Making it big : how characean algae use cytoplasmic streaming to enhance transport in giant cells

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6 CONCLUSIONS AND OUTLOOK

At the start of this thesis, we made the observation that the vast majority of cells found in nature have a size of less than 100 μm, whilst a large number of the cells with unusually large dimensions show forms of active internal transport. Most commonly this transport takes the form of cytoplasmic streaming, the steady and continuous circulation of the cellular fluid. The fact that streaming occurs precisely in larger cells suggests that this process may serve to help the cell maintain homeostasis by enhancing diffusive transport, which becomes prohibitively slow on long length scales.

In this work we sought to quantify this often raised hypothesis through a hydrodynamical treatment of rotational streaming in the characean algae, one of the best studied instances of this circulation. These weeds grow in shoots segmented into single-celled internodes with diameters as large as 1 mm and lengths that can exceed 10 cm. At the surface of these cylindrical cells, the fluid is transported up and down along two spiralling bands separated by two neutral lines where the flow reverses. The helicity of these bands varies over the course of development of the cell and is greatest during growth.

By analysis of the fluid mechanics of the internodal circulation we obtained a mode-expansion solution for the flow field, which shows excellent agreement with our measurements based on magnetic resonance velocimetry. Our solution also showed that helicity of these bands is in fact significant for the symmetry of the internal flow field. In a non-helical geometry, the flow field is parallel to the bands at every point in the cell. When the bands are helically shaped, a small transverse component is found, taking the form of two circulation vortices in the ascending and descending stream, driving an advection current between the two neutral lines. The amplitude of this secondary component increases with the helical pitch, suggesting that internal mixing could be improved by lowering the wavelength of the bands.

The effect of this circulation on transport can be quantified in a variety of ways. Our main analysis examines the response of the cell to a change in external concentration. We show that this response is enhanced in a manner determined by the Péclet number Pe^* of the circulation. At one of the neutral lines, the outward advection leads to the formation of a boundary layer at the periphery, whose thickness δ scales as $\mathrm{Pe}^{*-1/3}$. This behaviour is reminiscent of the situation in the spherical algae *Volvox*, where a similar

type of boundary layer scaling is observed due to the flows around the organism. This decrease of the boundary layer size implies an enhanced concentration gradient, and thereby an increased typical flux. Further analysis of the transient problem in the characean internode shows that this enhanced flux can be quantified by examining the rate of decay of the slowest eigenmode of the system of equations.

The role of this enhanced flux in cellular metabolism could be to increase rates of molecular exchange between the central vacuole and the layer of cytoplasm that surrounds it, thus aiding homeostasis by increasing the ability of the vacuole to serve as a buffering reservoir with respect to the metabolic processes that take place in the cytoplasm, particularly in the phase of cell development when growth is largest.

A key unknown surrounding this analysis is the appropriate choice of magnitude of the Péclet number associated with this circulation. At the height of cell development, the transverse velocity is only about 1% of the velocity at the wall. The Péclet numbers associated with this circulation would therefore lie somewhere in the range $\mathrm{Pe}^* = 0.1 - 10$, depending on the metabolite that is considered. While this indicates that the effects of the circulation could be just about significant, the benefits in terms of flux associated with this range of Péclet numbers would in fact be quite small.

In summary, it appears that the helical streaming found in characean algae does indeed provide a mechanism for enhancing metabolic rates, thus to some extent mitigating the diffusive limits associated with large cell sizes. However, given the relatively low magnitude of the observed enhancements of fluxes, these benefits would be restricted to slowly diffusing metabolites, like larger proteins, with a diffusion constant of $D \sim 10\ \mu\mathrm{m}^2/\mathrm{s}$.

Given this result it would be of interest to revisit the growth experiments performed by Green (1954), which track the evolution of the helicity of internodes during growth. We have set up a series of experiments that measure cell size, helicity and streaming rate in a growing shoot by imaging bottled plants with intervals of one day. With the results of these experiments we hope to make a more detailed assessment of the extent to which the observed enhancement of diffusion could play a role in cellular metabolism.

Returning to our initial question regarding the relation between diffusion and cell size, it is important to note that most of the discussion in this thesis has focused on flow and diffusion inside the vacuole. The thickness of the peripheral layer of cytoplasm, which houses all the cellular organelles, lies somewhere in the range 5-50 μm. Thus when considering

diffusion in the cytoplasm specifically, it appears that the diffusion times along the radial axis should not be all that different from those in typical cells. However diffusion should still be expected to be rate limiting along the axis of streaming and perhaps even more so along the transverse axis. A key question to be examined in future work is therefore how streaming affects diffusion inside the cytoplasm.

In order to develop a better understanding of how diffusion functions in the complex and highly crowded environment of the cytoplasm, a better understanding of cytoplasmic rheology is needed. Our experiments using injected beads suggest that the flow of the cytoplasm is noticeably non-Newtonian. This injection protocol has now been developed to a point where detailed studies of the interplay between streaming, diffusion and rheology are coming within reach of our capabilities. A combination of fluorescent staining of the cytoskeleton, flow measurements and microrheology experiments could allow this system to serve as a test case for models of myosin driving mechanics and cytoplasmic rheology.

The characean algae could present an excellent test-case for experiments on diffusion in the cytoplasm in the plant kingdom. Studies tracking diffusion of fluorescence-labelled proteins have largely focused on animal cells and prokaryotes, where length scales are much shorter. With its sheet-like structure, the characean cytoplasm has a comparatively simple geometry, potentially allowing new insight into how diffusive displacements scale with length in this crowded environment, ultimately helping answer the question of what physical factors limit the size of cellular life.