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Dye-coupling in the root epidermis of *Arabidopsis* is progressively reduced during development

Catherine M. Duckett[1,*], Karl J. Oparka[2], Denton A. M. Prior[2], Liam Dolan[1,†] and Keith Roberts[1,†]

[1]Department of Cell Biology, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK
[2]Department of Cellular and Environmental Physiology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

*Author for correspondence at present address: Department of Plant Sciences, Downing Street, University of Cambridge, Cambridge CB2 3EA, UK
†Joint last author

SUMMARY

Epidermal cells of the *Arabidopsis thaliana* root are derived from a ring of 16 or so initials (stem cells) that are located just below the quiescent centre of four central cells. Derivatives of these initials divide transversely in the meristematic region of the root to produce files of cells that subsequently elongate and differentiate. There are two kinds of cell file in the epidermis, hair cells and non-hair cells, that are organised in an ordered pattern and become distinguishable within a few divisions of the initials. It is likely that local intercellular communication plays an important role in the early establishment of this pattern, enabling cells to register their position and fate. We have therefore examined the extent to which cells in the root apex are symplastically coupled through plasmodesmata. Using a variety of dye loading methods we show that the undifferentiated cells are dye-coupled i.e. cells in the meristem and the elongation zone are symplastically connected, but that cells gradually become symplastically isolated as the cells differentiate. By the time that visible hair outgrowth is observed the cells of the epidermis are symplastically isolated. This resembles the pattern of gap-junction connectivity observed in many animal systems where cells are coupled at the stage that cell patterns become established, but become progressively more isolated as they differentiate.

Key words: dye-coupling, symplast, communication, *Arabidopsis*, root, epidermis, root-hair

INTRODUCTION

Local cell-cell interactions are an important feature of many developmental systems. In plants, plasmodesmata provide the symplastic intercellular junctions and are a route for the direct passage of small molecules from cell to cell. The basic architecture of plasmodesmata consists of a collar, a central core of ER and a cytoplasmic sleeve (Lucas and Wolf, 1993). They allow ions and small water soluble molecules, up to about 800-1000 M_r, to pass freely from cell to cell and thus couple the cells both electrically and metabolically. This molecular exclusion limit is very similar to that of the analogous communication channels in animals – the gap junctions (see reviews Robards and Lucas, 1990; Lucas et al., 1993; Oparka, 1993). Despite their very different structures, both gap junctions and plasmodesmata are thought to be dynamic structures that can alter their gating properties in response to stimuli thus allowing molecules of different sizes to diffuse through. However, despite recent advances in structural studies of plasmodesmata (Ding et al., 1992), little is known about how plants allow and regulate intercellular transport and what role this communication plays in the development, morphogenesis and functioning of the plant as a whole.

Tilney et al. (1990) have shown that during the development of the fern gametophytic prothallus the distribution of plasmodesmata between cells relates to the stage of the gametophyte's development. Earlier experiments by Nagai (1914) and Nakazawa (1963) demonstrated that disruption of symplastic communication between the cells of the prothallus induced each cell to differentiate into a new complete prothallus. This suggested that cells in a prothallus are inhibited from dividing by a signal that moves symplastically. Work on gamete formation in the alga, *Chara*, has detailed changes in plasmodesmata during antheridial development. When cells were 'differentiation-synchronised', i.e. at the same stage of development and differentiation, the plasmodesmata looked structurally 'open'. i.e. all cells were symplastically connected. However, when cells were at different developmental states the plasmodesmata looked 'plugged' and cells were symplastically isolated (Kwiatkowska and Maszewska, 1986; Kwiatkowska, 1988).

In animals complex patterns of compartments are defined by gap junctions that restrict cell communication to discrete domains. These functionally coupled compartments arise at many stages of development and have been shown to be important in pattern formation in embryogenesis of many species (see reviews Guthrie and Gilula, 1989; Warner, 1992). Domains of gap junction connectivity have been well characterised in early embryogenesis, for example in early mouse embryos (Lo and Gilula, 1979; Lee et al., 1987) and during imaginal disc differentiation in *Drosophila* (Weir and Lo, 1984; Fraser and Bryant,

1985) and also at very late stages in development such as the formation of feather buds in the skin of the late chick embryo (Serras et al., 1993) and hair follicle formation (Kam et al., 1986; Kam and Hodgins, 1992). In these systems, intercellular communication appears to be required during pattern formation but later becomes more selective. (Warner, 1992).

In the postembryonic root of *Arabidopsis thaliana*, the epidermis consists of two classes of cell files defined by whether their component cells do or do not develop root hairs. The root hair cells (trichoblasts) have a characteristic set of developmental traits that reflect a divergent pathway acting very early in epidermal cell development. Files of trichoblasts are positioned over the anticlinal walls of the eight underlying cortical cell files and are separated by either one or two intervening non-hair cell files (Cormack, 1947; Dolan et al., 1993). Thus, from 16 epidermal cell initials, two distinct final cell types are produced in an ordered and predictable pattern. Each cell file, followed back from the meristem, therefore, represents a time course of the cell differentiation sequence.

This system offers an excellent model for the analysis of how the local cell-cell interactions and plasmodesmatal communication are regulated during the establishment of a simple pattern of cells. It also provides an accessible set of cells with which to examine experimentally how cell coupling is regulated later during cell differentiation. Dye-coupling experiments monitor the movement of low-molecular-weight fluorescent probes through plasmodesmata and indicate the presence of symplastic communication between cells (Tucker, 1982; Goodwin, 1983; Oparka, 1991). Microinjection and other techniques have been used to label cells in vivo with these fluorescent probes and to explore the patterns of functionally open plasmodesmata (Erwee and Goodwin, 1985; Palevitz and Hepler, 1985; Goodwin et al., 1990). We have used a variety of methods, including both microinjection and non-invasive methods of dye loading, to assess the extent of dye coupling between epidermal cells at all developmental stages from their birth within the meristem, through the cell elongation phase, to their final differentiation into two distinct mature cell types in root tips of *Arabidopsis.*

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana ecotype 'Columbia' seeds were sterilised in 5% sodium hypochlorite and rinsed. They were allowed to germinate on plates containing 1× Murashige and Skoog (MS) salt mixture, 3% sucrose, pH 5.8, in thin-layer 1% agarose. After 24 hours at 4˚C, plates were incubated in a near vertical position at 25˚C in continuous light. In all cases the primary embryonic root of 4-day-old seedlings was used for dye coupling experiments.

Fluorescent probes

6-Carboxyfluorescein (M_r 376, dissolved in 0.3 M KOH or absolute ethanol) and Lucifer Yellow (M_r 457, Molecular Probes Inc., Eugene, OR, USA) were used at a concentration of 1 mM in distilled water and filtered and centrifuged before use. Dye was tested, as a control, by microinjecting *Tradescantia* stamen hair cells where it moves easily from cell to cell.

Microinjection

Microinjections were performed using a Narishige micromanipulator (model MO-204) hydraulically coupled to a four-axis joystick (model MO-202). Borosilicate glass capillaries (filamented for microinjection) (1×90 mm, Narishige, Tokyo, Japan) were pulled using a Narishige micropipette puller (model PP-83). Needle size depended upon whether they were used for microinjection or to ester load hairs. Needles were backfilled with dye (and low-viscosity silicone oil if used with the pressure probe). Intact plants for injection were removed from the Petri plates complete with a portion of the growth medium and placed in a moist chamber with a lid designed to permit both microscopy and microinjection whilst minimising dehydration of the plant. Control injections were also performed with the roots under Hoagland's solution. Probes were injected using either a pressure injection unit (model PL1-11 Medical Systems Corp. NY USA) or a pressure probe (Oparka et al., 1991). The pressure probe enabled the cell turgor pressure to be predicted (by recording previous levels of cytoplasmic 'backfire' into the needle). By raising the hydrostatic pressure in the pressure probe, prior to impalement of the cell, cytoplasmic disruption could be minimised, tip blockage reduced and smaller quantities of dye injected.

Microscopy

Microinjections were monitored using a Nikon Microphot SA microscope equipped for epifluorescence. It was essential to establish the exact position of the dye, which could be apoplastic, cytosolic or vacuolar. All experiments were observed with a confocal laser scanning microscope (CLSM) (BioRad MRC-600 and MRC-1000). Series of optical sections were made along the z-axis through the dye loaded cells which, when analysed, made possible the determination of the position of the dye. Photographs were taken using technical pan film.

Dye loading in the tip zone via the phloem

In these experiments, carboxyfluorescein was introduced into cells as its diacetate ester, which is uncharged at cell wall pH and thus membrane permeant. Once in the cell, esterases remove the acetyl groups, releasing carboxyfluorescein which, being membrane impermeant, is trapped in the cell (Thomas et al., 1979). Carboxyfluorescein diacetate (Molecular Probes Inc) was prepared as a 6 mg/ml stock in acetone, used at 30 μg/ml in distilled water and carefully administered to cotyledons that had had 1 mm of apical tissue removed. Released carboxyfluorescein was hence loaded into the phloem and transported down the root (see Oparka et al., 1994). In the root tip zone, the dye unloads symplastically. This provides an ideal non-invasive way to study dye-coupling patterns in this region. The dye occasionally entered the hypocotyl epidermis and symplastic domains in this region were also observed.

Ester-loading of root hairs

Carboxyfluorescein diacetate, concentration as above, was also used to ester load carboxyfluorescein non-invasively into individual hair cells. Specially pulled glass capillaries, which just fitted over the end of a hair were used to micro-apply the dye to the hair tips.

Electron microscopy

Seedlings were either fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7, for 1 hour at 4°C, washed and postfixed in 1% osmium tetroxide in veronyl buffer at 4°C (Burgess and Linstead, 1984). Alternatively, seedlings were fixed in 2.5% glutaraldehyde, 5 mM $CaCl_2$ in 50 mM cacodylate, pH 7, for 1 hour at 4˚C and postfixed in 2% osmium tetroxide 0.8% $K_3(CN)_6$ (potassium ferricyanide) 5 mM $CaCl_2$ in 50 mM cacodylate buffer pH 7 for 1 hour at 4˚C (Hepler, 1981). All samples were dehydrated through an ethanol series and embedded in LR white (London resin Company). Thin sections were cut on an Ultracut E microtome (Reichert Jung) and stained with uranyl acetate and lead citrate.

RESULTS

The epidermis of the *Arabidopsis thaliana* root is divided into

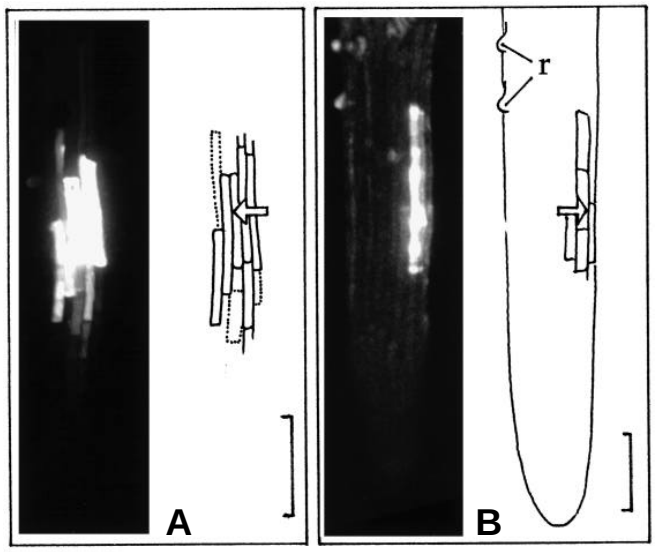

Fig. 1. CLSM optical sections show the dye distribution in the basal region of the *Arabidopsis thaliana* root after Lucifer Yellow was injected into single epidermal cells The accompanying diagram indicates the injected cell (arrow) and the extent of dye-coupling. (A) Injection into an epidermal cell in a hair file, dye coupling can be seen in surrounding epidermal cells and (dotted lines) in some underlying cortical cells. Bar, 100 μm. (B) Another similar injection shows dye coupling of epidermal cells within the same file. Bar, 100 μm.

files of cells, each file containing either hair cells or non hair cells as described previously (Dolan et al., 1994). The hairs form in a zone of differentiation when cell elongation has effectively ceased. The files of hair cells are separated by either one or two files of non-hair cells. The eight hair cell files overlie the radial walls of the underlying cortical cells. For this study, the term 'tip zone' is used to describe the cells in the combined meristematic and elongation areas. The 'differentiation zone' is the region above this where root hairs start to grow and finally mature.

Early in development epidermal cells are symplastically coupled

Communication between cells in the root tip area was investigated by microinjecting low molecular weight probes into the cytoplasm of one cell and observing their movement, via plasmodesmata, to neighbouring cells. Results were only recorded when an injection was considered to be 'clean'. This was defined as one where dye had clearly entered the cytoplasm rather than the vacuole and dye had not leaked to the apoplast. Injected cells also had to have been minimally damaged, with no cytoplasmic leakage and no damage to surrounding cells.

When injected into single epidermal cells in the tip zone, Lucifer Yellow was seen to move rapidly out of the injected cell into the surrounding cells (Fig. 1A). Similar results were obtained using carboxyfluorescein. Injections in this region are difficult because of the small cell size and because the lateral root cap can impede the injection by blocking the needle. The spread of dye between these cells was usually rapid, i.e. 1-3 minutes and in only 7% of injections was the dye restricted to the injected cell (Table 1). In several cases, injections into epidermal cells in the elongation zone revealed an asymmetrical pattern of dye coupling. Dye often moved preferentially to cells within the same file rather than between files (Fig. 1B). Analysis with a CLSM confirmed that dye movement was often confined to files of epidermal cells and tended not to spread transversely into the underlying cortical cell layer although this was not always the case (Fig. 1A).

Table 1. Quantification of dye movement after injection of *Arabidopsis* root epidermal cells at different stages of development

	Epidermal cell injected		
		Differentiation zone	
	Tip zone cells	Non-hair cells	Hair cells
% of injections where dye restricted to injected cell (n)	7.1 (14)	94.3 (35)	98.4 (61)

To ensure that these results were not affected in any way by the microinjection procedure itself, symplastic coupling in the tip zone was also monitored by non-invasively loading the cells with dye via the phloem. Carboxyfluorescein, loaded into the phloem of the shoot, is transported down the phloem strands to the protophloem in the root tip. Dye unloads from the protophloem as seen in Fig. 2A and is confined to the symplast. Dye movement among these young cells can then be observed and dye coupling patterns in this region can be mapped. After the cotyledons are treated with dye it is possible to see dye unloading in the root within 30 minutes.

Dye spreads in the epidermal cells back up to the boundary where cell elongation ceases and root hairs start to grow (Fig. 2A). This boundary was very distinct and was not caused by limiting amounts of dye unloading from the phloem. This was verified by observing the dye coupling pattern over time. Fig. 2A-C show roots left to grow after the communication boundary had been set up. All the new cells contained dye, probably due both to cell-cell communication and to cell division. There appeared to be no dilution of the dye suggesting that dye was still being unloaded from the phloem. However, the distribution of dye in the cells that bordered the boundary described previously did not alter even when the roots were left to grow for 19 hours (data not shown). Both epidermal cells with and without hairs showed this clear symplastic domain (Fig. 2D).

At the communication boundary, occasional hair bulges contained dye. This could be because these cells had very recently been within the area that was dye coupled to the rest of the cells in the elongation zone. Cells with bulges are newly differentiating cells and have just passed from the zone of elongation into the zone of differentiation. It is likely that this particular cell was located in the 'late' elongation zone when dye unloaded and therefore became labelled only to move into the differentiation zone later. The microinjection experiments described below reveal that cells that are in the differentiation zone are isolated.

Epidermal cells become symplastically uncoupled as they differentiate

After epidermal cells have progressed through the elongation zone they enter a region where they differentiate into hair cells or non-hair cells. Dye coupling experiments revealed that these differentiating epidermal cells become symplastically isolated.

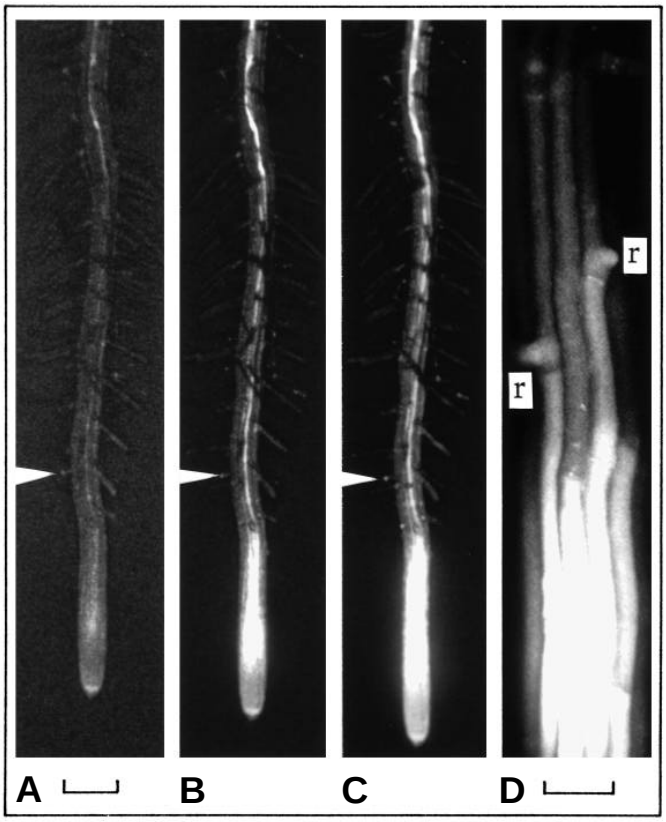

Fig. 2. Cells in the basal region of the root are dye coupled. This is demonstrated here by the symplastic movement of carboxyfluorescein unloading from the phloem in the root. (A-C) Unloading and symplastic movement of carboxyfluorescein in the root. Time elapsed since cotyledon ester-loaded: A, 43 minutes; B, 88 minutes; C, 163 minutes. Note that there appears to be a boundary beyond which the cells are not dye-coupled. This symplastic barrier to dye movement occurs where cell elongation ceases and root hairs start to grow. This region, the edge of the dye-coupled domain in the tip zone, is shown in close-up (D).The arrowhead in A-C indicate the same root hair at each time point which provides a marker against which to monitor the symplastic barrier as the roots grow. Bar, 300 μm. In D, 'r' indicates a root hair bulge. Bar, 50 μm.

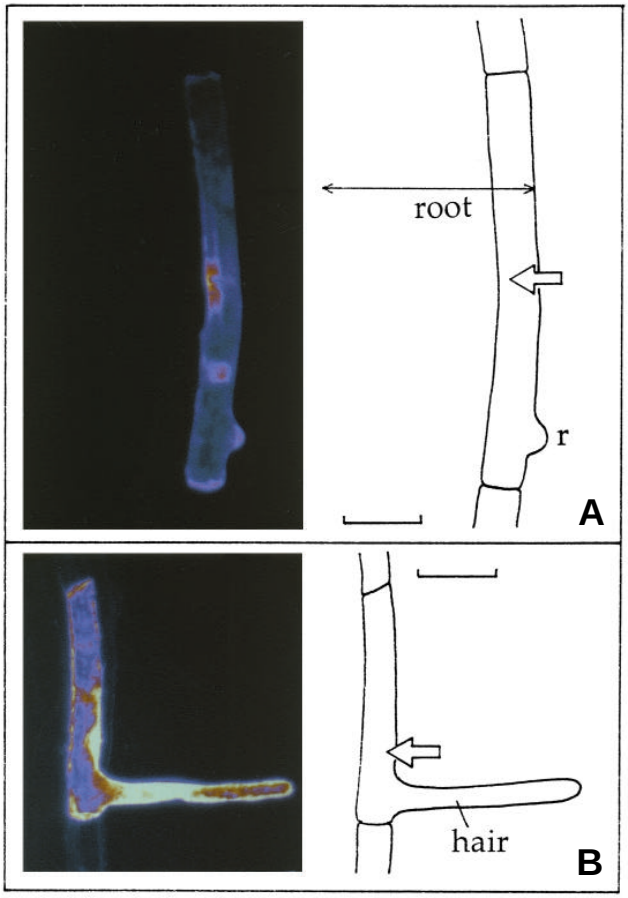

Fig. 3. Microinjection of carboxyfluorescein into root-hair cells. Note that dye does not move out of the cells into neighbouring cells. This lack of dye-coupling is evident at all stages of hair formation from a young bulge (A) to a mature and growing hair (B). Bar, 100 μm and 100 μm respectively. Root hairs are hence restricted in the symplastic communication that they have with other cells. Arrows indicate site of injection and 'r' indicates the bulge of the epidermal cell where a root hair will grow.

Fig. 3A,B show hair cells microinjected with carboxyfluorescein. There is no movement of dye out of the cells. These images, taken with a CLSM, clearly show the presence of the dye in the cytoplasm and absence of dye in the cytoplasm of all the adjacent cells. From Table 1 it can be seen that 98.4% of hair cells injected appear to be symplastically isolated from neighbouring cells. This was the case when epidermal cells were injected at all stages during hair formation, i.e. from epidermal cells with bulges (Fig. 3A) to epidermal cells with hairs (Fig. 3B). 94% of non-hair epidermal cells in the differentiation zone are also symplastically uncoupled from their neighbours. Microinjections with carboxyfluorescein and Lucifer Yellow both reveal the same pattern of symplastically isolated cells.

The possibility that the wounding effect of microinjection may cause plasmodesmata to shut down was addressed by ester-loading. The hair cells were non-invasively loaded with dye by micro-applying carboxyfluorescein diacetate to the hairs. Once inside the cell, this diacetate ester is cleaved by esterases to produce carboxyfluorescein. This is membrane impermeant and therefore the symplastic coupling of cells loaded in this way can be monitored. Esterase activity indicates cell viability. Fig. 4A,B show hairs whose tips have been dipped in carboxyfluorescein diacetate. It takes less than 5 minutes to load a cell with carboxyfluorescein. The dye remains in the cell bearing the hair that was dipped, i.e. there is no dye movement. This verifies the injection results that show that *Arabidopsis* hair cells are symplastically isolated from other epidermal cells and from the underlying cortex cells. Dipping non-hair epidermal cells and even cells with very young hairs or bulges was unreliable as it was difficult to be sure that dye application had been localised to one cell.

These results suggest that epidermal cells become symplastically uncoupled late in their development. Fig. 5A-C show plasmodesmata between cells in this hair region and between cells of the tip zone. All of these cells are linked by typical primary plasmodesmata that appear structurally normal. Examination of plasmodesmata in the walls of young cells in the

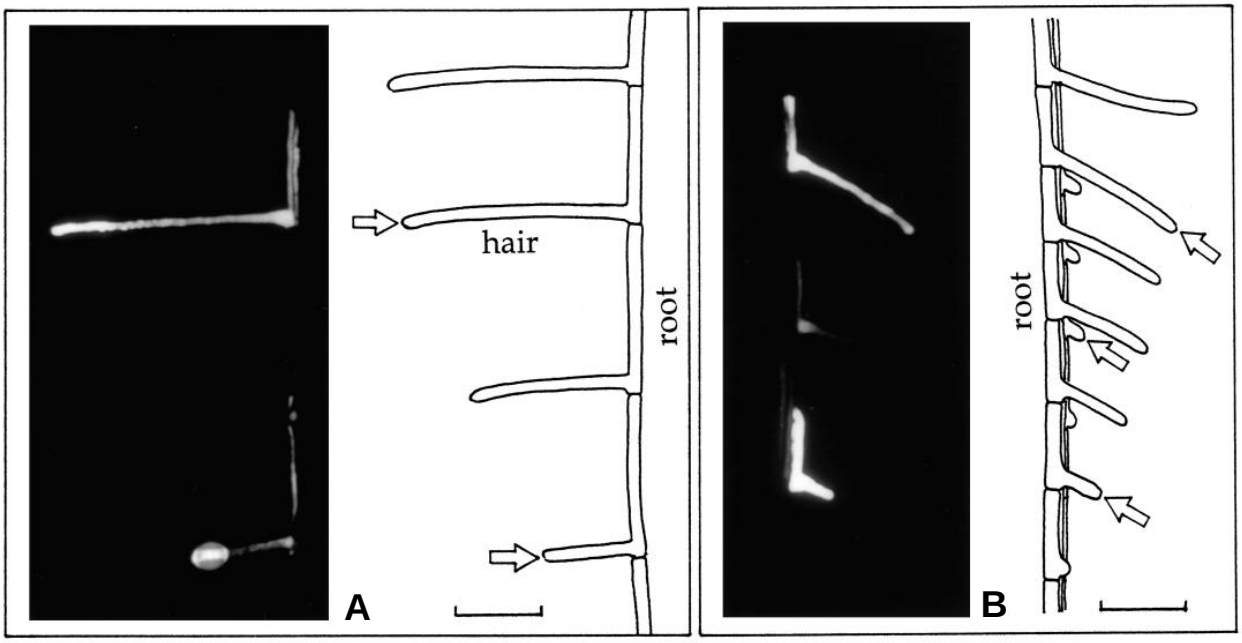

Fig. 4. Ester-loading of dye into individual root hairs also demonstrates a lack of dye coupling between root hairs and their neighbours. The microapplication of the diacetate form of carboxyfluorescein provides a non-invasive method to load dye into the hairs. The corresponding diagram with each figure shows clearly the position of neighbouring cells and an arrowhead indicates the labelled hair cell. The results for very young hairs were less reliable as it was harder to be sure that dye did not contaminate neighbouring cells. (A) Bar, 100 μm; (B) bar, 100 μm.

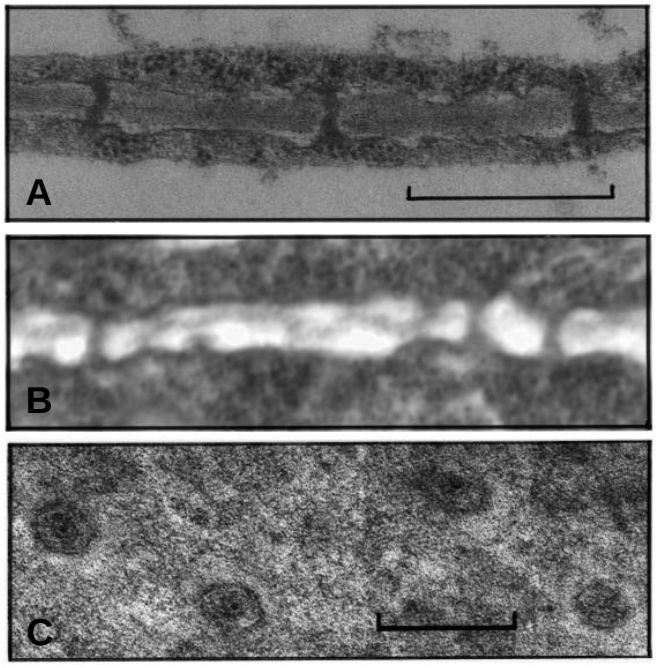

Fig. 5. Plasmodesmata in hair region (A) and meristematic region (B) in transverse walls. (C) Detail of the structure of plasmodesmata in cross-section. Bar A and B, 500 nm; C, 100 nm.

meristematic area revealed no apparent differences in structure compared to those in older cell walls although there appeared to be more of them in the transverse walls of epidermal files than in the longitudinal walls.

Other communication boundaries

Our results have also revealed other symplastic domains in epidermal cells in the hypocotyl of *Arabidopsis*. These were revealed when cut cotyledons were being loaded with dye. The dye entered the epidermal layer probably through the outer epidermal wall and then moved through this layer to the hypocotyl. Fig. 6A demonstrates the isolation of the hypocotyl epidermis from the rest of the hypocotyl. However, unlike the mature root epidermis the cells of the hypocotyl epidermis are still symplastically connected to one another. Fig. 6B shows the boundary at the root/hypocotyl junction. The hypocotyl epidermis is uniformly labelled with dye, but this does not move into the adjacent epidermal cells of the root.

DISCUSSION

The simple anatomy and patterning of the *Arabidopsis* root makes it an excellent model to study cell-cell interactions during development and differentiation in plants (Dolan et al., 1993). In this report, we have concentrated on the symplastic communications between epidermal cells. Communication, through plasmodesmata, in the *Arabidopsis* root has been investigated by observing coupling patterns of dye loaded into cells by a variety of methods. This approach enabled us to monitor whether small signalling molecules were capable of being exchanged between cells and hence whether they might, in principle, play a role in the early patterning of cell files in the epidermis. We have revealed that, in the meristem and elongation zone, cells are symplastically coupled but that, as the epidermal cells differentiate, they become isolated from their neighbours. This demonstrates a correlation between symplastic communication and cell differentiation in the epidermis of the *Arabidopsis* root.

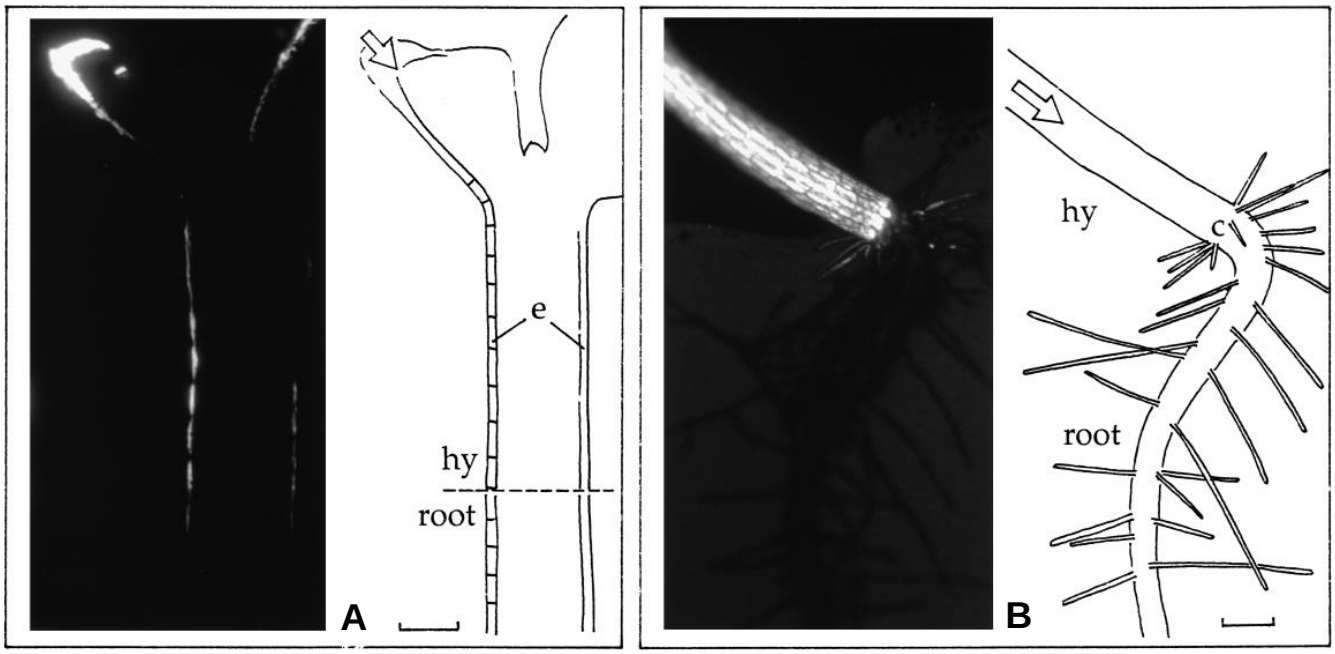

Fig. 6. Dye-coupling patterns in the hypocotyl of *Arabidopsis.* Cells of the cotyledons were labelled with carboxyfluorescein by ester loading and this revealed symplastic domains in the hypocotyl. The epidermis is isolated from underlying cells although epidermal cells are dye-coupled to each other (A). Communication between the epidermis in the root and that of the hypocotyl also appears to be restricted in the collet region (B). e, epidermis; hy, hypocotyl; r, root; c, collet. An arrow indicates the region where the dye was loaded into the cells. Bar, 100 μm and 200 μm for A and B, respectively.

In the postembryonic *Arabidopsis* root epidermis, the cells in the tip zone are symplastically coupled, allowing small molecules to pass from cell to cell. This may enable the cells to establish their position and pattern by exchanging positional cues. The communication boundary revealed by loading the dye into cells via the phloem demonstrates that a symplastic domain ends at the start of the zone of differentiation. Occasionally very young hairs at the boundary appeared to have some dye in them, contrary to the microinjection results that show that these cells are isolated. This was probably because the cells had been within the dye-coupled domain at the time of labelling and, therefore, had been symplastically connected to the rest of the tip zone, only to become symplastically isolated subsequently.

It is within this dye-coupled domain at the tip that the first differences between the hair cells (trichoblasts) and the non-hair cells (atrichoblasts), reported by Cormack (1947), become apparent. However, before further differentiation takes place, the epidermal cells become symplastically isolated as shown by the lack of dye movement after microinjection. We have taken care to ensure that our results have not been biased by the methods used. In particular, the possibility that the plasmodesmata may shut down as a response to the microinjection was rejected after passive dipping of the hairs produced the same response. It is known that wounding and large pressure changes can cause plasmodesmata to close (Reid and Overall, 1992; Oparka and Prior, 1992). Similarly the phloem loading experiments backed up the results of microinjection in the tip zone.

Few links between symplastic communication and development have been made in plants. There are, however, many studies on the transfer of low molecular weight probes between cells of mature tissues (e.g. Barclay et al., 1982; Oparka, 1991; Tucker, 1982; Tucker et al., 1989; Goodwin, 1983; Erwee and Goodwin, 1985). A detailed examination of symplastic domains has been made in the aquatic plant *Egeria densa* (Erwee and Goodwin, 1985; Goodwin and Erwee, 1985). In this species, the epidermis of the root and shoot was shown to be isolated from the underlying layers but cells within these tissues communicated freely with one another. However, the exclusion limit for these cells, i.e. the size of the largest molecules seen to transfer from cell to cell, was low. Only carboxyfluorescein could move, larger dyes were excluded. It was noted that, although dye-conjugates up to 665 M_r could move freely in the shoot apex, more mature cells had a molecular exclusion limit of just over 376 M_r. This observation supports the suggestion that, as cells mature, symplastic communication is restricted. Also of interest is the observation that the marginal tooth cells of the *Egeria* leaf appeared to have a lower molecular exclusion limit than the surrounding leaf epidermis (Erwee and Goodwin, 1985).

We have shown that, whilst cells of the hypocotyl epidermis are symplastically coupled, they are isolated from the underlying cortex thus confirming the observations of Erwee and Goodwin (1985). The hypocotyl epidermis, however, was also distinctly isolated from the root epidermis reflecting a developmental boundary set up very early in the embryo (Scheres et al., 1994).

The apparent isolation of the mature epidermis poses difficulties for the classical view of ion movement into the root as noted by Erwee and Goodwin (1985). Indeed the isolation of root hairs suggests an apoplastic pathway for ion uptake across the epidermis. Carboxyfluorescein, however, has a larger radius than many hydrated ions and it is possible that K^+ and

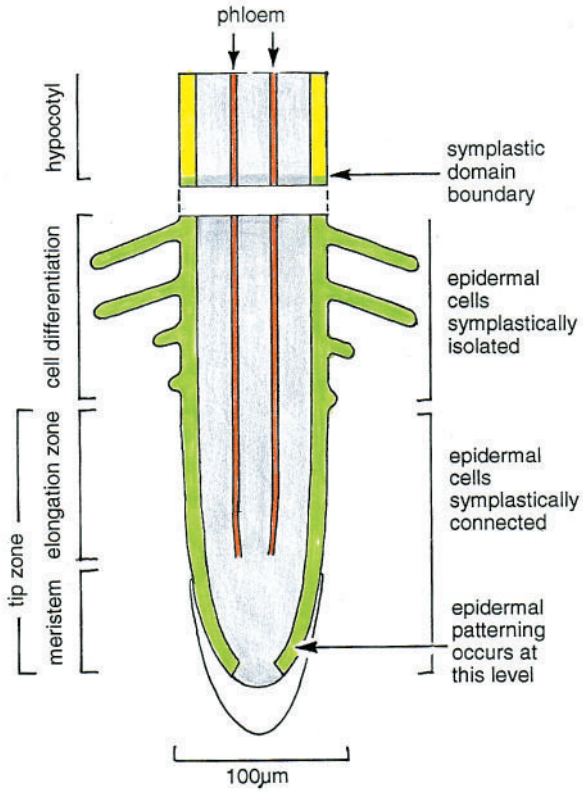

Fig. 7. Schematic diagram of the patterns of dye-coupling and symplastic communication in the *Arabidopsis* root.

Cl^-, for example, could still be transported in the symplast when carboxyfluorescein is excluded. Hence the cells could still be electrically coupled and have functional plasmodesmata (Overall and Gunning, 1982; Oparka, 1993). However, the recent results of Meharg et al. (1994) suggest that the root hair cells of *Arabidopsis* exhibit a high degree of electrical isolation supporting the results described here.

Since our results show that apparently normal plasmodesmata are present in walls that carboxyfluorescein is unable to pass, it suggests that either these plasmodesmata are occluded or their gating properties are developmentally regulated. Indeed in the *Arabidopsis* root, plasmodesmata were found in walls of all regions where symplastic connectivity was examined. Detailed studies on plasmodesmata of roots of other species have indicated higher frequencies in transverse walls than longitudinal walls and an age related loss of plasmodesmata during development (Robards, 1976; Gunning, 1978).

Our results show that mature hair cells of the *Arabidopsis* root are not dye-coupled to any neighbouring cell. An attractive hypothesis is that when cells finally undergo cell morphogenesis, during a differentiation pathway, that distinguishes them morphologically and possibly functionally from their neighbours, they may need first to become symplastically isolated. In the stems of higher plants, the sieve-element companion cell complex forms a symplastic domain. Although isolated from other tissues of the stem, the passage within the complex of probes up to 3000 M_r is still allowed (Kempers et al., 1993). Similarly symplastic domains in the shoots of maize seedlings and tomato internodes have been shown to organise different tissues into ‘symplastic units’ (Epel and Bandurski, 1990; Van der Schoot and Van Bel, 1990). Guard cells and surrounding epidermal cells are symplastically coupled during development but the guard cells become isolated at maturity (Palevitz and Hepler, 1985). Although the guard cell walls eventually lose their plasmodesmata as they mature, it is reported that the isolation of these cells occurs well before their plasmodesmata disappear. These results provide further evidence that symplastic uncoupling correlates tightly with cell differentiation relative to neighbouring cells.

In general, our results are compatible with similar events in animal cells where current evidence indicates that gap junction-mediated communication between cells is important in development (see reviews Guthrie and Gilula, 1989; Warner, 1992). Potter et al. (1966) suggested that embryogenesis could be controlled, in part, by the communication of signals between cells and that this had to be selective to establish and maintain differences between cells. Dye coupling experiments have since revealed many communication boundaries that match developmental domains (e.g. Guthrie, 1984; Guthrie et al., 1988; Dorrestigjin et al., 1983; Serras et al., 1989). Communication between animal cells is notably selective where differences between cells are established. In the larval epidermis of *Drosophila*, dye transfer is limited by the segment border, i.e. gap junctional communication changes at precisely defined developmental boundaries (Warner and Lawrence, 1982). Experimental perturbations with gap junction selective antibodies can severely disrupt patterning but allow normal differentiation to continue (Warner et al., 1984; Lee et al., 1987). Gap junction communication domains have been correlated with cellular domains defined by morphological and molecular markers, e.g. in skin patterning in mammals (Kam et al., 1986; Kam and Hodgins, 1992) and chicken (Serras et al., 1993). Spatiotemporal patterns of expression of genes of the multiple gap junction genes, connexins, also correlate with formation of communication domains during rat skin and hair development (Risek et al., 1992). Signalling domains generally encompass large groups of cells early in development and become gradually more restricted as development proceeds.

Our results indicate a clear relationship between symplastic communication and differentiation in the *Arabidopsis* root. The constant and simple anatomy of the *Arabidopsis* root and the communication patterns that we have revealed make this system a model in which the roles of symplastic communication and the structural and functional changes in plasmodesmata during development and differentiation can be explored. Fig. 7 shows a model of how symplastic communication restrictions could be involved in the development and differentiation of the *Arabidopsis* root epidermis. We suggest that cellular coupling becomes progressively restricted during both embryogenesis and postembryonic development, and that at least two distinct mechanisms may be involved. First, symplastic domains may be established as a consequence of developmentally significant early division patterns. Examples would be the boundary between root epidermis and hypocotyl epidermis, two contiguous groups of cells that arise in the globular embryo (Scheres et al., 1994). Second, domains may be set up within such large cell groups as a function of the developmental history of the tissue. The same epidermal cell in the tip zone moves during development from a region where it is coupled to its neighbours, to a region where it is not. The extreme version of this is the case where individual cells or

groups of cells become completely isolated when they differentiate with respect to their neighbours. Examples are the sieve-element/companion cell units, stomatal guard cells and the root epidermal cells described here. The mechanisms involved in these plasmodesmatal gating events remain obscure and will await a molecular description of plasmodesmatal structural and regulatory proteins.

The results of this study are compatible with a model of epidermal development in which local cell-cell interactions occur within the meristem to determine the cell fate of trichoblasts on the basis of positional value (Dolan et al., 1994) and in which a restriction of cell-cell communication is required to allow the final cell morphogenesis events to take place during differentiation.

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