) demonstrated that callose formation was dependent upon the presence of calcium ions. The requirement of this secondary signal molecule implicates the Ca++ binding protein, calmodulin, which, in turn, involves the major enzyme under calmodulin control, namely Ca++-ATPase, which is concerned with pumping Ca++ from the cytosol into cell walls.

Gating, and the interaction between calcium and calmodulin — closing plasmodesmas without cellular perturbation or damage

Many workers accept that the process involved in gating is considered one that allows for a degree of selectivity. The rate-controlling step must be associated with the outer, or neck regions, of individual plasmodesmas, but can be extended to include the mid regions of plasmodesma traversing interfaces delimited by suberin lamellae. Here plasmodesmas are constricted by the suberin lamella such as that which exists at the bundle sheath-vascular parenchyma interface in grasses (Botha et al. 1993).

Tucker (1988) demonstrated that D-my0inositol 1,4 bisphosphate (IP2) and D-my0-inositol 1,4,5 trisphosphate (IP3) could play an important role in regulatory mechanisms in plants. Tucker (1988) co-injected IP2 with buffered 5(6) carboxyfluorescein. Tucker (1988) reports that IP3 is hydrolysed to inositol 1,4,5 trisphosphate and diacylglycerol (IP3-DG) by the enzyme phosphodiesterase. The IP3-DG second messenger system (Berridge and Irvine 1984, Tucker 1988) seems to occur in many plants. Significantly, IP3-DG stimulates a release of calcium, most likely from the ER. Diacylglycerol has also been shown to elicit a response from protein kinase C. According to Tucker (1988) protein phosphorylation by these two kinases, evokes a cellular response. The lack of CF transport between cells and other evidence such as inhibition of cytoplasmic streaming (Allen 1987) and the stimulation of a Ca++ release from vacuolar membrane vesicles (Shumaker and Sze 1987) are strong evidence for an elicited cellular response from IP2 and IP3.

Yahalom et al. (1998) recently suggested that plasmodesmal conductivity may also be regulated by a phosphorylation mechanism. In a maize mesophyll cell wall fraction, a Ca++-dependent protein kinase (CDPK) is present that phosphorylates approximately 8 of 20 wall-associated proteins. The kinase is membrane-associated and is not easily extracted using routine methodologies. Two polypeptides in the cell wall fraction, with apparent molecular masses of 51 and 56kD, cross-react with an Arabidopsis CDPK anti-serum and undergo in situ Ca++-dependent autophosphorylation on nitrocellulose. These authors demonstrated that the molecular masses of the CDPKs extracted from the cell wall fraction are different from those extracted from the cell membrane fraction, suggesting that wall-associated CDPK is unique to the cell wall fraction. Using immunofluorescence microscopy, Yahalom et al. (1998) were able to localize CDPK to discrete punctate loci in isolated cell wall material. Isolated plasmodesmas challenged with CDPK anti-serum show a pattern of cross-reactivity similar to the cell wall fraction. This exciting data is evidence for the cell wall-associated CDPK being a putative plasmodesmal-associated membrane protein, which may be involved in regulating plasmodesmal conductivity. The question that remains unanswered is where exactly these CDPK molecules are located.
Is it at the neck region or is it in the plasmalemma cell wall complex, surrounding the neck region?

Lew (1994) used a voltage clamp to measure the voltage dependence of cell-to-cell coupling via plasmodesma between root hairs of Arabidopsis thaliana (L.) Heynh.). In addition, Lew (1994) used iontophoresis to introduce a variety of ions (Ca++, inositol-tris phosphate, Li+, K+, Mg++, ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), H+, and OH-) to examine whether they regulate cell-to-cell coupling. Electrical coupling showed high variability in this single cell type at the same developmental stage; the coupling ratio ranged from near 0% to about 90% (mean of 32%).

The coupling ratio appeared to be voltage-independent for intracellular transplasmodesmatal voltage gradients of -163 to 212mV. While Ca++ stimulated closure of the plasmodesmal connections (at concentrations higher than those causing cessation of cytoplasmatic streaming), inositol-trisphosphate and lithium were shown to be without effect. Apparently, inositol-trisphosphate may not cause increased concentrations of cytosolic Ca++ to appear in root hairs. Alkalisation by OH- iontophoresis caused a modest decline in cell-to-cell coupling, as did acidification by H+ iontophoresis, to an extent causing the cell to become flaccid. Interestingly, increases in cytosolic K+, Mg++, and the calcium chelator, BAPTA introduced by iontophoresis, had no effect on cell-to-cell coupling. The regulation (and lack thereof) reported here for plant plasmodesma is quite similar to that of gap junctions.

It should be clear from the above discussion that the literature unequivocally supports the notion that plasmodesmal closure is triggered by an increase in cytosolic calcium (Erwee and Goodwin 1993, Baron Epel et al. 1988, Tucker 1988, 1990, Tucker and Boss 1996, Holdaway-Clarke, Walker, Hepler and Overall 2000). It becomes tempting to suggest that cytosolic calcium concentration, its various interactions with calmodulin and Ca++ATP-ase, and related to this calmodulin activity, (Harper et al. 1996, Botha et al. 2000 and references cited) and IP3, must have an important role in selective gating capacity of this important cytoplasmic gateway.

Whilst there are many papers that deal with the interaction of calcium with calmodulin in the literature, there are less than 30 that deal with the interrelationship of plasmodesma with calcium in plant species, and only two relate cytosolic calcium concentration to calmodulin and plasmodesmal gating directly (Blackman et al. 1999, Harper et al. 1996). Blackman et al. (1999) demonstrated that antibodies against calmodulin, the ubiquitous calcium-binding contractile protein, recognised a 17kDa protein in extracts of onion root tips and cauliflower florets. Anti-centrin antibodies have been localised to the developing cell plate of onion and cauliflower root tip cells using immunofluorescence microscopy. In cauliflower florets, these antibodies localised to the walls in a punctate manner, consistent with the distribution of plasmodesma, as shown by colocalisation with callose. Anti-centrin antibodies were also localised to plasmodesma of onion root tips and cauliflower florets with immunogold electron microscopy, where this label was shown to be concentrated around the neck regions of the plasmodesma. In contrast, an antibody against calmodulin did not label plasmodesma. Blackman et al. (1999) and Harper et al. (1996) suggested that centrin is a component of calcium-sensitive contractile nanofilaments in the neck region of plasmodesma and somehow facilitates calcium-induced regulation of intercellular transport.

Clearly, the lack of plasmodesmal association with the antibody to calmodulin is strong evidence that the Ca++ binding protein is not directly associated with the plasmodesma, and may be spatially separated from them. Visualisation of immunofluorescence labelling of Arabidopsis roots has previously been limited to single cell layers. Using confocal microscopy, Harper et al. (1996) were able to visualise the whole root, after antibody penetration into several cell layers. Confocal optical sectioning allowed visualisation of the cellular arrangement of microtubules, callose, calmodulin and a phosphoprotein epitope.

Subsequently, Holdaway-Clarke et al. (2000) conducted a series of elegant experiments which persuasively showed that increased cytosolic Ca++ levels resulted in transient plasmodesmal closure, and that Ca++ cyt was capable of rapid change, which is in line with earlier experiments which demonstrated that membrane depolarisation was induced by cold treatment in corn coleoptiles (Nelles and Laske 1982) and in cucumber seedlings (Minorsky and Spanswick 1989). Cold shock induces an increase in extracellular Ca++ influx into maize roots (Zocchi and Hanson 1982) as well as in alfalfa (Monroy and Dhindsa 1995). K+ (Zocchi and Hanson 1982) and Ca++ influx across the plasmalemma has been shown to be a major component of the cold shock response in tobacco as well as in Arabidopsis (see Knight et al. 1996). Calcium has been identified as a major factor in the regulation of intercellular communication via plasmodesma (Beebe and Turgeon 1991). Holdaway-Clarke et al. (2000) state that although cold produced on average, a two-fold increase in cytosolic Ca++ (expressed as [Ca++] cyt) in corn suspension culture cells, Ca++ concentration could be influenced by leakage across the plasmamembrane. It is interesting to speculate that here perhaps is a reversal role for the recognised role of Ca++ ATP-ase, actively pumping Ca++ into the cytosol. Interestingly, Holdaway-Clarke et al. (2000) also suggest that increases in [Ca++] cyt at either physiological or non-physiological levels, can influence opening or closing of the plasmodesma in the corn suspension cultures.

Actin, myosin and centrin have been localised to higher plant plasmodesma (see White et al. 1994, Radford and White 1998, Blackman et al. 1999). The actomyosin interaction and contraction of centrin nanofilaments have been shown to be Ca++ dependent (Martindale and Salisbury 1990). However, Holdaway-Clarke et al. (2000) are quite convinced that the fast response times recorded during their experiments (recorded as changes in membrane potential (PD) to injected Ca++) means that callose is not involved in effecting closure of plasmodesma, as the process is, according to these authors, one which may take hours rather than seconds (Drake et al. 1978). This observation contrasts strongly with a supportive statement by Overall (1998).
act as a crude sphincter mechanism and that callose was deposited very rapidly but takes hours to disappear.

Van Bel et al. (1999) suggested that there could be several forms of gating involved, and that plasmodesmal closure may be a strongly energetic process and that it would be acceptable to regard the sealing of plasmodesma as a potential wounding response reaction, which is effected to prevent the loss of cytoplasmic material.

In addition to callose,pectins seem to be implicated in some way to callose deposition at plasmodesma, having been involved in plasmodesma in tomato pericarp cells (Cassero and Knox 1995). Low esterified pectin has been located around the plasmodesma in ripening apples but is apparently not involved in calcium cross-bridging. Pectins could result in the prevention of normal cell wall separation in regions containing plasmodesma by protecting the surrounding wall surrounding the plasmodesma from hydrolytic enzyme breakdown during ripening (Roy et al. 1997). Similar wall structures have been observed in barley aleurone plasmodesma (Taiz and Jones 1973). However, Overall (1999) states that it remains to be seen just how the pectin sleeve either modifies, or plays a role in altering the functional dimensions of plasmodesma.

So we have a number of intriguing possibilities associated with plasmodesmal function. Unequivocal evidence exists for a central role for the secondary messenger Ca^{2+} and coupled to this, calmodulin. Many supportive role players like actin, centrin, fimbrin IP3 and myosin clearly are...
involved. Time will tell which are the important ones and which are not.

**Plasmodesma in action**

We have chosen to illustrate this review with one example, *Tradescantia pallida* (Rose) DR Hunt, which we are currently using to model the effects of changing the plasmodesmatal microenvironment (e.g., changes in the [Ca$^{2+}$]cyt; osmolarity, effect of adding chelating agents and studying the resultant intercellular transport), using electrophysiological and reverse current microiontophoresis. Our work also involves examination of the structural characteristics and appearance of plasmodesma.

Figures 1-4 show aspects of the functionality (Figures 1-2) and ultrastructure of *Tradescantia pallida* staminal hair cells, and their associated plasmodesmal fields. Figure 1 shows part of an attached basal cell and the lowermost staminal hair filament cells, stained in 0.05% (w/v) aniline blue in water, and viewed under blue light, using an epifluorescence microscope. The bright fluorescence band is associated with large plasmodesmal fields. At higher magnification (Figure 2) the callose reaction is localised as punctate spots, each of which is an individual aniline blue-induced callose region. When viewed with the TEM (Figures 3-4), these plasmodesma show a variety of form — some are simple and unbranched and others are branched. The cell to the left in Figure 4 (and at higher magnification, below, in Figure 3) shows a relatively unperturbed state compared with the cell to the right in Figure 4 (and at higher magnification, above in Figure 3) where large aggregations of ER are interspersed with membranous and fibrillar material. Some plasmodesma (arrows, Figure 3) are associated with electron-dense material, which seems to form a collar surrounding the plasmodesma.

**Conclusions**

Plasmodesma clearly are important structures that we now understand to be involved in many more functions than simply providing the channels for intercellular symplasmic trafficking of sugars and other energy sources. Clearly, they are involved in intercellular communication, and are implicated in the communication of incompletely understood signalling agents. Whilst some would argue that we already know enough about the role of plasmodesma in cellular regulation and intercellular trafficking processes, it is clear to us that not all the questions have been answered yet. There remain many unanswered questions pertaining to the functional state of plasmodesma, and how the functional state of plasmodesma is maintained. Whilst there is evidence for a role for Ca$^{2+}$cyt and its interaction with calmodulin, there is no direct evidence of the actual plasmodesmal gating stimulus. If it is Ca$^{2+}$ then what levels of cytosolic calcium [Ca$^{2+}$]cyt are required? How do Ca$^{2+}$ amplification second messengers affect their roles in callose synthesis? Where are the effectors or locators for CDPK's situated? What is the role of changing osmotic potential?

We have chosen to highlight a few questions and have reviewed only part of the literature, but, we believe, these and other related questions can lead to significant answers to what could otherwise be described as a purely academic study. Plasmodesmal studies can provide more information about the as yet, poorly understood phenomenon of zone regulation which was highlighted recently by van der Schoot and Rinne (1999a,b) who demonstrated that plasmodesma can create regulating zones within the shoot apex, for example. Plasmodesmal gateability has become an area of great interest, as it is the relative degree of openness or closedness that governs intercellular trafficking. It is the trafficked signals which allow the formation of domains within the shoot and root apical meristems and which control synchronous and asynchronous cell division within these complex regions of the vegetative plant. Whilst is clear that these minute but complex structures are under the control of many interactions, such as the secondary messengers Ca$^{2+}$ and IP3, the elicited responses result in gating, regulation and control of intercellular transport. Clearly, the complexity of form, and the functional parameters associated with this form, and the interactions of these components, must be studied to provide answers about regulation and functionality. Until the questions listed above are answered, plasmodesma will remain incompletely understood structures. Perhaps when we understand these structures, we will be able to apply some of the answers in a more practical way. Pickard and Beachy (1999) so succinctly summed this up by stating that by understanding plasmodesmal form and function, we may get new insights into the control of viral movement and secondly, if we can control the flow of nitrogen-rich compounds, we could effect a significant increase in yield.

Clearly, the future of plasmodesmal research will continue to demand microscopy techniques, coupled with microinjection and, specifically, investigation of in situ experiments showing the effects of additions to, or manipulations of, the cytosol. These can only be accomplished and visualised with real-time digital fluorescence and confocal microscopy. Understanding plasmodesmal function remains the key.

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