Although it has long been known that plasmodesmata establish cytoplasmic continuity between most cells within the body of the plant, it is only recently that these special structures have been viewed as dynamic intercellular organelles (Lucas and Wolf, Trends in Cell Biology 3: 308–315, 1993) involved in the transport of macromolecules. Ultrastructural studies have provided important information on the formation of plasmodesmata during cytokinesis (primary plasmodesmata) and as post-cytokinetic events, where the new cytoplasmic bridges are inserted within the existing wall (secondary plasmodesmata). Modifications to plasmodesmal frequency, and presumably composition, probably reflect developmental and physiological requirements for symplasmic continuity/communication.

Pioneering studies on viral movement proteins provided the first direct experimental evidence that proteins and protein-nucleic acid complexes could traffic cell to cell, via plasmodesmata. This knowledge paved the way for the identification and characterization of endogenous proteins that also possess a similar capacity for cell-to-cell transport. Such proteins range from plant transcription factors, involved in orchestration of plant development, to proteins present in the phloem sap of angiosperms which are probably involved in maintenance of the enucleate sieve tube system. Information from viral infection studies and plant development support the concept that plasmodesmata play an essential role in the formation of developmental and physiological domains in which regulated trafficking of macromolecules establishes a non-cell-autonomous control network. Finally, the role of plasmodesmal trafficking of informational molecules is discussed in terms of providing a novel means for controlling cell differentiation. This concept is illustrated using current knowledge on root hair and trichome pattern formation.

**Key words:** Plasmodesmata, macromolecular trafficking, developmental patterning, root hairs, trichomes.

### INTRODUCTION

Plasmodesmata are unique intercellular transport complexes that connect neighbouring cells (Robards and Lucas, 1990; Lucas, Ding and Van Der Schoot, 1993; Lucas and Wolf, 1993; Lucas, 1995). Extensive ultrastructural studies have provided important information on the basic structure of the plasmodesma (Robards and Lucas, 1990; Ding, Turgeon and Parthasarathy, 1992b; Botha, Hartley and Cross, 1993; Overall and Blackman, 1996). It is now evident that, in contrast to other multicellular organisms, the ability to insert plasmodesmata across the cellulosic wall has allowed higher plants to develop an integrated cytoplasmic and endomembrane (endoplasmic reticulum-nuclear envelope) continuum that extends throughout the body of the plant. Interestingly, exactly 20 years ago, Esau (1977) wrote, ‘The relation of the structural features to the function of plasmodesmata as connections between protoplasts is not yet fully understood’.

As we will demonstrate in this review, plant biologists are currently providing the long-awaited details concerning the functional roles played by plasmodesmata.

### PLASMODESMAL BIOGENESIS

**Keeping up with cell division, expansion and differentiation**

Analyses of plasmodesmal distribution within such tissues as source and sink leaves provided important information on the symplasmic pathway available for the diffusion of photosynthates (Van Bel and Oparka, 1995, Evert, Russin and Botha, 1996), but yielded little insight into the underlying processes involved in the formation and regulation of such patterns. Certainly it has long been known that plasmodesmata are formed during the process of cytokinesis. The picture that has emerged is that this is a highly orchestrated process in which strands of the endoplasmic reticulum (ER) are first positioned across the plane of the advancing cell plate. Golgi vesicles that are being delivered to this region fuse in the division plane to provide both the initial matrix of the primary cell wall and establish the plasma membrane. Vesicle fusion in the vicinity of the ER provides the foundation for the establishment of primary plasmodesmata (Fig. 1). As the width of the new wall increases, individual plasmodesmata develop as simple, cylindrical-like structures: the central region is occupied by an appressed form of the ER (which is continuous with the fenestrated form of the ER within the cytoplasm of the daughter cells) and a cytoplasmic annulus forms a bridge between the daughter cells (Fig. 1; Hepler, 1982).
Plasmodesmata can also be established, de novo, across existing cell walls. As such plasmodesmata are post-cytokinetic in origin, they are normally referred to as secondary plasmodesmata (Ding and Lucas, 1996). The underlying mechanism(s) responsible for the establishment of these additional cytoplasmic bridges appears to depend upon cell position and/or developmental programme. In young, developing tissues, secondary plasmodesmata appear to be formed by a process in which the matrix of the wall is modified to allow a thinning to occur (Fig. 1). In concert with this thinning, ER cisternae begin to aggregate in this area and, simultaneously, Golgi-derived vesicles begin to fuse around the ER to deliver new plasma membrane and deposit the supporting wall material. A loosening of the original wall matrix then allows the formation of continuous cytoplasmic connections (Monzer, 1991). Secondary plas-
modesmata that form by this mechanism are often irregular and complex in morphology (Fig. 1).

Formation of secondary plasmodesmata across the periclinal walls located between the outer layers of the plant meristem(s) plays a vital role in plant development. These special intercellular transport complexes provide an essential pathway for the exchange of information molecules involved in non-cell-autonomous orchestration of developmental cascades (Lucas, 1995; Lucas et al., 1995). In addition, as a consequence of elongation growth, site-specific insertion of secondary plasmodesmata also appears necessary to maintain an adequate level of symplasmic continuity within tissues as they advance towards physiological competence (Robards and Lucas, 1990). An excellent example of such a role for secondary plasmodesmal formation was recently provided by Volk, Turgeon and Beebe (1996). Here the formation of secondary plasmodesmata was correlated with the sink-to-source transition in the minor veins of squash leaves. Furthermore, this form of plasmodesmata generally represents a significant proportion of the total number of plasmodesmata that interconnect most of the cells within the body of the plant (Ding et al., 1992a; Ding and Lucas, 1996). Thus, the role of secondary plasmodesmata in developmental and physiological processes cannot be overstated.

A complex symplasmic continuum: primary, modified primary and secondary plasmodesmata

It is interesting that the formation of primary and secondary plasmodesmata had often been considered to represent fundamentally different processes. The concept that plasmodesmata can undergo quite extensive structural modifications during the process of tissue differentiation is fully supported by ultrastructural studies (Ding et al., 1992a, 1993; Lucas et al., 1993). However, until recently, these changes were thought primarily to reflect modifications to secondary plasmodesmata (Ding and Lucas, 1996). An elegant study by Ehlers and Kollmann (1996) has now demonstrated that this viewpoint is in need of revision. Their studies on primary plasmodesmal formation in cells of Solanum nigrum revealed a dynamic aspect associated with these structures. Primary plasmodesmata were shown to undergo branching and this process was initiated from a region within the appressed ER which subsequently became bifurcated. Aggregation of vesicles at the plasmodesmal orifice, with the subsequent separation of plasmodesmal branches, gave rise to branched primary plasmodesmata (Fig. 1).

Collectively, the ultrastructural studies performed on primary and secondary plasmodesmata suggest that a common mechanism may well be involved in the formation of these cytoplasmic bridges. This mechanism probably involves the following steps: (a) strands of ER co-assemble with Golgi-derived vesicles; (b) the membrane from these vesicles forms the outer plasma membrane lining of the plasmodesmal channels; (c) wall material, delivered from within the lumen of these vesicles, reinforces the newly established plasmodesmal strands; (d) the arrangement of ER and membranes is precisely organized and always leads to a cytoplasmic annulus of the same dimension; (e) plasmodesmata appear to undergo rapid assembly into a supramolecular structure, as intermediate stages in their formation have rarely been observed. Unfortunately, little information is currently available on the cellular mechanisms involved in vesicle targeting, membrane fusion and protein integration/assembly to form the plasmodesmal supramolecular complex. This area clearly warrants immediate attention.

Any analysis of plasmodesmal dynamics during plant differentiation would be incomplete without a discussion of the processes involved in the sealing or removal/degradation of these cytoplasmic bridges. Analysis of plasmodesmal frequencies, within specific cells, indicates that plant cells have the capacity to regulate the degree to which individual cells are symplasmically connected to neighbouring cells. Thus, the plant must have the ability to remove, or seal off (temporarily or permanently), existing plasmodesmata. Insight into this process was recently provided by studies performed on secondary plasmodesmal formation in regenerating protoplasts. Immunocytochemical studies revealed the presence of high levels of ubiquitin in aborted plasmodesma that failed to establish secondary contacts between neighbouring cells (Ehlers, Schulz and Kollmann, 1996). Ubiquitination is well known as a signal for protein degradation and, thus, its accumulation within these half-plasmodesma may well reflect a central role for this enzyme in the targeted removal and subsequent elimination of specific cytoplasmic bridges between cells (Fig. 1).

A further important example of site-specific control over plasmodesmal structure/function can be found in differentiating xylem tissues. Immature xylem vessels and tracheids are connected to adjacent parenchyma cells by pits that contain a high density of plasmodesmata. As these water-conducting cells enter the final stage of programmed cell death, plasmodesmata within the pits become sealed (Lachaud and Maurousset, 1996). This process is carefully orchestrated, and is accomplished by the apposition of new wall material across the orifice at both ends of the plasmodesma (Fig. 1). At present nothing is known about the exchange of information between these special cells (Savidge, 1996), nor of the underlying molecular events that orchestrate this truncation of cytoplasmic continuity.

Genetic and molecular information on this and related processes may be forthcoming from studies on the sucrose export deficient 1 (sxd1) mutant of maize (Russin et al., 1996). In this mutant, plasmodesmata located within the bundle sheath-vascular parenchyma cell wall become blocked by wall apposition, thereby inhibiting sucrose movement into the loading region within these minor veins. Cloning of sxd1 should allow a dissection of the molecular events underlying the development of this intriguing mutant phenotype. As a similar process appears to be involved in the sealing of plasmodesmata between guard cells and epidermal cells it may well be that both processes share common elements. Obviously, identification of the genes involved in the truncation of plasmodesmata would potentiate a wide range of physiological and developmental studies.
Identification of plasmodesmal constituents

The isolation and biochemical characterization of the molecular constituents of plasmodesmata remains a major challenge. A number of workers have attempted to purify plasmodesmal components from cell wall preparations (Monzer and Kloth, 1991; Yahalom et al., 1991; Kotlízky et al., 1992; Turner, Wells and Roberts, 1994; Epel et al., 1996b). Unfortunately, to our knowledge no published report has provided unequivocal evidence for the localization of a cloned plasmodesmal protein within the cytoplasmic boundaries of the plasmodesma. The application of novel preparative techniques may yield the long-awaited breakthrough in this area. For example, as chimeric viral movement protein:GFP constructs have been shown to accumulate in mesophyll plasmodesmata during viral infection (Heinlein et al., 1995; Epel et al., 1996a), these fluorescently-tagged proteins may be of use in future attempts to isolate plasmodesmal components that interact with viral movement proteins (MPs). Our laboratory is also working on the development of an in vitro assay that will utilize the binding of endogenous transcription factors and/or viral MPs to plasmodesmal components in an attempt to identify binding motifs. Finally, the inhibitory effects of the herbicide, 2,6-dichlorobenzonitrile, on cellulose biosynthesis may also prove useful. Under the influence of this herbicide, newly formed division walls exist within cells as incomplete sheets that contain disoriented plasmodesmata (Vaughn et al., 1996). Thus, cell fractionation procedures could well be developed that would allow the isolation of these sheets of wall material which could then serve as a source for highly purified plasmodesmal proteins.

PLASMODESMATA TRAFFIC MACROMOLECULES

Viral MPs and plant proteins traffic through plasmodesmata

It has long been known that plant viruses spread throughout the plant by passing through plasmodesmata (Robards and Lucas, 1990). It is now clear that viruses encode proteins that mediate in the cell-to-cell trafficking of the infectious form of the virus. Based on microinjection studies, it has now been established that certain plant viruses, such as the tobacco mosaic virus (TMV), red clover necrotic mosaic virus and cucumber mosaic virus use a dedicated protein, termed the movement protein (MP) to traffic their infectious viral RNA through mesophyll plasmodesmata (Fujiwara et al., 1993; Waigmann et al., 1994; Ding et al., 1995). An extensive analysis of this viral MP literature can be found in recent reviews by Lucas and Gilbertson (1994), Gilbertson and Lucas (1996) and Ghoshroy et al. (1997). The salient features from this work are that viral MPs can interact with certain plasmodesmata, located in various plant tissues, to mediate their own cell-to-cell transport. In addition, these MPs have the ability to bind, in a non sequence-specific manner, to vRNA/DNA and presumably it is this MP-vRNA complex that moves through receptive plasmodesmata. During this trafficking process, MPs induce an increase in the size exclusion limit (SEL) of the plasmodesma. Normally, plasmodesmata permit the free diffusion of small molecules in the range of 850 Da, but this value increases to more than 10 kDa during the trafficking of macromolecules. Here it should be noted that in microinjection studies performed on trichomes, the TMV MP was reported to move along the length of the trichome without inducing a detectable increase in SEL (Waigmann and Zambryski, 1995). This finding is consistent with the notion that specific plasmodesmata can mediate in tissue-specific processes. Although much has been learned in terms of the way in which MPs interact with plasmodesmata, there is still a great deal yet to be elucidated, especially with respect to both the molecular constituents of the plasmodesmata involved in this transport as well as the nature of the cellular factors that restrict viral movement to particular domains within the plant (Wang, Gilbertson and Lucas, 1996).

The finding that viral MPs could move through plasmodesmata raised the important question as to whether endogenous proteins were also able to move between cells via plasmodesmata. That this was indeed the case was established by experiments performed with the maize KNOTTED1 (KN1) homeobox protein. Expression of KN1 within the maize meristem leads to the presence of KN1 mRNA within L2 cells, whereas KN1 is detected in almost all cells including the L1 layer (Sinha, Williams and Hake, 1993; Jackson, Veit and Hake, 1994; Lucas et al., 1995), suggesting that KN1 can move through the secondary plasmodesmata that interconnect the L1 and L2 layers of the meristem. Direct experimental proof that KN1 has the capacity to interact with and traffic through plasmodesmata was gained through microinjection studies. Lucas et al. (1995) showed that KN1 behaves in a manner almost identical to viral MPs, in that it increased plasmodesmal SEL (to approx. 40 kDa), mediated its own cell-to-cell movement and potentiated the trafficking of its own mRNA but not viral RNA; i.e. with KN1, mRNA trafficking was found to be sequence specific.

Additional evidence that endogenous proteins are transported cell-to-cell, via plasmodesmata, has been gained through studies on the phloem. In angiosperms, the enucleate sieve tube members are thought to be maintained by proteins synthesized in neighbouring companion cells and then transported through plasmodesmata into the functional sieve tube system (Lucas and Wolf, 1993; Lucas, 1995). Direct evidence has now been obtained that proteins within the phloem sap also have the capacity to increase plasmodesmal SEL and mediate their own cell-to-cell transport (Ishiwatari et al., 1996).

Recent studies performed using tobacco, potato and tomato demonstrated that the mRNA for the phloem specific sucrose transporter1 (SUT1) could be detected within the plasmodesmata connecting the companion cells to the functional sieve tube system (Kühn et al., 1997). This critical finding suggests that proteins within the companion cell can interact with SUT1 mRNA to allow it to gain entry into the sieve tube member where this integral membrane protein may be synthesized on residual ribosomes attached to the SER. In any event, these studies on phloem proteins and KN1 have provided insight into the extent to which the
plant is capable of utilizing plasmodesmata to traffic macromolecules between cells to regulate developmental and physiological processes.

**Plasmodesmal trafficking and the plant cytoskeleton**

Experiments with a TMV MP::green fluorescent protein (GFP) construct provided interesting results consistent with the hypothesis that this MP interacts with either microfilaments (actin) or microtubules during the process of viral infection. Plants or protoplasts inoculated with infectious TMV MP::GFP clones were found to accumulate MP::GFP within regions of the cytoplasm where tubulin and actin were also co-localized. These findings suggested that the delivery of proteins and protein-RNA complexes to plasmodesmata may utilize the plant cytoskeleton (Heinlein et al., 1996). The challenge remains to identify the molecular components that function in linking the cytoskeleton to this intercellular macromolecular trafficking pathway.

**Developmental domains established by protein trafficking through plasmodesmata**

The concept of non-cell-autonomous differentiation, during organogenesis, seems to hold for all multicellular organisms. At the molecular level, the intra- and intercellular mechanisms involved in controlling differentiation share striking homologies between animals and plants. Support for this viewpoint comes from the recent finding that, in maize, CRINKLY4, a homologue of the membrane-bound tumor necrosis factor receptor, appears to be involved in epidermal differentiation (Becraft, Stinard and McCarty, 1996). However, plants are clearly different from animals in one important aspect: they have cell walls and plasmodesmata through which regulators of differentiation can be exchanged. Obviously, this unique plant pathway for information exchange must have evolved highly selective mechanisms to regulate the trafficking of such proteins, otherwise the formation of developmentally different domains could not be maintained. A model for such regulation was recently proposed by Mezitt and Lucas (1996). Proteins, such as KN1, that can traffic through plasmodesmata and act as bona fide regulators of cellular development/differentiation were termed supracellular control proteins (SCPs). Cellular elements that act to regulate the delivery, binding, or plasmodesmal transport of such SCPs were called SCP cofactors.

Support for this SCP concept was provided by studies on KN1, the homeobox protein of maize, and more recently by genetic and microinjection studies conducted on the MADS-box carrying, homeotic proteins DEFICIENS (DEF) and GLOBOSA (GLO) of Antirrhinum majus. DEF and GLO act as heterodimers and control their own transcription as well as transcription of other genes. Perbal et al. (1996) employed periclinal chimeras expressing DEF and GLO in different cell layers to recover wild-type flower development. Here it is important to note that establishment of DEF and GLO expression in the L2 and L3 layers resulted in recovery of petaloid identity of the epidermal (L1) layer (derived from a def and glo mutant plant line). Immunocytochemical studies confirmed that both DEF and GLO were present in L1, whereas in situ analyses failed to detect the presence of DEF and GLO mRNA (Perbal et al., 1996). Finally, microinjection experiments performed on tobacco mesophyll cells demonstrated that both DEF and GLO have the capacity to increase SEL and traffic through plasmodesmata (Mezitt and Lucas, 1996). Thus, with the basic concept of SCPs in place, the challenge ahead will be the establishment of appropriate screens that will permit the identification of these SCP cofactors.

**ROLE OF PLASMODESMATA IN DIFFERENTIATION**

**Root development: A model system for plasmodesmal studies**

The primary root of Arabidopsis thaliana has a simple organization consisting of single-cell layers of the epidermis, cortex, endodermis and pericycle, which surround the central vascular cylinder (Fig. 2). The cortical and endodermal layers of the primary root consist of eight cells. This simple cellular architecture of the primary root is established in the embryo. Here, the first asymmetric cell division produces a smaller apical and a larger basal cell. The basal cell forms the hypophysis, which is located at the top of the suspensor. Later in embryogenesis the hypophysis divides to form a lens-shaped cell, the progenitor to four central cells (quiescent centre) and the initial cells directly adjacent to the meristem region (Fig. 2). The rest of the embryonic root derives from the apical cell. These two cell populations, having different clonal origins, act in concert to form the embryonic root, thus suggesting that cell-to-cell interaction is occurring to orchestrate the development of the root.

At first sight, the cellular architecture of the Arabidopsis primary root appears consistent with the hypothesis that cell fate is lineage dependent i.e. the cells of the epidermis and lateral root cap would appear to descend from a common set of initial cells, and the underlying cortical and endodermal cells appear to be derived from discrete initials (Dolan and Roberts, 1995). However, insightful laser ablation experiments performed on this root system provided unambiguous evidence that positional dependent information plays a critical role in the establishment (maintenance) of cell fate. Following ablation of an epidermal initial, a neighbouring cortical cell alters its fate and becomes a functional epidermal initial. Similarly, ablation of a cortical cell initial leads to the reversion of a
pericycle cell which then replaces the dead cell, leading to the progression of normal layered root tissue development. Additional evidence that continuous cell-to-cell communication is essential for cellular/tissue/organ development was provided by isolating a cortical initial cell from its cortical daughter cells. This isolated cell was shown to be competent to undergo cell division, but was now unable to divide asymmetrically to generate cortical and endodermal cell files. Thus, daughter cells within a cell layer appear to be involved in sending positional signals back to their initial cells to control cell fate and, hence, tissue differentiation (Van Den Berg et al., 1995).

Two pathways are available for the transmission of these putative regulatory signals: across the apoplasmic space of the adjacent cell walls, or cell-to-cell through plasmodesmata. Ablation experiments should cause little perturbation to the transmission of an apoplasmic signal, as the distance over which diffusion of the signal would occur is very short. In contrast, direct cell-to-cell communication would be significantly altered by killing adjacent daughter cells. Thus, it would appear that these ablation studies provide support for the involvement of signalling via the plasmodesmal pathway. This conclusion gains further support from an analysis of plasmodesmal distribution in the root meristem of *Arabidopsis*. As illustrated schematically in Fig. 2, cells within the ablation zone are highly interconnected by plasmodesmata. In addition, the transverse walls for cell files of common origin contain high numbers of plasmodesmata, presumably to ensure symplasmic continuity and the capacity to traffic information molecules within such cell layers. Furthermore, the density of plasmodesmata between epidermal, cortical, and the endodermal cell layers was found to be low, which would restrict symplasmic exchange but still permit cell-to-cell communication (Zhu, Lucas and Rost, unpubl. res.).

These interpretations of symplasmic connectivity are supported by dye-loading experiments in which it was shown that longitudinal movement of carboxyfluorescein, across transverse walls, was preferred over radial movement between different cell layers (Duckett et al., 1994; Zhu, Lucas and Rost, unpubl. res.). It is interesting to note that the observed slow radial movement of carboxyfluorescein was correlated with the density of secondary plasmodesmata within the longitudinal walls.

**Root hair cell patterning**: Role for plasmodesmal trafficking

In the Brassicaceae, root hair cells are arranged in files exclusively located over anticlinal walls of the underlying cortical cells (Fig. 3A). Root hair differentiation can be followed from a very early stage, as the progenitor cells are shorter and exhibit delayed vacuolation compared to their neighbouring non-hair epidermal cells (Benfey and Schiefelbein, 1994). As discussed above, a reduction in symplasmic continuity occurs later in development. Microinjection experiments performed on mid-to-late differentiating epidermal cells provided strong support for this change in symplasmic state, as carboxyfluorescein and Lucifer yellow failed to move into adjacent cells (Duckett et al., 1994). Hence, pattern formation occurs early while the epidermal cells are still symplasmically connected by plasmodesmata.

The critical unresolved question is how does the root epidermis establish the observed regular pattern of root hair differentiation? Clearly, at least two signals must be involved, one coming from the underlying cortical layer, which determines the exact positioning over the anticlinal cell walls, and a second, involved in suppression of the neighbouring non-hair cells. At a very early stage in epidermal cell differentiation, cells are considered to exist in a default state for root hair identity. An important gene involved in controlling (suppressing) this default state is the homeobox protein GLABRA2 (GL2) of *Arabidopsis thaliana*; this gene is expressed in non-root hair epidermal cells (Masucci et al., 1996). Another allele affecting the development of root hairs is the putative transcription factor, TRANSPARENT TESTA GLABRA (TTG). Mutants in either allele (gl2 or ttg) develop hair cells irrespective of their

![Diagram of cellular arrangement and degree of symplasmic continuity within the meristematic root of *Arabidopsis thaliana*. Model developed from plasmodesmal frequency and dye loading experiments (Zhu, Lucas and Rost, unpubl. res.). □, High number of plasmodesmata/symplasmic connections; □, low number of plasmodesmata/symplasmic connections.](http://example.com/diagram.png)
position relative to the underlying cortical cells (Masucci and Schiefelbein, 1996).

These results are consistent with the hypothesis that regulatory information must be exchanged between neighbouring cells, within or between these two layers. A model illustrating a likely scenario is presented in Fig. 3B. In this model, an as yet unidentified signal, generated from the cortex, activates the expression of a hair cell inducing factor (HCIF) which potentiates root hair differentiation. In addition, this HCIF controls the production of a hair cell suppressor factor (HCSF); note that HCSF would not have suppressor function in cells in which the HCIF programme has been activated. Cell fate of neighbouring cells is then controlled by HCSF, which in this model would have the capacity to mediate its own cell-to-cell transport through the plasmodesmata located within the radial, anticlinal walls of the epidermis. In the presence of TTG, this trafficking event would allow HCSF to induce GL2 expression, thereby leading to the suppressed state. Once this putative HCSF has been identified and cloned this hypothesis can be tested by in situ (mRNA), immunolocalization (protein) and microinjection (cell-to-cell movement) studies.

Fig. 3. Root hair differentiation in the Arabidopsis root. A, Cross section through the zone of root hair differentiation illustrating that root hair cells develop (grey shading) adjacent to the anticlinal walls of the inner cortical cells. B, Model for non-cell-autonomous, positional dependent control over root hair patterning. An unidentified signal (?) derived from the inner root tissue activates expression of a putative hair cell inducing factor (HCIF) which activates both root hair differentiation and expression of a hair cell suppressor factor (HCSF). The HCSF has the capacity to traffic through plasmodesmata that interconnect epidermal cells (double-headed arrows indicate direction of movement), to activate GLABRA2 (GL2; single-headed arrows indicate activation of GL2) thereby giving rise to the suppressed state.

Fig. 4. Role for plasmodesmal trafficking in trichome patterning in the epidermis of Arabidopsis leaf primordia. A trichome inducing factor (TCIF) activates the differentiation of the first priming trichome (A). The TCIF also activates expression of a trichome suppressing factor (TCSF) which can move cell-to-cell through epidermal plasmodesmata (A and B). In addition, this TCSF can mediate the plasmodesmal transport of TCIF mRNA (bold arrows indicate movement into neighbouring cells). Note that when the TCSF and TCIF are present in the same cell, TCIF function is suppressed (indicated by \( \perp \)). As TCSF moves out from the first priming trichome, its concentration is lowered due to binding to suppressor elements (B). Eventually the level of free TCSF falls below the threshold for plasmodesmal trafficking and only TCSF-mRNA (TCIF) moves into the next cell (A and B). Dissociation of the TCSF-mRNA (TCIF) complex allows translation of the TCIF mRNA which leads to induction of the next trichome (TRIC) and the activation of a second cycle (C). (Shading indicates the relative change in concentrations of TCSF and TCIF.)
Additional insight into the possible role of plasmodesmal trafficking of information molecules in cell patterning can be gained by examining data on trichome patterning in leaves. The positional distribution of trichomes on Arabidopsis leaves is not random, since newly initiated trichomes are normally separated by three to five cells. Furthermore, clusters of trichomes have been found to be extremely rare. In addition, leaves, an ordered distribution of trichomes, derived from cell lineages, can be excluded based on transposon-induced GUS expression within cells from a common origin (Larkin et al., 1996; Marks, 1997).

A model for non-random trichome patterning, based on the hypothesis that cells immediately bordering trichomes receive a non-translocatable suppressor signal, was proposed by Korn (1994). Recent advances in our understanding of the role played by plasmodesmata in the trafficking of plant transcription factors (Lucas et al., 1995) now provide a foundation for the development of a novel model to account for trichome patterning. This situation is highly analogous to root hair patterning, in that at least two antagonistic signals must be involved (Fig. 4A). One signal must induce trichome development (trichome inducing factor, TCIF), whereas the second signal suppresses trichome development (trichome suppressing factor, TCSF). These players act on the stage of the developing leaf primordia. The nature of the signal molecule responsible for initiating the first priming trichome remains to be identified. Thus, for the present discussion, we will make the assumption that the actual location of the first priming trichome within the epidermis is random.

Within the first priming trichome, TCIF is produced and initiates trichome differentiation. During this initiation process, the TCIF acts in concert with GLABRA1 (GL1, see below), GL2 and a number of other, as yet unidentified, regulators. Based on our studies with KN1, we propose that the putative TCSF binds and mediates the cell-to-cell transport of mRNA encoding TCIF. The stable form of this TCSF-mRNA (TCIF) complex is unavailable for translation. Both the TCSF-mRNA (TCIF) and free TCSF can interact with plasmodesmata to traffic cell-to-cell. As illustrated in Fig. 4A and B, the TCSF suppresses trichome development within a field of cells. The number of cells within a given field is determined by the threshold level of TCSF (free and complexed with TCIF mRNA) required for plasmodesmal transport (see, for example, Balachandran et al., 1997).

During the course of this movement, the concentration of TCSF declines as a result of its binding to either a nuclear or a cytoplasmic receptor involved in suppressor function. At the outer margin of this field of epidermal cells, a situation develops where free TCSF falls below the threshold for plasmodesmal transport; however, the stability (and level) of the TCSF-mRNA (TCIF) complex permits its entry into the next cell (Fig. 4B). Within this cell, the absence of free TCSF allows the dissociation of TCSF from the complex, thereby releasing the TCIF mRNA, which is immediately followed by translation to produce TCIF. Consequently, this cell is induced to form a trichome and therefore acts to propagate the next developmental field. As illustrated in Fig. 4C, the progression of this wave would then build an ordered pattern of trichomes across the surface of the leaf consistent with the non-random distribution of trichomes reported by Larkin et al. (1996).

The model presented in Fig. 4 can be tested against recent findings with mutant plants that displayed clustering of trichomes. Here it is important to note that TTG and GL1, which is a myc-like transcription factor expressed only in trichomes (Larkin et al., 1994), function as major elements in the regulation of trichome formation. Mutations in either TTG or GL1 significantly alter the number and distribution of trichomes. Clustering of trichomes was observed in heterozygous plants expressing ttg1/TTG and over-expressing GL1 (as a 3S::GL1 construct). Similar phenotypes were observed in homozygous plants expressing either the weak allele of tgg10 (relatively high numbers of trichomes) or the strong allele of tgg1 (low number of trichomes) (Larkin et al., 1994; Marks, 1997).

The formation of trichome clusters in plants carrying the tgg allele can be explained on the basis of an imbalance in the activities of TCSF and TCIF (Fig. 5). One scenario might be that tgg plants produce an altered form of TCSF (tcsf) (note that TTG may be the TCSF) having decreased TCIF mRNA binding activity; however, tcsf would still retain both suppressor and plasmodesmal transport functions. In the rare event that tcsf mediates trafficking of TCIF mRNA to the next cell, its reduced mRNA binding capacity would potentiate the dissociation of the tcsf-mRNA (TCIF) complex, thereby resulting in translation of TCIF. This
interpretation is consistent with the observation that these ttg mutants exhibit a decreased number of trichomes and the formation of clusters.

The influence of a weak allele, such as ttg10, on trichome patterning can be explained on the basis of this plasmodesmal macromolecular trafficking model. In this mutant, clusters of trichomes are found superimposed on a relatively normal background of trichome distribution. Relative to the situation with the ttg1 allele, binding of tcsf to TCIF mRNA would be less impaired. As a consequence, the clustering phenotype, as seen with the ttg1 allele, would still develop for all the same reasons advanced above. However, in the ttg10 mutants, a cluster of trichomes might cause a localized increase in both tcsf and the tcsf-mRNA (TCIF) complex. This would create a unique situation in which the high level of tcsf would stabilize the tcsf-mRNA (TCIF) complex, thereby reestablishing a near wild-type condition, but with clusters (Fig. 5).

Our explanation of the ttg10 phenotype can also account for the clustering that develops in plants constitutively expressing GL1, in a background of lower levels of functional TTG (i.e. heterozygous 35S::GL1) for the clustering that develops in plants constitutively expressing GL1. GL1 in these heterozygous plants could sensitize epidermal cells for trichome development and, in conjunction with perturbed TTG levels, establishes a concentration-dependent positional signalling system that would lead to clustering of trichomes.

CONCLUSIONS

Katherine Esau’s pioneering studies on the processes involved in plant virus infection established the important concept that plasmodesmata provide the conduit for cell-to-cell movement of the infectious agent. In the ensuing years, plant scientists have built upon this foundation to provide important insight into the processes by which many plant viruses traffic their RNA/DNA through plasmodesmata. The finding that viral proteins (MPs) potentiate the trafficking of infectious material, as viral ribonucleoprotein complexes, led to the discovery that endogenous proteins also move from cell-to-cell via plasmodesmata. This paradigm now provides a novel conceptual basis for analysis of the way in which plant cells communicate to control developmental and physiological processes. Clearly, plasmodesmal transport must be a highly regulated process. In order to gain a full understanding of the events involved in cell-to-cell signalling it will be essential to focus on the identification of both structural and regulatory constituents. This knowledge will provide important insight into the relationship between plant hormones and plasmodesmal-mediated control (non-cell-autonomous) over cell fate. Finally, it will be intriguing to see whether the translocation machinery of the plasmodesmata reflects homology to constituents of the nuclear pore complex, or if it represents a functional complex unique to plants.

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LITERATURE CITED


Kragler et al.—Plasmodesmata: Dynamics, Domains and Patterning


