Microfilament orientation constrains vesicle flow and spatial distribution in growing pollen tubes.

Jens H. Kroeger
Ernest Rutherford Physics Building, McGill University.
Montréal, Québec. Canada H3A 2T8

Firas Bou Daher
Institut de recherche en biologie végétale,
Département de sciences biologiques,
Université de Montréal. Montréal, Québec. Canada H1X 2B2

Martin Grant
Ernest Rutherford Physics Building, McGill University.
Montréal, Québec. Canada H3A 2T8

Anja Geitmann
Institut de recherche en biologie végétale,
Département de sciences biologiques,
Université de Montréal. Montréal, Québec. Canada H1X 2B2

1Corresponding author. Address: Ernest Rutherford Physics Building, McGill University. 3600 rue University, Montréal, Québec. Canada. H3A 2T8.
Tel.: (514)398-7025, Fax: (524)398-8434
Abstract

The dynamics of cellular organelles reveals important information about their functioning. The spatio-temporal movement patterns of vesicles in growing pollen tubes are controlled by the actin cytoskeleton. Vesicle flow is crucial for morphogenesis in these cells as it ensures targeted delivery of cell wall polysaccharides. Remarkably, the target region does not contain much filamentous actin. We model the vesicular trafficking in this area using as boundary conditions the expanding cell wall and the actin array forming the apical actin fringe. The shape of the fringe was obtained by imposing a steady state and constant polymerisation rate of the actin filaments. Letting vesicle flux into and out of the apical region be determined by the orientation of the actin microfilaments and by exocytosis was sufficient to generate a flux that corresponds in magnitude and orientation to that observed experimentally. This model explains how the cytoplasmic streaming pattern in the apical region of the pollen tube can be generated without the presence of actin microfilaments.

Key words: Cytoplasmic streaming, actin microfilament orientation, exocytosis, vesicle flow, pollen tube growth, tip growth.
**Introduction**

Cells are highly compartmentalised structures and specific cellular activities are spatially confined to certain types of organelles. The dynamics of cellular organelles reveal important information about their functions and mutual interactions. One important role of organelle movement is the transport and delivery of material from the site of synthesis to the site of usance or release. This type of targeted long distance transport is often carried out by vesicles, which are small, membrane bound organelles. Their small size, typically between 50 and 150 nm, makes the quantification of their movements a challenge for optical microscopy, especially when they are densely packed. However, the combination of high temporal resolution confocal microscopy and spatio-temporal image correlation spectroscopy has recently demonstrated that the movement patterns of densely packed vesicles can be quantified in space and time (1).

An example for dense and extremely dynamic vesicle trafficking occurs in rapidly growing plant cells. Expansion of cellular surface in walled cells necessitates the delivery of cell wall material and membrane to the site of expansion. Much of the required material is delivered in the form of secretory vesicles whose motion requires spatial and temporal coordination to ensure targeted discharge at the location of growth (1–4). In general, organelle transport is mediated by the cytoskeleton, and motor proteins linking the organelles with the cytoskeletal elements provide the propelling force. In plant cells, vesicle transport is mainly actin-myosin driven.

**Vesicle trafficking in growing plant cells**

Among the fastest growing plant cells is the pollen tube, a cellular protrusion formed by a pollen grain upon contact with a receptive stigma. The function of the pollen is to transport the male gametes from the anther of the donor flower to the female gametes located in the ovule of the receptor flower. Similar to other cells with an invasive life style such as fungal hyphae, root hairs and neuronal growth cones (5–8), pollen tubes display tip growth. In this type of growth all growth activity is confined to a very small area on the cellular surface, the apex (9). Continuous addition of cell wall material and turgor driven expansion of the existing cell wall at the apex result in the formation of a rapidly elongating, cylindrical tube. Because of the rapid growth rate and the spatial confinement of growth activity, vesicle trafficking in these cells is extremely dense and dynamic thus making them a very suitable system for the study of vesicle transport.
Mechanics of pollen tube growth

From a mechanical point of view, pollen tube growth is defined by two simultaneously occurring processes: the continuous addition of cell wall material and the mechanical deformation of the existing viscoplastic cell wall, driven by the hydrostatic turgor pressure. The spatial confinement of the growth activity to the apex is reflected in a polar distribution of the cytoplasmic contents (Fig. 1). The apical region, i.e. the growing region of the cell, beginning with the hemispherical tip of the tube and reaching to a distance of about one tube radius behind the tip, is almost exclusively filled with vesicles. The absence of bigger organelles gives it a “clear” appearance in the optical microscope (Fig. 1A), as compared to the granular shank of the cell that is densely packed with various types of organelles such as mitochondria, plastids, Golgi stacks, and endoplasmic reticulum. Labelling the vesicles with the lipophilic styryl dyes FM 4-64 or FM 1-43 has revealed that in angiosperm pollen tubes the space they occupy in the apical region has the shape of an inverted cone filling the extreme apex and pointing towards the rear of the cell (Figs. 1E and F; (1, 10, 11)). This cone shaped apical region is also relatively free of prominent filamentous actin cables, whereas the cylindrical distal portion of the cell is filled by longitudinally arranged actin arrays (Figs. 1B and C; (12)).

In the transition zone between the two regions, or the “subapex”, these arrays become finer and form a fringe like configuration at the shoulder region of the apical dome (Fig. 1D, Fig. 3). This fringe is always in close proximity to the continuously advancing apex of the cell. The position of this fringe is believed to be controlled by signalling cascades involving the subapical cytoplasmic alkaline band and the cytosolic $Ca^{2+}$ gradient present in the tube apex. Both regulate the rate of assembly of G-actin into F-actin mediated by $Ca^{2+}$ and pH activated proteins. These ion gradients thus limit the polymerisation and bundling of the actin-cytoskeleton in a space dependent manner (13–18). While generally the term “actin fringe” in pollen tubes denotes only the ring-shaped arrangement of actin filaments in the subapical cortex of the cell (12, 19), for the purpose of the present model we define it as the complete actin array bordering the inverted vesicle cone (marked in grey in Fig. 3). The role of the fringe in the control of cytoplasmic streaming, vesicle delivery and actin polymerisation is the subject of this paper. It should be noted that ”cytoplasmic streaming” is a term used in the biology community to designate the intracellular movements of organelles which in the optical microscope resemble
MF orientation constrains vesicle flow.

a streaming process. In reality this process is the sum of individually controlled movements of organelles through the cytoplasmic space. Most of these movements occur along cytoskeletal arrays. The cytosol, the liquid surrounding the organelles, is not the cause for the organelle movements, but it is likely to be dragged along passively. We discuss below the role of the surrounding liquid.

Time lapse imaging has revealed that in the shank of the pollen tube, the spatial pattern of organelle motions results in a bidirectional movement along parallel bundles of F-actin. Forward movement occurs in the periphery of the tube and rearward flow occurs in the centre of the shank (19, 20). Bigger organelles that can easily be observed in the optical microscope were found to reverse their movement direction at a certain distance from the growing tip to enter the rearward flow thus generating a “reverse fountain” pattern (21). This flow pattern also applies to the relatively smaller vesicles, with the exception that they actually enter the cytoplasmic region closest to the tip before flowing rearward (Figs. 1G; (1, 2)).

High temporal resolution confocal microscopy of living pollen tubes revealed that in the shank of the tube, vesicles display rapid, long distance movements that are clearly guided by the longitudinally oriented actin filaments. In contrast, in the apical clear zone of the tube, the vesicles show more erratic, random motion. Spatio-temporal image correlation spectroscopy revealed that in the apex the average velocities of the vesicles vary over small distances (1).

Both the absence of prominent actin cables and the erratic character of vesicle dynamics in the tip suggest that vesicle movement is not propelled or guided by an actin-myosin mechanism in this region. Our objective was to model the dynamics of both the actin cytoskeleton and the vesicle movement, in order to better understand their combined role for pollen tube growth. To have predictive value such a theoretical model needs to be able to reproduce the characteristic distribution of vesicles in the apical region as well as the experimentally observed relative movement rates and spatial patterns. One of the principal goals was to demonstrate that while vesicle delivery towards the apical region is mediated by an actin-myosin mechanism, movement through the cone region does not require active transport or cytoskeletal guidance.

Actin polymerisation

It is unknown where in the pollen tube and by which mechanism actin polymerisation takes place. However, in order be able to advance together
MF orientation constrains vesicle flow.

with the elongating pollen tube tip, it is safe to presume that the apical actin arrays need to polymerise continuously. Inhibitor studies have shown that actin polymerisation activity in the pollen tube is more sensitive than and thus independent from the capacity of actin to guide organelle movement (22).

In angiosperm pollen tubes, actin filaments are oriented with their barbed (plus) ends pointing toward the apex in the cortical cytoplasm and away from the tip in the centre of the cell (23). This explains how organelle transport can occur in opposite directions in these two regions as myosin-driven transport occurs mainly towards the plus end of actin filaments (21, 24–27). However, while this configuration of the actin array is consistent with enhanced polymerisation activity towards the apex in the cytoplasm, it is not quite clear how the central actin arrays keep up with the advancing tip.

Both fluorescence micrographs and transmission electron images (23) suggest, however, that the orientation of individual actin filaments in the subapical region is not necessarily parallel to the longitudinal axis of the cell and that microfilaments can even form curved shapes in this region. This change of orientation along the radial axis is crucial to explain both actin microfilament growth through polymerisation of G-actin monomers and direction of the vesicle flux towards the apical vesicle cone. Actin polymerisation and vesicle motion are therefore closely related since both have to satisfy the constraints set by the polarity of the actin microfilaments.

From a physical point of view one can therefore ask how the polymerisation of the G-actin monomers, i.e. a diffusion problem with sink and source terms at the boundary, creates the observed funnel shape. While not having the exact same symmetry, this funnel shape has certain similarities with the finger shape created by dendritic crystal growth in a channel (28, 29). The two problems have many common origins since dendritic crystal growth is essentially the diffusion process of a solute in a channel which, by preventing the solute from escaping from the setup, plays a role analogous to the cell wall in the pollen tube. Here we exploit this analogy and adopt a strategy for modelling that is similar to that used in the past for dendritic crystal growth.

Objectives and outline

Our objectives are to demonstrate that i) the spatial and temporal patterns of vesicle motion in the apical region of the pollen tube can be explained without cytoskeletal guidance in the clear zone, and ii) the constant shape of the apical actin fringe can be explained by its continuous advancement.
and the varying orientation of its polymerising ends.

We establish a theoretical model that helps understanding how the apical actin fringe advances through actin polymerisation while at the same time delivering and removing vesicles to and from the apical cone thus controlling the movements of these organelles through the apical cytoplasmic space. We adopt the following strategy: First, we obtain the geometry of the cell wall forming the expanding apex using a viscoplastic model of a steadily growing tube (based on the model for root hair growth by Dumais et al. (30)). The details of the calculation are found in the Supporting Material. Then, we model the aggregation of actin filaments to obtain their orientation at the border of the apical cytoplasmic space. We use this orientation, and a steady state assumption, to model the protrusion of the actin fringe array in 2-D from which we deduce the geometry for the inverted apical cone (Profile of the actin fringe and Supporting Material). Finally, we model the vesicle flux based on the assumption that the organelles diffuse freely in the inverted apical cone. The addition and removal of vesicles via actin mediated transport and the removal by fusion with the apical plasma membrane (exocytosis) are taken into account by imposing suitable boundary conditions (Vesicle diffusion and cytoplasmic streaming).

**Theory**

**Profile of the actin fringe**

In this section, we present a calculation of the shape of the actin fringe, based on the assumption that it maintains a steady profile while advancing in the y-direction and that it satisfies the constraints of the tread-milling model (31, 32) for microfilament polymerisation. **Furthermore, we assume that the fringe advances forward at a steady rate equal to the pollen tube growth rate.** We begin by using a model for the actin filament aggregation (33–38) to calculate the polarity, or orientation $\Theta(x)$, of the microfilaments along the actin front, in the fringe (23). For this purpose, we fix our coordinate system such that the tube grows in the positive y-direction. As detailed in the Supporting Material section, we obtain the following filament angle $\Theta(x)$ between the barbed (plus) ends of the filaments and the x-axis

$$\Theta(x) = -\frac{\pi}{L} x - \frac{\pi}{2}. \quad (1)$$

As an initial condition for the actin filament aggregation model, we set the filaments with plus ends pointing towards the apex at the
MF orientation constrains vesicle flow.

The periphery of the tube and with plus ends pointing away from the apex in the centre of the tube. These orientations are consistent with the orientations measured in the centre and the periphery of angiosperm pollen tubes (23). We can now use this variable orientation of the filaments along the actin front to understand the variable protrusion rate of this front and how it forms a stable V-shape. The elongation rate, or polymerisation rate, $v_{MF}$ of a single actin microfilament, according to the tread-milling model (32), is given by

$$v_{MF} = v(k_{on}G - k_{off})$$

and depends on the local G-actin concentration $G$, the length per monomer $v$ and the net rates $k_{on}$ and $k_{off}$ at which actin monomers polymerise at the barbed end of the actin microfilament. There are other proteins and factors such as branching, capping and un-capping that contribute to the polymerisation of individual actin filaments and actin fronts pushing a membrane (31, 32). In the absence of experimental quantification of the spatial distribution of such agents in the pollen tube, we neglect those factors and focus on the effect of the orientation change along the actin front observed in pollen tubes (23). Actin monomers are added along the orientation of the microfilament, described by the vector $n$. Consequently, the profile of the actin network will protrude at a rate

$$\frac{dr}{dt} = v_{MF} (n \cdot r)$$

in a direction along $r$, that is normal to the profile (Fig. 3). The multiplicative factor $n \cdot r$ ensures that the normal growth rate is maximal when the microfilaments are at a right angle to the actin profile and point out of the network. The profile stays in place when the microfilaments are parallel to the profile and retracts when the microfilaments are at a right angle to the profile but point with barbed ends into the network. Here $r$ is a unit vector normal to the profile and $n$ is a unit vector giving the average orientation of the actin microfilaments. The dependence of the protrusion rate of an actin front on the filament orientation has been observed in lamellipodia (39). As discussed in the previous section, we assume that the microfilament orientation along the x-axis in the network is given by $\Theta(x) = \frac{\pi}{2} x - \frac{\pi}{2}$. We will use this model to derive a profile $y(x)$ for the actin network advancing at a steady rate equal to the growth rate of the pollen tube. Using the relation $n = \hat{i} \cos \Theta + \hat{j} \sin \Theta$ for the vector describing the orientation of the microfilaments and

$$r = \frac{1}{\sqrt{1 + (y')^2}} \left( -y' \hat{i} + \hat{j} \right),$$

we can calculate the profile of the actin network.
MF orientation constrains vesicle flow.

we obtain

\[ \frac{dr}{dt} = \frac{v_{MF}}{\sqrt{1 + y'^2}} \left( -y' \cos \Theta(x) + \sin \Theta(x) \right). \] (5)

We will now use the assumption that the cytoskeleton profile advances at a constant rate \( v_p \) in the y direction. This constant growth or advancement rate in the y direction can be related to the protrusion rate \( dr/dt \) in the direction normal to the profile. Using the angle \( \theta \) between the normal vector \( r \) and the y direction, one obtains

\[ \frac{dr}{dt} = |r|v_p \cos \theta. \] (6)

This expression has been used for the calculation of the steady growth profile in the case of diffusion-limited dendritic crystal growth and fluid finger propagation (28, 29). Equating the left hand side of eq. 6 to the right hand side of eq. 3 gives

\[ \frac{dr}{dt} = v_{MF}|n||r|\cos \phi = |r|v_p \cos \theta. \] (7)

This relation has been obtained by expressing the right hand side of eq. 3 as \( v_{MF}|n||r|\cos \phi \) where \( \phi \) is the angle between the normal vector and the actin microfilament orientation. Since both \( n \) and \( r \) have unit length, we can interpret the equation as follows: the profile of the actin fringe must be such that the angle \( \phi \) between the normal vector and the actin microfilament orientation is equal to the angle \( \theta \) between the normal vector and the y-axis, the direction of the overall actin cytoskeleton growth. This condition is illustrated in Fig. 3. It is important to note that for these regions of low profile curvature we neglect any surface tension between the cytoplasm and the actin network. Using \( \cos \theta = r_y = (1 + y'^2)^{-1/2} \), we can express eq. 7 in terms of \( y' \) and \( \Theta(x) \):

\[ \frac{v_{MF}}{\sqrt{1 + (y')^2}} \left( -y' \cos \Theta(x) + \sin \Theta(x) \right) = \frac{v_p}{\sqrt{1 + (y')^2}}. \] (8)

We obtain an ordinary differential equation for the profile of the actin fringe

\[ y' = \tan \Theta(x) - \frac{\lambda}{\cos \Theta(x)}. \] (9)

which has the solution

\[ y(x) = -\frac{1}{m} \ln(\cos \Theta(x)) - \frac{\lambda}{m} \ln \left( \tan \left( \frac{\pi}{4} + \frac{\Theta(x)}{2} \right) \right). \] (10)
MF orientation constrains vesicle flow.

Here $\lambda = v_p/v_{MF}$ and $m$ is the slope in the expression $\Theta(x) = mx + b$. The profile velocity $v_p$ and the maximum filament growth rate $v_{MF}$ must be similar such that $v_p/v_{MF} \simeq 1$. The approximation of $\lambda$ being constant on the fringe is based on the assumption that the concentration $G$ of G-actin monomers, and thus $v_{MF}$, is a constant. The profile of the actin fringe for different values of $m, b$ in the function $\Theta(x)$ is shown in Fig. 2C. By adding the left hand side and separating the two halves of the profile by a distance corresponding to one fifth of the cell radius, we obtain an actin fringe that recovers the funnel shape with “shoulders” observed experimentally. Once the profile on the actin fringe is found, the vesicle flux at the fringe is obtained by evaluating eq. 14 with eqs. 7,8

$$j \cdot r = v_{ve} V \left( \frac{v_p}{v_{MF}} \frac{1}{\sqrt{1 + (y')^2}} - a \right).$$  \hspace{1cm} (11)

For various values of $m$ and $b$, this flux is shown in Fig. 2D.

Vesicle diffusion and cytoplasmic streaming

In the pollen tube shank, the vesicles are pulled along actin filaments by motor proteins (40, 41) and the cytosol is dragged along by this active movement of suspended particles. However in the apical inverted cone, there is not much filamentous actin that could serve to guide actin-myosin driven vesicle movement. And while the vesicles clearly display Brownian dynamics in this region (42–44), it is unknown whether the cytosol, the fluid surrounding the vesicles, is moving in the actin-free zone. Technical limitations have precluded quantitative measurements of individual vesicle dynamics in the densely packed apex hitherto.

Therefore, we resort to the calculation of various dimensionless numbers to determine whether bulk fluid movement or diffusion dominates the motion of vesicles in the apex. The Reynolds number is the ratio of inertial to viscous forces. For a mass density $\rho = 10^3 \text{kg/m}^3$, a tube radius $r = 6.5 \mu \text{m}$, a velocity $v = 0.45 \mu \text{m/s}$ and a dynamic viscosity $\eta = 10^{-3} \text{kg/m/s}$ (45), the Reynolds number $Re = \rho vr/\eta = 2.9 \times 10^{-6}$. Accordingly, inertial (convective) forces are negligible (40, 45–47), such that viscous (advection due to the surrounding fluid) and diffusive (vesicle collisions) forces determine the motion of vesicles. This regime is called Stokes flow, and in this regime the movement of the cytosol (the “solvent”) is described by the Stokes equation $\nabla p = \eta \nabla^2 v$ (46). The question
MF orientation constrains vesicle flow.

remains whether the movement of the bulk fluid cytosol or the collision of vesicles dominate vesicle movement. The Peclet number \( Pe = vr_v/D \), where \( r_v \) is the vesicle radius and \( D \) is the diffusion constant, gives us the ratio of the adjective (due to the surrounding fluid) to diffusive forces. The vesicle diffusion constant can be estimated from the evolution of the vesicle staining density-density correlation function (1). A broadening of the correlation function of \( 0.5\mu m \) occurred in \( 0.1s \), which is consistent with a translational diffusion coefficient of \( D_T = \langle r^2/4\Delta t \rangle = 0.625\mu m^2/s \) (46). For a vesicle radius \( r_v = 0.075\mu m \), the Peclet number is \( Pe = 0.054 \). Based on this number, we neglect the motion of the surrounding fluid and assume that the motion of vesicles in the apex is dominated by collision between vesicles (Brownian dynamics or diffusion). A mathematical analysis of the velocity field in the surrounding cytosol would require information on the pressure and stresses in the cytosol but also the proper treatment of the boundary conditions formed by the outer surfaces of the individual vesicles (in addition to the cell wall and the actin fringe) (46). This difficult problem has been addressed with the boundary integral approach (48, 49) and the boundary element method (50) but its solution is beyond the scope of this manuscript.

In our model the vesicle flux is constrained by the following sources and sinks: a) There is continuous flow of vesicles in the direction of the plus ends of actin filaments in the polymer network modelled previously, resulting essentially in an addition of vesicles in the periphery and a removal in the centre; b) A certain number of vesicles are absorbed by the fusion process (exocytosis) at the plasma membrane located in an annular region around the very tip of the tube.

We model the vesicle flow using Fick’s law \( \mathbf{j} = -D\nabla V \) on the domain bounded above by the cell wall calculated in the Supporting Material and below by the fringe calculated in the previous section. The average vesicle flow velocity \( \mathbf{v} \) can be related to the flux \( \mathbf{j} = V\mathbf{v} \) where \( V \) is the local vesicle density. Furthermore, the requirement of vesicle number conservation (continuity equation) leads to the diffusion equation 12. This description is justified by the observation that organelles in pollen tubes display Brownian motion (42–44). The change in vesicle density \( V(x,y) \) is given by the diffusion equation

\[
\frac{\partial V}{\partial t} = -\nabla \cdot \mathbf{j} = D\nabla^2 V = 0
\]

(12)
in the clear zone of the apex. In order to solve this equation, the boundary
MF orientation constrains vesicle flow.

conditions must be specified on the cell wall and the fringe. The flux of vesicles normal to the cell wall \( \mathbf{r} \cdot \mathbf{j} = \mathbf{r} \cdot -D \nabla V \) is given by the absorption of vesicle due to the fusion process

\[
\mathbf{j} \cdot \mathbf{r} = -D \nabla V \cdot \mathbf{r} = \frac{R}{V_{ol}},
\]

(13)

where \( V_{ol} \) denotes the volume of one vesicle and the net deposition rate \( R \) is calculated in the Supporting Material (Fig. 2B). We can estimate the average vesicle flux normal to the cell wall due to fusion at \( \langle j_R \rangle = 0.156 \text{s}^{-1} \mu \text{m}^{-2} \) from \( V_{ol} = 0.0026 \mu \text{m}^3 \) and \( R = 0.0244 \mu \text{m/minute} \). The net flux of vesicles normal to the actin fringe is generated by the addition of vesicles to the clear zone (inverted cone) from actin filaments with barbed ends oriented toward the tip and by vesicle recovery onto centrally located filaments that are oriented with the barbed ends pointing rearward.

\[
\mathbf{j} \cdot \mathbf{r} = -D \nabla V \cdot \mathbf{r} = v_{ve} V (\mathbf{n} \cdot \mathbf{r} - a).
\]

(14)

The quantity \( v_{ve} \) denotes the maximal rate at which vesicles are delivered into the apical cytoplasm. We can estimate the normal vesicle flux at the actin fringe (in the tube centre) by \( j_F \approx v_{ve} < V_{3D} > = 27.0 \text{s}^{-1} \mu \text{m}^{-2} \). Here \( < V_{3D} > = 62.0 \mu \text{m}^{-3} \) is the average 3-dimensional vesicle density in the apical cone (1). Comparing the numbers reveals a difference of two orders of magnitude between the vesicle flux at the plasma membrane and that normal to the actin fringe. This difference explains why the vesicle flow pattern predicted by our model is largely controlled by the orientation of actin filaments. Since the orientation of the microfilaments also controls the normal protrusion rate of the actin cytoskeleton, the right hand side of eq. 14 is proportional to the protrusion rate of the actin cytoskeleton described by eq. 3 (Fig. 2D). The constant term \( a \) represents myosin-mediated vesicle binding onto a microfilament that is oriented parallel to the fringe profile. The constant \( a \) is adjusted in such a way that the total number of vesicles entering the clear zone equals the total number of vesicles leaving the clear zone, i.e. the net flux is zero.

Results

A steady growth analysis (30) was used to calculate the shape of the apical cell wall during its viscoplastic expansion. The resulting cell wall shape, that minimises the mechanical stress induced by the turgor pressure, is shown in
MF orientation constrains vesicle flow.

Fig. 2A. The material necessary for the steady elongation of the cell wall is supplied by vesicles. The cell wall thus constitutes a target (i.e. a sink) for these organelles (Fig. 2B).

The orientation of the barbed (plus) ends of the actin microfilaments varies continuously along the radial axis in order to adopt the observed configuration (23) which minimises the mechanical stress in the actin polymer network (51). Once the steady shape of the advancing actin fringe is assumed, its profile is a direct consequence of the orientation of the actin microfilaments (Fig. 2C). This constraint is illustrated in Fig. 3. The orientation of the microfilaments also determines the direction in which the vesicles are delivered to or removed from the apical cone and the magnitude of their velocity (Fig. 2D). Addition (positive values in Fig. 2D) occurs in the periphery of the cell, removal (negative values) in the centre. The fringe thus constitutes a source and sink for the vesicles. The motion of the vesicles in the apical cone is modelled with the diffusion equation together with the boundary conditions described above, which are solved with Matlab (The Mathworks). After an integration time of 10 seconds, the vesicle density reaches a steady state shown in Figs. 4A and C. The average density from the simulations is rescaled to \(209 \mu m^{-2}\). This average vesicle density is obtained by dividing the number of vesicles present in a typical lily pollen tube apex (average of 81247 vesicles; (1)) by the area of the clear zone (389 \(\mu m^2\)). Our model indicates a clear density gradient from the front of the cell to the tail of the vesicle cone. This spatial profile of vesicle density is consistent with observations in the fluorescence microscope (1, 11) and the transmission electron microscope (4).

In addition to providing information on vesicle density, our model yields the relative speed and direction of vesicle motion at each coordinate in the vesicle cone. The resulting vesicle flux \(\mathbf{j}\) (Fig. 4B) is in excellent agreement with experimental data. Quantitative analysis of vesicle dynamics (1) revealed a vesicle flux with a direction field described by a reverse fountain pattern, qualitatively identical to the one our model produces. The microscopic observations showed very slow vesicle motion at the immediate tip of the pollen tube whereas vesicles move rapidly in the tail region of the cone. Our model is consistent with this change in the vesicle motion.

The absence of significant vesicle motion at the very tip of the cell is due to the small value of the vesicle fusion rate at the cell wall. Since the average vesicle fusion rate is directly proportional to the pollen tube growth rate, we can model the change in the flux pattern due to an increase in pollen tube growth rate. Fig. 4E shows the vesicle motion in a rapidly growing tube. The pattern was obtained by multiplying the net vesicle fusion rate by 50
MF orientation constrains vesicle flow. (R = 1.22µm/min when averaged over the cell wall). While this value of \( R \) corresponds to a tube growth rate (\( v = 350\mu m/min \)) that is much higher than any value observed in vitro (22), the numerical simulation displays the observed robustness of the streaming pattern to changes in the growth rate.

Our model, and especially the V-shape of the apical zone, relies on the fact that actin microfilaments are oriented with their barbed ends forward at the periphery and rearward in the centre of the tube, a typical configuration in angiosperm pollen tubes (23). In gymnosperm pollen tubes the flow direction of cytoplasmic organelles is reversed, it forms a fountain-like streaming pattern (52). However, it is unknown whether this flow pattern is due to an inversion of the orientation of actin filaments (i.e. barbed ends towards the rear in the periphery and toward the front in the centre), or whether a different type of myosin moves vesicles from the barbed ends of the actin filaments towards their pointed ends (53).

In order to find out which of the two alternatives is more likely we tried to model both. First we let vesicles move in the opposite direction to the actin polarity on actin filaments that are oriented and polymerise according to the conditions mentioned above for angiosperm pollen tubes. The vesicle flow now displays a fountain pattern (Fig. 4G), but the shape of the vesicle cone remains identical to that of the angiosperm pollen tube. Importantly, a high density of vesicles is now present in the tail of the cone, whereas the density is low close to the plasma membrane (Fig. 4F).

Next, we inverted the orientation of the actin filaments. Actin arrays in the periphery now point forward with their barbed ends, and the central array points rearward. We chose

\[
\Theta(x) = -\frac{\pi}{L}x + \frac{\pi}{2}
\]

for this approach. Not only do these inverse initial conditions result in a fountain-like flow pattern (Fig. 4H), they also lead to a very different shape of the apical vesicle population (Fig. 4I). Instead of an inverted cone, the apical vesicle population is now crescent-shaped. Inspection of available fluorescence micrographs reveals that vesicles in gymnosperm pollen tubes indeed accumulate in such a crescent shaped conformation (44, 52), whereas the configuration shown in Fig. 4F does not correspond to any phenomenon found in living pollen tubes.
Discussion

The goal of the present work was to model the dynamics of vesicles in the apical region of growing pollen tubes and to relate it to the polymerisation of the actin arrays bordering the apical vesicle cone. Our data show that a viscoplastic model of the cell wall and a steady state model for the actin polymerisation provide adequate inlet and outlet boundary conditions for the diffusive motion of the vesicles. When solved together, these constraints lead to a vesicle flux whose magnitude and direction are in agreement with the vesicle motion observed experimentally (1, 11, 54). The continuity of the vesicle motion at the apical fringe, i.e. the conservation of the total volume of cell wall material, was used to solve the model.

The robustness of our model is demonstrated by its applicability to a system that operates quite differently, the gymnosperm pollen tube. Inversion of the actin filaments in our model produces exactly the streaming and vesicle distribution patterns that are observed experimentally (44, 52). By contrast, inversion of the movement direction of the organelles, putatively mediated by a myosin motor protein operating in the opposite direction, does not produce any patterns that can be observed experimentally. This is consistent with the fact that no myosin molecules operating in unconventional direction have been identified in plants hitherto. It must be mentioned, however, that microtubules seem to be more important in gymnosperm pollen tubes, compared to their role in angiosperm counterparts (55). Drug induced microtubule depolymerization inhibits elongation in gymnosperm pollen tubes and changes the motion patterns of organelles. However, the authors postulate that this effect is mediated by the microtubules’ control of the actin array. This is corroborated by the finding that the microtubule disruption causes a reversal of organelle streaming in gymnosperm pollen tubes (52). This reversal from fountain-to inverse fountain-streaming is accompanied by a rearrangement of the actin array. Hence, these experiments are consistent with the results of our modelling. No information on the orientation of actin filaments in gymnosperm pollen tube is available, but our model predicts that actin arrays are oriented with their barbed ends towards the apex in the central cytoplasmic region, and rearward in the periphery. Vesicles are predicted to move towards the barbed ends of the arrays. Together these conditions result in the flow and distribution patterns observed experimentally.
MF orientation constrains vesicle flow.

In view of the simplistic assumptions of the model the agreement with experimental observations is encouraging. A very important test of the vesicle diffusion picture would be the prediction of the vesicle flux after a disruption of the tubular shape of the pollen tube, e.g. through a mechanical constriction of the tube or by the application of an agent causing swelling of the apex without interfering with actin functioning. However such tests require modelling beyond the limits of our current steady state cell wall analysis. Refinements of the model should include a better calculation of the granular flow of the cytoplasm, a heterogeneous and polydisperse medium (i.e. containing components of different sizes). Furthermore, the spatial variation of G-actin and calcium concentrations as well as their effects on the actin polymerisation process were not taken into account. Given the cytoplasmic calcium gradient in the clear zone of the pollen tube apex (56) and the role played by calcium during actin polymerisation (57), calcium and G-actin concentrations should be considered in a future model of the polymerisation process. Experimental validations of this model include a detailed determination of the polarisation of the actin microfilaments in the subapical region.

Acknowledgements

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References


MF orientation constrains vesicle flow.


MF orientation constrains vesicle flow.


MF orientation constrains vesicle flow.


MF orientation constrains vesicle flow.


MF orientation constrains vesicle flow.


Tables
**MF orientation constrains vesicle flow.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
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<tr>
<td>Vesicle flux</td>
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<tr>
<td>Average vesicle concentration</td>
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<tr>
<td>Actin monomer concentration</td>
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<td>Diffusion constant</td>
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<td>(60, 61)</td>
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<td>Average net vesicle deposition rate</td>
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<td>Unit vector of actin microfilament polarity</td>
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<td>Angle between the r and the tube growth direction</td>
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<td>Angle between n and r</td>
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<td>Strain rate in the cell wall</td>
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<tr>
<td>Vesicle delivery rate</td>
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<td>0.45 µm/s</td>
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<tr>
<td>Length of microfilament per added monomer</td>
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<td>2.2 nm</td>
<td>(32)</td>
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<tr>
<td>Steady growth rate, in the y-direction, of the cytoskeleton</td>
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<td>(63)</td>
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<td>Profile of the actin fringe</td>
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Table 1: Explanation and typical value of different variables used in the model. Values labelled by * have been calculated in the present article.
Figure Legends

Figure 1.
Cytoarchitecture of the apical region of *Lilium longiflorum* pollen tubes. (A) DIC micrograph revealing the difference between the smooth appearance of the apical cytoplasm (asterisk) and the granular texture of the shank. (B-D) Filamentous actin forming the apical fringe (arrowhead) revealed by label with rhodamine phalloidin. (B) Single optical section. (C) Projection of z-stack of the same tube as in (B). (D) Surface rendering of 3D z-stack reconstruction, tilted slightly to reveal spatial configuration of the apical actin fringe. (E-G) Vesicles visualised by label with FM1-43. (E) Single optical section. (F) Surface rendering of 3D z-stack reconstruction revealing spatial configuration of the inverted vesicle cone. (G) Vector map of vesicle flux resulting from STICS analysis of a time series of confocal laser scanning micrographs. Figure G, details of the experiment and STICS analysis, were first published by Bove et al. 2008 (reprinted with permission; copyright American Society of Plant Biologists). Fluorescence micrographs are false coloured. The images in this panel do not show the same tube. Bar = 10 µm. Pollen culture, fluorescent label and image acquisition for all figures are detailed in the Supporting Materials.

Figure 2.
Geometry of the apical cone and vesicle delivery and absorption rates at its boundaries. (A) Shape of the apical cell wall during steady viscoplastic orthogonal growth. (B) Cell wall vesicle deposition rate necessary to sustain the steady viscoplastic growth. The details of their calculation are found in the Supporting Material. (C) Right hand side of the actin profile given by eq. 10. Full line: \((m, b) = (-\pi/L, -\pi/2)\). Dotted line: \((m, b) = (-3\pi/2, -\pi/2)\). Crosses: \((m, b = -5\pi/6L, -4\pi/6)\). The inset shows two symmetric halves of the actin fringe profile. The half circle at the tail end of the profile is due to capillary effects (Supporting Material).

(D) Vesicle flux normal to the actin fringe given by eq. 11. Full line: \((m, b) = (-\pi/L, -\pi/2)\). Dotted line: \((m, b) = (-3\pi/2, -\pi/2)\). Crosses: \((m, b = -5\pi/6L, -4\pi/6)\).

In all cases \(\lambda = 1\). The units of the x- and y-axis are multiples of the pollen tube radius.

Figure 3.
Left: Schematic drawing illustrating the principal directions of vesicle motion (left half of the tube) and orientation of the actin filaments bordering the vesicle cone (white arrows in right half) in the apical region of a pollen tube. Following delivery into the apical region on the actin filaments forming the fringe, vesicles are released into the apical cytoplasm in an annulus shaped zone (vesicle delivery zone). Vesicles that succeed in contacting the plasma membrane undergo exocytosis. Vesicles that do not succeed in contacting the plasma membrane stream rearwards within the cone shaped vesicle pool. Many of these vesicles are recirculated back into the forwards stream immediately in the subapical region (not shown). Solid arrows indicate actin-myosin-guided vesicle movement, dashed arrows indicate movements that are presumably governed by diffusion. Objects are not drawn to scale. For clarity, except for vesicles, no other organelle or the cell wall are drawn. This figure is based on results by Bove et al. (2008); Zonia (2008). Right: Orientation of the
MF orientation constrains vesicle flow.

Vectors along the actin fringe profile. \( \mathbf{r}, \mathbf{n} \) and \( \mathbf{v} \) are the vector normal to the profile, the microfilament orientation vector and the growth vector of the cytoskeleton, respectively. The profile of the actin fringe is such that the angle \( \phi \) between the normal vector and the actin microfilament orientation is equal to the angle \( \theta \) between the normal vector and the growth direction, the y-axis. Once the orientation vector \( \mathbf{n} \) is fixed, the shape of the fringe profile can be determined.

Figure 4.
(A) Vesicle density in the clear zone of the pollen tube. Lighter shades indicate low density whereas dark shades indicate high density. The units of the x- and y-axis are multiples of the cell radius. (B) The vesicle flux in the cell apex reveals the reverse fountain pattern. The relative magnitude of the flux velocity is given by the length of the red arrows. (C) Full line: Vesicle density as a function of the distance from the tip, along the axis of symmetry of the tube (the y-axis). The vesicle density from the simulation is rescaled such that its average is 209 \( \mu m^{-2} \) (1). Dashed line: Vesicle density for a rapidly growing tube. The growth rate and the net vesicle fusion rate at the cell wall are 50 times larger. (D) Vesicle density in a rapidly growing pollen tube. (E) Vesicle flux in a rapidly growing pollen tube. (F) Vesicle distribution for an actin orientation identical to an angiosperm pollen tube but with inverted vesicle delivery at the fringe due to reverse myosin activity. (G) Vesicle flux for an actin orientation identical to an angiosperm pollen tube but with inverted vesicle delivery at the fringe due to reverse myosin activity. (H) Geometry of apex and density of vesicle in a gymnosperm pollen tube. This geometry is obtained by inverting the microfilament orientation at the fringe boundaries. The MF orientation profile used is \( \Theta(x) = \frac{\pi}{L} x + \frac{\pi}{2} \). (I) Vesicle flux in a gymnosperm pollen tube. For figures A,B,F,G,H and I a tube growth rate of 7 \( \mu m \) per minute (\(< R > = 0.0244 \mu m/\text{min} \)) and a cell radius of 6.5 \( \mu m \) were used. For figure D,E, we used a growth rate of 350 \( \mu m \) per minute and a cell radius of 6.5 \( \mu m \) (\(< R > = 1.22 \mu m/\text{min} \)).

Figures
MF orientation constrains vesicle flow.
MF orientation constrains vesicle flow.

Figure 2:
MF orientation constrains vesicle flow.

Figure 3:
MF orientation constrains vesicle flow.
Supporting material for

*Microfilament orientation constrains vesicle flow and spatial distribution in growing pollen tubes.*

Jens H. Kroeger¹
Ernest Rutherford Physics Building, McGill University.
Montréal, Québec. Canada H3A 2T8

Firas Bou Daher
Institut de recherche en biologie végétale,
Département de sciences biologiques,
Université de Montréal. Montréal, Québec. Canada H1X 2B2

Martin Grant
Ernest Rutherford Physics Building, McGill University.
Montréal, Québec. Canada H3A 2T8

Anja Geitmann
Institut de recherche en biologie végétale,
Département de sciences biologiques,
Université de Montréal. Montréal, Québec. Canada H1X 2B2

¹Corresponding author. Address: Ernest Rutherford Physics Building, McGill University. 3600 rue University, Montréal, Québec. Canada. H3A 2T8. Tel.: (514)398-7025, Fax: (524)398-8434
Cell wall expansion

Pollen tube elongation is due to stress induced expansion of its cell wall. Although the physical relations between stress $\sigma$ and viscoplastic deformation rate $\dot{\varepsilon}$ are firmly established, the precise role of the turgor pressure in the growth regulation remains ill defined. Decreasing the turgor below a critical level stops growth (1) and modulating the osmolarity induces variations in the tube growth rate (2). While expansion rate and stress variations can be caused by many different factors such as cell wall thickness, extensibility and turgor, the viscoplastic expansion rate and stress in the plant cell wall generally obey Lockhart’s equation $\dot{\varepsilon} = \Phi(\sigma - \sigma_y)$ (3–5). Here $\sigma_y$ is the yield stress and $\Phi$ is the cell wall extensibility. Consequently, we model the cell wall expansion following a viscoplastic analysis based on Lockhart’s equation (6).

The pollen tube cell wall is treated as a thin shell of viscoplastic material (6). The pollen tube is assumed to have axial symmetry and to grow at a steady rate. The growth is assumed to be orthogonal (7), meaning that a marker particle placed on the cell wall moves in a direction normal to the cell wall during growth. The turgor pressure inside the cell creates a tensile stress in the cell wall, which as a result is deformed in a viscoplastic manner. The expansion of the cell wall is described by the following stress-strain rate relations characteristic of a viscoplastic material and previously published by Dumais et al. (2006) (6)

$$\dot{\varepsilon}_i = \Phi(\sigma_e - \sigma_y) \frac{1}{H} \frac{\partial H}{\partial \sigma_i},$$

where $\Phi$ is the extensibility, $H = \sigma_e^2/2$ the stored elastic energy and $\sigma_e$ and $\sigma_y$ are the effective and constant yield stresses. When eq. 1 is written explicitly in terms of $s, n$ and $\theta$, the strain rate components are given by

$$\dot{\varepsilon}_s = \Phi(\sigma_e - \sigma_y) \left( \frac{\sigma_s - \nu \sigma_\theta}{K} \right),$$

$$\dot{\varepsilon}_\theta = \Phi(\sigma_e - \sigma_y) \left( \frac{\sigma_\theta - \nu \sigma_s}{K} \right),$$

$$\dot{\varepsilon}_n = \Phi(\sigma_e - \sigma_y) \left( \frac{(\nu - 1)(\sigma_s + \sigma_\theta)}{K} \right).$$

The stress components are given by

$$\sigma_s = \frac{p}{2\tau K_\theta},$$
MF orientation constrains vesicle flow.

\[ \sigma_\theta = \frac{P}{2\pi\kappa_\theta} \frac{(\kappa_s)}{\kappa_\theta}. \]  

Here, \( \kappa_s \) and \( \kappa_\theta \) are the cell wall curvatures along the curvilinear coordinates, \( \tau \) is the cell wall thickness, and \( p \) the hydrostatic pressure. Since the tube is axysymmetric, the curvature with respect to \( \theta \) can be expressed in terms of the curvature as a function of the position \( s \) along the arc of the tube. The cell wall curvature and shape are calculated in two dimensions. However, this shape contains all the information necessary to extract the 3-dimensional shape. The strain rates can be written in terms of the velocity vector component normal to tube, \( v_n \), and component tangential to the cell wall, \( v_t \),

\[ \dot{\varepsilon}_s = v_n\kappa_s + \frac{\partial v_t}{\partial s}, \]  

\[ \dot{\varepsilon}_\theta = v_n\kappa_\theta + \frac{v_t \cos \varphi}{\tau}, \]  

and

\[ \dot{\varepsilon}_n = -(\dot{\varepsilon}_s + \dot{\varepsilon}_\theta) + \frac{R}{\tau} = 0. \]  

Here, \( R \) is the rate at which new material is deposited on the cell wall.

A simple algorithm for the shape of a cell growing according to the viscoplastic model can be derived in the simple case of orthogonal growth, i.e. when every point on the cell wall moves exclusively in a direction normal to the cell wall such that \( v_t = 0 \). One can define the strain rate anisotropy \( \lambda(s) = (\dot{\varepsilon}_s - \dot{\varepsilon}_\theta)/(\dot{\varepsilon}_s + \dot{\varepsilon}_\theta) \). By substituting the equations for the strain rates into this relation, one obtains

\[ \lambda(s) = \frac{(\sigma_\theta - \sigma_s)(1 + \nu)}{(\sigma_\theta + \sigma_s)(1 - \nu)}. \]  

By defining the stress anisotropy \( \gamma(s) = (\sigma_\theta - \sigma_s)/(\sigma_\theta + \sigma_s) \), eq. 10 yields

\[ \nu = \frac{\lambda - \gamma}{\lambda + \gamma}. \]  

Putting \( v_t = 0 \) in eqs. 7,8 yields \( \lambda = (\kappa_\theta - \kappa_s)/(\kappa_\theta + \kappa_s) \) while substituting eqs. 5,6 into eq.11 for the stress anisotropy yields

\[ \gamma = (\kappa_\theta - \kappa_s)/(3\kappa_\theta + \kappa_s). \]  

Substituting eqs. 10,12 in terms of the principal curvatures into the eq.11, the relation

\[ \kappa_s = 1 - 2\nu(\kappa_\theta) \]  

(13)
MF orientation constrains vesicle flow.

is obtained. Since the pollen tube has axial symmetry, there is a second direct relation between the two principal curvatures. Given a function \( \nu(s) \), the principal curvatures can be found by iterating the following three equations

\[
\varphi(s) = \int_0^s \kappa_s^{(i)} ds,
\]

\[
\kappa_\theta^{(i)} = \frac{\sin \varphi}{r} = \sin \varphi \left( \int_0^s \cos \varphi(s) ds \right),
\]

\[
\kappa_s^{(i+1)} = 1 - 2\nu(\kappa_\theta^{(i)}).
\]

During the iterations, denoted by the superscript \( i \), the following boundary conditions must be met. At the tip, or pole, of the tube, the two principal curvatures must be equal thus \( \nu(0) = 0 \). At the equator of the tube, \( \kappa_\theta \) must be constant along the shank \( (d\kappa_\theta/ds)|_{s=0} \) and \( \kappa_s = 0 \). These two conditions are met by enforcing \( \varphi(S) = \pi/2 \) and \( \nu(S) = 1/2 \). Once the curvature \( \kappa_s \) is calculated along the arc position \( s \), the cell wall can be reconstructed, and the vesicle. Finally, the curvatures \( \kappa_s \) and \( \kappa_\theta \) are inserted into eqs. 5 and 6 for the different stresses. Fig. 2A in the main part of the manuscript shows the shape of the cell wall during the steady viscoplastic growth that satisfy Lockhart’s equation (eq. 1) and eq. 17.

Since the cell wall becomes thinner when stretched, vesicle deposition supplies the material necessary to maintain a constant thickness during the continuous elongation of the cell. The net rate \( R \) at which the vesicles fuse with the membrane (Fig. 2B in the main part of the manuscript) is assumed to be such that the thickness of the cell wall stays constant and the normal strain rate \( \epsilon_n = 0 \) (6). This is expressed by

\[
\dot{\epsilon}_n = -\dot{\epsilon}_s - \dot{\epsilon}_\theta + \frac{R}{\tau} = 0,
\]

where \( \tau \) is the thickness of the cell wall and the \( \dot{\epsilon}_i \) denote the strain rates in the curvilinear coordinates. The average net fusion rate can be estimated from measured quantities (8). Multiplying the average number of vesicles per minute needed to sustain a typical growth of \( 7\mu m/min \) \( (N_V = 3939 min^{-1}) \) by the average vesicle volume \( (V_{vl} = 0.0026 \mu m^3) \) and dividing by the total apex surface \( (S_A = 415 \mu m^2) \) yields an average net fusion rate \( R = 0.0244 \mu m/min \). Furthermore, in order to achieve steady growth, the turgor pressure and the rheological parameters such as the extensibility \( \Phi \) must remain constant. While the precise location of the exocytosis activity at the apex remains a subject of debate (9–12), a comparison of its average value (calculated above) with the average
vesicle flux at the fringe (see below) supports the claim that the vesicle flux pattern is only marginally affected by the localisation of this activity. While we use a consistent model (eq. 17) for this process, our results do not depend on this particular model of the average vesicle secretion rate.

**Actin microfilament orientation**

The spatial distribution and orientation of actin microfilaments is very distinct in the shank and apical fringe of the pollen tube. Long parallel, relatively thick bundles of F-actin occur along the shank of the pollen tube (13–16). However, in the region bordering the apical cone, actin forms a dense network of less bundled filaments (15, 17–19). This region of the actin cytoskeleton is called the apical fringe. The formation and stability of the fringe as well as the filament orientation can be studied with a statistical model of actin filament aggregation (20–25). The actin filaments in the pollen tube can be classified into two distinct groups: the filaments bound to the stable array and those free to move in the cytoplasm. Free actin filaments aggregate and diffuse rotationally and translationally until they bind to the network filaments. Network filaments cannot diffuse but they aggregate and re-orient into bundles of common orientation. This can be expressed with the following reaction-diffusion model (23). We fix our coordinate system such that the tube grows toward the positive y-direction, and will model the change in orientation and density of the actin filaments along the x-direction using

\[
\begin{align*}
\frac{\partial N(x, \Theta, t)}{\partial t} &= \beta_1 FK \ast F + \beta_2 NK \ast F - \gamma N, \\
\frac{\partial F(x, \Theta, t)}{\partial t} &= -\beta_1 FK \ast N - \beta_2 FK \ast F + \gamma N + \mu_1 \frac{\partial^2 F}{\partial \Theta^2} + \mu_2 \frac{\partial^2 F}{\partial x^2}.
\end{align*}
\]

Here \(N(x, \Theta, t)\) and \(F(x, \Theta, t)\) represent the network and free filaments densities. \(\Theta\) is the orientation of the filaments, i.e. the angle between the filaments’ barbed ends and the x-axis. \(\beta_1\) and \(\beta_2\) are the association rates, \(\gamma\) the dissociation rate and \(\mu_1, \mu_2\) the rotational and translational diffusion coefficients. We use the following values in the numerical simulations (\(\beta_1, \beta_2, \gamma, \mu_1, \mu_2\)=(0.5,0.5,0.1,1.0,0.5)). The binding of filaments occurs at a

---

1 Unless specified otherwise, all numbers refer to equations, figures or the bibliography of the Supporting material section
rate that depends on relative configuration and is described by the convolution terms of the form $K * F$ (25) where

$$K * F = \int_{-\pi}^{\pi} \int_{\Omega} K(\Theta - \Theta', x - x') F(\Theta', x') d\Theta dx.$$  \hspace{1cm} (19)

The kernel $K = K_1(x)K_2(\Theta)$ was introduced to model the re-orientation of actin fibres due to the action of cross-linking proteins (20). We use

$$K_2(\Theta) = 0.4 \text{ if } \frac{\pi}{2} - |\Theta - \Theta'| < \frac{\pi}{2},$$  \hspace{1cm} (20)

and else

$$K_2(\Theta) = 0.$$  \hspace{1cm} (21)

The spatial dependence of the kernel is $K_1(x) = \exp(-(x/20)^2)$. Our analysis is simplified by the assumption that the free filaments at the tip were just moved there whereas the filaments in the distal region of the shank have spent a certain amount of time re-arranging and bundling. Accordingly, the state of the filament population at the tip of the apex is well represented by the solution of eqs. 19 at early times. The state of the filament population in the shank is described by the solution at late times, when $N(x, \Theta, t)$ and $F(x, \Theta, t)$ have reached their equilibrium configuration. This assumption allows us to ignore the $y$ variable. In other words, we assume that the change in time of eqs. 19 describe the change along the $y$-axis of the pollen tube. The state of the filament population at the fringe is thus described by the solution of eqs. 19 at intermediate times. We set the initial distribution of network filaments to be $N(x, \Theta, t = 0) = \exp(-0.1(x^2 + (\Theta + \pi/2)^2)) + \exp(-0.1((x - L)^2 + (\Theta - \pi/2)^2)) + \xi$ and $F(x, \Theta, t = 0) = \xi$ where $\xi$ is a random variable that is uniformly distributed around a positive mean. These initial conditions and the model are chosen such that the filament population at late stages adopts the configuration observed in the pollen tube shank (26). The filaments in the shank form bundles in two regions: the centre ($x=0$) and the cytoplasmic cortex ($x=L$). This is equivalent to a filament distribution presenting one density peak in the centre ($x=0$) and one density peak at the periphery ($x=L$). It is known (26) that the bundle in the centre of the shank is composed of filaments with barbed ends pointing toward the back ($\Theta = -\pi/2$), while the bundles at the periphery are composed of filaments with barbed ends pointing towards the front ($\Theta = \pi/2$).

From Fig. 1, we see that the filament density profile along the fringe is uniform, unlike it is in the shank described above. Also, the fraction of the
filament population attached to the network is greater than in the apex but smaller than in the shank. Thus, the state of the filament population in the fringe is described by the solution of eqs. 19 at a time characterised by a sudden increase in the total network filament population and by a rather uniform density profile along the x-direction (Fig. 1A). Fig. 1 shows the solution of eqs. 19. From Fig. 1C, we set the time at which eqs. 19 describe the fringe to be $t_i = 10000\Delta t$. At this time, the average network filament orientation $\Theta(x)$ along the x-direction is evaluated by $\overline{\Theta}(x) = \frac{1}{C} \int_{0}^{L} \Theta N(x, \Theta, t_i) d\Theta$ where $C$ is a normalisation constant. The numerical solution (Fig. 1B) at $t = 10000\Delta t$ can be fitted to the linear orientation profile

$$\overline{\Theta}(x) = \frac{-\pi}{L} x - \frac{\pi}{2}$$  \hspace{1cm} (22)

From now on, we will drop the bar and refer to the average filament orientation along the fringe as simply $\Theta(x)$. The orientation profile could also be fitted to a sigmoid function which would yield a sharper fringe shape, as calculated in the section Profile of the actin fringe. The physical principle that allows to interpret this gradual change in filament orientation is a local elastic interaction between filaments. This elastic interaction re-orientates the filaments in order to minimise the local elastic stress caused by mismatched orientation between neighbouring filaments. It is thus not surprising that eq. 22 satisfies the laws of elastic polymer networks. Given the simplified case of an elastically isotropic polymer network, the stress energy is $E = \nabla^2 \Theta$ (27). Eq. 22 is a solution that minimises this stress.
MF orientation constrains vesicle flow.

Figure 1: Dynamic aggregating and re-orientation of the actin filaments. For (A) and (B) the thick full line represents the solution at $t = 4\Delta t$, the dashed line represents $t = 10000\Delta t$ and the dotted line represents $t = 20000\Delta t$. (A) Total density of network filaments $\int N d\Theta$ along the fringe (x-direction) at three different times. (B) Average filament orientation profile along the x-direction at three different times. The thin full line is a linear fit to the orientation profile. (C) Total density of network filaments $\int \int N d\Theta dx$ as a function of time. The steepest increase is found at a time of $t = 10000\Delta t$. We identify the configuration of the filament population at this time with the configuration in the fringe.
Surface tension at the tail of the cone

At the tail of the apical cone, the two halves of the profile shown in Fig. 2C in the main part of the manuscript must join at a very acute angle. At that position, the curvature of the profile is very large and the surface tension $\gamma$ between the actin fringe and the cytoplasm leads to capillary effects that cannot be neglected. Since the profile is parallel to the actin microfilaments, eq. 3 (in the main part of the manuscript) predicts that the normal profile velocity is zero at that location. That means that the tail of the clear zone becomes thinner and longer as the pollen tube grows, leading to an even sharper profile at its tail. The surface tension reduces the sharpness of the profile by increasing the protrusion rate as a function of the profile curvature. The pressure difference $\Delta p = \gamma K$ generated at the profile is given by the Gibbs-Thompson relation (28). Here $K$ is the curvature given by $K = \frac{y''}{1 + y'^2}^{3/2}$. This pressure difference acts as an effective increase in the local G-actin concentration to increase polymerisation. We approximate this effect by adding a term, valid only in regions of high curvature, to the profile velocity eq. 3 in the main part of the manuscript

$$\frac{dr}{dt} = v_{MF} (\mathbf{n} \cdot \mathbf{r}) + \frac{k_{on} \gamma K}{k_B T},$$

(23)

where $k_B T$ is the energy of the thermal fluctuations. Since the microfilaments are oriented parallel to the profile at the end of the tail, the first term in the previous expression is negligible. Assuming that the end of the tail advances at the same rate $v_p$ as the whole profile yields the equality $k_{on} \gamma K = v_p$. Expressing $K = 1/L$ where $L$ is the radius of curvature at the end of the tail, and $k_{on} = v_{MF}/G \simeq v_p/G$, yields the chemical capillary length $L \simeq \frac{\gamma}{G k_B T}$. We estimate the order of magnitude of the capillary length to be $L \simeq 1.5 \mu m$ from the width of the apical cone in Fig. 1F in the main part of the manuscript. For $G \simeq 10 \mu M$ (29), the surface tension is of the order of $4 \times 10^{-5} J/m^2$. The condition that the curvature $K$ is a constant at the end of the tail is met by a profile in the shape of a half circle

$$y = \left( L^2 - x^2 \right)^{1/2}.$$

(24)

The contribution of the surface tension is visible at the funnel end of the actin fringe, where the two symmetric halves of the profile meet and where its curvature is maximal. The full profile is shown in the inset of Fig. 2C in the main part of the manuscript.
MF orientation constrains vesicle flow.

Effect of arrest of pollen tube growth on vesicle streaming patterns

Vidali et al. (13) used biochemical (profilin and DNase) and pharmacological agents (latrunculin B and cytochalasin D) to perturb actin microfilament polymerisation and structure in growing pollen tubes. They found that these actin polymerisation inhibitors have a much stronger effect on pollen tube growth than on cytoplasmic streaming, i.e. that the concentration of inhibitor needed to stop streaming is much higher than that required to stop growth. To imitate the arrest of pollen tube growth, we set the values for $R=0$ for all positions along the cell wall and set the advancement and protrusion rate of the fringe to zero. The resulting vesicle movement patterns and density distribution are not significantly different from the normally growing tube (Fig. 2).

Therefore, according to our model, inhibiting the polymerisation process while maintaining the acto-myosin mediated vesicle transport would not alter the vesicle flux pattern significantly as long as the shape of the clear zone remains the same. In the same way, an inhibition of vesicle secretion at the plasma membrane would not disrupt the vesicle flux pattern but only disrupt cell wall elongation and thus pollen tube growth. This behaviour
MF orientation constrains vesicle flow.

was observed upon inhibition of Rho-GTPases (30). The treatment blocks vesicle exocytosis while maintaining the cytoplasmic streaming. Essentially, our model explains how the vesicle flux pattern is maintained as long as the orientation of the microfilaments and the funnel shape of the clear zone are maintained, even if the rates of actin polymerisation or pollen tube growth are affected.

**Pollen culture, fluorescent label and image acquisition**

This section explains the experimental methods and techniques used to obtain the pollen tube micrographs shown in Fig. 1 of the main article.

**Actin label**

After two hours of growth, pollen tubes were fixed for 40 seconds in 3% formaldehyde, 0.5% glutaraldehyde and 0.05% Triton X-100 solution in a buffer composed of 100 mM PIPES, 5 mM MgSO$_4$ and 0.5 mM CaCl$_2$ at pH 9. Pollen tubes were then washed 3 times for one minute each in the same buffer followed by an incubation overnight at 4°C in rhodmine phalloidin (Molecular Probes) diluted (1:30) in a buffer composed of 100 mM PIPES, 5 mM MgSO$_4$, 0.5 mM CaCl$_2$ and 10 mM EGTA at pH 7. Subsequently, the pollen was washed 5 times for one minute each in the same buffer, mounted on glass slides in a drop of citifluor (Electron Microscopy Sciences), covered with a cover slip, sealed and immediately observed with a Zeiss Imager-Z1 microscope equipped for structured illumination microscopy (apotome) and with a Zeiss AxioCam MRm camera. A filter set of BP 546/12 excitation, FT 560 beamsplitter and BP 575-640 was used. All fixation and washing steps were conducted in a PELCO cold spot biowave 34700 at 150 Watts and 26°C.

**Vesicle label**

Vesicles in living pollen tubes were labelled by adding 160 nM of the lipophilic styryl dye FM1-43 (Molecular Probes, Invitrogen) to the growth medium for five minutes. Then pollen was filtered and mounted in fresh growth medium between slide and coverslip for microscopic observations with a Zeiss LSM 510 META / LSM 5 LIVE / Axiovert 200M system. A 488 nm argon laser was used with an emission filter LP 575. Z-Stacks of 1 μm interval were taken and image reconstruction and surface rendering were performed using AxioVision Release 4.5 software.
MF orientation constrains vesicle flow.

References


MF orientation constrains vesicle flow.


