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A mesoscopic description of contractile cytoskeletal meshworks

K. Doubrovinski¹, O. Polyakov³, and M. Kaschube^{2,3,a}

¹Department of Molecular Biology, Princeton University, Princeton, NJ, USA

²Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA

³Physics Department, Princeton University, Princeton, NJ, USA

Abstract

Epithelial morphogenesis plays a major role in embryonic development. During this process cells within epithelial sheets undergo complex spatial reorganization to form organs with specific shapes and functions. The dynamics of epithelial cell reorganization is driven by forces generated through the cytoskeleton, an active network of polar filaments and motor proteins. Over the relevant time scales, individual cytoskeletal filaments typically undergo turnover, where existing filaments depolymerize into monomers and new filaments are nucleated. Here we extend a previously developed physical description of the force generation by the cytoskeleton to account for the effects of filament turnover. We find that filament turnover can significantly stabilize contractile structures against rupture and discuss several possible routes to instability resulting in the rupture of the cytoskeletal meshwork. Additionally, we show that our minimal description can account for a range of phenomena that were recently observed in fruit fly epithelial morphogenesis.

1 Introduction

Morphogenesis through the folding of epithelial sheets is ubiquitous in development [1]. In this process, cells in precisely defined epithelium regions constrict, folding the epithelial sheet into the required shape. For example, gastrulation in the fruit fly begins with the formation of the ventral furrow and the invagination of the prospective mesoderm, a process driven by cell constriction in the ventral part of the embryo [2,3]. Other examples include the formation of the cephalic furrow which segregates the prospective head of the fly larva from the rest of its body [4], the generation of dorsal appendages that serve as breathing tubes for the fly embryo [5], and wound healing in vertebrates [6]. All of these undergo epithelial sheet deformations and are likely to share similar physical mechanisms.

Like cell division and cell locomotion, cell constriction that is involved in epithelial morphogenesis is driven by the cytoskeleton, an intracellular meshwork of filamentous proteins [7,8]. The principal constituent of the cytoskeleton is actin, a globular protein that can polymerize into filaments forming a meshwork beneath the plasma membrane, referred to as the actin cortex [9,10]. Actin filaments have polarity: each actin monomer within a polymer chain is oriented with its cleft toward the same end. Actin filaments may undergo treadmilling, in which filament length remains approximately constant, while monomers add at one end and dissociate from the other. Cortical actin undergoes rapid turnover, depolymerizing into monomers that are nucleated into new filaments. Individual actin filaments are crosslinked by motor proteins that utilize chemical energy stored in the bonds

of Adenosine-Tri-Phosphate (ATP) to generate stress in the actin mesh-work that may drive cell constriction.

Understanding epithelial morphogenesis requires a physical description of force generation by the cytoskeleton. In this article we present a minimal model of molecular-motor-dependent filament aggregation. Our description is an extension of earlier work on the motor-filament systems [11–14], accounting for filament turnover as well as the transport of motors by the filaments they crosslink. We compare the mean-field dynamics to the corresponding stochastic system consisting of a finite number of particles and show that the mean-field dynamics are valid in the limit of high particle copy number. We demonstrate that our description can naturally account for a range of recent experimental findings on the role of cytoskeletal dynamics in the ventral furrow formation of the fruit fly *Drosophila melanogaster*.

2 The minimal model

2.1 The dynamics equations

We describe the system consisting of filaments and motors by the filament density ρ , the density of bound motors μ , and the density of freely diffusing motors μ_f . Their time evolution is determined by the continuity equations

$$\begin{aligned}\partial_t \rho &= -\nabla \cdot \mathbf{j}_\rho + S_\rho, \\ \partial_t \mu &= -\nabla \cdot \mathbf{j}_\mu + S_\mu, \\ \partial_t \mu_f &= -\Delta \cdot \mathbf{j}_f + S_f,\end{aligned}\tag{1}$$

where the $\mathbf{j}$ -terms and the S -terms denote the corresponding fluxes and sources, respectively. Assuming constant rates of filament nucleation and degradation, the source term due to filament turnover is given by

$$S_\rho = v - v_d \rho,\tag{2}$$

where v and v_d are the filament nucleation and degradation rates, respectively. A free motor is assumed to attach to a filament at a constant rate v_b and to detach exclusively upon depolymerization of the filament to which it is bound. Thus

$$S_\mu = -S_f = v_b \rho \mu_f - v_d \mu.\tag{3}$$

To specify the filament flux $\mathbf{j}_\rho$, we consider the force density on the filaments due to the motors. The motor-filament interactions are depicted schematically in fig. 1. We describe the motors as consisting of a binding site rigidly attached to the filament meshwork and a head that transiently attaches to filaments within some distance from the binding site. The head pulls the filaments with a force whose direction and magnitude is determined by the vector from the binding site to the head. Thus the motor is a force dipole whose magnitude depends on the displacement of the head from the binding site. We assume that both the binding site and the head interchange their associated filaments on a time scale that is much shorter than the time scale at which the motor and filament densities evolve. Hence, the force density on the filaments is

$$\phi(\mathbf{r}) = \int d\mathbf{r}' \frac{1}{\rho(\mathbf{r})} P(\mathbf{r}') \mathbf{f}(\mathbf{r}') \rho(\mathbf{r}+\mathbf{r}') \frac{1}{T(\mathbf{r})} \mu(\mathbf{r}) + \int d\mathbf{r}' \mu(\mathbf{r}+\mathbf{r}') \frac{1}{\rho(\mathbf{r})} \rho(\mathbf{r}) P(\mathbf{r}') \mathbf{f}(\mathbf{r}') \frac{1}{T(\mathbf{r}+\mathbf{r}')},\tag{4}$$

where $\mathbf{f}(\mathbf{r}')$ is the force exerted on the meshwork by the head whose separation from the binding site is given by vector $\mathbf{r}'$, $P(\mathbf{r}')$ is the probability of this separation, and $T(\mathbf{r}) = \int d\mathbf{r}' P(\mathbf{r}')\rho(\mathbf{r} + \mathbf{r}')$ is the total number of sites on the ambient filament meshwork where the head may attach. Since both the binding site and the head of the motor quickly interchange their associated filaments, the force exerted on those filaments is equally shared, and hence the denominators ρ and T in the integrands in eq. (4). It follows that the filament and motor fluxes are given by

$$\begin{aligned} \mathbf{j}_\rho &= -D\nabla\rho + \eta^{-1}\rho\phi, \\ \mathbf{j}_\mu &= -D_\mu\nabla\mu + \eta^{-1}\mu\phi, \\ \mathbf{j}_f &= -D_f\nabla\mu_f, \end{aligned} \quad (5)$$

where η is an effective filament friction coefficient stemming from the viscous drag with the ambient cytoplasm, and D , D_μ , and D_f are the effective diffusion constants. We assume that the effect of the bound motors on the mobility of their associated filaments is negligible. The second line of eq. (5) implies that the speed of a bound motor is given by the speed of the filament to which it is attached. Equations (1)–(5) are a closed set specifying a minimal model of molecular-motor-dependent filament aggregation. Our formulation is an extension of the earlier work on the motor-filament systems, see, *e.g.* [11–15]. We have extended the former approaches to describe the “hitch-hiking” of the motors on the filaments to which they are attached as well as to consider filament turnover.

2.2 Analysis of the minimal model

We begin the analysis of eqs. (1)–(5) by investigating the linear stability of the homogeneous steady state $\rho = v/v_d$, $\mu = v_b\mu_f/v$, and $\mu_f = \mu_f^0 = \text{const.}$

The stability boundary of the homogeneous steady state in the case of a one-dimensional system with periodic boundary conditions is presented in fig. 2. Consistent with [11], we found that the homogeneous steady state is destabilized upon increasing the strength of the motor-mediated inter-filament attraction α . Interestingly, decreasing the rate of filament turnover has a similar effect. Intuitively this behavior may be understood as follows. Motor-mediated filament interaction tends to aggregate the filaments, making the homogeneous filament density profile reorganize into sequential regions of high (“bumps”) and low (“holes”) filament concentration. Filament turnover, on the other hand, tends to even-out the density fluctuations, filling the holes and dissolving the bumps before they get a chance to fully develop. Furthermore, since filament depolymerization is assumed to lead to detachment of the associated motors, faster turnover results in a decrease of the average bound motor density, further stabilizing the homogeneous steady state against the formation of bumps. Both effects contribute to the overall stabilization of the homogeneous state through the filament turnover. Their relative contributions may depend on the parameters.

On length scales that are large compared to the range of motor-mediated inter-filament interactions, eqs. (1)–(5) may be replaced with a local continuum description. Taylor-expanding the integrands in (4), performing the integration with respect to $\mathbf{r}'$ in one spatial dimension, one arrives at

$$\begin{aligned} \partial_t \rho &= D\partial_x^2 \rho + \partial_x^2 \sigma + v - v_d \rho, \\ \partial_t \mu &= D_\mu \partial_x^2 \mu + \partial_x \left(\frac{\mu}{\rho} \partial_x \sigma + v_b \mu_f \rho - v_d \mu \right), \\ \partial_t \mu_f &= D_f \partial_x^2 \mu_f - v_b \mu_f \rho + v_d \mu, \end{aligned} \quad (6)$$

where stress σ is given by

$$\sigma = A_1 g \rho + A_2 \left(\partial_x^2 \rho g + \partial_x^2 g \rho - \partial_x \rho \partial_x g \right), \quad (7)$$

with $g = \mu/T$, $A_1 = \int_{-\infty}^{\infty} dx' x' P(x') f(x')$, and $A_2 = \int_{-\infty}^{\infty} dx' (x')^3 P(x') f(x') / 6$. Since to the leading order in the coarse-grained limit $T \sim \rho$, to the lowest order the stress is proportional to the motor density whereas it does not depend on the density of filaments. In the coarse-grained limit the wavelength of the fastest growing eigen-mode in the vicinity of the instability of the homogeneous steady state may be derived analytically. The corresponding expression is rather lengthy and is not given here. In the limit of fast motor and slow filament diffusion ($D_\mu, D_f \rightarrow \infty, D \rightarrow 0$), the wavelength of the fastest growing eigenmode is determined by $\sqrt{A_2/A_1}$, *i.e.* by the interaction range of the particles, see fig. 3. This is also true in the opposite limit when the diffusion of filaments is fast and the diffusion of motors is slow.

2.3 Simulations of the mean-field equations

In this section, we go beyond the linear stability analysis presented in the previous section and describe dynamics in the unstable region. Figures 4a,b present a simulation in the unstable regime. Initially, the density profiles are homogeneous. The dynamics upon the onset of instability is dominated by the fastest growing eigenmode. For the parameter values in fig. 2 the fastest growing eigenmode has a wavelength one quarter of the system length, therefore four density maxima form initially. Subsequently the pattern coarsens, smaller bumps coalescing into ever larger density aggregates, see fig. 4f. The wavelength of the target pattern that forms through coarsening is much harder to derive, since it is determined by the nonlinearities.

2.4 Stochastic description

In the previous section we considered a mesoscopic mean-field description where every motor effectively interacts not with a particular filament but with an ambient field of filaments. Mean field is a good description of the corresponding stochastic system with a finite particle copy number as long as the number of particles is large. In order to assess the validity of the mean-field approximation as well as the possible effects of the low copy number noise, in this section we shall analyze the stochastic version of eqs. (1)–(5). The derivation of the corresponding Langevin equations is straightforward and is deferred to appendix A. Figures 4c,d present the results of the stochastic simulations. Although, typically, both the average filament copy number and the number of motors were chosen to be as low as a few hundreds, the stochastic dynamics was little different from the mean field, see fig. 4e. In particular, in the unstable region we observed the formation of several peaks of motor density, co-localized with the peaks of filament concentration. The inter-peak spacing (*i.e.* the intrinsic wavelength of the pattern), the details of the transients, the coarsening dynamics as well as the phase boundaries agreed well with the corresponding deterministic description.

3 Experimental relevance

In this section we discuss the possible experimental implications of our minimal model.

We begin by noting that the form of the eigenvalue spectrum given in fig. 3 implies that the homogeneous steady state may be destabilized by interfering with the filament turnover. In principle, there are at least two possible routes to instability. For one, the homogeneous state may be destabilized by decreasing the rate of the filament degradation v_d . This route might correspond to the treatment of the actin meshwork with the drug phalloidin that blocks

filament disassembly. Alternatively, one can cross into the unstable regime by decreasing the average filament density $\bar{\rho}$. In this case the motor-generated force is distributed over a smaller number of filaments resulting in rupture of the cytoskeletal meshwork. This might correspond to treatment with cytochalasin, a drug that blocks actin polymerization thereby likely reducing filament density.

During *Drosophila* gastrulation, cells in the prospective mesoderm of the ventral part of the embryo constrict and invaginate. Cell constrictions depend on the contraction of the actin meshwork that is localized to the apical part of the constricting cell. Interestingly, if the embryo is injected with cytochalasin the contractile apical actin meshwork disintegrates into separate actin foci that grow with time [3,16]. Furthermore, cytochalasin-treated embryos do not gastrulate. Our minimal model may account for both the initial disintegration of the meshwork upon treatment with cytochalasin as well as the subsequent mergence of the actin foci. In particular, cytochalasin treatment may decrease the average actin density by blocking actin polymerization. In this case, myosin-generated force density is distributed over a smaller number of filaments resulting in rupture, *i.e.* disintegration of the actin meshwork into disjointed foci. Subsequently, the foci may merge through coarsening as was experimentally demonstrated. A network of loosely connected foci cannot generate considerable stress, implying cessation of gastrulation.

4 Conclusion

Physical descriptions of many vital biological processes such as, *e.g.*, epithelial morphogenesis and cell migration require an understanding of stress generation by the cytoskeleton. In this article we studied a minimal model of molecular-motor-dependent filament aggregation. We showed that our description can account for a number of phenomena associated with the formation of the ventral furrow in the fruit fly. In particular, our equations suggest an explanation for the disintegration of the actin mesh upon cytochalasin treatment, and the subsequent formation of the foci as well as for their subsequent growth.

In the future it would be interesting to quantitatively compare the predictions of our physical description with experimental data on the cytoskeletal dynamics in the ventral furrow. In particular, by varying the amount of injected cytochalasin one might be able to control the rate of turnover and thus study its influence on the course of instability as well as on the subsequent coarsening dynamics. The influence of drug treatment on the actin dynamics may be assessed by means of the standard method of fluorescence recovery after photobleaching (FRAP).

A clear advantage of employing *Drosophila* as a model organism to study the force generation by the cytoskeleton is that of the exceptionally well developed fly genetics. A number of interesting fly mutants that might prove useful for the study of the cytoskeletal dynamics in the ventral furrow have been identified in the past. For example, it recently proved possible to engineer acellular embryos that remain a single syncytium throughout their development. In these embryos the cytoskeletal mesh in the ventral part of the egg is not partitioned into multiple cells. The analysis of the cytoskeletal dynamics in such systems is expected to be less complicated by the boundary conditions.

Finally, it will be interesting to combine our descriptions of the force generation in the cytoskeleton with a description of the supposedly passive ambient visco-elastic cytosole in order to understand the dynamics of the cell shape changes during gastrulation.

Appendix A. Langevin equations

In this appendix we consider Langevin equations corresponding to eqs. (1)–(5). Figure 1 illustrates a system consisting of five filaments and two motors. The spatial coordinate of filament i and motor j are x_i^ρ and x_j^μ , respectively. The force acting on, *e.g.*, filament 2 has two contributions corresponding to the two terms of eq. (4): interaction with the motor binding sites and interaction with the motor heads. The total force is given by

$$\frac{1}{2} \frac{\sum_{i=1}^5 P(x_i^\rho - x_1^\mu) f(x_i^\rho - x_1^\mu)}{\sum_{i=1}^5 P(x_i^\rho - x_1^\mu)} + \frac{P(x_2^\mu - x_1^\rho) f(x_2^\mu - x_1^\rho)}{\sum_{i=1}^5 P(x_2^\mu - x_i^\rho)}. \quad (\text{A.1})$$

The one-half in front of the first term is because the force from motor 1 is shared equally between filaments 2 and 3. More generally, the force on a filament at x_i^ρ is

$$\sum_j \frac{1}{S(x_j^\mu)} \frac{\sum_{i=1}^N P(x_i^\rho - x_j^\mu) f(x_i^\rho - x_j^\mu)}{\sum_{i=1}^N P(x_i^\rho - x_j^\mu)} + \sum_{j=1}^M \frac{P(x_j^\mu - x_i^\rho) f(x_j^\mu - x_i^\rho)}{\sum_{i=1}^N P(x_j^\mu - x_i^\rho)}. \quad (\text{A.2})$$

The outer sum in the first term is taken over all motors whose binding site overlaps with a filament at x_i^ρ , $S(x)$ is the number of filaments that overlap with point x , M and N are the total numbers of motors and filaments, respectively. In every time step the force on each filament is computed according to (A.2). Filament velocity v is given by the product of this force and some effective mobility η^{-1} , see eqs. (5). At each subsequent time step a filament is shifted by amount $vdt \pm \sqrt{2Ddt}$, where dt is the time step and D is the corresponding diffusion coefficient. Accordingly, the speed of a motor binding site is the average speed of all filaments with which it overlaps. Finally, binding, unbinding, and nucleation are stochastic events whose probabilities per time are given by their respective kinetic rates.

Appendix B. Numerical methods

Numerical integration of eqs. (1)–(5) was carried out using a pseudospectral method by calculating the convolutions in the Fourier space. The pseudospectral method was also used to facilitate the time-integration of the stochastic dynamics. To this end, we binned the spatial domain into N evenly spaced subvolumes. The density of particles in each subvolume is the number of particles it contains divided by its length. The approximate force density on a particle within a given subvolume was calculated from the particle density by means of the fast Fourier transform in the same way as when integrating the mean-field equations. We checked that our results are independent of the spatial discretization used for computing the convolutions.

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Fig. 1.

Schematic sketch of the physical description: Motors consist of a binding site that attaches to filaments, and a motor head that transiently attaches to filaments some distance away with probability $P(x')$. The head pulls the filaments with a force $f(x')$ towards the binding site. The top and the bottom curves illustrate the choices of $P(x') \propto \exp(-x'^2)$ and $f(x') = \alpha x' \exp(-x'^2)$ used in all simulations.

Fig. 2.

a) Stability diagram of the homogeneous steady state within a minimal description of motor-mediated filament aggregation. Solid line without symbols indicates the linear stability boundary of the homogeneous steady state. The dashed line is the stability boundary in the corresponding coarse-grained equations. The back transitions in the mean-field description (circles) and with stochastic dynamics (diamonds) were computed by adiabatically decreasing α in the corresponding simulations. The inset is a magnification of the phase space in the vicinity of $\beta = 0$. Parameters are: $D_f/vbL^2 = D_\mu/vbL^2 = D_f/vbL^2 = 0.1$, $v/vb = 40\beta$, $vd/vb = \beta$, $f(x') = \alpha x' \exp(-x'^2/L^2)$, $P(x') \propto \exp(-x'^2/L^2)$. The number of particles in the stochastic simulations used to compute the back transition is 400.

Fig. 3.

The spectrum determining linear stability of the homogeneous steady state of the minimal model in the limit of fast motor diffusion. Here, $B_2 = \int_{-\infty}^{\infty} dx' (x')^2 P(x') / 2$, and $\bar{\rho}$ and $\bar{\mu}$ are the steady-state motor and filament densities, respectively. The eigenvalue corresponding to the zero wave number $k = 0$ is $-v_d$.

Fig. 4. Simulation of the minimal model. a), b) Mean-field dynamics determined by eqs. (1)–(5). c), d) Stochastic simulations with the same parameters as in a) and b). e) Motor density profile of the asymptotic state in a simulation with the same parameters as in a)–d). The domain length is one-fourth that in a) and b), chosen in order to obtain a single peak. The profile corresponding to the stochastic simulation is averaged over 150 time points. Parameters are: turnover rate $\beta = 1$, $\alpha = 0.020$, the total number of motors as well as the average filament copy number are 20000. All other parameters are as in fig. 2. f) Coarsening dynamics. Shown is the filament density. Note the logarithmic scale of the time axis. Parameters are: $D = D_\mu = D_f = 0.2$, $v = 2 \cdot 10^{-3}$, $v_d = 2 \cdot 10^{-6}$. All other parameters are as in a)–e).