A Brief Summary of Cytoplasmic Streaming (Cyclosis)

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Cytoplasmic streaming, or cyclosis, is the name given to the fluid-like motion of the cytoplasm of cells, particularly in eukaryotic cells. Starting with the seminal work by Kamiya and Kuroda,[1] who studied the alga *Nitella*, it is now known to be driven in large part by the motion of the motor protein myosin along filamentary actin strands. The ratcheting myosin drags along much larger organelles, including the endoplasmic reticulum, which in turn entrain the cytoplasm. [2–8]

Myosin translocation is powered by the hydrolysis of ATP, and in plants is considerably faster than in muscles.[9] Indeed, sliding velocities can reach up to 100 μ/s. As summarized by Pickard,[10] the motion of protoplasmic granules entrained in the flow has a stochastic component, likely due to Brownian motion, but also includes unidirectional "streaming," and "fountain streaming," in which the motion near the central axis of the cell is opposite to that near the periphery. Even spiral "rotational streaming" is known. The velocity of cytoplasmic streaming has been studied by direct visualization and also by such techniques as laser doppler spectroscopy,[11] which has provided an early, localized measure of the width of the velocity distribution and the rather strong temperature dependence to the streaming velocity in *Nitella*.

I suggest four key questions remain to be answered in the kind of systematic, quantitative manner central to modern biological physics. These relate to the fundamental role played by cytoplasmic streaming.[12,13]

- *What determines the speed of cyclosis?* Given that the motion of organelles carried by myosin is the fundamental driving force, a still-unanswered question is how the speed and area (or volumetric) density of those forcing elements determines the basic velocity scales of cyclosis. Note that myosin motion along actin can be rather stochastic, with motors attaching and detaching, and thus there is not necessarily a simple relation between the average motor velocity and the fluid speed.

- *What determines the flow profile?* Observations suggest that the velocity profile need not look like the standard Pouiseuille flow we expect from pipe flow driven by bulk forces. And we know that there can appear bidirectional flow. It is quite likely that the inherent viscoelasticity of the cytoplasm plays a role in determining the detailed flow. Historically, there are very few in-depth theoretical studies of the velocity fields.[14,15]

- *How is the flow pattern regulated?* Since the flow pattern can change over time even within a given cell the orientation of myosin must change as well. How is this controlled? This also relates to the proposed role of cyclosis in gravitational sensing.[16]

- *What is the nature of the molecular transport controlled by cyclosis?* Pickard[10] emphasized four areas in need of further investigation: "(i) visualization of the real-time transport of messages and metabolites; (ii) enumeration of the entities trafficked; (iii) elucidation of the routing of the messages and metabolites within the cytoplasm; and (iv) transference of the trafficked entities from cytoplasm ...." It should be emphasized that even the problem of thermal diffusion within the cytoplasm is highly complex, as seen in recent studies of bacteria[17] and yeast.[18] We need to develop methods to tackle these questions

As we know, the Peclet number

$$Pe = \frac{UL}{D}$$

is the appropriate dimensionless measure of the competition between advection and diffusion, where U is a characteristic fluid velocity, L a characteristic length scales, and D the diffusion constant.. As we have seen, $U \sim 10 - 100\mu$m/s and cellular length scales give $L \sim 100\mu$m. The most conservative estimate of the diffusion constant D for the purposes of estimating the Peclet number is $D \sim 10^{-5}$ cm^2/s, appropriate to a *small* molecule like O_2. Since the diffusion constant is inversely proportional to the radius of a particle, a value of $D \sim 10^{-6}$ cm^2/s is probably more appropriate for the more important proteins or other chemical messengers. Thus, it is possible for $Pe \sim 10^2$, at which advection strongly dominates diffusion. The dynamics of molecular transport in the presence of such stirring is largely unstudied in this context.

In order to achieve a true quantitative understanding of the dynamics of cyclosis I propose a two-pronged attack. First, an appropriate *in vitro* model should be developed using microfluidic fabrication techniques. One possible realization involves actin filaments localized along the walls of a microfluidic chamber, with myosin attached to microspheres carried along, thus dragging the fluid. This is very much like the "gliding motility assay" used to investigate myosin dynamics[19,20] in which a bed of myosin is created on a solid substrate (e.g. glass) and filamentary actin is allowed to settle on those motors and be pushed along. A similar setup has been used to study kinesin translocating along microtubules.[21–23] Such a system allows systematic control over the size of the spheres carried along, control over the local ATP concentration (e.g. through flash photolysis methods), etc.

As made clear by the theoretical investigations cited above,[14,15] the interplay between the localized forcing of organelles carried by myosin moving along actin and the larger-scale fluid motion of the cytoplasm as a whole requires control over the dimensions of the cytoplasm container on the scale of tens to hundreds of microns. This can easily be achieved with PDMS technology.[24] Quantification of the velocity profiles of entrained fluid can be achieved with the technique of Particle Imaging Velocimetry (PIV), using suspended fluorescent microspheres as has been done recently with flagella-driven flows around *Volvox*.[25,26] This will help answer the fundamental question of what the fluid is doing independent of the organelles.

It is worthwhile mentioning that this kind of model system is naturally the most straightforward to model theoretically, as the problem of spheres moving near a wall and entraining fluid is well-studied.[27] The likely effects of viscoelasticity have not been incorporated, but may be very important. These can be studied experimentally by the method of microrheology,[28,29] by which the time-resolved positional fluctuations of suspended microspheres reveal the relevant dissipative and elastic components of the fluid response. Models of the rheology of suspensions of motor proteins have recently been developed from very general symmetry considerations.[30] There is also a fairly long history of more microscopic approaches to transport within the cytoplasm.[31–36]

Our *in vivo* investigation would naturally would use *Acetabularia*, for which we have so much local expertise. Through micro-injection of tracer particles (e.g., quantum dots or fluorescent microspheres) we can quantify the fluid velocity profile, the dynamics of diffusion in the presence of those shearing flows, the effects of light of various wavelengths on the streaming velocity (as it may influence the production of ATP), etc. It might also be of interest to investigate a hybrid *in vitro* and *in vivo* system by studying extracted cytoplasm in microfluidic chambers or even lipid vesicles.[37]

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