Protoplasma (2010) 240:99–107
DOI 10.1007/s00709-009-0088-x

NEW IDEAS IN CELL BIOLOGY

Cytoplasmic streaming enables the distribution of molecules and vesicles in large plant cells

Jeanmarie Verchot-Lubicz · Raymond E. Goldstein

Received: 18 May 2009 / Accepted: 2 November 2009 / Published online: 25 November 2009

Abstract Recent studies of aquatic and land plants show that similar phenomena determine intracellular transport of organelles and vesicles. This suggests that aspects of cell signaling involved in development and response to external stimuli are conserved across species. The movement of molecular motors along cytoskeletal filaments directly or indirectly entrains the fluid cytosol, driving cyclosis (i.e., cytoplasmic streaming) and affecting gradients of molecular species within the cell, with potentially important metabolic implications as a driving force for cell expansion. Research has shown that myosin XI functions in organelle movement driving cytoplasmic streaming in aquatic and land plants. Despite the conserved cytoskeletal machinery propelling organelle movement among aquatic and land plants, the velocities of cyclosis in plant cells varies according to cell types, developmental stage of the cell, and plant species. Here, we synthesize recent insights into cytoplasmic streaming, molecular gradients, cytoskeletal and membrane dynamics, and expand current cellular models to identify important gaps in current research.

Keywords Cytoplasmic streaming · Cytoskeleton · Intracellular transport · Cyclosis · Peclet · Diffusion · Advection · Endoplasmic reticulum · Actin · Myosin network · Calcium

J. Verchot-Lubicz (✉)
Department of Entomology and Plant Pathology,
Oklahoma State University,
Stillwater, OK 74078, USA
e-mail: Verchot.lubicz@okstate.edu

R. E. Goldstein
Department of Applied Mathematics and Theoretical Physics,
Centre for Mathematical Sciences, University of Cambridge,
Wilberforce Road,
Cambridge CB3 0WA, UK

Cytoplasmic streaming is a vital force

Although the phenomenon of cytoplasmic streaming was reported first in 1774 by Bonaventura Corti in his celebrated work on aquatic plants, the precise role of streaming in plant physiology remains somewhat of a mystery to this day. Cytoplasmic streaming, also termed cyclosis, is a very widespread phenomenon, occurring in organisms ranging from amoebae, algae, and terrestrial plants to fungi (Allen and Allen 1978a; Cole et al. 1998; Shimmen and Yokota 2004). Fundamentally, it is driven by organelle-associated myosin motors moving along actin filament bundles. Streaming is directional and often rotational movement that may play a significant role in promoting the exchange of molecules and proteins across organelle membranes. Cytoplasmic streaming is responsible for mobility of the ER, golgi, peroxisomes, and mitochondria. Vesicles and organelles are carried along by myosins (Kachar 1985; Kachar and Reese 1988) and as that cargo has a significantly larger hydrodynamic size than the motors themselves, considerable fluid is entrained by their motion. Whether in regular or disordered patterns, this entrainment leads to large-scale fluid motion within the cell. In plants, the patterns of flow include unidirectional streaming, "fountain" streaming (with motion near the central axis of the cell opposite to that at the periphery), and spiral "rotational" streaming (Allen and Allen 1978a; Allen and Allen 1978b).

The physiological consequences of streaming have implications for metabolism, development, and disease. In early studies, streaming was often associated with cell viability. Cells that are undergoing senescence or are damaged by disease show increased membrane permeability and loss of cyclosis, followed by condensation of the cytoplasm prior to cell death (Hancock 1968; Mertz and

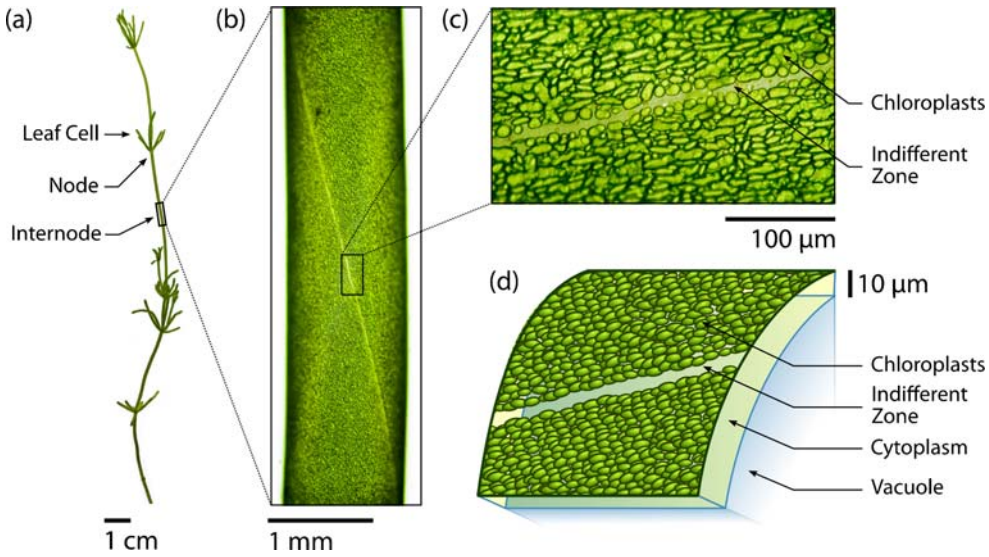

Fig. 1 Geometry of *Chara corallina*

Arntzen 1977; Nieman and Willis 1971). The possible physiological consequences of streaming fall naturally into the two categories of intra- and intercellular roles. These potential roles are easily seen using as a model the alga, *Chara corallina*, which has historically been among the organisms of choice for studies of cytoplasmic streaming due to its large size and simple geometry. As shown in Fig. 1, this plant consists of a sequence of long cylindrical internodal cells that form the stem, meeting at nodes from which emanate branches. Each internodal cell has two helical bands of chloroplasts that meet at two "indifferent zones" visible as light lines spiraling along the cell. The actin–myosin system responsible for streaming resides in the ~ 10 µm thick layer of cytoplasm adjacent to the chloroplasts, with the actin filaments arranged in helical paths along the chloroplasts. Just below, the tonoplast encloses the vacuole. The internodal cells are connected by the plasmodesmata, providing a mechanism for molecular transport from one cell to the next via the cytoplasm. Inside the large multinucleated internodal cells, each of which can reach lengths of 10 cm, is found rotational streaming, with upward motion along one spiraling half of the cell cylinder and down along the other. This is the so-called "barber pole" flow (Goldstein et al. 2008; van de Meent et al. 2008; van de Meent et al. 2010). The early measurements of velocity profiles showed that not only does the actin–myosin system produce fluid flow within the cytoplasm, but the vacuolar fluid is also set in motion with a velocity profile indicated by the arrows in Fig. 2.

The recent literature on streaming involves the two very different perspectives of molecular/cellular biology on the one hand and physics/fluid dynamics on the other. As authors representing these two areas, our goal in this review is to bridge the gap between these disciplines in the hope of stimulating a much deeper understanding of the role of cytoplasmic streaming in plant physiology.

This review begins with a brief explanation of the hydrodynamic flow in plant cells, a theoretical discussion of the competition between advective and diffusive transport, followed by a summary of recent work on the actomyosin network driving streaming and its regulation by Ca^{2+}.

Hydrodynamic flow enhances the mobilization of molecules in plant cells

With streaming velocities that can reach 100 µm/s, the time it takes a fluid parcel to move longitudinally by the length of the *Chara* cell is 10^3 s or about ~ 17 min (Hochachka 1999), whereas the time it takes a small molecule to diffuse that same distance of 10 cm, in the absence of fluid flow, would be 10^7 s or nearly 3 months. While *Chara* is perhaps an extreme case because of its

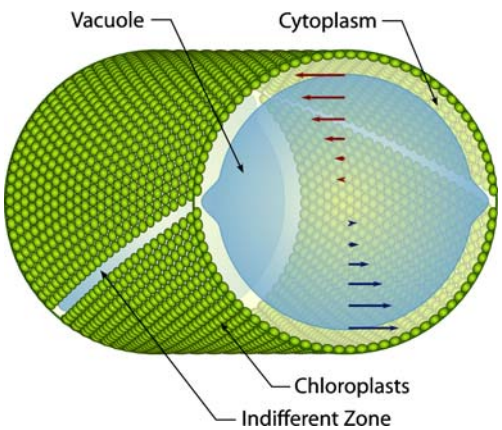

Fig. 2 Cross-sectional view of *C. corallina*, with *arrows* indicating the flow within the vacuole

unusual size, it is nevertheless natural to expect that one role of streaming is simply to accelerate molecular transport within a given cell. Yet, dating back to the original studies of streaming flows, we know that the entire vacuolar fluid is set in motion by forces generated in the cytoplasm (Kamiya and Kuroda 1956). Recent theoretical work discussed below has lent support to the notion espoused some time ago that this stirring of the vacuolar fluid by streaming may also enhance mixing within a given cell, enabling the mobilization of metabolites and contributing to physiological homeostasis. In a separate line of investigation (Pickard 2006), streaming has also been conjectured to enhance transport of molecular species to and from individual organelles within a cell and across membranes of chloroplasts or mitochondria.

In angiosperms, cytoplasmic streaming is greatest in elongated cells such as trichomes, cortical cells of the hypocotyls, pollen tube cells, and phloem-associated cells. Streaming is also reported in BY-2 suspension cells, which, though not giant cells as in *Chara*, can be up to 1 mm in length. Therefore, the notion that cytoplasmic streaming provides the means for transport of organelles, vesicles, and solutes over longer distances and especially toward the growing points is also applicable to angiosperm species. In all cases, streaming is bidirectional and rotational. The intensity of streaming in various cells can vary with plant species. For example, rapid cytoplasmic streaming is described in onion epidermal cells (Jedd and Chua 2002), however, streaming in leaf epidermal and mesophyll cells of *Nicotiana benthamiana* and *Arabidopsis* is reported to be less intense or difficult to detect. Reports of organelle and vesicle movement in leaf epidermal and mesophyll cells have failed to link these events to large-scale fluid motion within the cell or a clear pattern of flow, which constitute cytoplasmic streaming (Avisar et al. 2008; Nebenfuhr et al. 1999). Since vesicles and organelles move shorter distances in *Arabidopsis* leaf epidermal cells, it is arguable that there is less of a requirement for intense cytoplasmic mixing. On the other hand, cortical cells in *Arabidopsis* hypocotyls show streaming rates of 3 μm/s. In root hairs, the subapical region displays streaming velocities of 2 μm/s, while the shank shows velocities of 8–14 μm/s (Emons 1987; Miller et al. 1999; Sieber and Emons 2000). In *Arabidopsis* roots, streaming is greatest in zone of elongation, whereas movement is diminished in the meristem and the zone of differentiation. The root phloem shows rapid movement of peroxisomes with velocities reaching 9 μm/s (Jedd and Chua 2002). Comparison of the streaming velocities in various root cells suggests that there is a positive correlation with cell length. Further research is needed to determine if streaming may be important for phloem function.

An interesting study was recently conducted using 10-day-old BY-2 suspension cultures expressing cytosolic GFP (Esseling-Ozdoba et al. 2008). FRAP was employed to quench GFP expression and record the rate of recovery as a measure of hydrodynamic flow in the cell. The myosin inhibitor 2, 3-butanedione monoxime (BDM) was employed to slow organelle movement and impede cytoplasmic flow. The comparison of fluorescence recovery confirmed a role for organelle movement in creating cytoplasmic flow, which aids the dispersal of GFP in the cytoplasm. The rate of GFP recovery was comparable to the rate of organelle movement. Upon BDM treatment, the rates of both organelle movement and GFP recovery were comparably reduced. In young cells that were approximately 40 μm in diameter, the rates of GFP recovery were similar (and near zero) in untreated and BDM treated cells, a result that supports the argument that cytoplasmic streaming aids the dispersal of molecules in the cytoplasm of large cells (Esseling-Ozdoba et al. 2008).

Advection versus diffusion

A key distinction necessary in the discussion of transport of molecular species is between diffusion and advection (Goldstein et al. 2008). Fickian diffusion is associated with flux of molecules driven by a concentration gradient, whereas advection is mass transport due to fluid flow. Advanced physical measurements of transport processes go beyond the simple determination of distance versus time to quantify the relative strengths of competing processes. Since only pure numbers ("dimensionless quantities") can be said to be large or small, the competition is defined in these terms. One such quantity is the Reynolds number *Re* measuring the importance of inertia to viscous drag, with turbulent flows occurring at very high *Re*. In contrast, most processes that occur within cells or involving motility of individual cells occur without inertia, the world of very small *Re*. Here, external body forces (such as gravity) or internal forces (such as the thrust due to bacterial flagella) are balanced by the drag on particles due to fluid viscosity. In this regime, the transport of molecular species occurs by a combination of diffusion and "advection," the latter arising from the existence of fluid flow such as cytoplasmic streaming. The quantity measuring the relative importance of these processes is known as the Péclet number *Pe*, and it can be viewed as the ratio of two characteristic time scales. Suppose there is some characteristic length L over which the concentration of a molecular species varies (e.g., the diameter of a cell) and a typical velocity U of the flow (e.g., the cytoplasmic velocity of streaming) and a molecular diffusion constant D. The first time of interest is $T_{\text{advection}} = L/U$, the time it takes for the flow to cover a distance L. The

second is the time $T_{\text{diffusion}}=L^2/D$ for diffusive spreading over a distance L. The ratio defines the Péclet number,

$$Pe = \frac{T_{\text{diffusion}}}{T_{\text{advection}}} = \frac{L^2/D}{L/U} = \frac{UL}{D}.$$

The diffusion-dominated regime $Pe << 1$ holds at small length scales, large diffusion constants and/or slow flows. Swimming bacteria provide a good example, for with $L \sim 1$–5 μm, $U \sim 1$–20 μm/s, and a molecular diffusivity $D \sim 10^3$ μm^2/s, one finds $Pe \sim 0.001$–0.1, implying that fluid motion is not important for transport. On the other hand, cytoplasmic streaming can readily involve cell sizes reaching $L \sim 1{,}000$ μm, with fluid velocities $U \sim 100$ μm/s, which yield $Pe \sim 100$. Of course, for the transport of larger species, with correspondingly smaller diffusion constants, Pe will be even larger (Goldstein et al. 2008; van de Meent et al. 2008).

In this flow-dominated regime, the transport and mixing of molecular species can be very complex, involving the formation of "boundary layers" of high concentration gradients which accelerate the transport across surfaces compared to that by diffusion alone. This effect is very much like the increased heat loss on a windy day, as measured by the wind–chill factor.

In the case of cytoplasmic streaming, the most obvious advective transport is parallel to the long axis of a cell (such as the internodal cells of *Chara*), but the particular geometric features of rotational streaming, which is driven by helical forcing in the cytoplasm, can give rise to flows across the diameter of the cell (Fig. 3) within the vacuole (Goldstein et al. 2008; van de Meent et al. 2008). This intrinsically three-dimensional aspect of transport depends on the tightness of the cell helix and can lead to a transport process that competes with diffusion. The result is mixing of the vacuolar contents on a time scale much shorter than required by diffusion acting alone.

Actomyosin network provides internal mechanical force for cytoplasmic streaming and organelle movement

In *Chara* and *Nitella* it is the movement of actin tethered organelles that drives cytoplasmic flow (Nagai and Hayama 1979). Cells treated with cytochalasin B, latrunculin B, or BDM, which disrupt the actomyosin network, show cessation of cytoplasmic streaming and organelle movement (Foissner and Wasteneys 2007). Actin is a polar polymer and its orientation determines the direction of myosin motion. In *Chara* and *Nitella*, myosin XI is responsible for organelle movement along actin filaments. Actin polymerization and myosin motion generate the force that drives cytoplasmic streaming. The myosin motors in *Chara* are among the fastest myosins, causing obvious rapid movement of organelles and cytoplasmic mixing (Ito et al. 2007; Sumiyoshi et al. 2007; Yamamoto et al. 2006).

In angiosperms, studies exploring the growth characteristics of pollen tubes have revealed a vital role for actin polymerization and cytoplasmic streaming (Fig. 4a). One function of streaming is to maintain steep ion gradients that are also essential for driving polar cell growth (Hepler et al. 2001). Studies using lily, tobacco, and *Arabidopsis* pollen tubes reveal that growth characteristics are conserved among angiosperms. Specifically, growth occurs mainly at the apical dome in a forward direction. A large portion of the cell is taken up by the vacuole and nucleus with a thin cytoplasm along the periphery and the bulk of the cytoplasm seems to push forward into the apical region (Hepler et al. 2001). Actin filaments are arrayed longitudinally and polymerization drives tube elongation. Vesicles traffic along the microfilaments and accumulate at the extreme apex to deposit cell wall material that enables apical elongation. Organelles are found in the subapical region and are absent from the apex (Hepler et al. 2001). Unlike the helical streaming in *Chara*, the cytoplasmic motion in pollen tubes is referred to as "reverse fountain"

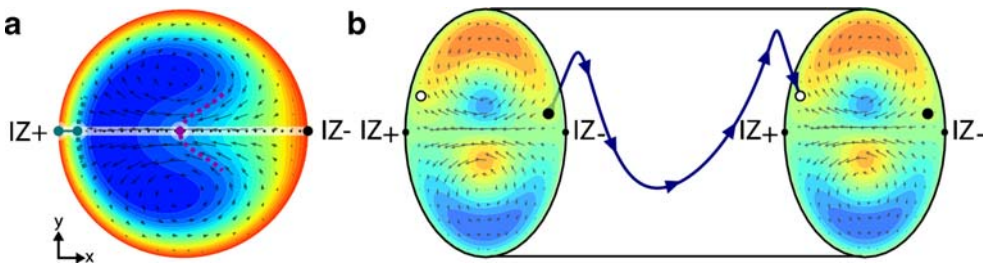

Fig. 3 Theoretical flow patterns of a cell with rotational cytoplasmic streaming. **a** Viewed in cross section, the two indifferent zones ($IZ_{\pm}$) of an internodal cell of *Chara* are not equivalent with one having local fluid flows away from the cell periphery and the other toward the periphery. *Arrows* indicate calculated velocity profiles (van de Meent et al. 2008) and *colors* show concentration of a molecular species diffusing inward from the cell wall. The flows send a tongue of enhanced concentration across the cell diameter, leading to mixing of the vacuolar contents. **b** A depiction of the three-dimensional character of the flow showing how a parcel of fluid that starts near one indifferent zone is carried to the other zone as it is advected in a helical path along the length of the cell. This provides an alternative illustration of the mixing properties of rotational streaming

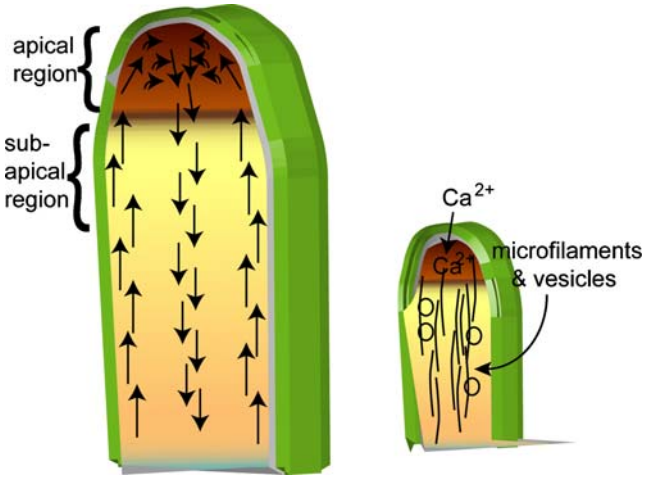

Fig. 4 Cytoplasmic streaming in angiosperm pollen tubes. **a** The cylindrical body of the pollen tube is similar among most plant species. There is an elongated shaft and a rounded apex. Growth is mainly along the apex. Apical and subapical regions are indicated. *Arrows* in the larger diagram on the left shows the pattern of reverse fountain streaming. *Acropetal arrows* show forward cytosolic movement toward the apex while the basipetal movement is represented by *interior arrows*. The *interior shading* represents ionic gradients with brown at the apex representing high concentration of Ca^{2+}. The smaller version of the pollen tube depicts the longitidunal microfilaments which drive cell elongation. Vesicles are carried along the filaments to the *tip* and deposit newly synthesized cell wall materials needed during elongation. The interior Ca^{2+}gradient can be fed by external Ca^{2+} taken up at the apex

streaming (Fig. 4a; Hepler et al. 2001). The cytoplasm moves forward along the edge of the cell toward the apex and then basipetal movement is central. The actin network is longitudinally arrayed and carries vesicles toward the apex, and the network is disorganized at the pollen tube apex (Cardenas et al. 2008; Vidali et al. 2001). Thus, the actin network provides the driving force for growth and cytoplasmic circulation.

It is worth noting that studies characterizing the directional flow in pollen tubes are primarily collected from in-plane data, and thus, a three-dimensional analysis is of interest to understand whether there are mixing components in reverse fountain streaming like those associated with the helical flow seen in *Chara* species. Clearly pollen tube cytoplasm flows in two bands of opposite polarity, which implies that there may be an indifferent zone with high shear (Fig. 4). A study of the interplay between calcium gradients and cytoplasmic streaming (Lazzaro et al. 2005) provides an important step in unraveling the competition between advective and diffusional transport of ions and microscopic particles in cell growth. The movement of vesicles within pollen tubes is itself also a fascinating example of inertialess motion in which motor activity balances against viscous drag, yet, there is little theoretical understanding of the details of this process.

Organelle and vesicle transport in angiosperms is also tightly linked to the actomyosin network and in particular, the myosins VIII and XI. Class VIII myosins are responsible for regulating conductivity across plasmodesmata, as well as endocytic trafficking, ER tethering, and plasma membrane dynamics (Sattarzadeh et al. 2008; Volkmann et al. 2003). Class XI myosins tether chloroplasts, mitochondria, Golgi, and peroxisomes, and it is their motion that is essential for cell elongation and vitality (Prokhnevsky et al. 2008).

In higher land plants, particular attention has been paid to myosin XI isoforms, which are responsible for organelles sliding along actin filaments because they have provided evidence alluding to cytoplasmic circulation (Avisar et al. 2008; Li and Nebenfuhr 2008; Peremyslov et al. 2008). Gene knockouts for the 13 class XI myosins in *Arabidopsis* showed that inactivation of certain isoforms causes profound changes in mitochondria, Golgi, and peroxisome mobility as well as root hair growth. Further analysis in leaves showed a similar role for myosins in organelle movement, arguing that myosin motors are primarily responsible for organelle repositioning in higher land plants. This is based on significant variability in the movement of individual peroxisomes or Golgi bodies while verifying the relationships of myosins and organelle movement. A broader and more in depth analysis of large cells in the roots, phloem-associated tissues, and trichomes are needed to learn if the same myosin isoforms drive streaming in various tissues and plant species.

Furthermore, physical scientists agree that microfilament-driven organelle movement perpetuates flow and can be used to measure the dimensions of cytoplasmic flow for the dispersal of molecules that are not tethered to the actomyosin network but may reside in the cytoplasm or even inside the endomembrane network. For example, distribution of cytosolic Ca^{2+} and calmodulin is driven by cytoplasmic streaming in both algae and angiosperms (Cardenas et al. 2008; Hayashi and Takagi 2003). Ca^{2+} is vital to transduction of environmental stimuli into physiological adaptations including cell elongation. In pollen tubes, microinjected calmodulin spreads evenly throughout the cell with some binding behind the apical region (Obermeyer and Weisenseel 1991). However, once growth is arrested the specific concentration of calmodulin is dispersed through the cytosol, providing evidence to support the notion that the destination of cytosolic free proteins such as calmodulin, as well as, vital ion gradients are directly linked to active cytoplasmic streaming, driven by actin polymerization for polarized cell growth (Moutinho et al. 1998; Obermeyer and Weisenseel 1991).

It remains an open problem to understand the implications of spatial inhomogeneities in streaming profiles for molecular transport in elongated plant cells. High-resolution studies of streaming patterns in various model organisms

(esp. *Chara*) with appropriate molecular and large tracer particles should help address the issue of how local variations in streaming speeds interact with molecular diffusion to modify transport. The recent development of genetic tools, GFP fusions and myosin inhibitors has prepared the way for a new era in research to measure the cytoplasmic dynamics and its implications for intracellular and intercellular dispersal of organelles, vesicles, and vital macromolecules (Sparkes et al. 2008; Walter and Holweg 2008). Theory, too, must be developed to help address such questions. To join theory and experiment, a truly three-dimensional map of the velocity and concentration fields in the appropriate cells of model organisms is needed.

Ca^{2+} and environmental cues regulate cytoplasmic streaming in higher aquatic plants

Unlike the less evolved *Chara* and *Nitella*, which show persistent streaming, *Elodea* and *Vallisneria* show light and environmental cues induce movement (Takagi et al. 2003). *Vallisneria* plants show significantly greater streaming in the mesophyll cells with little or no movement in epidermal tissues. In one study, the types of motion that occur in *Vallisneria* plants as they transition from quiescent to active streaming were characterized (Takagi et al. 2003). Plants were maintained in the dark, and then chloroplast movement in mesophyll cells was studied following treatment with the Ca^{2+}-chelating agent, EGTA. The initial response was local random movement, followed by saltatory movement, and after 2 min the chloroplasts formed a line and showed unidirectional motion. The velocity increased to a maximum within 10 min of EGTA treatment. Interestingly, the number of streaming cells in a tissue examined microscopically increased with increasing EGTA concentrations and with time. At most, 50% of the cells treated with 1 mM EGTA were active between 20 and 30 min following treatment while 100% showed activity within the same time frame following treatment with 20 mM EGTA. However, cytoplasmic streaming declined after 30 min when treated with 1 mM EGTA or after 3 h following treatment with higher concentrations. These experiments clearly show that chemical treatments are sufficient to alter cytoplasmic circulation in a manner that can antagonize plant cellular transport functions (Takagi et al. 2003).

Tests employing varying qualities of light determined that continuous cytoplasmic streaming occurs under lighting that is between 450 and 600 nm, but that far red light is inhibitory. Research has also shown that cytoplasmic Ca^{2+} is lower in streaming *Vallisneria* mesophyll cells than in quiescent cells. In fact, high concentrations of cytoplasmic Ca^{2+} can cause cessation of streaming in *Vallisneria* (Hayashi and Takagi 2003; Takagi et al. 2003).

Importantly, these *Vallisneria* studies focused on organelle movement, specifically chloroplasts, as a measure of cytoplasmic streaming in plant cells. However, in some organisms, chloroplast movement is unrelated to streaming. Cytoplasmic streaming has been studied for 50 years in the cylindrical-shaped giant cells of algae such as *Nitella* and *Chara*. As mentioned previously, studying the movement of cytoplasmic granules has provided the crucial measure of cytoplasmic flow. In fact, the movement of cytoplasmic fluid past fixed chloroplasts can increase the exchange of molecules across the membrane boundary (Pickard 2003), and so, extensive and coordinated movement of organelles might not be as necessary as some researchers suggest (Avisar et al. 2008). The spiraling flow in *Chara* is represented by ascending and descending bands of cytoplasm separated by indifferent zones where there is little movement (Figs. 2 and 3). Given the contrast between results obtained by studying chloroplast dynamics and those from cytoplasmic granular motion, it is important to develop assays that can measure directly the flow of cytoplasmic fluid along with the motion of particles of various types, keeping in mind that some particles are passively carried with the streaming flow, some particles are actively transported by myosins (thus, participating in the generation of the flow), while others are fixed or not systematically in motion.

Ca^{2+} provides a driving force for cytoplasmic streaming in angiosperms and is a second messenger in response to cellular stimuli

For most pollen tubes that have been studied, cytoplasmic streaming enables formation of a Ca^{2+} gradient (Fig. 1) that focuses toward the pollen tube apex to promote polar cell growth (Cardenas et al. 2008; Cardenas et al. 2005; Chen et al. 2008; Vidali et al. 2001). The Ca^{2+}-specific buffer dibromo-BAPTA as well as brefeldin A were shown to dissipate the gradient and inhibit pollen tube elongation, although, streaming and growth are recovered when the Ca^{2+} gradient is revived (Parton et al. 2003). Moderate concentrations of Ca^{2+} drive cytoplasmic circulation throughout the pollen tube shaft. Moderate levels of Ca^{2+} are also essential for longitudinal growth of microfilaments, thereby driving actin assembly toward the tip and creating forward motion (Cardenas et al. 2008; Justus et al. 2004). This also promotes reversal of streaming in the apex and central regions. High concentration of Ca^{2+} at the apex is sufficient to disassemble microfilaments at the apex (Fig. 4), which causes the cell to continue actin polymerization from the opposite pole for cellular elongation. Thus, it is the self-propagating relationship between Ca^{2+} and the microfilament network that drives cytoplasmic streaming and polar growth (Chen et al. 2008; Yokota et al. 1999).

Importantly, if cytoplasmic Ca^{2+} along with the actin network are sufficient to drive cytoplasmic streaming in pollen tubes, then it is possible that external or physiological stimuli, which result in the increase of cytoplasmic Ca^{2+} in other types of cells such as leaf mesophyll or root cells, induce cytoplasmic motion that is an important component of the physiological response (Shimmen and Yokota 2004; Pike et al. 2005). Specifically, drought and the stress hormone ABA cause elevated levels of cytosolic free Ca^{2+} in plants and play a central role in physiological adaptation (Navazio et al. 2001; Pickard and Ding 1993). Changing concentrations of cytosolic Ca^{2+} are also linked to programmed cell death mediated by hypoxia or ethylene (Ihara-Ohori et al. 2007; Romeis et al. 2001). Ca^{2+}-dependent protein kinases are active in cell death involving a hypersensitive response to pathogen attack (Romeis et al. 2001). A specific example is the bacterium *Pseudomonas syringae* AvrRpt2 protein that triggers membrane depolarization and Ca^{2+} entry into the cell.

Another aspect of Ca^{2+} based metabolism is the role of Ca^{2+} in the ER. A resting cell has low levels of Ca^{2+} in the cytosol and higher levels in the ER, which enables the plethora of Ca^{2+}-dependent chaperones in the ER to stabilize protein-folding intermediates following translation (Navazio et al. 2001). Various cellular disturbances cause accumulation of unfolded proteins in the ER, prompting a response that is conserved across kingdoms: the unfolded protein response (UPR). Stress exerted upon the ER, including events relating to UPR, can lead to release of ER Ca^{2+} stores to the cytoplasm. Aberrations of ER Ca^{2+} can occur as the result of environmental factors such as pathogen attack, nutrient depletion, or osmotic stress, leading to oxidative stress and ROS production (Ellgaard and Helenius 2001). Depletion of ER Ca^{2+} stores and consequential rise in cytosolic Ca^{2+} can interfere with proper protein glycosylation, disulfide bond formation, or protein transfer to the Golgi leading to the accumulation of misfolded proteins in the ER and upregulating protein degradation pathways to enable turnover of the defective proteins (Zhang and Kaufman 2006).

Architectural features of the ER change in response to stress and Ca^{2+} mobilization. The ER in a resting state cell is a tubular network at the surface and cisternae lying deep in the cell. There is flow within the ER between the cortical cisternae and tubular strands. It is postulated that the cisternae sequester Ca^{2+} more effectively than the tubules. Depletion of Ca^{2+} from the ER can cause a decline in cisternal ER and longer tubules. Researchers have reported (Navazio et al. 2001) that when the levels of cytosolic Ca^{2+} are extremely high, the ER is constricted to thin tubules. It would be interesting to determine whether signal transduction of external stimuli via Ca^{2+} mediated pathways leads to changes in ER architecture and increase cytoplasmic streaming by altering cytosolic Ca^{2+} concentrations.

Conclusion

Cytoplasmic streaming is vital for plant development and growth as demonstrated by examples of algae, aquatic plants, and angiosperms. *Chara* provides an example of streaming in internodal cells, *Vallisneria* shows streaming in the mesophyll but not epidermal cells, and lily provides an example of streaming in the pollen tubes. Recent cloning of myosins and cytoskeleton-associated proteins has led to new insights into organelle movement and vesicle transport. These create new opportunities to explore cytoplasmic streaming in other tissues such as leaf mesophyll or root cells in higher land plants. In addition, efforts to understand the factors regulating streaming in cells may lead to new insights into cellular responses to external stimuli such as invading pathogens.

We wish to emphasize again the importance of quantitative measures of the three-dimensional movement patterns of various components of the cytoplasm (lipid droplets, mitochondria, vesicles, and the fluid itself). It is likely that molecular species will be transported differently from objects with much larger hydrodynamic scales, but there is little relevant information of this type yet available. Experiments to characterize cytoplasmic mixing, using fluorescent proteins such as GFP, caged dyes, or suitable tracer particles are also urgently needed. Extension of these techniques to land plants will help validate the hypothesized connections between such plants and the aquatic plants outlined here. Finally, a systematic determination of the Péclet number in different species will help make contact with emerging theoretical ideas regarding the interplay of advection and diffusion.

Acknowledgments We thank an anonymous reviewer for excellent and careful edits and suggestions and are grateful to J. Carr, J. Davies, E. MacRobbie, I. Tuval, J.-W. van de Meent, and A. Webb for important discussions. This work was supported in part by a USDA CSREES-NRI-2007-01530 Research Sabbatical Grant and OCAST ONAP08-018 (J.V.L.), the Leverhulme Trust and the Schlumberger Chair Fund (R.E.G.), and Oklahoma Center for Advancement of Science and Technology Contract number 7141.

Conflict of Interest The authors have no conflict of interest. We have no financial relationship with the organization that sponsored the research.

References

Allen N, Allen R (1978a) Cytoplasmic streaming in green plants. Annu Rev Biophys Bioeng 7:497–526

Allen R, Allen N (1978b) Cytoplasmic streaming in ameboid movement. Annu Rev Biophys Bioeng 7:469–495

Avisar D, Prokhnevsky AI, Makarova KS, Koonin EV, Dolja VV (2008) Myosin XI-K Is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. Plant Physiol 146(3):1098–1108

Cardenas L, Lovy-Wheeler A, Wilsen KL, Hepler PK (2005) Actin polymerization promotes the reversal of streaming in the apex of pollen tubes. Cell Motil Cytoskeleton 61(2):112–127

Cardenas L, Lovy-Wheeler A, Kunkel JG, Hepler PK (2008) Pollen tube growth oscillations and intracellular calcium levels are reversibly modulated by actin polymerization. Plant Physiol 146 (4):1611–1621

Chen KM, Wu GL, Wang YH, Tian CT, Samaj J, Baluska F, Lin JX (2008) The block of intracellular calcium release affects the pollen tube development of Picea wilsonii by changing the deposition of cell wall components. Protoplasma 233(1–2):39–49

Cole L, Orlovich D, Ashford A (1998) Structure, function, and motility of vacuoles in filamentous fungi. Fungal Genet Biol 24:86–100

Ellgaard L, Helenius A (2001) ER quality control: towards an understanding at the molecular level. Curr Opin Cell Biol 13 (4):431–437

Emons AMC (1987) The cytoskeleton and secretory vesicles in root hairs of Equisetum and Limnobium and cytoplasmic streaming in root hairs of Equisetum. Annu Bot 60:625–632

Esseling-Ozdoba A, Houtman D, Van Lammeren AAM, Eiser E, Emons AMC (2008) Hydrodynamic flow in the cytoplasm of plant cells. J Microscopy 231:274–283

Foissner I, Wasteneys GO (2007) Wide-ranging effects of eight cytochalasins and latrunculin A and B on intracellular motility and actin filament reorganization in characean internodal cells. Plant Cell Physiol 48(4):585–597

Goldstein RE, Tuval I, van de Meent JW (2008) Microfluidics of cytoplasmic streaming and its implications for intracellular transport. Proc Natl Acad Sci USA 105(10):3663–3667

Hancock J (1968) Effect of infection by Hypomyces solani f. sp. Cucurbitae on apparent free space, cell membrane permeability, and respiration of squash hypocotyls. Plant Physiol 43:1666–1672

Hayashi T, Takagi S (2003) Ca^{2+}-dependent cessation of cytoplasmic streaming induced by hypertonic treatment in *Vallisneria* mesophyll cells: possible role of cell wall-plasma membrane adhesion. Plant Cell Physiol 44(10):1027–1036

Hepler PK, Vidali L, Cheung AY (2001) Polarized cell growth in higher plants. Annu Rev Cell Dev Biol 17:159–187

Hochachka PW (1999) The metabolic implications of intracellular circulation. Proc Natl Acad Sci USA 96:12233–12239

Ihara-Ohori Y, Nagano M, Muto S, Uchimiya H, Kawai-Yamada M (2007) Cell death suppressor *Arabidopsis* bax inhibitor-1 is associated with calmodulin binding and ion homeostasis. Plant Physiol 143(2):650–660

Ito K, Ikebe M, Kashiyama T, Mogami T, Kon T, Yamamoto K (2007) Kinetic mechanism of the fastest motor protein, *Chara* myosin. J Biol Chem 282(27):19534–19545

Jedd G, Chua NH (2002) Visualization of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding. Plant Cell Physiol 43(4):384–392

Justus CD, Anderhag P, Goins JL, Lazzaro MD (2004) Microtubules and microfilaments coordinate to direct a fountain streaming pattern in elongating conifer pollen tube tips. Planta 219(1):103–109

Kachar B (1985) Direct visualization of organelle movement along actin filaments dissociated from characean algae. Science 227:1355–1357

Kachar B, Reese T (1988) The mechanism of cytoplasmic streaming in characean algal cells: sliding of endoplasmic reticulum along actin filaments. J Cell Biol 106:1545–1552

Kamiya N, Kuroda K (1956) Velocity distribution of the protoplasmic streaming in *Nitella* cells. Bot Mag Tokyo 69:544–554

Lazzaro MD, Cardenas L, Bhatt AP, Justus CD, Phillips MS, Holdaway-Clarke TL, Hepler PK (2005) Calcium gradients in conifer pollen tubes; dynamic properties differ from those seen in angiosperms. J Exp Bot 56:2619–2628

Li JF, Nebenfuhr A (2008) Inter-dependence of dimerization and organelle binding in myosin XI. Plant J 55(3):478–490

Mertz S, Arntzen C (1977) Selective inhibition of K^+, Na^+, Cl^-, and PO_4^{3-} uptake in *Zea mays* L. by *Bipolaris (Helminthosporium) maydis* Race T pathotoxin. Plant Physiol 60:363–369

Miller DD, deRuijter NCA, Bisseling T, Emons AMC (1999) the role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. Plant J 17:141–154

Moutinho A, Trewavas AJ, Malho R (1998) Relocation of a Ca^{2+}-dependent protein kinase activity during pollen tube reorientation. Plant Cell 10(9):1499–1510

Nagai R, Hayama T (1979) Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. J Cell Sci 36:121–136

Navazio L, Mariani P, Sanders D (2001) Mobilization of Ca^{2+} by cyclic ADP-ribose from the endoplasmic reticulum of cauliflower florets. Plant Physiol 125(4):2129–2138

Nebenfuhr A, Gallagher L, Dunahay T, Frohlick J, Mazurkiewicz A, Meehl J, LA S (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. Plant Physiol 121 (14):1127–1142

Nieman R, Willis C (1971) Correlation between the suppression of glucose and phosphate uptake and the release of protein from viable carrot root cells treated with monovalent cations. Plant Physiol 1971:287–293

Obermeyer G, Weisenseel MH (1991) Calcium channel blocker and calmodulin antagonists affect the gradient of free calcium ions in lily pollen tubes. Eur J Cell Biol 56(2):319–327

Parton RM, Fischer-Parton S, Trewavas AJ, Watahiki MK (2003) Pollen tubes exhibit regular periodic membrane trafficking events in the absence of apical extension. J Cell Sci 116(Pt 13):2707–2719

Peremyslov VV, Prokhnevsky AI, Avisar D, Dolja VV (2008) Two class XI myosins function in organelle trafficking and root hair development in *Arabidopsis*. Plant Physiol 146(3):1109–1116

Pickard BG, Ding JP (1993) The mechanosensory calcium-selective ion channel: key component of a plasmalemmal control centre? Aust J Plant Physiol 20:439–459

Pickard W (2003) The role of cytoplasmic streaming in symplastic transport. Plant Cell Environ 26:1–15

Pickard WF (2006) Absorption by a moving spherical organelle in a heterogeneous cytoplasm: implications for the role of trafficking in a symplast. J Theor Biol 240:288–301

Pike SM, Zhang XC, Gassmann W (2005) Electrophysiological characterization of the *Arabidopsis* avrRpt2-specific hypersensitive response in the absence of other bacterial signals. Plant Physiol 138(2):1009–1017

Prokhnevsky AI, Peremyslov VV, Dolja VV (2008) Overlapping functions of the four class XI myosins in *Arabidopsis* growth, root hair elongation, and organelle motility. Proc Natl Acad Sci USA 105(50):19744–19749

Romeis T, Ludwig AA, Martin R, Jones JD (2001) Calcium-dependent protein kinases play an essential role in a plant defence response. Embo J 20(20):5556–5567

Sattarzadeh A, Franzen R, Schmelzer E (2008) The *Arabidopsis* class VIII myosin ATM2 is involved in endocytosis. Cell Motil Cytoskeleton 65(6):457–468

Shimmen T, Yokota E (2004) Cytoplasmic streaming in plants. Curr Opin Cell Biol 16(1):68–72

Sieber B, Emons AMC (2000) Cytoarchitecture and pattern of cytoplasmic streaming in root hairs of Medicago truncatula during development and deformation by nodulation factors. Protoplasma 214:118–127

Sparkes IA, Teanby NA, Hawes C (2008) Truncated myosin XI tail fusions inhibit peroxisome, Golgi, and mitochondrial movement in tobacco leaf epidermal cells: a genetic tool for the next generation. J Exp Bot 59(9):2499–2512

Sumiyoshi H, Ooguchi M, Ooi A, Okagaki T, Higashi-Fujime S (2007) Insight into the mechanism of fast movement of myosin from *Chara corallina*. Cell Motil Cytoskeleton 64(2):131–142

Takagi S, Kong SG, Mineyuki Y, Furuya M (2003) Regulation of actin-dependent cytoplasmic motility by type II phytochrome occurs within seconds in *Vallisneria gigantea* epidermal cells. Plant Cell 15(2):331–345

van de Meent JW, Tuval I, Goldstein RE (2008) Nature's microfluidic transporter: rotational cytoplasmic streaming at high Peclet numbers. Phys Rev Lett 101(17):178102

van de Meent JW, Sederman AJ, Gladden LF, Goldstein RE (2010) Measurement of cytoplasmic streaming in single plant cells by magnetic resonance velocimetry. J Fluid Mech. (in press)

Vidali L, McKenna ST, Hepler PK (2001) Actin polymerization is essential for pollen tube growth. Mol Biol Cell 12(8):2534–2545

Volkmann D, Mori T, Tirlapur UK, Konig K, Fujiwara T, Kendrick-Jones J, Baluska F (2003) Unconventional myosins of the plant-specific class VIII: endocytosis, cytokinesis, plasmodesmata/pit-fields, and cell-to-cell coupling. Cell Biol Int 27(3):289–291

Walter N, Holweg CL (2008) Head-neck domain of *Arabidopsis* myosin XI, MYA2, fused with GFP produces F-actin patterns that coincide with fast organelle streaming in different plant cells. BMC Plant Biol 8:74

Yamamoto K, Shimada K, Ito K, Hamada S, Ishijima A, Tsuchiya T, Tazawa M (2006) *Chara* myosin and the energy of cytoplasmic streaming. Plant Cell Physiol 47(10):1427–1431

Yokota E, Muto S, Shimmen T (1999) Inhibitory regulation of higher-plant myosin by Ca^{2+} ions. Plant Physiol 119(1):231–240

Zhang K, Kaufman RJ (2006) The unfolded protein response: a stress signaling pathway critical for health and disease. Neurology 66(2 Suppl 1):S102–S109