Protoplasma 113, 193–201 (1982)

PROTOPLASMA

Translocation in the Staminal Hairs of *Setcreasea purpurea*. I. A Study of Cell Ultrastructure and Cell-to-Cell Passage of Molecular Probes

E. B. TUCKER*

Department of Biology, Vassar College, Poughkeepsie, New York

Received December 22, 1981
Accepted in revised form June 22, 1982

Summary

Investigations into plant intercellular communication were initiated through an examination of plasmodesmata and cell-to-cell passage of molecular probes in the staminal hairs of *Setcreasea purpurea*. Plasmodesmata connecting staminal hair cells of small buds are filled with an electron-opaque homogenous material. To examine the permeation selectivity of plasmodesmata, molecular probes made up of fluorescein isothiocyanate (FITC) complexed with amino acids and peptides were injected into the staminal hair cells and the spread of these fluorescent molecules through the symplast, was monitored. Molecules composed of FITC complexed to single amino acids with polar and aliphatic R groups travel rapidly, while those which include peptides travel slowly. Dye molecules composed of an amino acid with an aromatic side group do not pass from cell to cell at all. It is hypothesized that the material occluding the plasmodesmata constitutes the diffusion barrier, by presenting a hydrophilic environment which allows passage of molecules with maximum molecular weights of 700-800 daltons, but which retains those with aromatic side groups.

Keywords: Intercellular communication; Plasmodesmata; Symplastic transport; *Setcreasea purpurea*.

1. Introduction

Extensive studies of cell-to-cell communication via permeable cell junctions have been reported for many animal systems (for review see DE MELLO 1977, LOEWENSTEIN 1977, EVANS 1980). It has been suggested that direct cell-to-cell communication via these junctions plays a crucial role in both normal cell growth and differentiation (LOEWENSTEIN 1968, FURSHPAN and POTTER 1968). In contrast, studies of direct communication between plant cells and the extent to which such communication may be involved in normal growth and development have only recently been initiated. VAN WENT *et al.* 1975, TYREE and TAMMES 1975 and MOGENSEN 1981 used uranin (disodium fluorescein) to study intercellular movement in plant tissues. These researchers reported that uranin was translocated through the staminal hairs of *Tradescantia virginica* and into ovules and/or embryos of *Nicotiana tabacum*, *Polygonum capitatum* and *Torenia fournieri*. GOODWIN (1976) reported intercellular passage of the anionic dyes Procion Yellow and Procion Brown between *Elodea* leaf mesophyll cells. More recently the same author injected FITC labelled peptides into leaf tissue of *Elodea canadensis* and found that of the compounds tested, those of m.w. 665 and less passed freely from cell to cell, while that of 749 had very restricted movement (GOODWIN 1981). It was concluded that for *Elodea*, there is an intercellular passage exclusion limit of 800 daltons.The work reported in this present communication extends these studies to provide additional information on what properties, *i.e.*, size, charge or chemical nature, limit the movement from cell to cell. In plants, the most likely pathway for direct cell-to-cell movement of molecules are the plasmodesmata (for review see ROBARDS 1976), fine structural elements which connect neighboring cells at their areas of contact. Plasmodesmata vary greatly in size and structure, but in higher plant tissue, a desmotubule – which

* Correspondence and Reprints: Department of Biology, Vassar College, Poughkeepsie, NY 12601, U.S.A.

0033-183X/82/0113/0193/$ 01.80

is assumed to be connected to endoplasmic reticulum – is usually present. This tube, presumably derived from endoplasmic reticulum, may be open or occluded, and it may contain a median nodule or a central rod. The space between the plasma membrane and desmotubule may be continuous with the cell cytoplasm or, if the plasma membrane and desmotubule fuse to form a seal in the neck region, this space may be isolated. Although this plasmalemma-lined desmotubule-containing plasmodesmata is the "typical type", those containing no desmotubules have been reported (ROBARDS 1976, p. 48). In these "typical type" plasmodesmata the precise space through which organic molecules migrate from one cell to another is unknown, but one may speculate that it is the central region of the desmotubule, or between desmotubule and plasma membrane, or both.

In the present report, staminal hairs of of *S. purpurea* were chosen to study the passage from cell to cell of fluorescein-tagged molecular probes. The linear arrangement of cells of these hairs provide for the visual determination of the direction and transfer time of molecular probe movement between cells.

Using FITC, fluorescein can be complexed to amino acids and peptides, thus providing fluorescent molecules of various molecular weights, charges and steric configurations. Such FITC fluorescent probes were chosen because they are hydrophilic, do not significantly permeate nonjunctional animal cell membranes, are not degraded inside the cell, are non-toxic and do not bind significantly to the cytoplasm of animal cells (LOEWENSTEIN 1979). GOODWIN (1981) reported that these compounds could not pass the plasmalemma of *Elodea* leaf cells nor be degraded by *Elodea* leaf tissue homogenates. The cytology and ultrastructure of the staminal hair cell of *S. purpurea* are presented as well as data on molecular probe passage as determined by microinjection, fluorescent spectroscopy and low-level light detection, which allow determination of the intercellular permeation limits.

2. Methods and Materials

2.1. Electron Microscopy

Pots of *S. purpurea* synonym *S. pallida* were maintained in a greenhouse which had an average daily temperature of 25.7 °C and natural lighting. Stamens with hairs were removed from small unopened buds and fixed in a modified Karnovsky's solution (KARNOVSKY, 1965) containing 1% formaldehyde, 2.5% glutaraldehyde, 0.1 M sodium cacodylate (pH 7.3), 0.005% $CaCl_2$ and 2.5% sucrose before being postfixed in 1% OsO_4 and embedded in Spurr resin (SPURR 1969). Sections of staminal hairs were stained with 7.5% uranyl acetate and lead stain (SATO 1968) before being observed with a JEOL JEM 100B electron microscope. For studies using interference microscopy, the hairs of freshly removed stamens were observed with an Olympus BH microscope equipped with Nomarsky optics. Photographs of this material were used for calculating the average thickness of the cytoplasm.

2.2. Fluorescent Tracer Studies

Fluorescein probes were synthesized following the method reported by SIMPSON (1978). Ten mg FITC (Sigma Chem. Co., St. Louis, Mo.) dissolved in 1.0 ml acetone was added to a solution containing 1.25×10^{-5} mole amino acid or peptide (Sigma Chem. Co.) and 20 mg $KHCO_3$ in 9.0 ml H_2O. This solution was held in the dark at room temperature for 48 hours after which it was lyophilized. The powder was redissolved in a small amount of water and applied as a streak to Whatman 3 MM paper. The yellow product which separated out from the orange hydrolyzed dye during descending chromatography, using 0.05 N $KHCO_3$ as the eluant, was collected and lyophilized. The purity of the yellow product was tested by spot descending chromatography (Whatman paper # 1, 0.05 N $KHCO_3$) and by paper electrophoresis (Whatman paper # 3, 0.05 N NH_4HCO_3). If fluorescent impurities remained, they were removed by re-chromatographing and by preparative paper electrophoresis. R_f values and migration in an electrical field relative to uranin, were used to characterize and help identify a probe.

The tips of respective micropipettes were loaded with aqueous solutions of fluorescent probes of various molecular weights and containing amino acids of differing solubility and steric configuration (Table 1). A micromanipulator was used to carefully impale a cell with the loaded micropipette. Upon entrance of the tip into the cytoplasm and vacuole of a specific staminal hair cell from these young buds, a small volume of the fluorescent probe simply flowed in. A Leitz Orthoplan fluorescent microscope, equipped with a SIT television camera containing an image intensifier (Dage-MTI, Inc., Michigan City, In.) and connected to a Panasonic Omni Vision II video tape system and television screen was used to monitor the movement of the injected probe through the symplast. The effect of residual $KHCO_3$ and NH_4HCO_3 in the probe solution is probably insignificant because the image intensifier system allows detection of and thus microinjection of infinitesimal volumes. SIMPSON *et al.* 1977 and ROSE *et al.* 1977 did not find it necessary to remove $KHCO_3$ from probes used in their permeability studies.

3. Results

3.1. Cytology and Ultrastructure

Floral buds of *S. purpurea* 11.36 ± 1.0 mm long by 3.58 ± 0.4 mm wide, containing an average of 7.1 staminal hairs, 10.16 ± 8.0 mm long on each of the 6 stamens, develop within 24 hours to a mature bud which undergoes anthesis to senescence within 6 hours. The staminal hair cells of these immature buds, which were used in all the studies reported in this communication, are composed of a large vacuole surrounded by a layer of cytoplasm around 0.97 μm thick (Figs. 1 *A* and *B*). Larger amounts of cytoplasm (0.62 μm to 1.7 μm) are observed in the area of connecting cell walls and

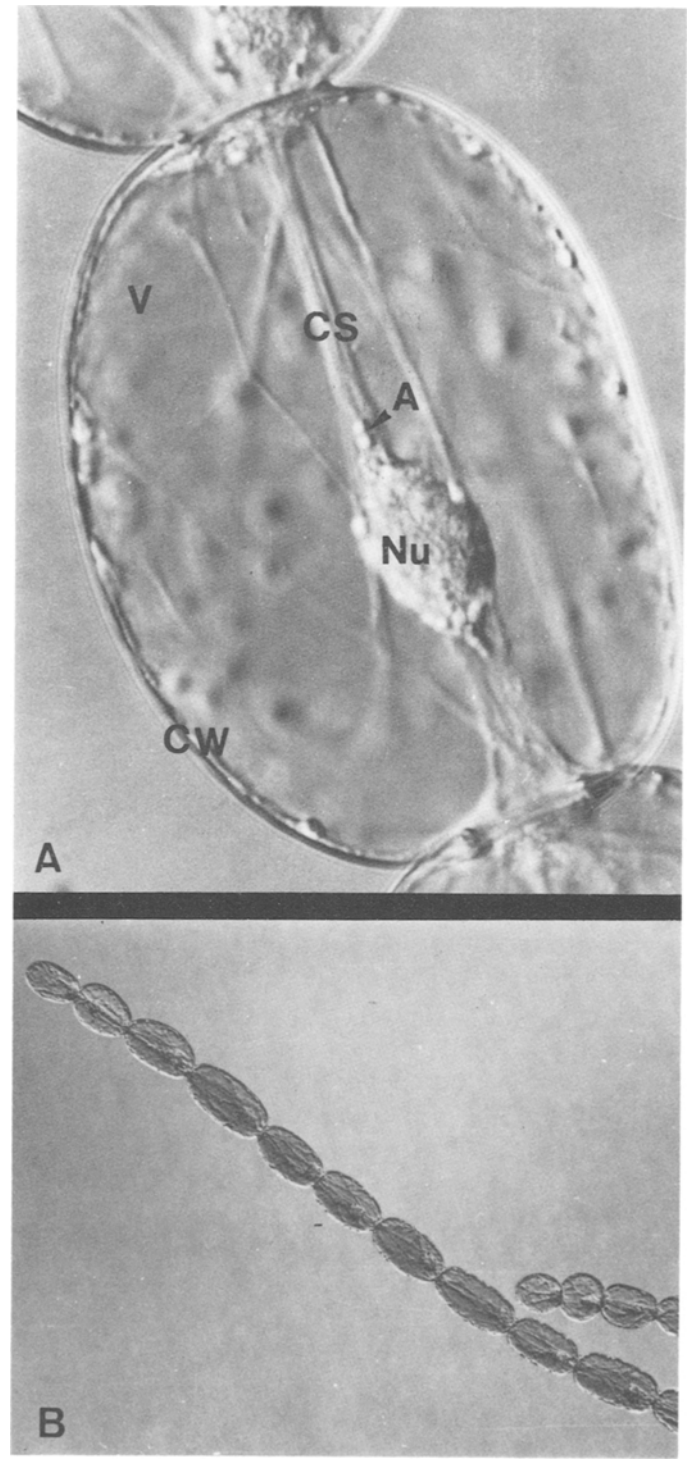

Fig. 1. Light micrographs obtained from interference optics microscopy of *Setcreasea purpurea* (*A*) staminal hair cell ×250. *A* amyloplast, *CS* cytoplasmic strand, *CW* cell wall, *Nu* nucleus, *V* vacuole and (*B*) staminal hair, ×25. Photograph taken by D. O'LEARY

around the nucleus while strands 2.3 μm average diameter traverse the vacuole. The average number of cells per hair was 15.25 and they ranged from a length of 0.83 to 0.33 mm (Fig. 1 *B*). Only cells midway along the hair which varied in length between 0.50 to 0.75 mm were used in these studies.

The staminal hairs of *S. purpurea* are difficult to fix which is partially due to a lack of fixative penetration through the cuticle and outer cell wall into the protoplasm. When a stamen in placed in a pool of fixative, cytoplasmic streaming is observed to occur in the hair cells until the fixative enters the most basal hair cell. Cytoplasmic streaming then stops in this and successive cells proceeding towards the tip of the hair. Good fixation in terms of an intact tonoplast, absence of myelin figures and distinct mitochondrial cristae was obtained when the "Karnovsky" buffer (modified to contain 0.005% instead of 0.05% $CaCl_2$) was used. GLAUERT (1975) suggests that the addition of calcium decreases the extraction of membrane lipids during tissue preparation for electron microscopy.

The ridged cell wall surrounds the cytoplasm which contains a nucleus, mitochondria, proplastids, amyloplasts, smooth and rough endoplasmic reticulum, dictyosomes, microfilaments, microbodies and a large vacuole (Figs. 2 and 3 *A*). The layer of electron opaque material which covers the cell walls is thought to be cuticular in nature. The plasmodesmata between adjacent cells are plasmalemma-lined and appear occluded with densely staining material (Figs. 3 *B* and *C*). Plasmodesmata are cylinders 37.6 ± 10 nm in diameter by 288.8 ± 100 nm long and they are surrounded by a cylinder of material 29.8 ± 15 nm in thickness which stains less dense than cell wall material. Amyloplasts are round to oval in shape containing one or two large starch granules in their stroma and may contain a membrane bound intraplastid body (Fig. 3 *A*). These starch containing organelles are very numerous in staminal hair cells of young buds (see Fig. 1 *A*) and can be observed under the light microscope to move rapidly through strands of cytoplasm. The large vacuole is surrounded by a tonoplast and often contains densely staining granular material.

3.2. Molecular Probe Passage

Aqueous injection solutions of FITC $(Glu)_2$, FITC $(Met)_2$, FITC $(Ser)_3$ and FITC $(Gly)_6$ were mixed for 3 hours at room temperature with stamen homogenates. Samples of these mixtures were chromatographed and electrophoresed along with the respective untreated probe solutions. In all cases the homogenate-probe mixtures migrated at the same rate as the untreated probe. These results indicate that FITC-peptide complexes are not degraded in *S. purpurea* cells.

The results of at least 15 to 20 injections each of fluorescein labelled molecular probes are listed in Table 1. When a probe which passes from cell to cell was injected into the vacuole and cytoplasm of a cell, it passed bi-directionally into the cytoplasm of its sequential neighbors (Figs. 4 and 5). Three classes of molecule can be described: rapidly passing (*e.g.*, Fig. 4), those probes which were noted in their third neighbor 2–3 minutes after injection; slowly passing, those probes which were noted in their third neighbor 5–8 minutes after injection; not passing, those probes which either remained in the injected cells or were seen only in their first neighbor.

Fluorescein isothiocyanate (FITC) molecules containing amino acids with polar R groups (Ser, Cys, Asp, Gln, His, Lys) and alipatic R groups (Met, Leu) travel rapidly through the symplast. With an increasing number of amino acids in the side chain, [*e.g.*, $(Glu)_2$, $(Met)_2$, Arg-Asp, $(Ser)_3$, $(Ala)_6$] the passage becomes slower. In fact, in a percentage of cases (see Table 1) these probes would not pass at all. An exception to this is FITC $(Gly)_6$ which passes rapidly. If the amino acids containing an aromatic ring are complexed to FITC [*e.g.*, Phe, $(Phe)_3$, $(Phe)_4$, Tyr, $(Tyr)_3$, Trp, $(Trp)_2$], the synthesized probe will not pass. When one of these aromatic amino acids is part of any fluorescein dipeptide probe (*e.g.*, Ala-Phe, Ser-Phe, Met-Phe, Trp-Leu), passage no longer takes place.

4. Discussion

An ultrastructural examination of staminal hair cells of *S. purpurea* was performed concomitant with studies of cell-to-cell molecular probe passage to attain a clearer understanding of translocation in these cells. The fine structure of these cells is similar to that reported by VAN WENT *et al.* 1975 for another member of the *Commelinaceae*, *T. virginica*. For example, the depth of cytoplasm at the cross walls in *S. purpurea* is 0.62–1.7 μm while in *T. virginica* it is an average of 0.9 μm. The hair cell vacuoles of both species contains densely staining granular material on electron micrographs. Both species have plasmodesmata which are surrounded by an electron translucent ring and are occluded with electron-opaque material. A noticeable contrast is that the amyloplasts found in *S. purpurea*,

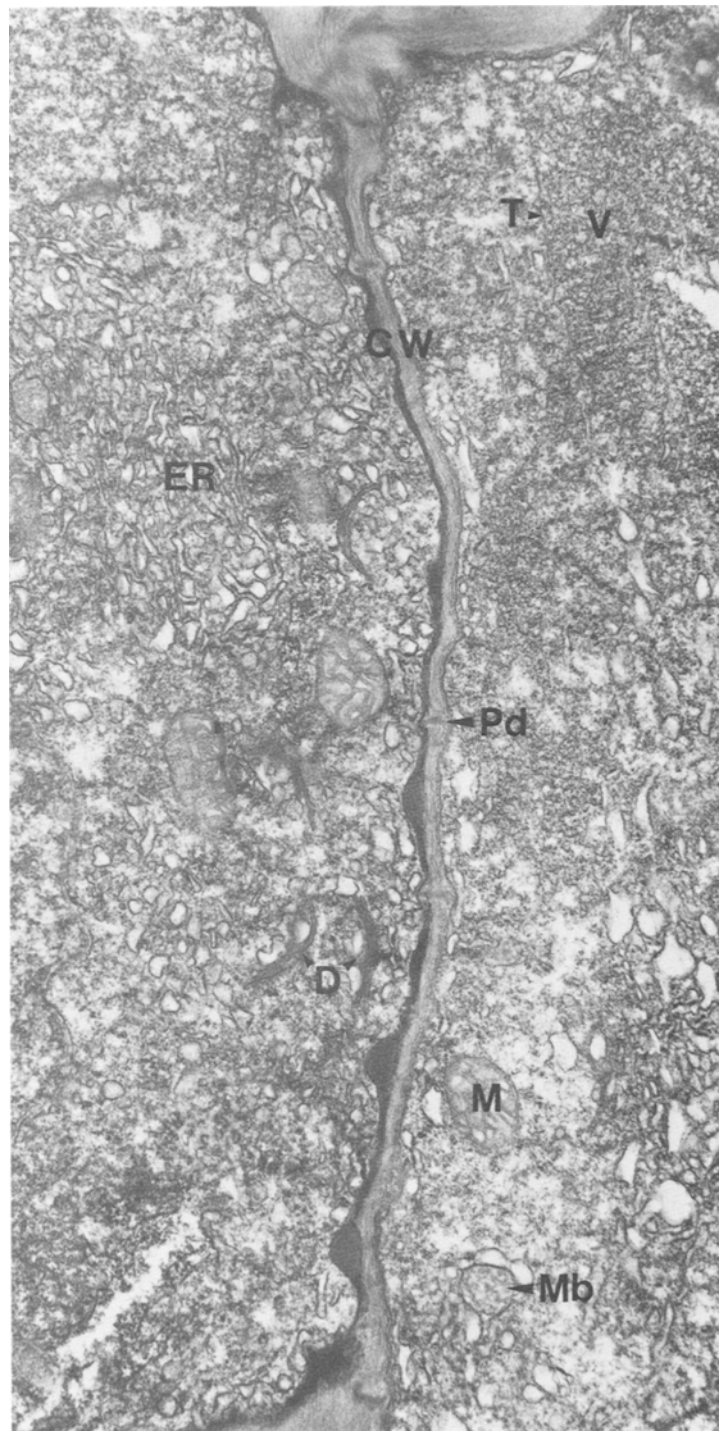

Fig. 2. Electron micrograph of staminal hair cell protoplasm and interconnecting cell wall area, ×15,000. *CW* cell wall, *D* dictyosomes, *ER* endoplasmic reticulum, *M* mitochondrion, *Mb* microbody, *Pd* plasmodesma, *T* tonoplast, *V* vacuole

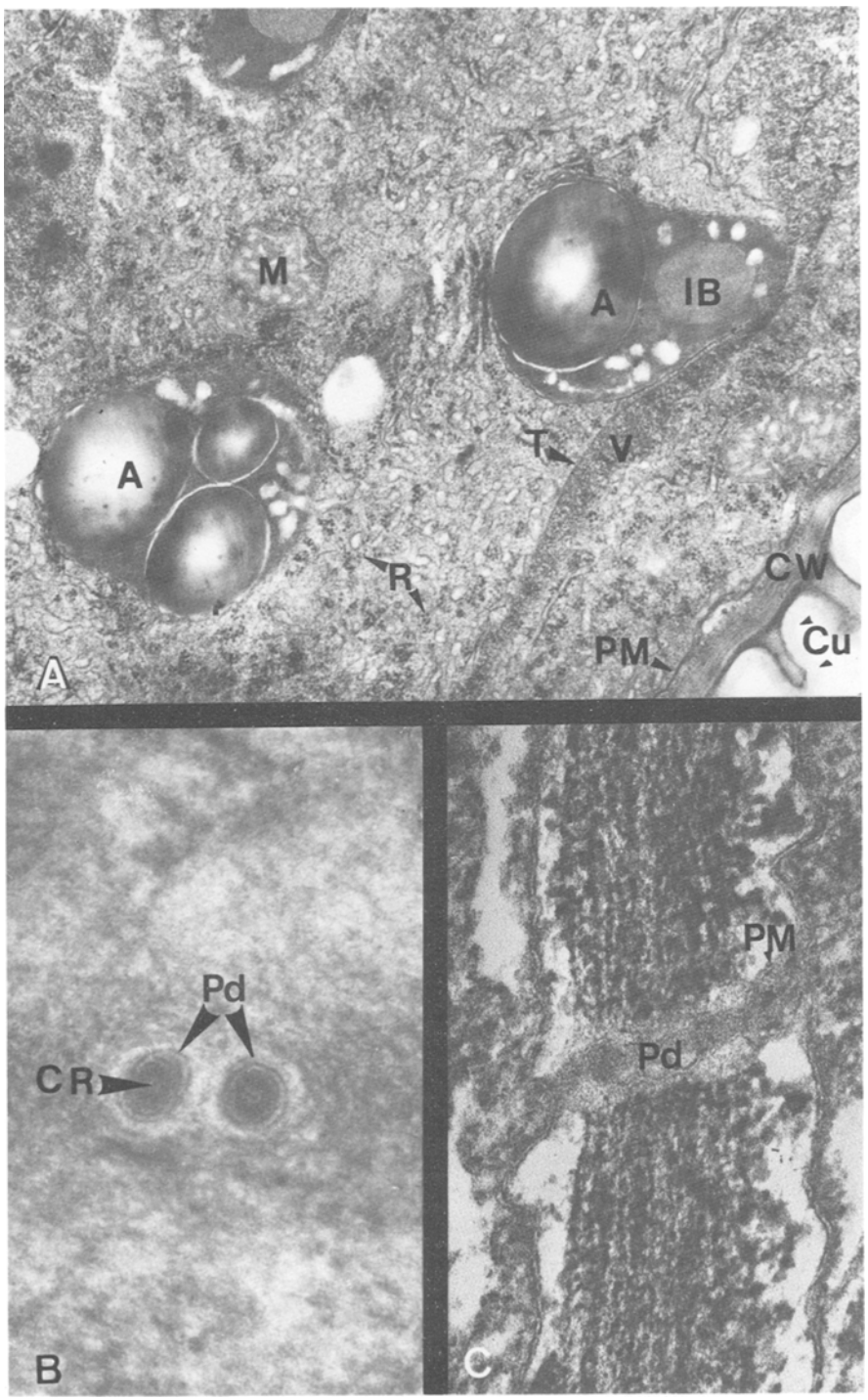

Fig. 3. (*A*) Staminal hair cell cytoplasm in the region along the outer cell wall, × 30,000 (*B*) Cross section, × 245,000 and (*C*) longitudinal section, × 136,000 of plasmodesmata. *A* amyloplast, *CR* central rod, *Cu* cuticle, *CW* cell wall, *IB* intraplastid body, *M* mitochondrion, *Pd* plasmodesma, *PM* plasmalemma, *R* ribosomes, *T* tonoplast, *V* vacuole

Table 1

Molecular probe	Molecular weight	Polarity[1]	R_f	Migration[2]	Passage[3]
1. Disodium Fluorescein	376	P	0.619	1.00	+ +
2. Serine	494.40	P	0.621	1.20	+ +
3. Cysteine	510.50	P	0.531	1.13	+ +
4. Asparagine	521.40	P	0.605	1.21	+ +
5. Glutamine	535.40	P	0.600	1.15	+ +
6. Lysine	535.50	P_i	0.530	0.63	+ +
7. Methionine	538.50	N	0.628	1.21	+ +
8. Histidine	544.50	P_i	0.502	1.08	+ +
9. Phenylalanine	554.59	N_a	0.589	1.11	0
10. Tyrosine	570.59	$P_{a,i}$	0.532	1.09	0
11. Tryptophan	593.59	N_a	0.389	0.85	0
12. Ala-Phe	625.60	P-N_a	0.614	1.03	0
13. Ser-Phe	641.60	P-N_a	0.640	1.05	0
14. $(Lys)_2$	663.60	P_i	0.550	0.19	+ 38
15. $(Glu)_2$	665.59	P_i	0.920	1.96	+ 80
16. $(Ser)_3$	668.60	P	0.621	1.06	+ 23
17. $(Met)_2$	669.79	N	0.688	1.07	+ 65
18. Arg-Asp	678.69	P_i-P	0.421	0.52	+ 78
19. Met-Phe	685.70	N-N_a	0.627	1.04	0
20. Trp-Leu	706.79	N_a-N	0.565	0.85	0
21. Trp-Phe	740.79	N_a-N_a	0.468	0.77	0
22. $(Leu)_3$	746.99	N	0.824	1.17	0
23. $(Gly)_6$	749.99	P	0.657	1.06	+ +
24. $(Trp)_2$	779.79	N_a	0.458	0.69	0
25. $(Ala)_6$	833.90	P	0.755	1.03	+ 90
26. $(Phe)_3$	848.99	N_a	0.566	0.85	0
27. $(Tyr)_3$	896.99	N_a	0.482	0.71	0
28. $(Phe)_4$	996.19	N_a	0.490	0.65	0

[1] P = polar, N = non-polar, subscript a = aromatic, i = ionic.
[2] Migration of probe (mm)/migration of disodium fluorescein (mm).
[3] + + = rapid, + = slow, 0 = no movement, Number = percentage of injections where passage occurred.

which often contain two starch granules and an intraplastid body, are absent from the electron micrographs published for *T. virginica* staminal hair cells. An electron opaque covering on cell walls referred to by VAN WENT *et al.* (1975) as cuticle is observed in both species. Cuticle is expected on the *S. purpurea* cells used in these studies (hairs on stamens 1 day pre-anthesis) since its formation on the same cells in *Tradescantia sp.* begins about 10 days prior to anthesis (MERICLE and HAZARD 1980). Furthermore, upon the eventual wilting of *S. purpurea* flowers an outer layer of material is observed which is similar in appearance under the light microscope to that described by ITERSON (1937) and ROELOFSEN and HOUWINK (1951) and referred to by these authors as cuticle.

The average diameter of the plasmodesmata (*i.e.*, the presumed permeation channel) bound by the plasmalemma in *S. purpurea* staminal hair cells is 376 Å which is approximately 20 times larger than the hydrophilic pore of a gap junction (SCHWARZMANN *et al.* 1981), and thus should be large enough to allow passage of large macromolecules. However, our results suggest that in these staminal hair cells, the effective passageway must be much smaller since at least for anionic-hydrophilic molecules, the molecular weight limitation for cell-to-cell passage lies at 700–800 daltons. From space filling molecular models, probes of this weight have a size of around 14 Å (LOEWENSTEIN 1979). FITC $(Gly)_6$ with a molecular weight of 750 daltons may pass rapidly, because the peptide chain is long and thin. FITC

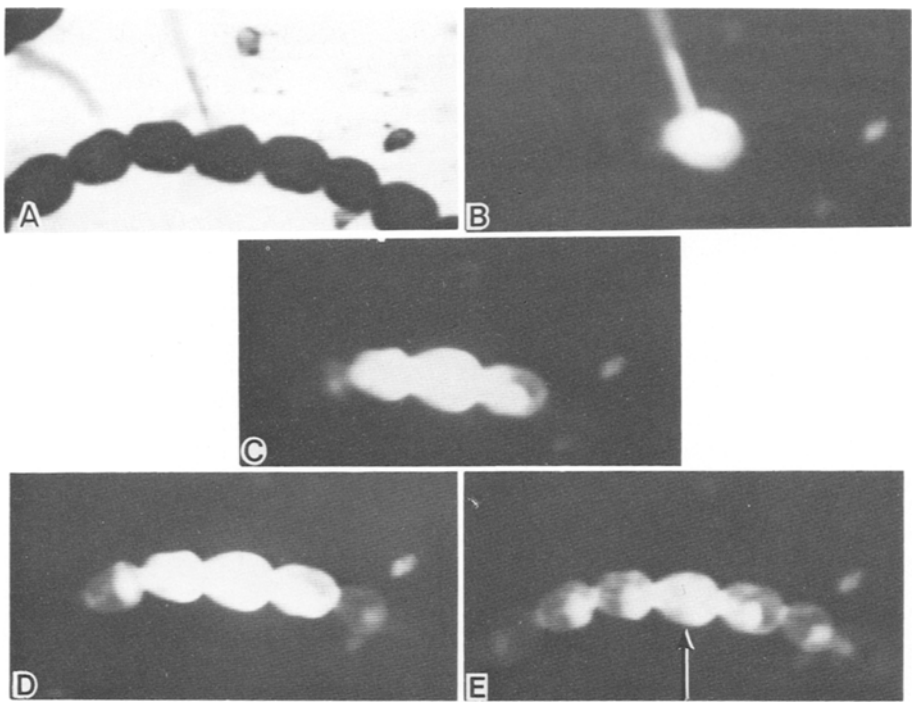

Fig. 4. Monitor image photograph illustrating fluorescent molecular probe injection and temporal passage through staminal hair of *S. purpurea*. Uranin intercellular passage (*A*) before injection, (*B*) at injection (0 time), (*C*) 30 seconds, (*D*) 1 minute, (*E*) 2 minutes. Photograph taken by J. CALVIN

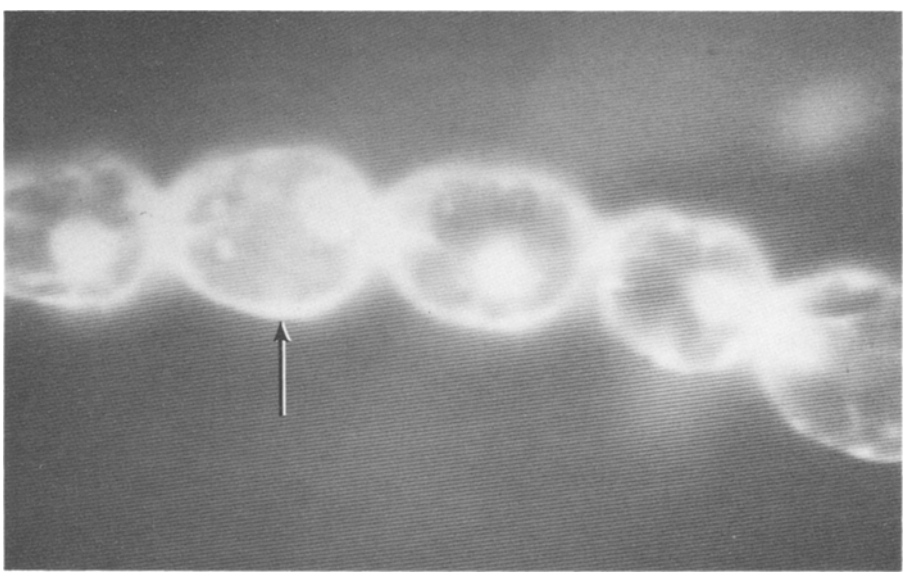

Fig. 5. Monitor image photograph illustrating fluorescence in symplast. Same hair as in Fig. 4, but five minutes after injection, under water and magnified 2.5 times. White arrow is used to identify the injected cell

molecules with molecular weights below 650 daltons but which are complexed to an aromatic amino acid will not pass at all.

Restriction to movement through plasmodesmata has been reported by SPANSWICK and COSTERTON (1967), SPANSWICK (1972) and TYREE and TAMMES (1975). Their conclusion that this is due to occlusion of plasmodesmata is supported by the results presented in this communication. In addition, the 700-800 dalton exclusion limit for anionic-hydrophilic molecules is precisely that reported by GOODWIN (1981) for *Elodea* leaf mesophyll cells. From our results we extend these conclusions and suggest that the rate of movement between cells of these molecules is dependent upon their size. Furthermore, certain other characteristics of a molecule – in *S. purpurea*, an aromatic amino acid complexed to FITC – will completely inhibit passage even though the molecular weight of this molecule is below the exclusion limit for the tested anionic-hydrophilic fluorescein probes.

Preliminary data indicate that qualitative passage of these fluorescent labelled probes microinjected into mature flowers, differs from that reported here for small unopened buds and thus we theorize that during development of the flower, cell to cell communication is altered. Studies of molecular probe passage through staminal hairs of flowers at various stages of development are presently being performed.

Acknowledgement

I thank Dr. BIRGIT ROSE for discussion, Dr. PATRICIA JOHNSON for critical review and ELIZABETH WILSON for technical assistance. The work was supported by NSF-URP grants, NSF-Equipment grant No. CDP-8011271 and a Research Corporation Cottrell College Science Grant.

References

DE MELLO, W. C., 1977: Intercellular Communication (DE MELLO, W. C., WALMOR, C., eds.). New York: Plenum Press.

EVANS, W. H., 1980: Communication between cells. Nature **283**, 521–522.

FURSHPAN, E. J., POTTER, D. D., 1968: Low resistance junctions between cells in embryos and tissue culture. Curr. Topics Devel. Biol. **3**, 95–127.

GLAUERT, A. M., 1975: Fixation, dehydration and embedding of biological specimens. In: Practical Methods in Electron Microscopy (GLAUERT, A. M., ed.), pp. 37, 41. New York: American Elsevier Pub. Co.

GOODWIN, P. B., 1976: Physiological and electrophysiological evidence for intercellular communication in plant symplasts. In: Intercellular Communication in Plants: Studies on Plasmodesmata (GUNNING, B. E. S., ROBARDS, A. W., eds.), pp. 121–129. New York: Springer.

– 1981: Short distance transport in the plant symplast. XIII. International Botanical Congress. Abstracts p. 35.

KARNOVSKY, M. J., 1965: A formaldehyde – glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**, 137 A.

LOEWENSTEIN, W. R., 1968: Some reflections on growth and differentiation. Perspectives in Biol. and Med. **11**, 260–272.

– 1977: Membrane Channels – intro remarks Symposium. Amer. Physio. Soc.

– 1979: Junctional intercellular communication and the control of growth. Biochim. biophys. Acta **560**, 1–65.

MERICLE, L. W., HAZARD, R. M., 1980: Stamen hair initiation and development in *Tradescantia*, clone 02. Environ. exp. Bot. **20**, 233–241.

MOGENSEN, H. L., 1981: Translocation of uranin within the living ovules of selected species. Amer. J. Bot. **68**, 195–199.

ROBARDS, A. W., 1976: Plasmodesmata in higher plants. In: Intercellular Communication in Plants: Studies on Plasmodesmata (GUNNING, B. E. S., ROBARDS, A. W., eds.), pp. 15–57. New York: Springer.

ROELOFSEN, P. A., HOUWINK, A. L., 1951: Cell wall structure of staminal hairs of *Tradescantia virginica* and its relation with growth. Protoplasma **50**, 1–22.

ROSE, B., SIMPSON, I., LOEWENSTEIN, W. R., 1977: Ca^{+2} ion produces graded changes in permeability of membrane channels in cell junction. Nature **267**, 625–627.

SATO, T., 1968: A modified method for lead staining of thin sections. J. Electron Microsc. **17**, 158–159.

SCHWARZMANN, G., WIEGANDT, H., ROSE, B., ZIMMARMAN, A., BEN-HAIM, D., LOEWENSTEIN, W. R., 1981: Diameter of the cell-to-cell junction membrane channels as probed with neutral molecules. Science **213**, 551–553.

SIMPSON, I., 1978: Labeling of small molecules with fluorescein. Analytical Biochem. **89**, 304–305.

– ROSE, B., LOEWENSTEIN, W. R., 1977: Size limit of molecules permeating the junctional membrane channels. Science **195**, 294–296.

SPANSWICK, R., 1972: Electrical coupling between cells of higher plants: A direct demonstration of intercellular communication. Planta **102**, 215–227.

– COSTERTON, J., 1967: Plasmodesmata in *Nitella translucens*: Structure and electrical resistance. J. Cell Sci. **2**, 451–464.

SPURR, A. R., 1969: A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43.

TYREE, M. T., TAMMES, P. M. L., 1975: Translocation of uranin in the symplasm of staminal hairs of *Tradescantia*. Can. J. Bot. **53**, 2038–2046.

VAN ITERSON, G., 1937: A few observations on the hairs of the stamens of *Tradescantia virginica*. Protoplasma **27**, 190–211.

VAN WENT, J. L., VAN AELST, A. C., TAMMES, P. M. L., 1975: Anatomy of staminal hairs from *Tradescantia* as a background for translocation studies. Acta. Bot. Neerl. **24**, 1–6.