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Cytoplasmic Streaming Does Not Drive Intercellular Passage in Staminal Hairs of *Setcreasea purpurea***

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Summary

The effect of inhibition of cytoplasmic streaming on intercellular passage of carboxyfluorescein (CF) in staminal hairs of *S. purpurea* was examined. Tip cells of staminal hairs were microinjected with buffered-CF. Cytoplasmic streaming was then inhibited by addition of KCN or NaN_3 to the external bathing solution. In separate experiments, cytoplasmic streaming was inhibited by microinjection of cytochalasin D along with the buffered-CF. CF passage over a 5 minutes treatment period was monitored by video fluorescence microscopy and video intensity analysis. Cytoplasmic streaming ceased within 1 minute of inhibitor agent treatment, however, little change in the kinetics of intercellular passage was noted over the 5 minute experimental period. Thus, cytoplasmic streaming plays no major role in the regulation of intercellular passage of the hydrophilic, negatively charged molecule CF.

Keywords: Intercellular transport; Cell-to-cell communication; Plasmodesmata; Cytoplasmic streaming; *Setcreasea purpurea.*

1. Introduction

In plants, intercellular passage involves the transfer of metabolites and nutrients through the symplast (OLESEN 1975, EVERT *et al.* 1977, MOGENSEN 1981, ERWEE *et al.* 1985), and is thought to be involved in both the regulation of developmental processes (CARR 1976, JUNIPER 1977, PALEVITZ and HEPLER 1985) and in the symplastic spread of viruses (GIBBS 1976). Intercellular passage was reported to be a process of simple diffusion; that is, the distance travelled by disodium fluorescein in staminal hairs of *Tradescantia* and trichomes of *Lycopersicon esculentum* was proportional to the square root of time (TYREE and TAMMES 1975, BARCLAY *et al.* 1982). Plasmodesmata constitute a diffusion barrier which allows passage of molecules with a maximum molecular weight of 700–800 daltons (GOODWIN 1981, 1983, TUCKER 1982). Cytoplasmic streaming is another fundamental process in plants. Although the molecular mechanisms underlying this process have been the subject of much study (ALLEN 1980, KAMIYA 1981), the function of the process remains elusive. However, some plant cell biologists speculate that streaming plays a role in cytoplasmic "stirring" (WOODS *et al.* 1984 a) and in intercellular passage (TYREE 1970, GUNNING and OVERALL 1983).

The major problem in studying intercellular passage has been the lack of quantitative methodologies. A method whereby even slight changes in passage can be measured, was used to determine that cytoplasmic streaming has little influence on intercellular passage of (CF) in staminal hairs of *S. purpurea*.

2. Methods and Materials

2.1. Plant Material

Pots of *S. purpurea* 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, secured to a microscope slide and microinjected with buffered-CF (see 2.3. below). Staminal hairs composed of cells. 50 ± 7 µmeters in diameter were used.

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** The work is dedicated to professor Saal Zalik, Department of Plant Science, University of Alberta, on his 65th birthday.

2.2. Measure of Intercellular Transport

The system developed to study kinetics is described by TUCKER and SPANSWICK (1985). Briefly, CF was microinjected into the nucleoplasm of the tip staminal hair cell (cell 1). The cell-to-cell movement of this fluorescent molecule was monitored in 5 sequential cells and video taped through a SIT television camera situated on a Leitz Orthoplan microscope. A model 321 video analyzer processed the signals. This information was stored on a diskette and then graphed using a Lotus (TM) program.

2.3. Treatment

Experiments were designed to note changes in intercellular passage over time for each respective treatment. The experimental sequence used was: (1) hair placed on microscope slide and covered with 0.2 ml water, (2) cell 1 impaled with the tip of a micropipette containing 50 mM CF in 0.1 M citrate pH 7.0 (buffered-CF simply diffused into the cell), (3) treatment (0.2 ml 10.0 mM NaN_3 or 1.0 mM KCN) applied 30 seconds after microinjection, (4) video taping continued for 5 minutes, (5) micropipette removed and experiment terminated. At least 20 trials for each treatment were performed.

Cytoplasmic streaming was also inhibited by cytochalasin D (CD) microinjected into the cytoplasm. This drug, dissolved in dimethyl sulfoxide (DMSO), was mixed with buffer-CF and microinjected into the nucleoplasm of cell 1. The mixture composition was: 0.25 mg CD in 5 µl DMSO in 100 µl buffered-CF.

3. Results

In untreated cells, CF permeated well past the 5th cell during the 5 minute experimental time. The steady increase in fluorescence intensity for the first 5 cells, as noted in Fig. 1, illustrates that the CF diffused through the symplast of staminal hairs in an unimpeded manner. Fluorescence was first observed in cell 2 (the first neighbor) about 4 seconds after it was injected into cell 1. Intensity rose exponentially in cell 2 and, with a lag, in cells 3, 4, and 5. The time required to reach a plateau was dependent upon the size of the cells (presumably the cytoplasmic volume), and the amount of dye which continued to diffuse from the micropipette into cell 1.

Cytoplasmic streaming stopped within 1 minute of the NaN_3 and KCN treatment. This inhibition was fully reversible: cytoplasmic streaming resumed within 5 minutes when water replaced the inhibitor solution. With CD treatment, cytoplasmic streaming was inhibited sequentially in cells progressively further away from the CD-buffered-CF microinjected cell. After the 5 minute treatment period, streaming was not detected for a distance of 6 or 7 cells distal from the injected cell. In these cells, the transvacuolar cytoplasmic strands remained intact. 5% DMSO-buffered-CF had no effect on streaming.

The kinetic curves for cells treated with KCN (Fig. 1 *b*), NaN_3 (Fig. 1 *c*) or microinjected with CD (Fig. 1 *d*), were similar to those of untreated cells. However, the curves contained fewer fluctuations of fluorescence intensity, and thus appeared smoother. This may reflect stationary cytoplasm or unregulated intercellular passage.

4. Discussion

Cytoplasmic streaming and its response to azide had been examined using high resolution video-enhanced light microscopy (TUCKER and ALLEN 1986). It was suggested that streaming in *S. purpurea* staminal hair cells (and probably other higher plant cells) is not bulk movement of cytoplasm as commonly presumed, but rather discrete movement of particles through a relatively stationary cytoplasm. Particles and organelles became stationary, that is, they stopped moving along well defined pathways when cells were treated with azide.

The effect of azide and cyanide on streaming was presumably due to depleted levels of ATP (HAYASHI 1960, SHIMMEN and TAZAWA 1983, WOODS *et al.* 1984 b) and/or acidification (TAZAWA and SHIMMEN 1982, SHIMMEN and TAZAWA 1985) and/or elevated free calcium (WILLIAMSON 1975, HAYAMA *et al.* 1979, DOREE and PICARD 1980, WILLIAMSON and ASHLEY 1981, KIKUYAMA and TAZAWA 1982, WOODS *et al.* 1984 b). Depleted ATP levels may result in elevated Ca^{2+} levels, since sequestering of calcium is inhibited by cyanide in some animal tissue (ROSE and LOEWENSTEIN 1975). Cytoplasmic pH was expected to decrease in azide treated staminal hair cells as it did in azide treated *Nitella* cells (SPANSWICK and MILLER 1977) and root hair cells of *Sinapis alba* (BERTL and FELLE 1985). Cytochalasin D was presumed to inhibit cytoplasmic streaming, as did cytochalasin B (NAGAI and KAMIYA 1977, WILLIAMSON 1975, SEAGULL and HEATH 1980), by disrupting microfilaments (WESSELLS *et al.* 1971).

Intercellular passage of CF was found to be much faster in staminal hairs of *S. purpurea* (5 cells in 2 minutes) than was intercellular passage in staminal hairs of *Tradescantia* (5 cells in 35 minutes) (TYREE and TAMMES 1975) and trichomes of *Lycopersicon esculentum* (5 cells in 30 minutes) (BARCLAY *et al.* 1982). The microinjection technique and younger tissue is presumed to account for these observations in staminal hairs of *S. purpurea*.

The chain of cells making up the staminal hair may be thought of as a chain of chambers interconnected by channels, the plasmodesmata. As can be observed on the TV monitor, the time required for CF to permeate plasmodesmata is greater than the time required for it

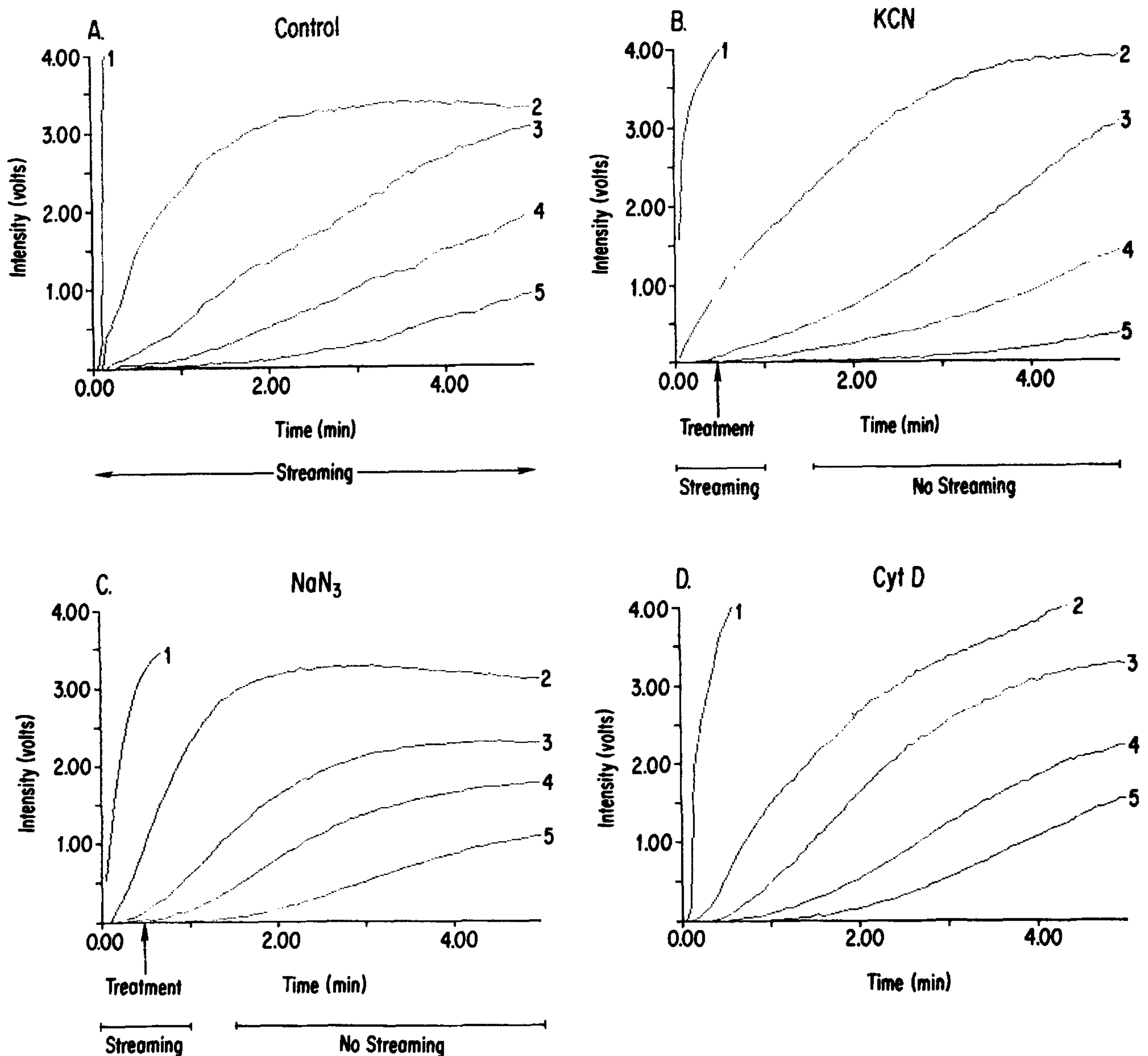

Fig. 1. Kinetics of intercellular passage. Buffered-CF was microinjected into the nucleoplasm of the most distal cell (*1*) and the intensity (volts) of fluorescence due to CF was analyzed in respective cells (*1, 2, 3, 4, 5*) over 5 minutes. Passage was through *2, 3, 4*, and *5* in less than 2.0 minutes. *A* Control; *B, C* Hairs treated with 1.0 mM KCN or 10 mM NaN_3, respectively, 30 seconds after buffered-CF was microinjected into *1*; *D* Cell 1 microinjected with CD-buffered-CF

to diffuse through the cytoplasm. This agrees with the conclusions of TYREE (1970), TYREE and TAMMES (1975) and BARCLAY *et al.* (1982). However, since the temporal appearance of fluorescence was influenced by several factors, including the degree of coupling between cells (TUCKER and SPANSWICK 1985), a simple correlation between distance and square root of time was not always obtained.

The present study focuses on determining if there is any correlation between cytoplasmic streaming (particle movement) and CF intercellular passage. Since large changes in the kinetics of intercellular passage were not observed when cytoplasmic streaming was inhibited by metabolic inhibitors (cyanide and azide) and a microfilament disrupting agent (cytochalasin D); it was concluded that cytoplasmic streaming plays no major role in regulating intercellular passage of CF. These results agree with the findings that cytoplasmic streaming is not required for polar transport of auxin in oat and maize coleoptile tissue (CANDE *et al.* 1973) and

fluorescein transport in detached tomato trichomes (BARCLAY *et al.* 1982). Microinjection of CD into the nucleoplasm of *S. purpurea* staminal hair cells, rather than the long extracellular treatments required to inhibit streaming in tomato trichomes, appeared to disrupt the cytoplasm less. That is, aggregation of the cytoplasm as noted by BARCLAY *et al.* (1982) did not occur. As noted above, intercellular passage of CF in *S. purpurea* staminal hairs was much faster than reported in tomato trichomes.

These results appear to differ from the findings of DRAKE (1979) that 1 mM NaN_3 and 1 mM KCN decreased electrophysiological coupling between oat coleoptile cells. However, further analysis using mathematical modeling may illustrate slight decreases in intercellular passage in hairs treated with these metabolic inhibitors.

It appears that "stirring", due to cytoplasmic streaming, of hydrophilic, negatively charged molecules analogous to CF has little effect on their passage from cell to cell. Symplastic transport must be regulated at the plasmodesmata and/or by cytoplasmic processes other than cytoplasmic streaming. Calcium may be involved in this regulation (ERWEE and GOODWIN 1983).

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