

Pulsating Ion Fluxes and Growth at the Pollen Tube Tip

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The arrival of an angiosperm pollen grain on a compatible stigma triggers germination of a tube. This tube must grow down the style to the ovule, where it ruptures and delivers the two sperm cells that effect double fertilization of the egg and the central cell. The style may be several centimeters long, and each pollen tube is competing with others in the race to the ovary, where a limited number of eggs await, so speed matters. Pollen tubes elongate by tip growth—that is, by the directed addition of new membrane and cell wall material to the extending tip. Growth rates in excess of 1 $\mu\text{m/s}$ are achieved in some species. Within the cytoplasm, the extensive array of actin filaments that support cytoplasmic streaming must be continuously remodeled to accommodate the growth. The major components of the cytoplasm, including the mitochondria, endoplasmic reticulum, and Golgi apparatus, maintain their position with respect to the ever-moving tip. The machinery that supports this dramatic, directed mobilization of resources has been the focus of considerable recent attention, and an impressive array of physiological, biochemical, and molecular techniques have been brought to bear upon the investigation. Perhaps not surprisingly, many of the usual suspects of signal transduction cascades are involved, including Ca^{2+} , pH, the actin cytoskeleton, adenosine 3',5'-monophosphate (cAMP), and small guanosine triphosphatases (GTPases). Here, we will focus on the newly revealed world of ion dynamics at the pollen tube tip. (Fig. 1)

The central importance of calcium for germination and growth of pollen tubes has been long known (1). With the advent of ratiometric Ca^{2+} indicators and digital imaging techniques, the obligatory existence of elevated cytosolic Ca^{2+} at the tip of lily pollen tubes was established (2, 3). The concentration of cytosolic Ca^{2+} is elevated in a tip-to-base gradient, and if that gradient is disrupted, growth ceases (2-5). The directional information contained in the Ca^{2+} gradient is required for normal function of the secretory machinery. Calcium gradients appear to be a universal feature of pollen tube growth and are found in all species that have been examined (6-8). Perturbation of the Ca^{2+} gradient by various means (6, 7, 9, 10) reveals that the gradient can determine the direction of growth.

The discovery that pollen tube growth is not steady, but oscillatory, has added an exciting temporal dimension to the field. Pierson *et al.* (8) showed that lily pollen tubes, upon reaching a length of about 1 mm, begin to exhibit regular oscillations in growth rate, with maximum growth rates that are three to four times greater than the minimum. Subsequent work showed that Ca^{2+} oscillations accompany the growth oscillations at precisely the same frequency (5, 11), but slightly out of phase, with the peak of cytosolic Ca^{2+} lagging the peak of growth rate by about

4 s out of an oscillatory period of around 40 s (12). The Ca^{2+} concentration just inside the plasma membrane typically reached 10 μM at the peak. There is uncertainty about the source of Ca^{2+} for the elevated level at the tip. Although it has been suggested that Ca^{2+} influx occurs through plasma membrane Ca^{2+} channels that are mechanically coupled to membrane tension, there is no direct information about the nature of these channels (gating, selectivity, distribution, and so forth). Measurement of the net fluxes of extracellular Ca^{2+} reveals that Ca^{2+} influx at the growing tip is oscillatory, but substantially out of phase with both growth and changes in intracellular Ca^{2+} concentrations (11, 13). The lack of temporal correlation among these three oscillatory variables—growth rate, tip Ca^{2+} concentration, and Ca^{2+} influx—is puzzling and raises questions regarding the source of Ca^{2+} for the oscillations and how secretion is coupled to growth. Various models have been proposed, but no resolution is at hand.

Although there is considerable agreement among investigators about the spatial and temporal aspects of Ca^{2+} dynamics, the situation with regard to pH is quite different. Feijó *et al.* (14) reported the existence of cytosolic acidification at the tip and a “constitutive alkaline band,” where the cytosolic pH reached 7.8 or more, centered about 15 μm behind the growing tips of lily pollen tubes. To detect these regions, they reported that it was necessary to lower the concentration of the fluorescent pH indicator, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF), to 0.5 μM , apparently because higher indicator concentrations buffered out the band even though higher concentrations did not disturb growth. This is surprising, given that the typical cytoplasmic H^+ buffering capacity is orders of magnitude greater than 1 μM . They also measured a large net efflux of protons from this region, which presumably generates the observed alkaline zone. However, other measurements of intracellular pH obtained with a different indicator at higher concentration failed to detect the alkaline band (15). Instead, tip-focused oscillations of acidification, with intervening periods that lacked pH gradients, were reported. Extracellular measurements of proton fluxes detected large-amplitude oscillations of H^+ influx, but no region of efflux adjacent to the tip (13). Although the disagreement about the distribution of protons in the cytoplasm may be explained by differences in the techniques (different pH indicators used at different concentrations imaged by different types of optical systems), the two groups used identical methods to measure proton fluxes. Adding to the confusion are earlier experiments from two other groups that report no pH gradients at all in growing pollen tubes (16, 17). It is important that the disagreement about the nature and location of pH gradients in the cytoplasm be resolved, because protons are known to affect many biochemical processes.

Fluxes of two other ions have also been measured at the pollen tube tip. A large oscillatory influx of K^+ accompanies,

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temporally and spatially, the influx of H^+ and Ca^{2+} (13). The magnitude of the potassium influx is not significantly different from that of the proton influx, and the two fluxes are temporally coincident, leading to the suggestion that potassium uptake is coupled to and powered by proton influx. However, the sum of these ion fluxes did not match the previous current measurements (15, 18). The peak K^+ influx of about $700 \text{ pmol cm}^{-2} \text{ s}^{-1}$ (13) amounts to $70 \text{ } \mu\text{A cm}^{-2}$ of ionic current, yet the peak values of net ionic current are less than $1 \text{ } \mu\text{A cm}^{-2}$ (15, 18). This led to the suggestion of electroneutral transport in which H^+ and K^+ uptake was either coupled to release of an unknown cation or uptake of an anion. Recently, Zonia *et al.* (19) reported large oscillatory fluxes of Cl^- leaving, not entering, the tips of growing tobacco and lily pollen tubes. The net Cl^- efflux in lily is at least an order magnitude larger than the influx of any other ion, thus greatly adding to the discrepancy between the summed individual ionic fluxes and the measured net current. Interestingly, the Cl^- efflux was found to be in phase with the growth oscillations. This is the first oscillating variable to be shown to match growth oscillations in both frequency and phase, suggesting the possibility that it might be closely linked to the growth mechanism. Disruption of the Cl^- efflux by 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) or microinjected inositol 3,4,5,6-tetrakisphosphate [$Ins(3,4,5,6)P_4$] also disrupted growth. Two aspects of Cl^- fluxes are surprising. Lily pollen tube growth is completely independent of inorganic anions, including Cl^- (20), so massive Cl^- efflux would not be expected to play a central role in volume regulation and growth, raising the question of the source of this anion. Can the pollen grain contain the reserves of Cl^- necessary to sustain the efflux in the absence of extracellular sources? It is also surprising that pollen tubes lose large amounts of an osmotically important ion during each growth oscillation. Growth involves continuous uptake of water, and the pollen tube must maintain sufficiently low water potential to drive uptake. Resolving these inconsistencies will be an important task for future study, because the inconsistencies imply that the ionic fluxes are accompanied by the movement of counterions that have not yet been detected.

There remain many gaps in our understanding of how the

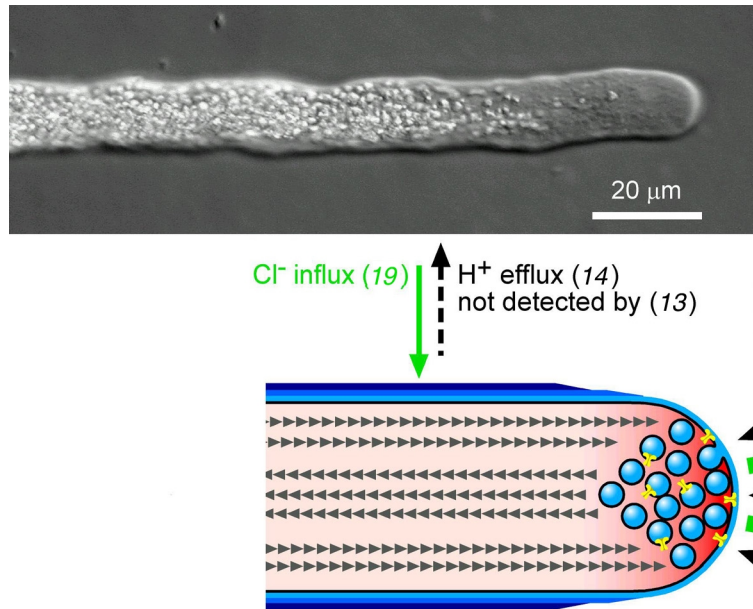


Fig. 1. Ion fluxes in a growing pollen tube. The diagram depicts the growing tip of a pollen tube with the measured ion fluxes shown as arrows. The black arrows at the apex indicate the oscillatory inward movement of H^+ , K^+ , and Ca^{2+} , whereas the green arrow shows the recently reported massive efflux of Cl^- . Behind the tip are shown the reported influx of Cl^- , as well as the efflux of H^+ that has been reported by one group (14) but was not detected by another (13). The red shading within the tube indicates the tip-focused Ca^{2+} gradient that is assumed to regulate the secretion of the growth vesicles, which are shown in blue. The yellow structures represent the hypothetical calcium channels that we imagine are inserted into the plasma membrane by secretory vesicles. The black triangles indicate the location and polarity of the actin cables that support cytoplasmic streaming. Shown in the inset is a differential interference contrast micrograph of a growing lily pollen tube.

rapid, oscillatory growth of pollen tubes is generated. Although a number of measurable quantities have been correlated with growth, including the ones discussed above and others such as short actin bundles (under the control of a Rop GTPase) (21), the causal relation between any two of these or between one of them and growth is not understood. Thus, we do not understand the feedback network that coordinates these variables and sets the frequency of the oscillations. To gain this understanding will require simultaneous, multivariable measurements. Another essential consideration is the behavior of pollen tubes in the transmitting tract of the style. The tract through which the pollen tubes grow varies enormously among species, from an open conduit to a dense cellular matrix. Unlike the situation in culture, which is the source of nearly all of the physiological data, there may be important external cues interacting with the internal machinery to direct growth. With the proper choice of biological material and clever molecular engineering, it may be possible to carry out physiological measurements in the intact or semi-intact style.

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