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**THE MECHANICS OF CYTOPLASMIC STREAMING IN *DROSOPHILA*
OOCYTES:
HYDRO-MECHANICAL COUPLING OF MICROTUBULES AND KINESIN
MOTOR PROTEINS**

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Abstract

The Mechanics of Cytoplasmic Streaming in *Drosophila* Oocytes:
Hydro-mechanical Coupling of Microtubules and Kinesin Motor Proteins

by

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Cytoplasmic streaming is the widespread motion of viscous cytoplasm fluid occurring in some large Eukaryotic cells. In a developing *Drosophila* egg, termed oocyte, both the slow, poorly ordered and fast, self-organized forms of streaming require microtubule filaments and connected kinesin motor protein walkers. These flows are implicated in two respective processes: polarity establishment and fluid mixing. The fast streaming state is particularly intriguing as microtubules have been observed to generate long-ranged, wave-like motions. In utilizing fundamental hydrodynamic and physical principles, Joshua Deutsch et al. (2012) have developed a model of this system which successfully demonstrates both slow and fast streaming states. The simulations of their model are a primary topic of discussion in this paper, as they also elucidate to the mechanics behind the slow to fast transition. This paper aims to show that in combination with additional data and experiments, there is strong evidence supporting their hypothesis for the mechanism behind cytoplasmic streaming. In brief, the microtubule bending and directional fluid flows observed during streaming are a result of a force transfer from kinesin to the microtubule, and to surrounding fluid. In total, this review discusses the underlying mechanism of streaming, explores results of the model generated by Deutsch et al. (2012), and considers what we know about the critical transition from slow to fast streaming.

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1

Introduction

1.1 Background

Fluid flows are ubiquitous and play key roles in all biological systems. All living organisms are made up of one or more cells, and the fluid dynamics inside these cells represents a large part of the shaping of that organism. Although cells can differ greatly in their size, structure, and function, all share a common feature, namely, “a large proportion of their volume is a gel like fluid: cytoplasm” (Cartwright et al., 2008, p. 79). The cytoplasmic space is home to all intracellular activities that are not sequestered within membrane-bounded organelles (e.g. the nucleus) (Luby-Phelps, 2013). And transport processes which deliberately move chromosomes, organelles, and other objects through cytoplasm are fundamental to the reproduction, growth, and development of eukaryotic cells (cells having a nucleus enclosed within a nuclear envelope and membrane-bound organelles) (Monteith et al., 2016). Found in many large eukaryotic cells, cytoplasmic streaming, also

termed cyclosis in plant species, is an important activity that accomplishes many of the necessary transport processes. It involves the persistent circulation and mixing of the cell's viscous fluid contents driven by cytoskeleton and motor proteins (Goldstein et al., 2008). Although this transport process has been thoroughly studied and proven essential in the development of proper plant and animal cells, it has not been until recently that the underlying mechanism and its hydrodynamic underpinnings have been quantitatively understood.

In the late 1700s, cellular streaming was first discovered in plant cells, specifically in the large cells of the freshwater plant *Chara* (similar to green algae), by Italian botanist Bonaventure Corti (Verchot-Lubicz & Goldstein, 2010). This rotational fluid motion was experimentally observed and found to enable materials such as oxygen and other nutrients to reach all parts of the cell. More recently, streaming has been observed in the relatively large oocytes (immature egg cells) of many multicellular metazoan animal species. For example, *Drosophila* (fruit flies), *Xenopus* (amphibians), mice, and humans have oocytes ranging from $100\mu m$ to greater than $1mm$ in diameter, and undergo this streaming process during some stages of oocyte development (Quinlan, 2016). Many theorize that fluid flows are more important in large cells rather than smaller cells. One plausible reason for this is that the streaming process is used as a mechanism for long-distance transport within these large cells (Quinlan, 2016). The cell's cytoplasm is viscous and thick, and although this is useful in supporting its organelle and cellular molecules, it makes for very slow diffusion processes. Therefore the ability for a large cell to properly mix its contents at certain times throughout its development is important and is believed to be aided by this cytoplasmic streaming process.

Eggs are highly organized cells that contain genetic material and nutrients to sustain

embryogenesis, the formation and development of an embryo. Throughout its developing process, increasing levels of complexity are imposed on the basic system that first is the oocyte, and then eventually the egg. *Drosophila melanogaster*, better known as the fruit fly, has been intensively studied over the last century for many reasons. This complex multicellular organism has a rapid life cycle, wide geographic range, and relatively simple genetic structure. Interestingly, it also shares many aspects of development and behavior parallel to those in humans. These combined advantages have “permitted research in *Drosophila* to make seminal contributions to the understanding of fundamental biological processes” (Beckingham et al., 2005). This literature review will focus specifically on the development of the Drosophila oocyte and the observed cytoplasmic streaming process briefly described above. It is a unique topic of interest how and why exactly these swirling and stirring-like motions occur inside the oocyte during different stages of oogenesis. What is behind this cytoplasmic streaming mechanism and how can we best model it using the laws of physics and hydrodynamics?

1.2 Drosophila Oocyte

Drosophila oocytes develop within a structure termed an egg chamber, which originates from a single germline stem cell (Quinlan, 2016) (Figure 1.1). The chamber consists of a 16-cell cyst, or sack. One cell is the oocyte, and the other 15 are nurse cells connected to each other by cytoplasmic channels as well as to the anterior end of a developing oocyte (Monteith et al., 2016). During oogenesis, the nurse cells synthesize maternal components for selective transport

to the developing oocyte by means of connecting ring canals (Cooley & Theurkauf, 1994). These important maternal ingredients include mRNAs, protein complexes, and organelles (Monteith et al., 2016). Egg chamber development past the germarium is divided into 14 stages of morphological evolution. It is common to refer to stages 1-6 as early oogenesis, stages 7-10A as mid oogenesis (the period of slow streaming), and stages 10B-14 as late oogenesis (the period of fast streaming) (Quinlan, 2016). Stage 10 is divided in two because this is precisely where the transition from slow to fast cytoplasmic streaming occurs. In late oogenesis, nurse cells dump their remaining cytoplasm into the oocyte anterior. To mix this new cytoplasm with the viscous ooplasm, streaming accelerates and becomes much faster and more coherent (Monteith et al., 2016). These are the last functions of the nurse cells as they then degenerate, eventually leaving a mature egg (Quinlan, 2016). It has been experimentally shown that these fluid flows, specifically the fast fluid flow towards the end of oogenesis, are necessary for the correct formation of a *Drosophila* egg. The dynamic mixing of these large oocyte vesicles is incomplete in the absence of cytoplasmic streaming.

1.3 Important Components in the Oocyte

1.3.1 Microtubules

Figure 1.2 shows an image and schematic of a *Drosophila* oocyte in stage 9, one of the latter stages of development. During oogenesis, proteins, mRNAs, and organelles synthesized in the nurse cells are delivered to the oocyte through the ring canals. **Microtubules** are long, flexible, and hollow polymers made of tubulin, emanating from the non-centrosomal microtubule organising

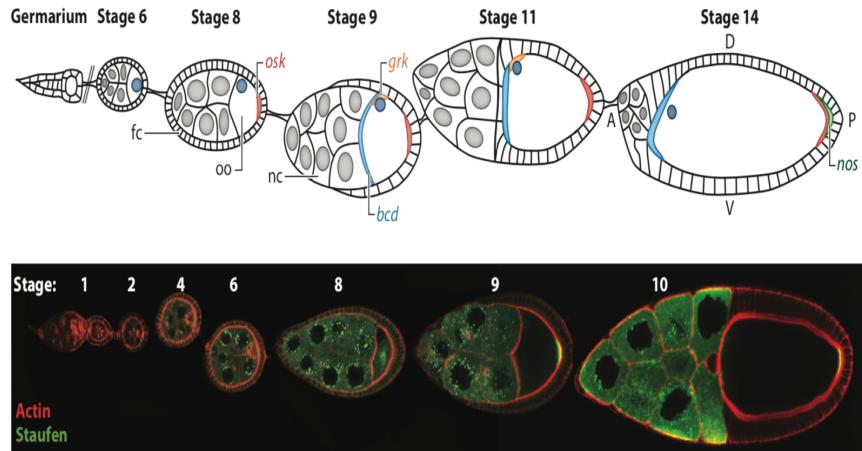


Figure 1.1: Diagram, and fluorescent images of *Drosophila* oogenesis stages. Top diagram shows snapshots from all three stages of oogenesis (early, mid, late). Nurse cells and the oocyte can be seen, as well as the oocytes increased size throughout maturation. Highlighted in dark blue is the oocyte nuclear location, and in lighter blue and red, landmarks established by polarity determinants. Abbreviations included: A, anterior; *bcd*, *bicoid* mRNA; D, dorsal; fc, follicle cells; *grk*, *gurken* mRNA; nc, nurse cells, *nos*, *nanos* mRNA; oo, oocyte; *osk*, *oskar* mRNA; P, posterior; V, ventral. Bottom images show the evolution of a fluorescently marked live oocyte. Fluorescence resembles the distribution of actin and Staufen, a component of *osk* ribonucleoproteins. Actin filaments form a network that provides mechanical support and cell motility, and the Staufen protein is required for the localization of mRNA species in the oocyte (Quinlan, 2016).

centres (ncMTOCs) connected to the cortex of the oocyte, and extend through the ring canals into the nurse cells allowing for important microtubule-based transport into the oocyte (Saxton, 2001; Deutsch et al., 2012). These microtubule filaments are not only used for material transport, but have been observed to undergo undulating and synchronous motions, forming metachronal waves during certain stages of the cytoplasmic streaming process (Martin et al., 2019; Serbus et al., 2005). The wave-like motion of microtubules has also recently been observed in a simple in vitro system, meaning outside of the normal biological context. When microtubule bundles are isolated with kinesin-1 motor proteins (discussed below) between glass plates, sustained self-oscillations

and coherent movements are observed (Sanchez et al., 2011). The fact that this simple system can develop such complex microtubule dynamics is strong evidence of their importance in the closely related fluid motions observed during Drosophila oogenesis. The leading theory is that each microtubule acts as a stirrer, helping mix the viscous oocyte solution in a clever and efficient way. And there is a primary force providing these microtubules with the ability to flow around and mix the solution; these are the walking motor proteins.

1.3.2 Motor Proteins

Molecular motor proteins, in this case dynein and **kinesin-1**, carry important cellular ingredients (cargo) such as polarity determinant mRNAs along the microtubules (Serbus et al., 2005). These motor proteins are a primary contributor to the dispersal of components delivered to the oocyte anterior from adjoining nurse cells, and play a major role in polarity establishment during oogenesis (Quinlan, 2016; Serbus et al., 2005). Kinesin-1 moves cargoes toward the fast growing plus-end of the microtubule, while dynein moves cargoes towards the slower growing minus-end, the end connected to the MTOCs at the cortex of the oocyte (Serbus et al., 2005) (Figure 1.3). Kinesin-1 and dynein provide a unique ability to transport cellular structures which need to be distributed throughout the cell; those that are too large to mix purely by diffusion processes. One of the most important molecules kinesin-1 carries towards the posterior pole of the oocyte is *oskar* mRNA, a key player in the embryonic axis specification process (Lasko, 2012). It has also been shown through the use of a kinesin null that kinesin-1 can transport dynein as their cargo away from the anterior of the oocyte (Brendza et al., 2002).

These motor proteins are able to ‘walk’ along the microtubules by converting chemical energy in the organic compound adenosine triphosphate (ATP) into mechanical energy. This type of system, one that is able to consume energy and turn it into organized motion, is known as an “active matter” system. Unlike systems that always seek equilibrium, active matter systems often remain out of equilibrium as long as their energy source remains available, consuming and dissipating energy at all times (Marchetti et al., 2013). The underlying mechanism of cytoplasmic streaming in *Drosophila* oocytes is one unique example of an active matter system. In this case, the primary role of kinesin-1 motor proteins is not only to transport important molecules within the oocyte. In doing this, there is a resulting force transfer from the motor to the microtubule filament and the surrounding fluid. This specific hydro-mechanical coupling or pairing of microtubules and kinesin-1 is, in essence, what generates fluid flows and drives the cytoplasmic streaming process, allowing nurse cell cytoplasm and ooplasm to be properly mixed in a *Drosophila* oocyte (Monteith et al., 2016).

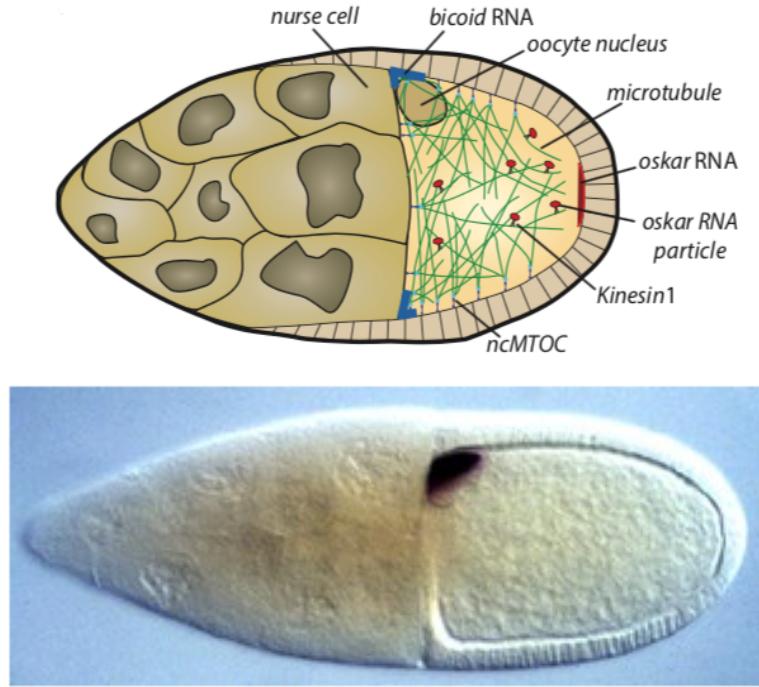


Figure 1.2:

(top): Schematic representation of a stage 9 Drosophila egg chamber showing both the nurse cells and major body axes in the oocyte. Microtubules are attached at their minus end to or near the cortex of the oocyte at non-centrosomal microtubule organising centres (ncMTOCs), connecting to the nurse cells through the ring canals. Kinesin-1 motors (red) can be seen ‘walking’ along the microtubules (green), away from the tethered minus ends.

(bottom): Image of a stage 9 Drosophila oocyte and its polarity. Can see similarities to the schematic. Width of the egg is on average $200\mu m$ (González-Reyes et al., 1997; Nieuwburg et al., 2017).

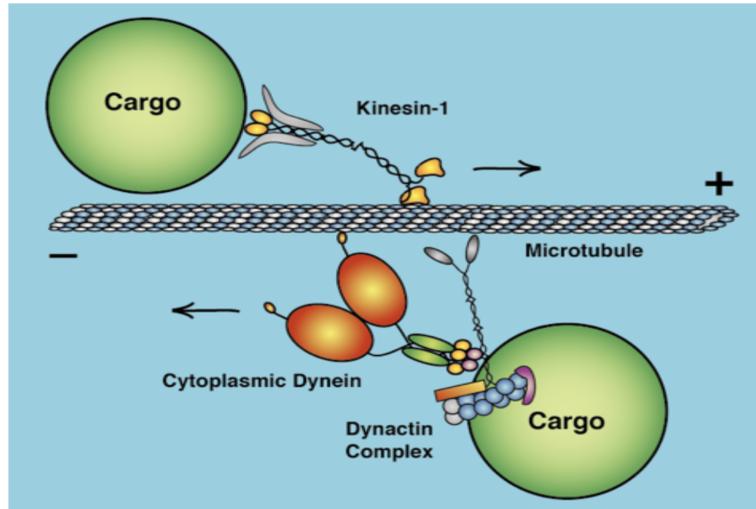


Figure 1.3: Schematic of a microtubule with cargo-carrying kinesin-1 and dynein motor proteins. Microtubule is made up of tubulin, which is a dimer consisting of alpha-tubulin and beta-tubulin, represented in the figure as blue and white spheres respectively. Kinesin-1 and dynein walk in opposite directions along the microtubule (Monteith, 2015).

1.4 Types of Cytoplasmic Streaming

There are two forms of microtubules-based streaming in the *Drosophila* oocyte. Slow, poorly ordered streaming occurs during mid oogenesis, helping disperse new ingredients injected into the oocyte by the nurse cells. During which pattern formation determinants such as oskar and bicoid mRNA are being localized and anchored at specific sites on the cortex, establishing the major body axes of the future organism (Figure 1.2) (Serbus et al., 2005; Steinhauer & Kalderon, 2006). At the time of slow streaming, main ingredients in the oocyte include microtubules, kinesin-1, dynein, and f-actin (an abundant protein, making cytoplasm more viscous). However, in comparison to dynein, it has been specifically shown that kinesin-1 is a required ingredient for proper slow streaming (Palacios & St Johnston, 2002). After slow streaming is complete, a transition to fast,

well-ordered streaming begins during stage 10B (Figure 1.4), increasing the mixing velocity tenfold just before nurse cell cytoplasm is transported into the oocyte and mixed with the viscous ooplasm.

Concurrent with this shift from slow to fast streaming is a shift in the microtubules orientation such that they are aligned parallel to the cortex wall (Serbus et al., 2005). It has been found that the plus-end-directed kinesin-1 motors are required for, and solely (along with microtubules) capable of driving fast cytoplasmic streaming (Serbus et al., 2005). Both types of streaming are completely abolished when kinesin mutants known to reduce kinesin motility are present (Palacios & St Johnston, 2002). Experiments also show that premature fast flows are induced when anti-dynein chain antibodies are injected into the oocyte during stage 9 (Serbus et al., 2005). This allows the kinesin to move without obstruction, hence why fast streaming starts early. These mutations that allow premature fast streaming disrupt the proper formation of body-axis polarity, and mutations that allow no fast streaming prevent this necessary mixing process and can cause embryonic lethality (Monteith et al., 2016). A collection of different mutation and oogenesis studies have shown that fast streaming plays a crucial role, more so than slow streaming, in the development of a healthy *Drosophila* egg. Therefore it's most important to address questions and test hypotheses regarding the biophysical mechanism and purpose of this self-organized fast streaming process.

The following sections will provide a detailed analysis of the force behind fast cytoplasmic streaming, that is, the wavelike microtubule motion and fluid flow in the oocyte due to drag from kinesin-1 motor proteins walking along microtubule filaments. In section 2, a simple system of one microtubule and kinesin-1 walkers is quantitatively discussed and modeled. In section 3, a more

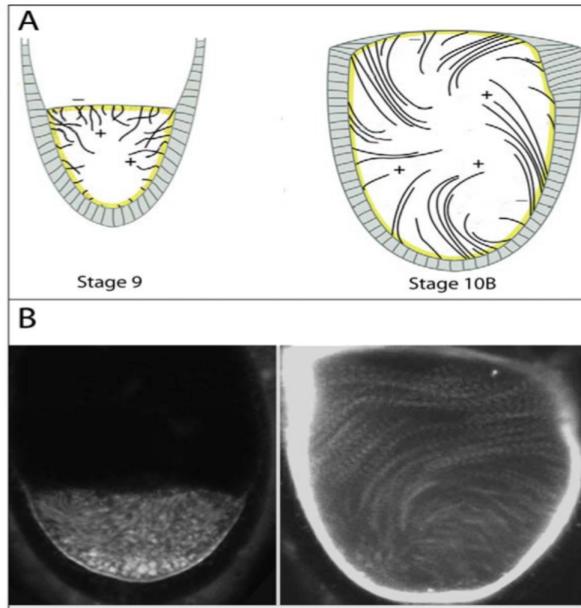


Figure 1.4: Model and time-lapse projections of slow and fast streaming in the *Drosophila* oocyte. Slow cytoplasmic flows are short and randomly directed as in stage 9 of oogenesis. Fast cytoplasmic flows, starting in stage 10B, are organized and long-ranged, distributed throughout the whole oocyte. The schematic depicts microtubules with their minus ends embedded in the cortex and plus ends free to move around. In the time-lapse projections, stationary yolk endosomes appear as dots, while streaks represent endosomes that are flowing. (Serbus et al., 2005; Moua, 2009).

realistic and complex system of multiple microtubules with kinesin-1 walkers is discussed. The system is modeled both with and without an applied external velocity field. The latter case is a more ideal and realistic model, in which the full hydrodynamic many-polymer theory must be considered. In section 4, the theory behind slow to fast cytoplasmic streaming is explored as it is a critical transition point in oogenesis that is still being understood. Lastly, section 5 will summarize this review in total and discuss the implications of the studies and results that have been deduced, as well as provide ideas for future experiments which will further help us understand cytoplasmic streaming and its importance.

2

A Hydrodynamic Streaming Model: Kinesin-1 Driven Dynamics of a Single Microtubule

Initially it might seem intuitive that the time-dependent and wave-like motion of the microtubules during fast streaming is due to turbulence of the surrounding fast-moving fluid (Deutsch et al., 2012b). This would be the case if, for example, we were examining the correlated movements of seaweed in the turbulent regions of the ocean. However, when considering a viscous, microscopic system such as an oocyte, this is not the case. The reason has to do with a quantity known as the Reynolds number, which represents the ratio of inertial forces to viscous forces in the flow, and is generally very small in such a microscopic developmental system. This means that

viscous forces dominate, inertial effects are negligible, and turbulence (chaotic changes in pressure and flow velocity) cannot be a natural characteristic of such a system (Deutsch et al., 2012). Low Reynolds number fluid systems are thus characterized by a smooth and laminar flow. This is why much investigation has been spent studying the microscopic mixing mechanism of such systems, and how dynamic and chaotic fluid patterns can still emerge. In considering a *Drosophila* oocyte, mid-oogenesis cytoplasm has a viscosity similar to that of glycerol ($\sim 1.4 \text{ Pa} \cdot \text{s}$), and fluid flows are considered Newtonian (Ganguly et al., 2012), meaning the viscosity remains constant. Both a single stirrer and diffusion techniques have proven insufficient for complete mixing on a timescale of oogenesis (hours). The steady state flow fields inside this closed chamber are much more efficient when chaotic flows are generated by several stirrers (Boyland et al., 2000).

To computationally model the fast streaming process in a *Drosophila* oocyte, Deutsch et al. (2012) consider the enclosed system, consisting of a stationary cortical barrier, an array of minus-end tethered microtubules, kinesin-1 motor proteins, and viscous cytoplasmic fluid. This model assumes that the microtubule can be modeled as an elastic semi-flexible string in the viscous medium (Deutsch et al., 2012). We first consider a simple system of only one microtubule, kinesin-1 motor complex, and the resulting forces that act at any point on the microtubule (Figure 2.1). During the fast streaming stage, kinesin-1 motors walk towards the outward direction of the microtubule, carrying cargo away from the cortex wall. The kinesin-1 cargo complex serves as an *impeller*, driving fluid motion away from the microtubules minus end, which in turn generates an opposing force tangentially along the microtubule.

Stokes' law can be used to quantify the drag force exerted on a spherical object moving

through a viscous fluid with a very small Reynolds number. Applying this to a kinesin impeller, we find that it transfers a force to adjacent cytoplasmic fluid by viscous drag. With a motor complex of linear dimension a , this drag force is $3\pi\eta av$, where η is the fluid viscosity, assumed to be 8 times that of water, and v is the velocity of the motor complex, calculated during in vivo experiments to be 780nm/s (Luby-Phelps, 2000; Monteith et al., 2016). According to Newton's third law, this drag force will transfer an equal and opposite force (\mathbf{F}_k) to the microtubule that is tangent to its long axis, i.e. pointing down the microtubule (Monteith et al., 2016). However, conservation of momentum tells us that kinesin-1 moving along a *free floating* microtubule will apply little to no net force to the surrounding fluid. The opposing tangential force generated by the moving impeller would only serve to move the microtubule in the opposite direction, creating at most a small local fluid displacement (Monteith et al., 2016). A free floating microtubule model is therefore one that will not produce efficient and long-ranged cytoplasmic streaming.

In accordance with experimental findings (Monteith et al., 2016), the model discussed here assumes that the minus end of the microtubule is anchored and not free flowing like the positive end. Thus the equal and opposite force applied to the microtubule generates dynamic buckling along its length (Deutsch et al., 2012). An intuitive comparison would be someone holding a flexible cord from the top end while applying a tangential upward force with their fingers somewhere below. This results in a wave-like bending of the cord because the top end is tethered and has no where to move. This force applied along the assumed inextensible microtubule creates a tension, T , acting on nearby components in opposing directions. The net tension force is denoted as ΔT . The microtubule has an elastic bending constant C that produces a force \mathbf{F}_e perpendicular to the

microtubule long axis as it buckles (Monteith et al., 2016). The sum of the above forces represents the total local force acting on an element of fluid next to a microtubule,

$$\mathbf{f} = \mathbf{F}_k + \mathbf{F}_e + \Delta T. \quad (2.1)$$

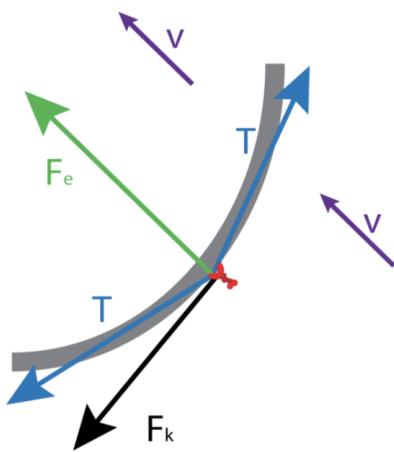


Figure 2.1: Force diagram for a microtubule and kinesin-1 system. Shown is a section of a microtubule (gray) with an attached kinesin-1 motor protein (red) and the forces (arrows) acting along its length which cause microtubule bending. Kinesin-1 exerts a force tangential to the long axis of the microtubule (\mathbf{F}_k). The microtubule has an elastic bending constant that produces a force (\mathbf{F}_e) which opposes the microtubule bending. Tension forces (T) act on neighboring components of the microtubule in opposite directions tangent to the long axis at the point where kinesin-1 lies. Force is transferred from the microtubule to the surrounding fluid, which moves at velocity v (Monteith et al., 2016).

In the low Reynolds number regime, hydrodynamic theory tells us that a force applied on an element of fluid has a long-range disturbance on velocities far from that point. The velocity of the fluid due to a force acting on a point is given by the second rank tensor field known as the

Oseen tensor, $\mathbf{J}(\mathbf{r}, \mathbf{r}')$. This tensor describes how small or thin objects interact in fluid and with each other, and dictates that the fluid velocity will fall off as the inverse of distance from the point of the applied force, $1/r$, with an additional pre-factor of order unity depending on the force's direction (Monteith et al., 2016). The Oseen tensor is defined as,

$$\mathbf{J}(\mathbf{r}) = \frac{1}{8\pi\mu} \left(\frac{\mathbf{I}}{|\mathbf{r}|} + \frac{\mathbf{r}\mathbf{r}^T}{|\mathbf{r}^3|} \right). \quad (2.2)$$

Conversely, the velocity, $\mathbf{u}(\mathbf{r})$ of fluid at an arbitrary point \mathbf{r} is a weighted sum over all forces (Monteith et al., 2016). The Oseen tensor is this weight factor, which connects the force density $\mathbf{f}(\mathbf{r}')$ at point \mathbf{r}' in the fluid to the velocity at another location \mathbf{r} , such that

$$\mathbf{u}(\mathbf{r}) = \int \mathbf{f}(\mathbf{r}') J(\mathbf{r} - \mathbf{r}') d^3 \mathbf{r}'. \quad (2.3)$$

So if a kinesin-1 complex applies a force, f , at some point in the fluid, we can calculate what the velocity flow field will look like throughout the entire oocyte. We know that this force will move the surrounding fluid such that flow velocities are inversely proportional to the distance from the impeller. Therefore, the force from a single impeller can affect neighboring fluid, but will not be able to create such fast mixing velocities as measured farther away from the microtubule. Fortunately, this phenomena is possible when we consider multiple impellers. Fluid-flow analysis done by Deutsch et al. (2012) has shown that long range hydrodynamic forces couple the assumed spherical individual kinesin-1 complexes moving along the same microtubule. Independent of the exact shape of the impellers, their effect on the velocity field only starts to decrease at a distance of order the length of the microtubule L (Deutsch et al., 2012b). In summary, this hydrodynamic

coupling increases the ability for few impellers to produce high velocity fluid flows far from the microtubule, making bulk cytoplasmic streaming possible.

Let us go back to considering, in more detail, the force applied from a single impeller. Euler's theory of elastic column buckling for a long column rod of length L with elastic constant C tells us that the force \mathbf{f}_K applied from a load tangentially along the microtubule has the ability to buckle it, causing the microtubule the bend in a wavelike fashion. The microtubule has a critical buckling force which is inversely proportional to the length of the microtubule, $f_B = C(\pi/L)^2$. This is correct if the direction of the microtubule end segments are not constrained. If there are such end constraints or if the load is distributed evenly over the rod, the critical buckling force will differ by a factor of order unity (Monteith et al., 2016). The force per unit length generated by a train of kinesin-1 complexes walking along the microtubule at constant velocity is proportional to η , v , and the ratio of the diameter of each complex to the distance between them, such that $f_k = \eta v a/d$. The total longitudinal force due to the kinesin-1 complex is proportional to L , $\mathbf{f}_L = \mathbf{f}_K * L$, and if greater than the critical buckling force, will cause the microtubule to buckle with a certain radius of curvature,

$$R = (C/\beta f_k)^{1/3} \quad (2.4)$$

where β is a constant, determined from simulations to be $\beta \approx 0.05$ (Monteith et al., 2016). However, this buckling analysis is simplified as it assumes a static load. In reality, the kinesin-1 is moving and buckling the microtubule, causing it to bend over time, which therefore redirects the succeeding buckling forces. This implies the load on the microtubule is dynamic and time dependent.

The dynamic motion of a microtubule is modeled more accurately when we consider the drag on it due to the surrounding fluid. Consider a single microtubule, with a local drag coefficient ν , but with no long-range hydrodynamics (Monteith et al., 2016). Deutsch et al. (2012) constructed a simplified simulation which assumes the only effect of all the other microtubules is to produce a fixed fluid velocity field, v_s . The forces acting on a point in the fluid can be written in more detail, similar to Equation 2.1, but this time modeled in three-dimensions with an external velocity field:

$$\nu \frac{\partial \mathbf{r}}{\partial t} = -C \frac{\partial^4 \mathbf{r}}{\partial s^4} + \frac{\partial}{\partial s} (T(s) \frac{\partial \mathbf{r}}{\partial s}) - f_k \frac{\partial \mathbf{r}}{\partial s} + \nu v_s \hat{k} \quad (2.5)$$

where $\mathbf{r}(s, t)$ is the position of the microtubule at arclength s and time t . Let $s = 0$ represents the location of the tethered microtubule base. The first term on the right hand side describes the microtubules stiffness as an energetic cost of bending proportional to the curvature squared, implying a local force at s proportional to $d^4 \mathbf{r}/ds^4$ (Martin, 2018). The second term is the tension term between length segments. The position dependent tension, $T(s)$ enforces inextensibility of the microtubule such that $|\partial \mathbf{r} / \partial s| = 1$ (Monteith et al., 2016). Microtubules also feel the tangential buckling force due to drag from kinesin-1, $F_k = -f_k d\mathbf{r}/ds$, directed toward the microtubule base with a magnitude proportional to the speed and size of the kinesin's cargo. The last term describes the velocity flow field, where ν is the local hydrodynamic drag coefficient (Deutsch et al., 2012). For this model, the flow field is in the \hat{k} direction, which is perpendicular to the surface and directed away from the microtubules base.

The mathematical model in Equation 2.5 is used to determine the 3-dimensional motion of a microtubule exposed to tangential kinesin forces in a constant external velocity field. The

microtubules minus end is tethered in free space near the cortex-like plane at which the velocity field becomes zero (Monteith et al., 2016). In accordance with observed microtubule motion, all numerical solutions appear to be travelling waves for a large enough arclength s (Deutsch et al., 2012a). And a specific family of solutions can appear at any scale, that is the solution forms are scale invariant. Figure 2.2 shows a series of snapshots in which we can see the developing motion of the microtubule through time. Starting from random initial conditions and large enough s , the equation rapidly evolves into a steady state helix that uniformly rotates with constant angular velocity ω . In fact, the modeled microtubule dynamics will always approach the same helical solution for any random initial configuration (Monteith et al., 2016). This model agrees both with the straightforward buckling analysis that produced Equation 2.4. The same radius of curvature for buckling can be derived from the fact that for the boundary condition, $\mathbf{u}(0) = 0$ (microtubule has a tethered minus end), a solution will only exist for discrete values of $\beta = C/(R^3 f_k)$, or $R = (C/(\beta f_k))^{1/3}$ (Deutsch et al., 2012b).

A more realistic oocyte cortex barrier can also be modeled by inserting a nonmoving plane parallel with the flow field. The adjacent microtubule bending patterns are still travelling waves but become two dimensional and are now parallel to the cortical plane. For small and large external velocity fields, Equation 2.5 has respective steady-state solutions which are flattened cycloidal or sinusoidal travelling wave configurations (Figure 2.3) (Monteith et al., 2016). The sinusoidal solutions correspond well with the motions of microtubules during fast streaming. All in all, the straightforward analysis and modelling of the forces present in this single microtubule and kinesin-1 system has shown to produce different travelling wave solutions which are in good agreement with

experimentally observed microtubule movements and patterns.

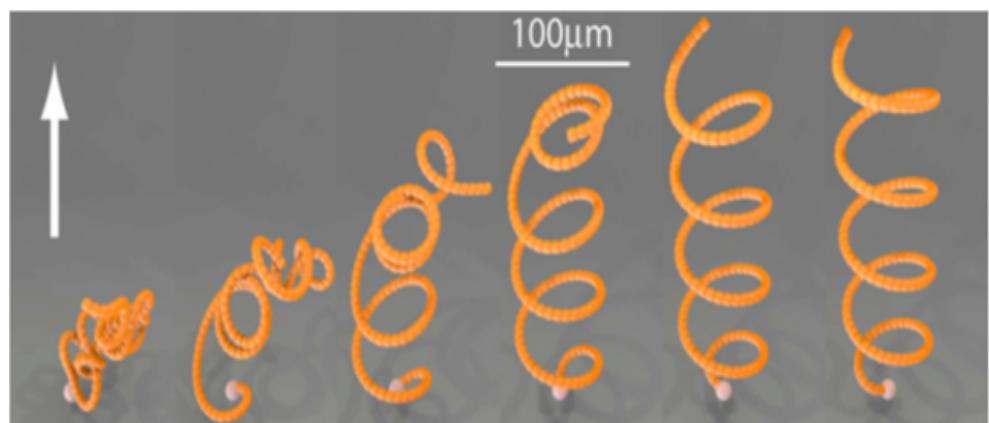


Figure 2.2: Behavior of a single microtubule-like filament subject to kinesin forces and placed in a constant external fluid flow. The microtubule can be seen to start in a random configuration (*left side*), and quickly evolves into a steady state helical wave pattern (*right side*). The minus end of the microtubule is fixed at a point in space (*white ball*) but is free to pivot. Chosen values for microtubule stiffness, kinesin force, and fluid viscosity produce a final radius of curvature between $25 - 54\mu m$ (Monteith et al., 2016).



Figure 2.3:

(left): Motion of a single microtubule-like filament subject to all the same parameters as in Figure 2.2, but now tethered to a plane with a velocity field parallel to that plane (pointing away from the microtubule's base). Motion of the microtubule now becomes two dimensional. For a large enough external velocity field, v_s , the motion becomes sinusoidal, similar to fast streaming microtubule motion experimentally observed near the cortex of the oocyte.

(right): Motion of a single microtubule-like filament subject to the same parameters as in Figure 6, again tethered to a plane with a velocity field parallel to that plane. This velocity field strength is much smaller than in Figure 7a. Steady state travelling wave solutions are similar to a cycloid shape, that which periodically loops back on itself (Deutsch et al., 2016).

3

An Organized Streaming Network of Many Microtubules

The next step towards a more complete cytoplasmic streaming model means considering the complex hydrodynamics of multiple interacting microtubules with kinesin-1 walkers. In the previous chapter we simplified things by saying the only effect of the other microtubules was to generate a constant external velocity field. In reality, long-ranged, time-dependent fluid flow is internally generated by the kinesin-1 driven movements of each microtubule, dynamically affecting all the other microtubules. Monteith et al. (2016) generated simulations of many microtubules, considering the same physical ingredients and governing equations as discussed in the previous chapter used to model the motion of a single microtubule. Now we consider an array of 100 microtubules with their minus ends connected near the cortex-like barrier plane at which both fluid

and microtubule velocities are zero (Monteith et al., 2016).

To model all of the microtubules motions, we need to include the previously described Oseen interaction tensor. Equation 2.3 utilizes this tensor to describe microtubule motion coupled to the surrounding velocity field. However that form of the Oseen tensor does not take into account the presence of the oocyte cortex wall. The presence of this stationary wall imposes a new boundary condition on the fluid motion; namely the velocity at the wall must equal zero (Monteith et al., 2016). In brief, we now need to consider this boundary condition when calculating the Green's function solution used to generate Equation 2.3, the equation for the fluid velocity field. The boundary geometry is considered to be that of an infinite plane hard wall at $z = 0$ (Monteith et al., 2016). Solving for a Green's function with such boundary condition utilizes the method of images (Dabros & van de Venand, 1992), and is analogous to a common electrostatics problem. That is, solving for the potential of point charge separated by a distance H from a conducting plate at which the potential is zero. The resulting solution includes an equally spaced image charge on the other side of the conducting plane with opposite sign. Because this solution for the potential includes two point charges, this has the effect of turning a monopole potential ($1/r$) into a dipole ($1/r^2$). Analyzing hydrodynamics in an oocyte is slightly more complicated, nevertheless the resulting modified Oseen tensor, $\mathbf{J}^w(\mathbf{x}, \mathbf{y})$, is given in Equation 3.1, which describes the interaction between a point force \mathbf{f} at location x and a fluid location y :

$$\mathbf{J}^w(\mathbf{x}, \mathbf{y}) \cdot \mathbf{f} = \frac{1}{8\pi\mu r^*} \left[-\left(\mathbf{I} + \frac{(\mathbf{r}^* \cdot \mathbf{f})\mathbf{r}^*}{\mathbf{r}^{*2}} \right) + \frac{2H}{\mathbf{r}^{*2}} \left(\left(\frac{3x_3(\mathbf{r}^* \cdot \tilde{\mathbf{f}})}{\mathbf{r}^{*2}} \mathbf{r}^* - \tilde{\mathbf{f}}_3 \right) \mathbf{r}^* - x_3 \tilde{\mathbf{f}} + (\mathbf{r}^* \cdot \tilde{\mathbf{f}}) \hat{\mathbf{z}} \right) \right] \quad (3.1)$$

where the reflected displacement vector is $\mathbf{r}^* = (x_1 - y_1, x_2 - y_2, x_3 + y_3)$, the distance of the fluid

from the fall is $H = y_3$, and the reflected force is $\tilde{\mathbf{f}} = (f_1, f_2, -f_3)$. The terms in this modified Oseen tensor now ensure that the fluid velocity will vanish at the cortex wall. In this hydrodynamic scenario, the fact that a monopole potential has the effect of a dipole potential means that a shift in the microtubules tethered point closer to the wall (reducing H), coincides with a faster decay of hydrodynamic coupling. This result is a key element to understanding the differences between slow and fast streaming which will be further discussed.

We consider each microtubule and its attached kinesin-1 to be in direct contact with the fluid, therefore the velocity of the fluid must match the velocity of the object at their interface and can be described using the modified Oseen tensor. Combined with Equation 2.1, which describes the transmitted forces from kinesin-1, this will generate a complete hydrodynamic model. The system evolves in time using Equation 2.3 combined with constant kinesin-1 velocity towards the free floating plus end of the microtubule. Constant kinesin-1 velocity is an appropriate representation of multiple kinesin-driven impellers, each applying tangential forces to the microtubule opposite their direction of motion. In summary, the velocity field of our system can now be computed by applying the modified Oseen tensor and integrating over the multiple force densities. Forces acting on the microtubule are calculated by integrating over its length. Forces acting on the kinesin-1 impellers are calculated by integrating over a cylindrical shell surrounding the microtubule. This leads to the equation for the fluid velocity field,

$$\mathbf{u}(\mathbf{r}) = \int \left[\mathbf{J}(\mathbf{r} - \mathbf{r}'(s)) \left[-C \frac{\partial^4 \mathbf{r}}{\partial s^4} + \frac{\partial}{\partial s} \left(T(s) \frac{\partial \mathbf{r}'}{\partial s} \right) - \mathbf{f}_k \right] + \int \mathbf{J}(\mathbf{r} - \mathbf{r}'(s) + \mathbf{a}(s)) \frac{\mathbf{f}_k}{2\pi|\mathbf{a}|} \frac{\partial \mathbf{r}'}{\partial s} d\hat{\mathbf{a}} \right] ds \quad (3.2)$$

where \mathbf{u} is the fluid velocity and \mathbf{a} is a vector pointing radially outward from the microtubule axis to the impellers location (Monteith et al., 2016). As showcased through Equation 3.2, numerical analysis of this system is more complex than directly adding the modified Oseen tensor (Equation 3.1) to the previous equation of motion (Equation 2.5). Although we still include the impeller force as tangent to the backbone of the microtubule, we now need to include the fact that long range hydrodynamic forces will transmit the reaction force produced against the impeller back towards that same microtubule (Monteith et al., 2016). The second integral in Equation 3.1 represents these additional forces we must consider.

Now that the velocity field of this system has been quantified, the focus shall be directed towards describing what Monteith et al. (2016) found in their simulations of an array of microtubule-like filaments. These filaments are equally spaced and floating in a viscous medium with zero initial velocity. Similar to the model of a single microtubule, they are parameterized with free-rotating minus ends tethered at a height H above the cortical barrier plane, and all start in an initial random configuration (Monteith et al., 2016). Results of this lengthy simulation can be seen in Figure 3.1, and show a system with two very recognizable states.

When the filaments are connected at a point close to the cortex plane (e.g. $H = 1.0$), their long-ranged movements are primarily uncorrelated and random, similar to what has been experimentally observed during slow cytoplasmic streaming (Figure 3.1 a/c). The filaments movements are still wave-like but do not move together in any coherent fashion. However, at certain times throughout the simulation, small portions of filaments did coherently move together over small distances (Monteith et al., 2016), which is also a behavior that has been observed during slow

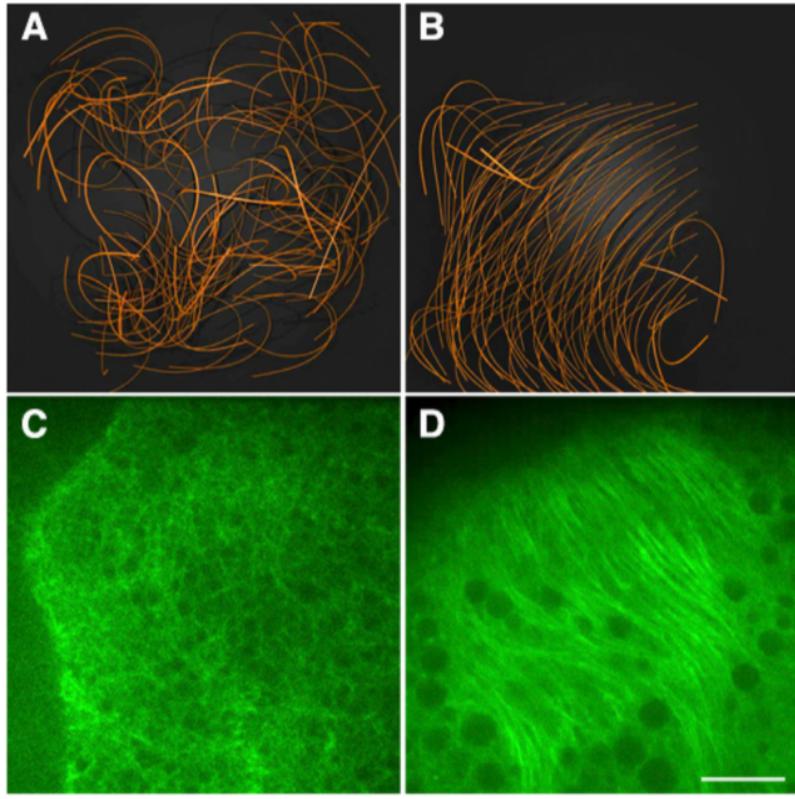


Figure 3.1:

(left): Simulated (a) and actual (c) images of stage 10A slow streaming. Simulation shows a network of 100 equally spaced microtubules bending in somewhat non-correlated movements due to the kinesin-1 driven tangential forces along the microtubules. Microtubules all start in random initial configurations with zero external velocity field. The microtubules minus-end pivot points are at a distance $H = 1.0$ from the cortex-like plane.

(right): Simulated (b) and actual (d) images of stage 10B fast streaming. Simulation considers a network of 100 microtubules constrained to the same conditions as in Figure 8a, except now their tethered end is a distance $H = 2.0$ from the cortex-like plane. Microtubule movements are now much more correlated and synchronous, as they should be during fast streaming (Monteith et al., 2016).

streaming. If we keep all other parameters the same, but now increase the distance of the filaments tethered point to $H = 2.0$ units from the cortex plane, we find intriguingly different dynamics (Figure 3.1 b/d). The main mechanism driving this H dependence is the form of the modified

Oseen tensor (Equation 3.1). As briefly discussed, in accounting for a zero velocity cortex wall, a result is that there is an even faster decay of hydrodynamic coupling as H decreases. Therefore an increase in H means an increase in hydrodynamic coupling between microtubules. As visualized in the simulation, the array of filaments now move in a much more correlated and self-organized manner, oriented such that they flow parallel to the cortex wall. As a result, there was an increase in the entire fluid velocity field. These behaviors are identical to those experimentally observed during fast streaming stages of a *Drosophila* oocyte (Serbus et al., 2005; Theurkauf et al., 1992). All filaments surrounded by a full set of neighbors exhibited these strongly correlated bending patterns which varied over time, promoting efficient mixing of the viscous solution. Near the edges of the array, filaments are more isolated and some displayed independent helical behaviors, similar to those of the single filament modeled in chapter 2 (Monteith et al., 2016).

Repeated trials of this simulation identified that certain parameter values favored correlated filament behavior and a high fluid velocity; qualities of fast cytoplasmic streaming which this model aimed to reproduce. To reiterate, the first parameter that produced this was an increased distance in microtubule minus-end pivot points above the cortex plane, equivalent to an increase in the hydrodynamic coupling between microtubules. Along with this, an intermediate filament stiffness value C (corresponding with that measured for microtubules) and an intermediate kinesin force density on the filaments generated a proper fast streaming model (Monteith et al., 2016). If parameters are shifted in order to create a higher force density on the microtubules, the uncorrelated, slow streaming state was reproduced. Those parameter changes include an increase in the kinesin velocity (v_0), kinesin-cargo diameter (a), or viscosity (η), as well as a decrease in the

spacing between successive kinesin-1 complexes (d). A decrease in microtubule stiffness (C) also reproduces the same slow streaming behavior. One might intuitively agree that it will be harder for microtubules to align if they are much more loose and floppy (decreased C). On the other hand, adjusting those parameters such that they create a much lower force density on the microtubules simulated fast streaming dynamics, but did not produce as large of a fluid velocity ($v(r)$) and delayed the attainment of self-organizing microtubules. Overall, if the microtubules are tethered far enough away from the cortex plane, parameter values which created an intermediate force density produced the best fast streaming model.

Results of these hydrodynamic simulations confirm the established kinesin-1 driven mechanism for cytoplasmic streaming. Monteith et al. (2016) were able to recreate both slow and fast streaming stages of oogenesis by adjusting certain system parameters. In doing so they have provided insight into the plausible reorganization mechanisms of the oocyte cytoskeleton which allow for a transition from slow and unordered to fast and synchronous streaming. Alongside the models' visually accurate representation of slow and fast streaming, Monteith et al. (2016) further compared their analytical solutions to measured bending dynamics of microtubule arrays during fast streaming in stage 10B-11 oocytes. For a microtubule with elasticity constants $C = 0.5$ to $2 \times 10^{-23} Nm^2$, a kinesin velocity of $v_0 = 780 nm/s$, a cargo diameter/spacing (a/d) between 0.4 and 1, and assuming a cytoplasmic viscosity in fast streaming oocytes of 8 times that of water, the radius of curvature of the microtubule in its steady state, R , is predicted to be between $25 - 54 \mu m$, with a wave period, τ , predicted to be $203 - 1094 s$ (Monteith et al., 2016). Quantifying live microtubule dynamics is difficult as each microtubule has slightly different trajectories and changing degrees of

curvature. Nevertheless, Monteith et al. (2016) fitted microtubule curvature radii with circles of known radius, and found the average minimum radius of curvature to be $R = 16.3 \pm 2.2 \mu\text{m}$. This is reasonably close to the models predicted minimum value, $R = 25 \mu\text{m}$. The average wave period of the roughly sinusoidal motions was determined to be $T = 370 \pm 42\text{s}$, which is within the predicted period range (Monteith et al., 2016). Both of these parameter comparisons help validate that this kinesin-1 driven streaming model is suitable and can accurately describe microtubule motion inside a *Drosophila* oocyte.

A similar experiment was conducted by Sanchez et al. (2011) which confirms that a system as simple as kinesin-1 motor proteins and microtubules can indeed form synchronous, large-scale oscillations. Sustained between two glass plates in a polyethylene-glycol solution with ATP (the energy source for kinesin motors), biotin-labeled kinesin-1 is bound into clusters which simultaneously bind and walk along bundles of microtubules (Sanchez et al., 2011). An array of microtubule bundles in the sample remained attached to the boundaries of the flow chamber or partially trapped under air bubbles, allowing them to separate from the bulk network of microtubules and undergo self-organized oscillating patterns. These active microtubule bundles exhibit oscillations only when attached to a fixed boundary (Sanchez et al., 2011), akin to microtubules attached by their minus ends to the cortex of an oocyte.

Figure 3.2 shows snapshots of one of these microtubule bundles undergoing wave-like oscillations. Figure 3.3 shows a larger scale image of an air bubble coated with a lawn of active microtubule bundles. The bundles appear to self-organize and flow together in a wave-like fashion, analogous to the oocyte simulations done by Monteith et al. (2016). However, one important

difference between oocytes and the in vitro experiment done by Sanchez et al. (2011) is that it was conducted between two glass plates. What must now be considered when simulating this experiment is the zero velocity boundary condition at each of these plates, rather than at a singular cortex boundary. As per the discussion of the modified Oseen tensor, this has the effect of further reducing hydrodynamic interactions (Blake, 1971), making it harder for microtubules to cohesively flow together.

To properly reproduce this experiment, Martin et al. (2018) were able to adapt the same kinesin-1 force model (Equation 2.1) which Deutsch et al. (2012) used in their oocyte simulations, except now both no-slip boundary conditions are considered. As expected, the distance between plates, H , has a considerable effect on the dynamics of the simulated microtubules due to the strong screening effect that these no-slip boundary conditions impose (Martin et al., 2018). More coherent, long-range motion was simulated when H is larger, as opposed to the disordered, short-ranged motion observed when H is small. Figure 3.4 shows a simulation snapshot of the array of 128 equally spaced microtubules, tethered in a circular geometry. Certain parameter values were chosen to simulate this oscillatory behavior. These include, $H = 1$, $k_{oseen} = 0.1$, and $C = 10.0$, where k_{oseen} is the strength of the interaction tensor, and C is the microtubules stiffness. Similar to simulation results from Deutsch et al. (2012), neighboring microtubules demonstrate cohesive and self-organized wave-like behavior. As can be seen by comparing Figures 3.4 and 3.3, there are strong similarities between these results and those of Sanchez et al. (2011). It is impressive that the cohesive, metachronal wave behavior observed can be simulated solely utilizing first-principle physics and hydrodynamics. Ultimately, this is a testament to the robustness of their model, and

serves to provide additional confirmation that kinesin-1 drives the important oscillatory movements of microtubules, whether it be in or outside a developing *Drosophila* oocyte.

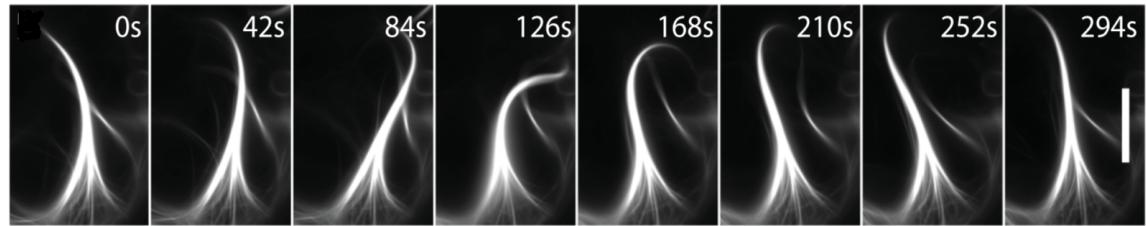


Figure 3.2: A sequence of zoomed in images showing the oscillating patterns of a bundle of microtubules with attached kinesin-1 walkers. This sequence captures an entire oscillation cycle of the microtubule bundle, attached to the boundary of the flow chamber by its base. The scale bar (*white*) to the right is $30\mu m$ (Sanchez et al., 2011).

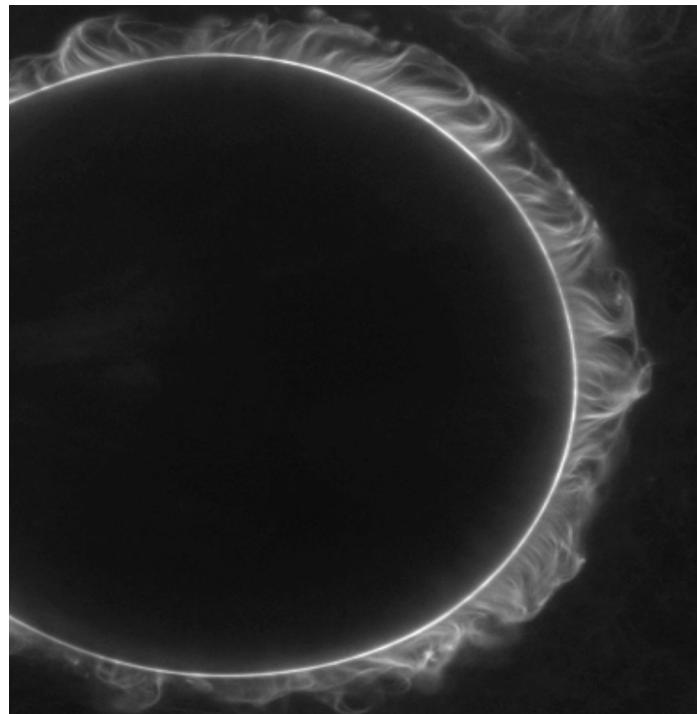


Figure 3.3: A zoomed out image of an air bubble between glass plates coated with active microtubule bundles and kinesin-1. Neighboring bundles appear to self-organize and flow together in a sinusoidal-like fashion. Approximate diameter of the air bubble is $200\mu m$, and the length of the bundles is on average $15\mu m$ (Sanchez et al., 2011).

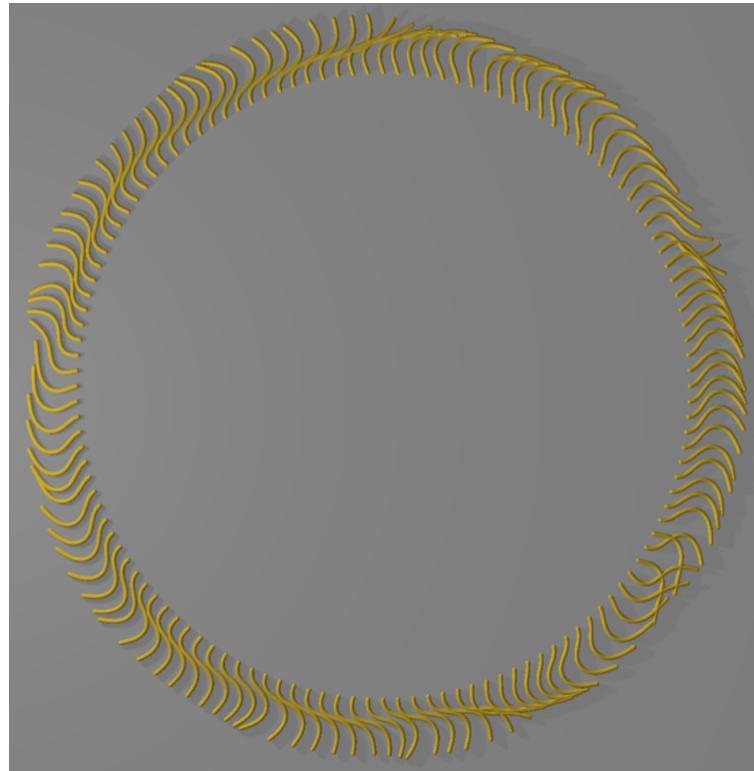


Figure 3.4: An image from a simulation of an array of 128 equally spaced microtubule-like filaments tethered in a circular geometry. For certain parameter values ($H = 1$, $k_{oseen} = 0.1$, $k_{stiff} = 10.0$), neighboring filaments move in a correlated, wave-like fashion, akin to the experiment shown in Figure 3.3. (Martin et al., 2018).

4

The Transition From Slow to Fast Streaming

4.1 A Shift in the Microtubules Tethered End

Microtubules and kinesin-1 have been shown to drive both slow and fast streaming (Serbus et al., 2005), and thus it is sensible to ask what exactly mediates this progression. There are multiple leading theories that explain changes in cytoskeleton structure which could accomplish a transition from slow to fast cytoplasmic streaming. One such theory is explored by Ying Wang and Veit Riechmann (2008). Their studies of Drosophila oogenesis suggest that a redistribution of the microtubules tethered minus ends from the cortex to subcortical regions occurs during the transition from slow to fast streaming (Wang & Riechmann, 2008). They were able to observe this through γ -tubulin, which belongs to the tubulin family of proteins found in Eukaryotic cells,

and has been shown to localize to the minus ends of microtubules at their organization/anchoring centers along the cortex (Moritz et al., 1995). To identify the location of the microtubules minus ends at different streaming stages, Wang and Reichmann (2008) used an antibody against γ -tubulin, which fluorescently labeled it for identification purposes. Throughout oocyte stages 9-10A, at which slow streaming occurs, γ -tubulin forms a continuous homogeneous layer within the cortex (Wang & Riechmann, 2008). However, during the fast streaming stage 10B, it was found that the thin and concentrated γ -tubulin layer is no longer present. Instead a more diffuse subcortical layer develops (Wang & Riechmann, 2008). Figure 4.1 shows the evolution of γ -tubulin distribution from stage 10A to 11. The plots suggest that γ -tubulin, and therefore the tethered microtubule ends are initially concentrated at the cortex, and then during the transition to fast streaming, are redistributed to a subcortical region, allowing microtubules to better couple together and align parallel with the cortex, generating correlated bending patterns.

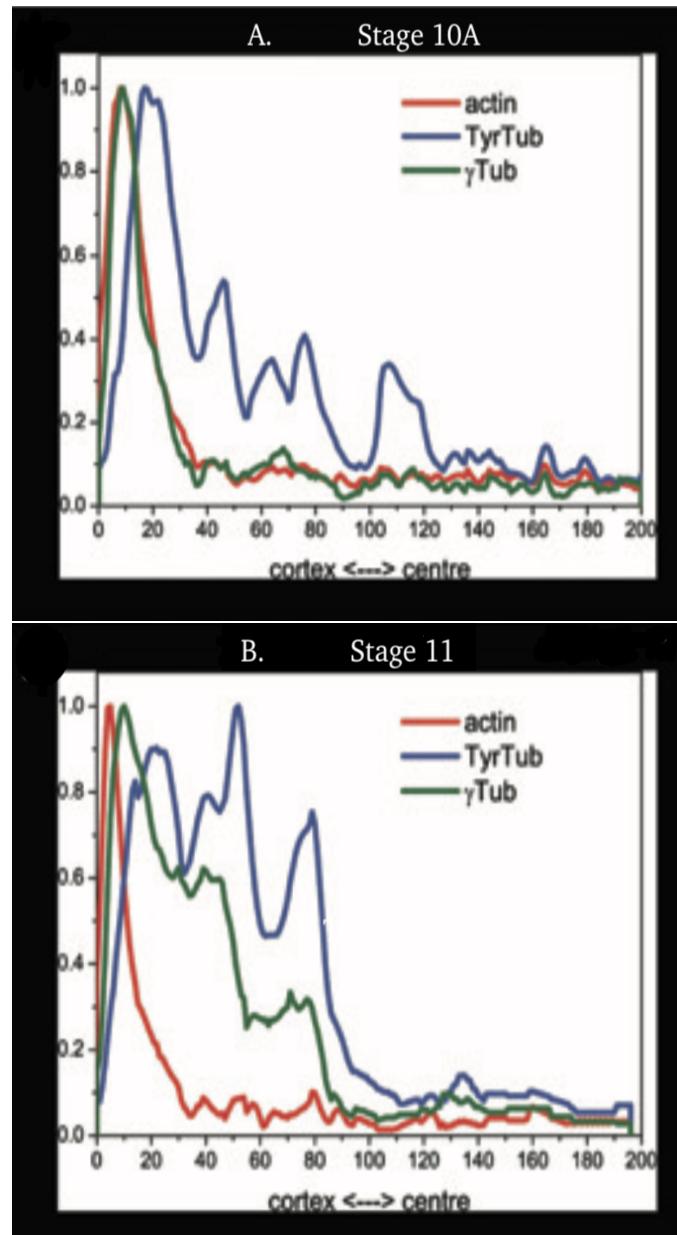


Figure 4.1: Plots showing distribution of fluorescent signal intensities of actin, tyrosinated tubulin, and γ -tubulin from the cortex to the interior of a Drosophila oocyte during stages 10A (*top*) and 11 (*bottom*). Data shows γ -tubulin (*green*) is much more locally concentrated at/near the cortex wall during stage 10A (slow streaming), and is distributed throughout a subcortical interior region of the oocyte during stage 11 (fast streaming) (Wang & Riechmann, 2008).

This proposed mechanism for the transition from slow to fast streaming agrees well with the model presented by Monteith et al. (2016). You may recall that they found when the microtubules tethered end was shifted from a distance $H = 1$ to $H = 2$ units away from the cortex wall, depicted bending behaviors switched from that of slow to fast streaming. The underlying reason for this has been discussed and is due to the fact that the velocity at the cortex wall must be zero, inducing the velocity of the fluid layer in direct contact with the wall to also be zero (no-slip boundary condition). This boundary condition is incorporated into the modified Oseen tensor, which says that a shift in the microtubules connected end towards the cortex wall (decrease in H) coincides with a faster hydrodynamic coupling decay. Hydrodynamic coupling is what allows the microtubules to align and flow together, and the range of this coupling between microtubules is proportional to their height above the cortex wall.

Tethering microtubules far enough away from the wall allows them to hydrodynamically couple and flow together because they are not being screened out due to the no-slip boundary condition (von Hansen et al., 2011). On the other hand, anchoring the microtubules closer to the wall will decrease the hydrodynamic coupling and self mobility of microtubules, therefore causing disordered arrays as seen in slow streaming. Agreement between analysis done by Wang and Riechmann (2008) and Monteith et al. (2016) suggests that a multi micron sized inward shift in microtubules tethering points would enable such a transition from slow to fast streaming. This shift allows microtubules to better interact and couple together hydrodynamically, producing the self-organized and cohesive dynamics observed during the fast streaming stage. However, further study is needed to answer questions such as how the microtubules can remain connected to such a

dispersed subcortical layer. Although this is a promising mechanism for the slow to fast streaming transition, we must not rule out other possibilities which have also been observed.

4.2 Dynein and the Actin Cytoskeleton

Both the repression of dynein motor proteins and the depolymerization of f-actin have been found to occur during the slow to fast streaming transition, suggesting that these changes are also contributing in some way. Serbus et al. (2005) have found that both dynein and the actin cytoskeleton have the ability to inhibit fast streaming. This is desired during the slow streaming state since the oocyte is not yet prepared for fast streaming. To reiterate, dynein is the motor protein walking with cargo along the microtubule opposite that of kinesin-1. Experiments which suppressed dynein induced a premature transition to fast streaming (Serbus et al., 2005). This is explained by the change in hydrodynamic interactions, as the plus-end directed forces on the microtubule are no longer opposing the minus-end directed forces from kinesin-1.

During the slow and unordered streaming state, the presence of dynein acts to resist and counteract the effects of kinesin-1. This resistance may be accomplished via: counteracting motions of opposing motor proteins attached to the same cargo (Theurkauf, 1994), dynein walking the opposite direction attached to separate cargo, or competition by dynein and kinesin-1 for the same binding site on microtubules (Mizuno et al., 2004). Regardless of how exactly dynein interferes with kinesin-1, observations show that dynein is suppressed just before nurse cell cytoplasm is dumped into the oocyte and prepared for fast streaming (Serbus et al., 2005). This would explain why the

minus-end-directed forces from kinesin-1 are now able to affect the microtubules in such a way that they can produce fast, well-ordered flows.

The f-actin cytoskeleton also plays a crucial role in suppressing premature fast streaming during early oogenesis stages. Fast streaming has been seen as early as stage 4 when F-actin is obstructed (Emmons et al., 1995). F-actin is a globular protein that is most abundant in the cortex and ring canals of the oocyte and nurse cells but also traverses the internal oocyte cytoplasm, giving it a higher viscosity (Quinlan, 2016; Serbus et al., 2005). The loosening or depolymerization of F-actin is equivalent to lowering the viscosity, and is coincident with the transition from slow to fast streaming (Serbus et al., 2005). It makes sense then, that premature depolymerization of the F-actin meshwork has been found to induce premature fast streaming (Serbus et al., 2005; Theurkauf et al., 1992).

This is in agreement with the model and simulations done by Monteith et al (2016), suggesting that the fluid's viscosity has an effect on the slow to fast streaming transition. Even though their modeled microtubules were tethered a distance away from the cortex plane such that fast streaming was enabled ($H = 2.0$), increasing the fluid viscosity resulted in disordered, slow streaming behavior (Monteith et al., 2016). Conversely, decreasing the viscosity allowed for a transition to correlated microtubule behavior and induced long-ranged ordering. Akin to parameter changes such as decreasing the velocity of kinesin-1 or increasing microtubule stiffness, lowering the fluid's viscosity (depolymerization of F-actin) has the effect of decreasing the force density on the microtubules. It is to note, however, that parameter changes resulting in a much lower force density delay the attainment of fast streaming, and do not permit as fast of fluid flows as

desired. If the depolymerization of the F-actin meshwork is to contribute to the transition to fast streaming, it is likely supplemented by other factors such as the inhibition of dynein or an increased distance between the microtubules tethered end and the cortex, allowing for a more timely transition to faster fluid flows. Serbus et al. (2005) suggest one possible connection between the F-actin mesh and dynein such that dynein inhibitory features are concealed by F-actin prior to the fast streaming stage. Then, just before the nurse cells final cytoplasm dump, F-actin is depolymerized, exposing those inhibitory features which consequently inhibit dynein and allow kinesin-1 to drive fast streaming.

The transition mechanisms discussed in this chapter all seem to be viable. It is equally possible that a combination of these and/or additional mechanisms is responsible for the slow to fast streaming transition. As experimental techniques and equipment continue to advance, additional detailed studies will be needed to confirm the proposed theories that have been explored here. Further analysis and tracking of microtubules, their tethered minus ends, and their relationship with the evolution of the oocyte cytoskeleton should provide additional valuable insights into how exactly the transition from slow to fast cytoplasmic streaming is controlled.

5

Conclusion

In summary, cytoplasmic streaming is the important hydrodynamic mixing and movement of fluid found inside many large Eukaryotic cells. An example of such crucial fluid flows is found inside premature egg cells, termed oocytes, which are structurally the very beginning of life. Once fertilized it gives rise to a complete individual, within a matter of days for some animal species. *Drosophila*, or fruit fly, oocytes are an abundant and established model system which have been used to gain valuable insights into the mechanisms of early stage egg development.

Advancements in microscopy and other techniques have allowed us to learn a great deal about these mixing mechanisms in such viscous, low Reynolds number systems. Both slow and fast streaming are driven by the active coupling of kinesin-1 motor proteins and the microtubule filaments which they walk along (Serbus et al., 2005). The ability for these same general components to generate two vastly different flow types has been of interest to many research groups. Deutsch

et al. (2012) have generated a model based on fundamental hydrodynamics and physical principles which has successfully described both slow and fast streaming. In summary, a viscous drag force is exerted on the kinesin-1 cargo complex which in return generates forces upon the microtubule and the surrounding fluid. The tangential force the microtubule feels results in buckling because its minus end is tethered at the oocyte's cortex wall (Monteith et al., 2016). Time dependent wave-like motion of the microtubule is indeed generated when considering the fact that kinesin-1 continuously transfers force to the microtubule as they walk towards its free floating plus end. In the fast streaming state, viscous drag on the microtubules due to the bulk cytoplasmic movement combined with a hydrodynamic coupling and force transfer between adjacent microtubules permits correlated bending patterns that are parallel to the cortex wall (Monteith et al., 2016). In comparison to the slow, disordered streaming state, the ability for these microtubules to couple and flow together generates fast and long-ranged fluid motions, which are much needed to facilitate mixing in such a viscous, microscopic regime.

The timing and ability of the oocyte to transition from slow to fast streaming is vital in its development. Further study must be done to narrow down which parameter changes cause this shift, but for now there are multiple compelling possibilities. Reducing the force density on the microtubule is one method to control the transition to fast streaming (Monteith et al., 2016). This can be accomplished in many ways, such as lowering the cytoplasmic viscosity, lowering the kinesin-1 velocity, or inhibiting the kinesin-1 opposed dynein motor protein. Although these parameter changes permit an evolution to correlated microtubule behavior, it is unfortunate that they also decrease the rate of fluid motion and delay the time it takes for simulations to evolve to

fast streaming. This is contrary to the biological purpose of fast streaming. If fluid flows are not fast enough or are generated too late in the development process, a proper oocyte will not be able to develop (Serbus et al., 2005). Therefore it is useful to consider other mechanisms which would allow the transition to occur in an efficient manner and produce fast enough fluid flows. The model generated by Monteith et al. (2016) also displays another promising streaming transition mechanism, namely, increasing the distance between the cortex barrier plane and the point at which the microtubules are tethered. This is coincident with an increase in hydrodynamic coupling between the microtubules, allowing for a suitable transition to self-organized, fast streaming behavior.

The question remains, which of these (or additional) methods are actually contributing to the streaming transition? The multiple techniques discussed here have all been individually observed at the normal transition from slow to fast streaming. This suggests that as of now, there is no single frontrunning theory to this transition mechanism. In terms of advancing computational models, integrating additional oocyte conditions such as the hemispherical geometry of the cortex boundary should yield further insights into the regulation and biophysical mechanism of slow and fast streaming. Although computationally demanding, it is important to model as realistic of an oocyte system as possible. Comparing these simulations to increasingly advanced oogenesis studies will undoubtedly bring us closer to confirming what we already know about both the mechanism and objective of streaming inside a *Drosophila* oocyte.

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