

Tissue to tissue symplastic communication in the shoots of etiolated corn seedlings

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Carboxyfluorescein, a symplastic probe, was applied to the cut mesocotyl base or coleoptile apex of etiolated *Zea mays* cv. Silver Queen seedlings and its transport measured and tissue distribution determined. Long-distance longitudinal symplastic transport of the carboxyfluorescein was mainly in the vascular stele. It moved laterally from the mesocotyl stele to the mesocotyl cortex but the presence of a weak barrier limited the movement. A partial symplastic barrier was also present near the coleoptile-mesocotyl node.

Key words – Carboxyfluorescein, carboxyfluorescein diacetate, cell to cell communication, maize, symplast, symplastic domains, symplastic subdomains, symplastic translocation, tissue to tissue communication, translocation barriers, *Zea mays*.

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Introduction

Orderly patterns of growth and development are probably dependent on a regulated transport of metabolites and growth regulatory molecules from source cells and/or tissues to specific target cells and tissues. In higher plants, translocation may be by one, or by a combination of two possible routes. The translocate may move symplastically via plasmodesmata, or it may be transported out of a cell and move to other cells and/or tissues via an apoplastic route which includes, cell walls and the various xylem elements (Gunning 1976). Transport via the apoplastic route could conceivably be controlled by changing the composition of the cell wall. Transport in the symplast could be controlled by regulating intracellular movement between different compartments, by altering membrane properties and/or by regulating the conductance of the plasmodesmata. The existence of barriers to transport, partial or total could have a profound effect on patterns of growth and development.

Plasmodesmata are complex structures which penetrate through cell walls and connect the cytoplasms of

adjacent cells (Gunning 1976). It has been suggested that the cytoplasm, the plasmalemma, and endoplasmatic reticulum of all cells in a plant, from root tip to shoot apex, are interconnected via the plasmodesmata, forming a cellular continuum, the so called symplast (Robards 1976). Morphological studies on the distribution of plasmodesmata indicate that, with only a few exceptions, all plant cells are linked together by these structures (Robards 1976). Evidence showing cytoplasmic continuity between contiguous cells has come from studies showing that compounds move between plant cells and tissues without being exchanged with molecules in a bathing solution (Arisz and Schreuder 1956, Arisz 1960, 1969); that there exists electrical coupling between cells (Goodwin 1976); and that low molecular weight non membrane-permeable hydrophilic fluorescent compounds, following their introduction into the protoplast of a cell, move between cells (Baron-Epel et al. 1988, Erwee and Goodwin 1985, Goodwin 1976, 1983, Mogensen 1981, Palevitz and Hepler 1985, Terry and Robards 1987, Tucker 1982, 1988). It should be noted, however, that evidence demonstrating functional linkages between all cells of a plant has not been pre-

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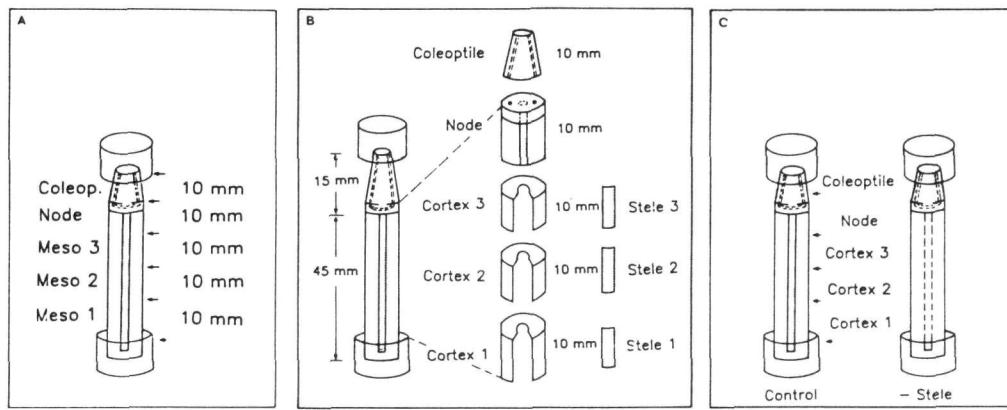


Fig. 1. Schematic drawing of etiolated corn shoots. Both ends of an excised shoot were immersed in agar blocks containing 10 mM potassium phosphate (pH = 5.5). Either the apical or basal block contained 22 μ M CFDA. After the indicated period, the shoot was dissected as shown. (A) The shoot, 60 mm long, (mesocotyl 45 mm, coleoptile 15 mm) was sectioned into 3 mesocotyl sections, Meso 1, 2, 3 and a nodal section, Node, and a coleoptile section, Coleop, each 10 mm long. (B) The shoot, 60 mm long, (mesocotyl 45 mm, coleoptile 15 mm) was sectioned into cortical sections Cortex 1, 2, and 3; stellar sections Stele 1, 2, and 3; and node and coleoptile sections, each 10 mm in length. (C) Shoot 50 mm long (mesocotyl 45 mm, coleoptile 5 mm); the stele of each shoot was either left intact or was removed from the base of the mesocotyl to within 5 mm of the node. After the indicated time, the shoot was sectioned into cortical sections Cortex 1, 2, and 3 and the nodal section (Node), as shown in Fig. 1B.

sented. Recently, it has been found that cell to cell communication via the plasmodesmata can be effectively gated (Baron-Epel et al. 1988, Erwee and Goodwin 1984, Tucker 1988). Thus, it is conceivable that even though adjacent cells or tissues are interconnected by plasmodesmata, these plasmodesmata may or may not be conducting.

In the present study, we have studied the movement of a symplastic probe, carboxyfluorescein (CF), in etiolated seedlings of *Zea mays*. Since CF is charged and cannot penetrate membranes, it was introduced into the tissue as its diacetate ester which is uncharged at cell wall pH and thus membrane permeable. Once in the cell, esterases remove the acetyl moieties leaving two ionized carboxyl groups and an ionized phenolic hydroxyl group, resulting in the CF becoming trapped within the cell (Thomas et al. 1979). Carboxyfluorescein diacetate (CFDA) thus diffuses from the apoplast into the cells near the point of application by passive diffusion driven by a concentration gradient and is hydrolyzed to CF where it is trapped as charged CF. Since the charged CF can only move from cell to cell via plasmodesmata, it serves as a probe for measuring symplastic domains (Baron-Epel et al. 1988). Fluorescein has also been classically used as a symplastic probe for measuring the long distance transport in phloem sieve elements (Schumacher 1933), but is less charged than CF and hence more prone to leakage. It is reported here that in the shoot of an etiolated corn seedling there are a number of kinetic symplastic sub-domains which determine the distribution pattern of simple symplastic translocates.

Abbreviations – CF, carboxyfluorescein; CFDA, carboxyfluorescein diacetate; KPi, potassium phosphate.

Material and methods

Plant material

Seedlings of *Zea mays* L. cv. Silver Queen were grown for 96–100 h and selected as previously described (Epel and Bandurski 1990). Shoots were severed from the kernel 45 mm below the coleoptile-mesocotyl node and the tip of the coleoptile was removed, leaving a coleoptile 15 mm, 5 mm, or 2 mm in length as indicated in the text. In one experiment, the entire coleoptile plus node was excised at a distance 2 mm below the node. Shoots from which their steles were removed were prepared from intact seedlings by nicking and ringing the mesocotyl cortex 45 mm below the node and then pulling the mesocotyl above and below the cut, resulting in the detachment of the mesocotyl-stele from the mesocotyl-cortex. The stele generally broke about 5 mm below the node. The cut shoots were washed as previously described (Epel and Bandurski 1990) and then inserted into agar containing the CFDA.

Carboxyfluorescein transport

Carboxyfluorescein diacetate (22 μ M; CFDA, Molecular Probes Inc., Eugene, OR) was presented to the base or tip of an excised shoot as indicated in the text in 2 ml of 1% agar containing 10 mM potassium phosphate (KPi), pH 5.5 and 8.2 μ M IAA. The probe was administered to the cut shoot as described elsewhere for Light Green (Epel and Bandurski 1990).

At the indicated time intervals, shoots were removed from the agar and 5 mm of the apex and base discarded. The shoots, generally 3 per sample, were dissected into 10 mm long sections as indicated (Fig. 1). Primary leaves were removed from the coleoptile. The nodal region consisted of the node plus about 8 mm of the mesocotyl and about 1 mm of the coleoptile base. The

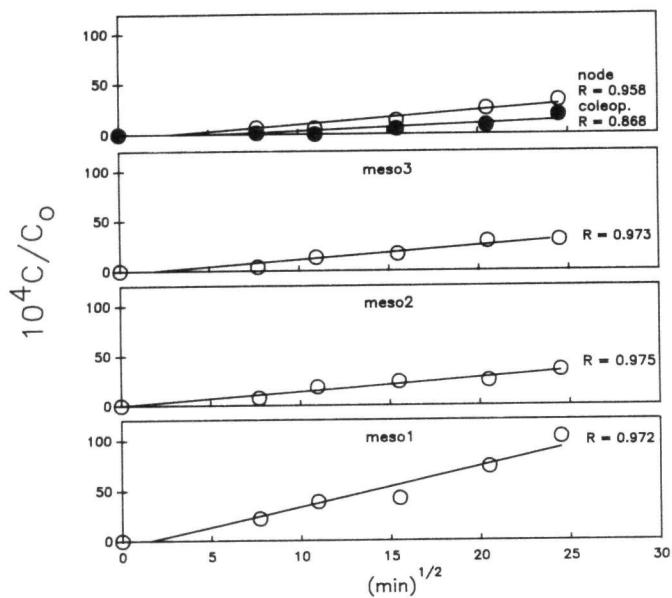


Fig. 2. Kinetics of translocation and accumulation of CF into mesocotyl sections, meso 1, meso 2, and meso 3, into node and into coleoptile sections of the shoot (see Fig. 1A) when CFDA was fed to the mesocotyl base and relative CF accumulation per g fresh weight (C/C_0) plotted as function of the square root of time, where C is mmol CF per g fresh weight and C_0 concentration of CFDA in the agar donor block. Lines are drawn as first order linear regressions with R being the square root of the coefficient of determination.

three contingent 10-mm sections of the vascular stele of the mesocotyl are denoted from the base upward as Stele 1, 2, and 3, while the three contingent 10-mm sections of the mesocotyl cortex plus epidermis, respectively, from the base upward are designated Cortex 1, 2, and 3 (Fig. 1B). Analogously, 10-mm sections of mesocotyl with stele included are designated Meso 1, 2, and 3, respectively, from the base upwards (Fig. 1A). Each sample was weighed and frozen until extraction and assay of the probe.

In order to extract CF for fluorimetric assay, the frozen samples were ground in 70% aqueous ethanol containing 10 mM KPi buffer, pH 8.0, since pH 8.0 is the optimum for CF fluorescence. The ethanol was used to denature proteins and nucleic acids in the extract and to precipitate them from the extract and to thus get a clear non-turbid extract which could be assayed spectrofluorimetrically. Extracts were clarified by centrifugation at 13 000 g for 3 min. CF was assayed spectrofluorimetrically with excitation at 460 nm and emission at 520 nm in a Perkin Elmer 650-40 spectrophotometer. A freshly prepared standard solution of CF was used to calibrate the measurement. All experiments were in duplicate and were repeated at least three times.

In order to determine whether CFDA moved appreciable distances in the apoplast and thus was present at detectable levels in the regions extracted, the following control was performed. CFDA was applied to shoots at the cut mesocotyl base for 4 h as described above. The stele and cortex where then separated as described

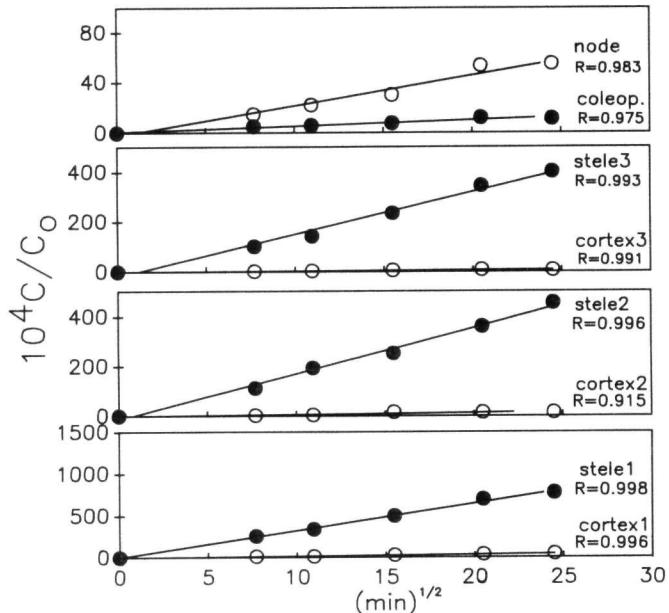


Fig. 3. Kinetics of translocation and accumulation of CF into mesocotyl-stellar sections stele 1, 2, and 3, mesocotyl-cortical sections cortex 1, 2, and 3 and into nodal section, node and coleoptile section, coleop. of the shoot (see Fig. 1B). CF was applied as the diacetate ester CFDA to the mesocotyl base. CF accumulation plotted as in Fig. 2.

above and either homogenized in 70% ethanol containing 10 mM KPi buffer pH 8.0 as described above or in 50 mM KPi buffer pH 8.0 without ethanol. The homogenate, prepared in the absence of the denaturant 70% ethanol, was incubated for 1 h at 25 °C in order to hydrolyze any CFDA which may be present in the apoplast. No CFDA was detected in the tissue.

Results

The kinetics of the uptake and transport of CF when applied as the diacetate ester to the mesocotyl base were linear when plotted as a function of the square root of time (Fig. 2). Thus, the kinetics of symplastic movement were that of simple diffusion. If the stele and cortex were separated and the concentration along the cortex and the stele determined, a much higher specific activity of CF was found in the stele than in the cortex (Fig. 3). No free CFDA was detected either in the stele or in the cortex. Fluorescence microscopy revealed that the CF behaved very similarly to fluorescein (Schumacher 1933) and moved mainly in the phloem tissue. Similar diffusion-type kinetics were obtained for CF when it was applied to the cut coleoptile (Fig. 4).

The relative distribution profile of this symplast probe along the axis of the seedling (the relative amount of CF per 10 mm section $\text{min}^{-1/2}$) for the case where it was applied to the cut mesocotyl base is presented in Fig. 5. At the node, there was an apparent reflectance pattern of the probe suggestive of a rate limiting step or partial barrier to the movement of the probe between the mesocotyl and coleoptile (Jacobs 1967). More direct

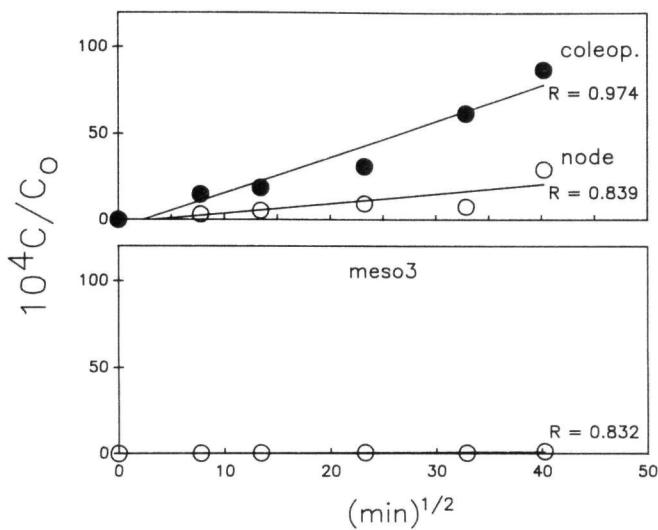


Fig. 4. Kinetics of translocation and accumulation of CF into coleoptile section and node section when CFDA was applied to cut apex of coleoptile (see Fig. 1A). CF accumulation plotted as in Fig 2.

evidence for a partial symplastic barrier at the node was provided by experiments in which CFDA was fed to the apex of shoots cut either 2 mm above node (+ node) or 2 mm below the node (- node), and the amount of CF uptake was measured in a 30-mm mesocotyl section taken 10 mm below the cut apex. In the absence of the node, uptake was more than 200% greater than in the presence of the node (Fig. 6).

Although the concentration of CF in the cortex was low relative to the stele, it was present in appreciable amounts considering that the mass of the cortex is 8 times that of the stele. We thus asked whether the presence of CF in the cortex was due to (a) cell to cell symplastic longitudinal transport within the cortex, or (b) longitudinal symplastic transport in the stele followed by lateral symplastic transport out of stele into cortex. To answer this question we performed the following experiment. The stele was pulled from seedlings and CFDA was applied to the base of the steleless

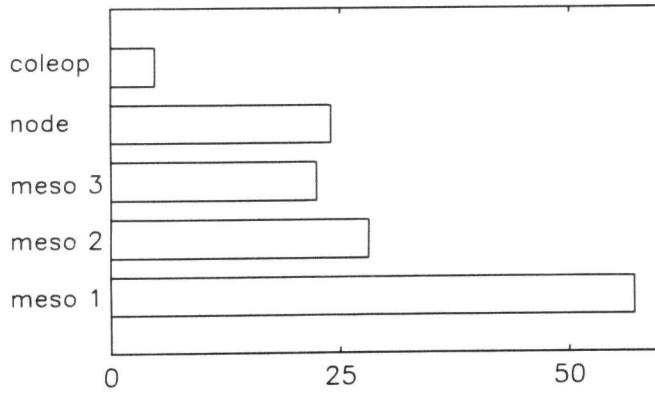


Fig. 5. Distribution coefficient (slope of curves in Fig. 2, $C_x/C_0 t^{-1/2}$) for CF along the axis of the seedling (see Fig. 1A). CFDA applied to a cut mesocotyl base.

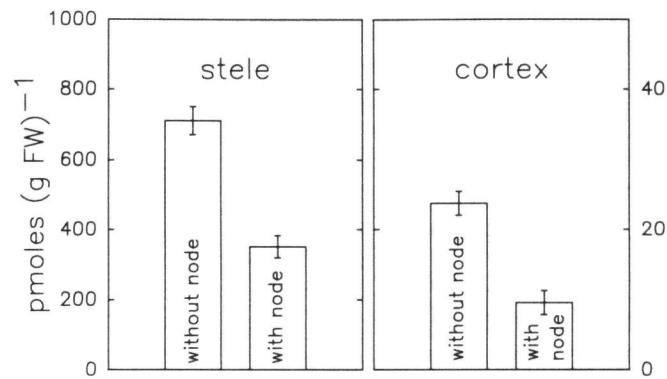


Fig. 6. CF uptake into a 30-mm mesocotyl section taken 10 mm below the cut apex following application for 4 h of CFDA to the apex of shoots cut either 2 mm above the node (with node) or 2 mm below the node (without node).

mesocotyl. Thus, the movement of the CF in the cortex could only occur symplastically via cortical and/or epidermal cells.

The relative distribution of the symplast probe CF following the application of CFDA to the mesocotyl base of seedlings with and without the vascular stele is presented in Fig. 7. Although CF moved longitudinally in the cortex even in the absence of the vascular stele, the relative distribution along the length of the tissue decreased much more rapidly in the absence of the stele than in its presence. These data indicated that the main route for longitudinal transport was the stele and that in an intact shoot, CF was translocated into the cortex by lateral symplastic movement from the stele.

Discussion

Our data with CF indicate the existence of a number of different sub-symplastic domains within the corn shoot. The node apparently acts as a partial barrier to the movement of the probe CF between the coleoptile and the mesocotyl. Although this barrier does not prevent the movement of the probe from one domain to the other, it limits the rate of movement and, thus, func-

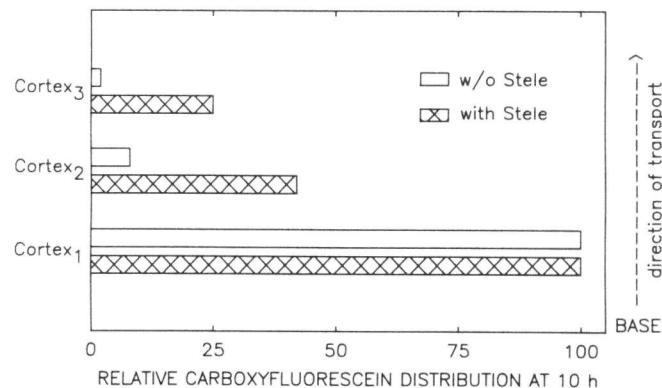


Fig. 7. Relative distribution of CF along the axis of the shoot in the cortex/epidermis (see Fig. 1C) following application of CFDA for 10 h to the mesocotyl base of shoots with or without (w/o) mesocotyl stele.

tionally creates two symplastic sub-domains. Thus, in the region adjacent to the node, between the source and the node there is a build-up of translocant, CF. Distal to this kinetic barrier, there is a large drop in concentration. In the absence of the node, longitudinal transport is doubled. This barrier is apparently due, at least in part, to the presence of much shorter cells in the nodal region (Iino 1982), resulting in the presence of more diffusional barriers per unit path length. This barrier could also be due, in part, to a decrease in concentration of plasmodesmata at the interface between the two sub-domains (most probably the node) or be due to a regulation of plasmodesmatal conductance. At present, experimental evidence with respect to these latter suggestions is unavailable.

A partial kinetic barrier to symplastic movement apparently also exists between the mesocotyl cortex and the mesocotyl stele. It should be emphasized that there is symplastic communication between the two tissues as is demonstrated in the experiments shown in Fig. 7. The large displacement from equilibrium between the two tissues (Fig. 3) may be due, in part, to the difference in the number of barriers presented to the probe in longitudinal versus lateral diffusional translocation. Firstly it should be pointed out that longitudinal symplastic translocation in the stele is kinetically more favorable due to the presence of very long sieve tube elements which present much fewer diffusional barriers than the shorter cells found in the cortex. Furthermore, the cortical cells have much less cytoplasm and, thus, their diffusional cross section is reduced. Hence, longitudinal symplastic diffusion in the phloem is kinetically the path of least resistance. Lateral symplastic diffusion, in contrast, exhibits the greatest number of diffusion barriers. This is a consequence of cell geometry, since lateral cell dimensions are much smaller than longitudinal dimensions. It must be kept in mind that the lateral diffusional barrier may also be due, at least in part, to a lower density of plasmodesmata on the lateral walls relative to the end walls. This latter point needs to be investigated with special attention to cells at tissue interfaces, such as the pericycle and the endodermis. Another possibility which must be considered is that between the two tissue or cell types there may be a down regulation of plasmodesmatal conductance.

Symplastic domains have been reported in studies made with *Egeria densa* using fluorescein conjugates of varying size, which were microinjected into a cell in a given tissue and the movement from cell to cell and tissue to tissue followed (Erwee and Goodwin 1985). Cell to cell communication via the symplast was demonstrated between cells within the cortex or within the epidermis of either the root or the stem. However, a barrier to dye movement was found between the epidermis and the cortex in both tissues. Barriers were also found at the nodes between expanding internodes. In the root tip, a barrier to the movement of dye was found between the root cap and the remainder of the root.

The barriers were not due to the lack of plasmodesmata between cells as plasmodesmata were found linking all cell types. This lack of symplastic communication may be explained by the findings of Erwee and Goodwin (1983, 1984), Baron-Epel et al. (1988), and Tucker (1988) that plasmodesmatal conductance can be regulated.

It is suggested that the existence of symplastic domains and sub-domains may be of major importance in determining patterns of growth and differentiation. The existence of symplastic domains will determine the patterns of translocation of growth regulators and of nutrients and at interfaces between sub-domains there could be large gradients in translocate concentration with resultant differential effects on growth and development. If plasmodesmatal conductance could be differentially regulated in some manner, then patterns of translocation could be altered. We now have evidence for such a situation: we find that light, through the phytochrome system, reversibly regulates symplastic communication between adjacent tissues (B. L. Epel and M. Erlanger, personal communication).

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