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Release of Ca^{2+} from intracellular pools stops cytoplasmic streaming in *Tradescantia* staminal hairs

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Summary. Ionophore A 23187 reversibly stops cytoplasmic streaming in *Tradescantia* staminal hairs. Cessation of streaming occurs even in the absence of free Ca^{2+} ions in the medium.

Cytoplasmic streaming occurs in many plant and protozoan cells[1,2]. The welldocumented suppression of streaming by cytochalasin B, which appears to weaken the actin filament structure[3–6], suggests that bundles of microfilaments are involved in the mechanism of streaming. The presence of such bundles has actually been reported in plant cells which demonstrate cytoplasmic streaming[4,7]. In non-muscle cells Ca^{2+} seems to be involved in the control of the formation of bundles of actin filaments[8] and actin-containing gels[9] as well as in the interaction of actin with myosin[10]. On the other hand it has long been known in *Charophyta* that streaming halts transiently when an action potential passes along a cell[11,12]. Barry found that replacement of Ca^{2+} in the medium with Mg^{2+} left *Nitella* giant cells excitable but abolished the blockage of streaming that normally accompanies excitation[12]. Although this finding supports the view that the action potential is normally accompanied by a net influx of Ca^{2+} which has an inhibitory effect on streaming, this explanation lacks clear experimental support, since Barry failed in his attempts to inhibit streaming by driving Ca^{2+} directly into the cytoplasm either ionophoretically or by microinjecting it. This led us to test the effect of the rather Ca^{2+}-specific ionophore A 23187[13] on cytoplasmic streaming in *Tradescantia* staminal hairs, a very favorable material for quantitative studies of streaming rates[14].

Material and methods. Staminal hairs were used on the day following opening of the flowers. They were mounted on a slide in a 100-µl chamber filled either with pure water, 1 mM $CaCl_2$ or 10 mM EDTA, with bubbling air for at least 1 h before starting the experiments. A Wild microscope equipped with a ×40 phase contrast objective under ordinary light was used, heating of the slide being avoided by the interposition of a water cell. Readings were made of the motion of the smallest visible particles (figure 1) in transvacuolar strands of a single cell, using a calibrated ocular micrometer and a stop watch. A 200-µM ionophore solution in methanol was used as stock solution: 1 µl was added directly into the 100-µl chamber. Controls with 1% methanol were run in parallel to the inhibitor experiments: no change in the rate of streaming was observed. Ionophore A 23187 was a gift of Eli Lilly.

Results. Figure 2, a, shows that, in our experimental conditions, streaming-rate remains constant for at least 1.5 h in aerated pure water. It is not altered when water is changed for 1% methanol. 10 mM EDTA or 50 mM $CaCl_2$ (nor when 50 mM $MgCl_2$ is substituted for 50 mM $CaCl_2$). Figure 2, b and c, show that 1 min after addition of 2 µM ionophore A 23187 a decrease of streaming rate is already noticeable. Within 5 min streaming completely stops. No recovery occurs within at least 30 min in the presence of ionophore. However, a rapid and complete restoration occurs after the chamber containing the staminal hair has been washed thoroughly several times either with 10 mM $CaCl_2$ (figure 2, b) or with 10 mM EDTA (figure 2, c). This result suggests that despite its hydrophobic nature, ionophore can be washed out relatively easily from the membrane, as already reported by others[15,16]. A 2nd ionophore application again suppresses cytoplasmic streaming. Comparison of figures 2, b, and 2, c, makes clear that the presence of Ca^{2+} in the medium is not required for ionophore A 23187 inhibition of cytoplasmic streaming. Even after standing 30 min in 10 mM EDTA, with several changes of medium, staminal hairs respond to ionophore as well as they do in the presence of Ca^{2+}. Inhibition is neither reversed nor enhanced when Ca^{2+} concentration is raised to 50 mM.

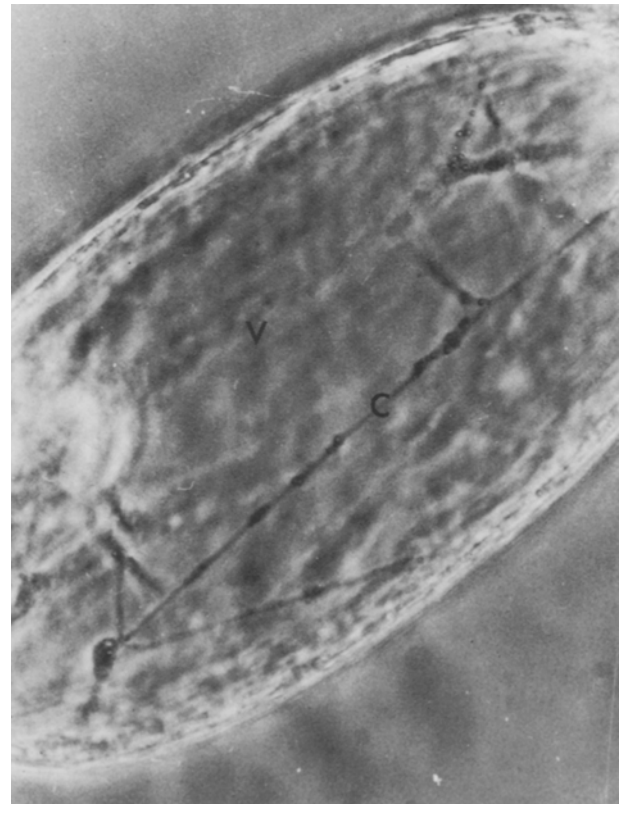

Fig. 1. *Tradescantia* staminal hairs. Arrows point to particles whose rate of rectilinear movement was measured in transvacuolar strands. v, vacuole; c, cytoplasm. ×700.

Discussion. The above results give direct experimental support to the hypothesis that calcium plays a key role in the control of cytoplasmic streaming in plant cells. Our finding that ionophore A 23187 stops cytoplasmic streaming even after free or loosely-bound calcium has been eliminated by thoroughly washing out cell walls with 10 mM EDTA suggests that a release of membrane-bound Ca^{2+} into the cytosol is required for cessation of streaming rather than influx of Ca^{2+} from outside into the cell. This does not conflict with Barry's finding that the action potential does not suppress *Nitella* cyclosis in the absence of external Ca^{2+} since release of Ca^{2+} from many excitable membranes does not occur in the absence of external free calcium[17,18].

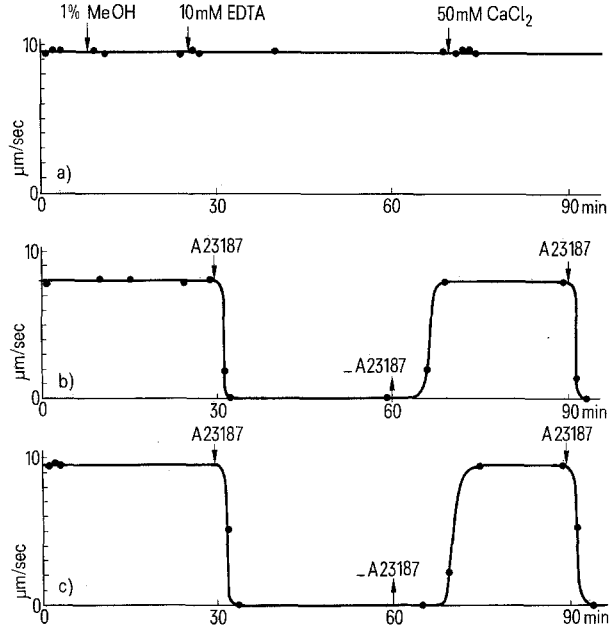

Fig. 2. Rates of streaming of staminal hair cells. *a* Staminal hair in aerated pure water. Arrow points to successive substitution of aerated 1% methanol, 10 mM EDTA and 50 mM $CaCl_2$ for pure water. *b* and *c* Arrows point to addition (+) or elimination (−) of ionophore A 23187. 1 µl of 200 µM methanolic solution of ionophore was added to 100 µl of 1 mM $CaCl_2$ (B) or 100 µl of 10 mM EDTA (C). In experiment C staminal hair was first washed rapidly several times with 10 mM EDTA.

Our results contrast, however, with Herth's findings that ionophore A 23187 stops tip growth, but not cytoplasmic streaming in pollen tubes of *Lilium longiflora*[19], which led him to conclude that A 23187 does not interfere directly with the contractile elements involved in cytoplasmic streaming. Growing pollen tubes, however, drive considerable currents which maintain a Ca^{2+} gradient along them[20,21]. Such currents, which antagonize ionophore action, would be expected to decrease sensibility to ionophore.

How Ca^{2+} redistribution within the cell can induce changes in cytoskeleton organisation remains largely speculative at present[22]. Cytoplasmic streaming, which allows rapid and quantitative studies of such changes, might provide a tool for elucidating the regulatory mechanisms which control the assembly of contractile proteins into supramolecular forms.

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Characterization of polyphenol-containing dense cells[1]

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Summary. Dense cells of *Rubus chamaemorus* L. (cloudberry) root meristems have been characterized by histochemical staining reactions at light and electron microscope levels. Presence of polyphenolic compounds in the cellular matrix contribute to the density of the dense cells.

In recent years dense cells have been reported from growing and differentiating plant tissues. Some of these cells have denser cytoplasm due to increased concentration of ribosomes in the matrix[2], thereby showing more basophilia and appearing more electron dense when viewed with the electron microscope. A different kind of dense cell has been also observed at the junction of the shoot apical meristem and leaf primordia in maize[3] and in the root meristems of cloudberry[4]. The density of these cells is uniformly distributed over the cytoplasm as well as the nucleus and it has been postulated[4] that polyphenolic compounds present in the cell matrix are responsible for the intense staining reaction. This communication presents evidence to support the above hypothesis.