To go with the flow: Molecular motors are a drag
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Chapter 5

Hydrodynamic Flow in the Cytoplasm of Plant Cells

In this thesis we test our hypothesis for explaining how transport takes place in cells by means of computer simulations. Our simulations show that the momentum transfer via hydrodynamic interactions between the active bound organelles and the suspended objects gives rise to a non-negligible flow in solution. In order to test these results in a real biological system Agnieszka Esseling-Ozdoba performed experiments in the group of Anne Mie C. Emons (Department of Plant sciences) at Wageningen Universiteit. This work was a close collaboration between both our groups and was published [150,162]. The text below is an adapted version of the publication. Copyright 2013 Wiley. Used with permission from Esseling-Ozdoba et al., Hydrodynamic flow in the cytoplasm of plant cells, Journal of Microscopy, Blackwell Publishing Ltd, 2008, 231, 274-283 [150]. The original section on materials and methods can be found in appendix C.

Introduction

The cytoplasm of eukaryotic cells consists of all cell material between the nucleus and the plasma membrane and contains membrane-bounded structures, organelles, which are embedded in the cytosol consisting of water, salts and organic molecules, including sugars, proteins, and many enzymes that catalyse reactions. The cytoskeleton of microtubules and actin filaments in the cytosol structures the cell by localising and transporting the organelles bound to these tubules and filaments. The plasma membrane, enveloping the cytoplasm physically, separates the cell content from the extracellular environment, which in plant cells is the cell wall. The latter consists of cellulose micro-fibrils embedded in a matrix of polysaccharides, glycoproteins and phenolics. The largest organelle in the plant cell is the vacuole [163–165], which functions as waste managing factory and also to maintain the hydrostatic pressure in the cell. Cytoplasmic strands of cytoplasm (also called transvacuolar strands) that are bounded by the vacuolar membrane traverse the central vacuole in mature plant cells. They connect
the peripheral cytoplasm to the cytoplasm that surrounds the cell nucleus [164, 166]. In Fig. 5.1-Left, a stamen hair cell of *Tradescantia virginiana* is shown and the main components of the cell are highlighted.

![Diagram of cell](image)

**Figure 5.1:** Left - Cytoplasmic streaming in *Tradescantia virginiana*. Right - Confocal laser scanning microscope (CLSM) images of young tobacco BY-2 suspension cells transformed with GFP:FABD ( fimbrin actin-binding domain) to visualise actin filaments (A and A1) and GFP:TUA6 (α-tubulin) (B and B1) to visualise microtubules. Actin filaments (A) but not microtubules (B) are present in the cytoplasmic strands. Both microtubules and actin filaments are present in the cell cortex (Figures A1 and B1). The fluorescence in cytoplasmic strands in B is possibly free GFP-tubulin, bar = 20 μm.

Under a differential interference contrast (DIC) microscope, the rapid transport of organelles in the cytoplasmic strands can be observed. This rapid transport is called cytoplasmic streaming (e.g. pollen tubes: [167]; root hairs: [168]; review: [169]). The organelle movement is driven by the molecular myosin motors that walk along actin filaments [5, 170–173]. In interphase plant cells, microtubules are not present in the cytoplasmic strands (this chapter) but radiate only from the nucleus during the transitions from interphase into mitosis [163, 174, 175], and just after cytokinesis [176]. In cytoplasmic strands, actin filaments are mainly present in bundles [169, 177–179] and can be considered to be cellular highways, on which organelle-associated myosin motors move their cargo [5, 48, 170, 171, 180]. The energy necessary for the movement of the myosin motors is generated by hydrolysis of ATP. In vitro, the maximum measured speed of the higher plant myosin XI is 7 μm/s [30]. Organelle movement in different plant cells can reach various velocities, see table 5.1.

The bulk of soluble molecules such as metabolites do not move with motor proteins in the cytoplasm. In a system in which particles, organelles here, move actively in-
Plant Speed | Reference
---|---
Pollen tubes | Nicotiana tabacum, Arabidopsis | ± 2 | [181, 182]
Root hairs | Medicago truncatula | 8-14* | [168]
Hypocotyl | Equisetum hyemale | 3.5-7* | [177]
Stamen hairs | Arabidopsis | 4.8 | [183]

Table 5.1: Motor velocities in different plant cells. *Organelle velocity dependent on the developmental stage of the cell. **Own observations, unpublished.

side an aqueous environment with suspended molecules, it is expected that the actively moving particles induce a flow in the surrounding medium, dragging along other particles and molecules. For this intra-cellular movement the term hydrodynamic flow may be used. In general, the term hydrodynamic flow is used when a fluid stream exerts a drag force on any obstacle placed in its wake, and the same force arises if the obstacle moves and the fluid is stationary [184].

A recent simulation study [149] showed that active transport of organelles gives rise to a hydrodynamic flow in the cytosol, which may be important for the fast distribution of proteins and nutrients in large cells. Here we show for the first time that actively transported organelles produce hydrodynamic flow in plant cells, which significantly contributes to the movement of the molecules in the cytosol. We show that in the cytoplasm of tobacco BY-2 suspension cells, constitutively expressing cytoplasmic GFP, free GFP molecules move faster in cells with active organelle transport than in cells where this transport has been inhibited. Furthermore, we show that the direction of the GFP movement is the same as that of the organelle movement. We conclude that hydrodynamic flow is a faster way than diffusion in distributing molecules inside plant cells.

Results

Choice of inhibitors of organelle movement

Our aim was to study whether actively moving organelles influence the movement of molecules in the cytosol. The cells of choice were tobacco BY-2 suspension cells expressing cytoplasmic GFP. In the cytoplasmic strands of those cells, actin filaments are present (Fig.5.1A), but microtubules are not (Fig.5.1B) showing that actin filaments are the highways for organelle movement in plant cells. Actin depolymerising agents stop streaming while microtubule depolymerising drugs do not influence organelle movement (data not shown). For this study we used Fluorescence Recovery After Photo-
bleaching (FRAP). In such experiments a cytoplasmic area is photobleached and the time of re-appearance of the bleached fluorescence molecule is measured. FRAP was measured in 2.2 \( \mu m \times 2.2 \mu m \) areas of cytoplasmic strands. Fig.5.2A shows the recovery of GFP fluorescence in a bleached area of a cytoplasmic strand of a young BY-2 tobacco suspension cell.

![GFP recovery in control cells](image1)

**Figure 5.2:** Recovery of GFP fluorescence after photobleaching in untreated control-cells (A), cells treated 50 mM BDM (B) and fixative: 2% paraformaldehyde (PA) and 0.2% glutaraldehyde (GA) (C). In cells treated with BDM fluorescence recovery of GFP was observed. In cells treated with fixatives no fluorescence recovery was observed. The recovery was measured in young BY-2 suspension cells.

We used 2,3 butanedione monoxime (BDM) as an inhibitor of active organelle transport in the cytoplasm of BY-2 suspension cells expressing cytoplasmic GFP. Our purpose was to inhibit organelle movement, but not the Brownian movement of GFP. BDM is a general myosin ATPase inhibitor [185–187]. BDM at a concentration of 50 mM stopped organelle transport, but recovery of GFP occurred (Fig.5.2B), similarly like in the control cells (Fig.5.2A). The recovery of GFP ceases in cells chemically fixed with 2% paraformaldehyde (PA) and 0.2% glutaraldehyde (GA). In those cells no GFP recovery occurred during a typical FRAP experiment (Fig.5.2C).
Free GFP molecules move faster in cells with active transport than in those treated with BDM

To test whether the moving organelles induce hydrodynamic flow in the cytoplasm, we used cells with active transport and compared the GFP recovery after photobleaching in control cells with cells in which this movement is inhibited with BDM. With this, we test if hydrodynamic flow is produced by moving organelles and to what extent this contributes to the movement of molecules in the cytoplasm. If our hypothesis is correct, then the recovery of GFP molecules in the cytoplasm of control cells should be faster than in the cells in which this movement is inhibited. Instead of using young small cells that hardly have visible organelle movement (Fig.5.3A) and consequently hardly any hydrodynamic flow, we used large elongated cells (Fig.5.3B) from 10-day-old subcultures of tobacco BY-2 suspension cells, expressing cytoplasmic GFP. Like in young cells from 3 to 4-day-old subcultures (Fig.5.1A), actin filaments but not microtubules are present in the cytoplasmic strands (Fig.5.4) and are responsible for active movement of organelles, since treatment with the actin depolymerising drug latrunculin stops cytoplasmic streaming (data not shown; [172, 188, 189]). In comparison with young small cells, 10-day-old cells have visible, vigorous cytoplasmic streaming (0.78 ± 0.17 μm/s). We measured the half time of GFP recovery after photobleaching in a cytoplasmic strand region of 2.2 μm x 2.2 μm. GFP recovers faster (within 0.31 ± 0.07 s) in untreated cells with active organelle transport, than in cells treated with 50 mM BDM (within 0.48 ± 0.12 s) (Fig.5.5). This shows that actively transported organelles contribute significantly to the movement of GFP in the cytoplasm of BY-2 cells. In theory, one would expect to see movement of the bleached area, since omnidirectional diffusion will be superimposed on the hydrodynamic flow, resulting in a distorted diffusion pattern. However, the speed of recovery, combined with the theoretical unevenness of the speed distribution of flow, being faster nearer the actin filament bundles than further away from them, is such that this cannot be observed. From our experiments we conclude that organelle movement causes a hydrodynamic flow that contributes significantly to the movement of free GFP in the cytoplasm.

Hydrodynamic flow is higher in cells in which the velocity of organelle movement is faster

If motor-driven organelle movement causes the hydrodynamic flow of cytoplasmic molecules, then the speed of GFP movement in the cytoplasm should positively relate to the speed of organelle movement. To show this, we measured the GFP recovery after photobleaching in cells with different velocities of organelle movement (Fig.5.6). In cells in which organelles moved with an average velocity of 0.21 ± 0.02 μm/s, the recovery of GFP fluorescence took 0.73 ± 0.19 s, which was slower than in cells hav-
BY-2 cells from a suspension culture: A. Typical cell from a 3 to 4-day-old culture, B. Typical cell from a 10-day-old culture. The cells in a 10-day-old culture show vigorous cytoplasmic streaming with easily visible moving organelles. Insert in B shows a cytoplasmic strand with organelles indicated by arrowheads. Bars in A and B correspond to 10 μm and in the insert to 5 μm.

Figure 5.4: CLSM images of 10-day old tobacco BY-2 suspension cells labelled with GFP:FABD (fimbrin actin-binding domain) to visualise actin filaments (A) and GFP:TUA6 (α-tubulin) to visualise microtubules (B). Actin filaments (A) but not microtubules (B) are present in the cytoplasmic strands; fluorescence in the cytoplasmic strands in B is due to free GFP-tubulin. Bar = 10 μm. A1 and B1 are images of cell cortex.

ing faster organelle movement of 0.75 ± 0.10 μm/s. In those cells, GFP recovery was 0.14 ± 0.04 s. This shows that the fluorescence recovery of GFP increases with the increasing velocity of organelles in the cytoplasm, and confirms that organelle transport induced hydrodynamic flow occurs in plant cells.

With FRAP we measured that GFP recovery, in a 10-day-old BY-2 cell, in an area of 2.2 μm×2.2 μm, is on average 0.31 s without BDM and on average 0.48 s with BDM (Fig. 5.5). If we consider the half time of GFP recovery as a time that GFP moves trough...
Figure 5.5: Recovery of cytoplasmic GFP after photobleaching in control BY-2 cells and cells treated with 50 mM BDM. GFP recovered faster in control cells with active organelle transport than in cells in which this transport was inhibited with the myosin inhibitor BDM. This shows that organelle movement causes movement of GFP in the cytoplasm. For FRAP experiments, we used large elongated cells from a 10-day-old culture. Half time of GFP recovery after photobleaching is shown in a region of 2.2 μm × 2.2 μm, data shown with SD.

Figure 5.6: Correlation between GFP fluorescence recovery after photobleaching and the velocity of organelles in the cytoplasm of BY-2 suspension cells. GFP recovery after photobleaching is faster in cells with faster organelle movement. Half time of GFP recovery is shown, measured in regions of 2.2 μm × 2.2 μm, data presented from 8-10 measurements and shown with SD.

a distance of 2.2 μm, then we could calculate that GFP needs 16.9 s without BDM (with speeding up by organelles) and 26.2 s with BDM (without speeding up by organelles) to travel a distance of 120 μm, which is on average a length of 10-day-old BY-2 cells. For this calculation, we also considered only one cytoplasmic strand that connects two ends of a cell. In young BY-2 cells, GFP recovery did not differ in cells treated or not treated...
with BDM. Without BDM, half time of GFP-recovery was on average 0.51 s and with BDM 0.56 s. Therefore we exclude that the difference in GFP mobility between BDM-treated and control 10-day-old cells was caused by a change in cytoplasmic viscosity. Taking into consideration that the length of young cells is 40 μm, GFP needs 11.8 s without BDM and 12.7 s with BDM to travel from one side of the cell to the other. We conclude that in large cells organelle movement is faster than in a small cells and that it is responsible for an efficient distribution of GFP molecules.

**Direction of GFP movement in a cytoplasmic strand is the same as that of moving organelles**

If moving organelles cause hydrodynamic flow of molecules, then the direction of flow should be the same as that of the organelle transport. To check this, GFP was photo-bleached in a region of 2.2 μm × 2.2 μm and its fluorescence recovery was followed in time. After photobleaching we checked the direction in which organelles move in the strand. We observed that after photobleaching GFP moves in the direction of the moving organelles (N = 4) (Fig.5.7). This confirms that hydrodynamic flow occurs in plant cells with moving organelles, which drag molecules, in this case GFP, in their wake, as theoretically predicted [149].

![Figure 5.7: After photobleaching, cytoplasmic GFP (white arrowheads) moved in the same direction (black arrow) as organelles (black arrowheads). GFP is visible as fluorescent speckles and an organelle as a black spot of about 1 μm. For this experiment we used 10-day-old elongated cells with an average organelle velocity of 0.78 μm/s (± 0.17); time is indicated in seconds after photobleaching; bar = 1 μm.](image)

**Synthetic lipid and stealth vesicles move in cytoplasmic strands**

To test if hydrodynamic flow could contribute to the movement of larger structures such as vesicles we injected synthetic lipid vesicles of size of 80 nm in diameter into *Trades-cantia virginiana* stamen hair cells and observed if they moved inside the cytoplasm. The vesicles were made only of phospholipids without any proteins and their membrane contained 98% of 1,2-Dioleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (DOPG) and 2% of fluorescent phosphocholine Bodipy FC12-HPC. Injected vesicles distributed in the
cytoplasm of the whole cell within 10-15 min after the injection (Fig. 5.8A). This result would suggest that these vesicles moved by hydrodynamic flow produced by moving organelles in the cytoplasm. We could not exclude that vesicles were coated with cytoplasmic motor proteins upon the injection and moved along the actin cytoskeleton. Therefore we injected so called stealth vesicles to which proteins cannot be attached. Stealth vesicles are phospholipid vesicles with addition of PEG (polyethylene glycol) phospholipids. The presence of PEG phospholipids prevents the attachment of proteins to the surface of vesicles. Stealth vesicles are often used in drugs therapy to prevent the attachment of proteins and lysis of vesicles that carry the drugs [190]. The concentration of PEG phospholipids used in those studies was 5-10%. The concentrations of PEG phospholipids that we used to make stealth vesicles varied from 2% up to 30% in combination with DOPG. All injected stealth vesicles distributed in the cytoplasm (Fig. 5.8B) similar to DOPG vesicles without PEG and moved in the cytoplasm but slower (0.8 ± 0.13 \( \mu \text{m/s} \)) than 1-2 microns organelles (1.4 ± 0.25 \( \mu \text{m/s} \), Fig. 5.8C). The fast distribution of stealth vesicles and their movement in the cytoplasm could be an indication that structures larger than molecules and possibly organelles move by hydrodynamic flow in the cytoplasm of plant cells.

Figure 5.8: Synthetic lipid (DOPG) vesicles (A) and stealth vesicles (B) of 80 nm in diameter distribute in the cytoplasm of T. virginiana stamen hair cells after injection. Stealth vesicles moved slower than cell organelles of 1-2 \( \mu \text{m} \) in diameter (C). Stealth vesicles were made of 15% of PEG phospholipids, 83% of DOPG and 2% of Bodipy FC12-HPC. Images were taken 15 minutes after injection, bar = 10 \( \mu \text{m} \).
Discussion

Transport in cytoplasmic strands

A major characteristic of mature plant cells is that they have large vacuoles that expand considerably during cell elongation and together become one central vacuole that takes up most of the cell volume. The vacuole confines the cytoplasm to a thin layer in the periphery of the cell and an area around the nucleus. The cytoplasm of these two regions is connected to each other by a few thin tunnels of cytoplasm: the cytoplasmic strands, also called transvacuolar strands [165, 166]. These cytoplasmic strands are essential transport routes for the distribution of organelles and metabolites [169,172]. The mechanism of this transport is organelle movement, caused by an ATP-driven movement of myosin motors attached to the organelles along the actin cytoskeleton [5, 169, 191]. Unbound structures move via diffusion, a passive process of molecule distribution. We raised the question whether what we see as cytoplasmic streaming includes a passive component of organelle drag induced by the actively moving organelles. Our experimental work indeed is in agreement with our theoretical considerations that the active transport induces hydrodynamic flow in the cytoplasm that surrounds the organelles, speeding up the transport of otherwise only diffusing molecules.

Difference between young and mature cells in organelle transport

The velocities of cytoplasmic streaming vary between different cell types, the developmental stage of the cell and the species. This is interesting, because these cells have similar myosins that belong to plant specific subfamilies of Myosin VIII and XI. Myosins XI are more abundantly expressed in plant cells than Myosin VIII. Myosin XI has been shown to be present on peroxisomes [192] and fragments of the tail may localise to Golgi and mitochondria [193]. Myosin VIII appears to be restricted to the plasma membrane and plasmodesmata [194]. This makes Myosin XI the candidate motor molecule involved in organelle transport [1]. We observed differences in velocities of organelle movement through cytoplasmic strands between small elongating and large fully elongated cells of a tobacco BY-2 suspension culture. This phenomenon is not specific for cells from this suspension culture. Also in other fully elongated plant cells, of for instance T. virginiana stamen hairs, the velocity of organelle movement increases during cell elongation and is maximal in fully elongated large mature cells (own observation, unpublished). The most obvious visible difference between these cells, besides their difference in size, is the larger volume that is taken up by the vacuole in the fully elongated cells. Molecules move faster for longer distances through thinner tubes. Differences in speed of cytoplasmic streaming have been observed within different parts of one and the same cell, namely, in root hairs and pollen tubes, that grow at the cell tip that contains only vesicles, which show little movement [168]. In the sub-apical cytoplasmic
dense region of these growing hairs the net speed of organelles is low up to 2 \( \mu m/s \) \((Equisetum hyemale [177], Vicia sativa [178], Medicago truncatula [168])\). In the shank of tip growing root hairs where the central vacuole is present, the net speed of organelles is 8-14 \( \mu m/s \) \((Equisetum hyemale [177], Vicia sativa [178], Medicago truncatula [168])\). In these cells, these differences can be linked to the configuration of the actin cytoskeleton, which consists of thick bundles in the hair tube but of thin bundles in the subapex \((Equisetum hyemale [177], Vicia sativa [178], Arabidopsis thaliana [195]; Eucalyptus globulus [196])\). In the sub-apex, organelles often stop for a short time and jump for a short distance from one place to the other [168]. Also the possible differences in the configuration of the actin cytoskeleton between the elongating and fully elongated BY-2 suspension cells are expected to be the underlying mechanism of the measured differences in velocities of organelle movement. If indeed the myosin type is the same in both cell structures, it cannot be the step size that determines organelle velocity, but the number of steps made per time unit.

**Need for hydrodynamic flow in large cells**

Molecules in the cytosol that are not attached to the cytoskeleton do not posses an active driving force for transport. They move by Brownian motion only. If a concentration gradient is produced for instance by the consumption of molecules at one side of the cell, such as the high rate of sugar use at the cell cortex to produce the cell wall, directional movement governed by Brownian motion is the result. Here we show that movement of molecules in a cell, and maybe also ribosomes, vesicles and small organelles, is faster than mere diffusion, and that the cause for this speeding up is the active transport of organelles along bundles of actin filaments, as theoretically predicted [149]. If the velocity of organelles in the cytosol of tobacco BY-2 suspension cells is three times faster, the GFP molecules move also three times faster (Fig.5.6). Why would this be useful for cells? Signalling and other proteins have to find a partner to bind to for their activity, and these activities have to be carried out at the right sites in the cell. If all of these molecules had to be produced at the site where needed, this would require precise targeting of ribosomes, including the free ribosomes and polysomes not attached to the endoplasmic reticulum. Now we show that random molecule movement inside the cytoplasm, can be speeded up by myosin-driven organelle movement along the actin cytoskeleton, increasing the chance for finding a partner. In the cells that have fast cytoplasmic streaming, hydrodynamic coupling between actively transported organelles and the surrounding solvent causes molecules to spread faster, with the result that those molecules will reach their target with greater efficiency.

The mature BY-2 suspension cells that we studied have velocities of cytoplasmic streaming 10-15 times lower than for instance some of the root hairs studied, or than mature Tradescantia stamen hair cells. We expect that in those cells also organelles, or at
least vesicles, could be dragged along with the actively moved motor-driven organelles. Also lipid droplets injected into hyphal extensions of *Neurospora crassa* moved without motor proteins [197]. The movement of fluorescent stealth vesicles injected into *T. virginianna* stamen hair cells in the cytoplasmic strands indicates that these vesicles and possibly organelles can also move by hydrodynamic flow in the cytoplasm of plant cells.