Dispatches

Asymmetric Division: Motor Persistence Pays off

A new study shows that an antagonistic force model can explain a number of complex mitotic spindle movements in the first mitosis of the *Caenorhabditis elegans* embryo by simply assuming that cortical force generators become increasingly persistent in their interaction with microtubules during mitosis.

Melissa K. Gardner and David J. Odde

Asymmetric cell division provides a mechanism for generating diversity amongst the progeny of individual cells in the developing embryo, and is mediated in part by the complex interplay between microtubules and cortically bound microtubule-based molecular motors. To achieve asymmetric cell division, an axis of polarity is established within the cell, and then the mitotic spindle is positioned asymmetrically along this axis, such that after completion of cell division the daughter cells are of unequal size and composition [1,2]. Exactly how cortical forces on microtubules are temporally and spatially regulated to achieve asymmetry during the first mitosis of the embryo is unclear [3]. In the first division of the *Caenorhabditis elegans* embryo, which has served as a key model system for the study of asymmetric division, a further puzzling feature is the appearance, and then disappearance, of spindle oscillations transverse to the spindle axis [4]. A combined mathematical and fluorescence microscopy analysis of the *C. elegans* embryo recently found that the asymmetry in spindle position along the anterior-posterior axis could be explained by an imbalance in the persistence of motor interaction with microtubules at the opposite ends of the embryo [5]. This model was then extended to establish a theoretical framework for also explaining the transverse oscillations by assuming that antagonistic motors work against each other and against an elastic load [6].

As they reported recently in *Current Biology*, Pecreaux *et al.* [7] tested a number of the predictions of this antagonistic motor model by quantitatively analyzing transverse oscillations of the mitotic spindle during asymmetric cell division. Remarkably, the antagonistic motor model successfully predicts the outcome of a series of observations, such as the build-up and die-down of transverse oscillations, by the monotonic decrease over time of a single model parameter: the off-rate constant of the motor from the microtubule. Thus, the axial movements and transverse oscillations are both explained by a single model where the only thing changing is that motors gradually become more persistent ('processive') during mitosis.

To understand why this simple explanation for oscillations suffices, consider first the case where cortical motors have a high off-rate constant, which means that the motors are less persistent in their force generation (Figure 1). In this case, motors are rarely attached and a spring-like centering force, possibly from the bending of microtubules contacting the cortex, resists the weak motor-based transverse forces to result in small transverse displacements, but no oscillations. This is assumed to be the case during metaphase.

If the off-rate constant is then gradually decreased during further mitotic progression, the motors will attach more persistently, and consequently will generate larger summed forces. These forces are antagonistic, and will be approximately equal and opposite with the upper half motors balancing the lower half motors. Because of the limited number of motors, however, there will be transient force imbalances due to stochastic fluctuations, and as a result the spindle pole will move transiently in the direction having the greater number of attached motors (Figure 1). As the spindle pole moves closer to the cell cortex, the centering spring force builds to resist the leading motors. The increased load will first slow and then detach the leading motors, which are assumed to detach more rapidly with increasing load. So the pole slows and eventually reverses direction, with the motors on the opposite side of the embryo now leading. These newly leading motors have the benefit of the spring force working with them, which ensures they are under relatively low load compared to the following motors. Thus, the lightly loaded leading motors have a relatively low off-rate compared to the still heavily loaded following motors, so that there are more leading motors engaged than following motors when the spindle pole returns to the centerline. The spindle pole will then shoot right on past the centerline due to this imbalance, and the cycle will repeat indefinitely.

How then do the transverse oscillations ultimately die out as the embryo progresses through anaphase? Importantly, the model predicts that the motor off-rate constant decreases monotonically as mitosis progresses. Initially, oscillations begin once motor off-rates decrease sufficiently to achieve a threshold force necessary for spindle pole oscillations, as described above. Similarly, as the motor off-rates continue to decrease during oscillations, a substantial number of motors remain attached on both the top and the bottom halves of the embryo, and thus the system sensitivity to individual motor force-dependent off-rates

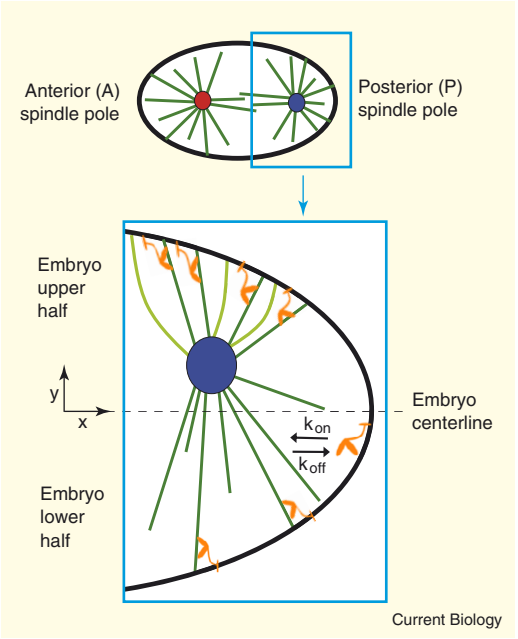

Figure 1. Model for transverse spindle pole oscillations.

Cortical force generators, which we call 'motors' (yellow), possibly dynein motors, pull microtubules (green) toward the cortex (black) in the direction of the y-axis, which is transverse to the spindle axis (x-axis). Motors bind and unbind at rates k_{on} and k_{off}, respectively, where k_{off} is assumed to increase with increasing load. Thus, as the upper half transiently has a larger number of force generators, it will transiently pull the posterior spindle pole (blue) upward. As the spindle pole is displaced from the centerline, a centering spring, possibly due to astral microtubule bending (light green) resists the movement and increases the load on the leading motors in the upper half. These motors eventually stall and detach, which causes the spindle pole to start moving toward the lower half. The lower half motors are now leading, and the upper half motors are following. The leading motors are under relatively low load because the spring force is assisting them, which means their effective off-rate is lower than it is for the following motors. Thus, the newly leading motors will increase in number relative to the following motors so that the spindle pole continues to move past the centerline, now into the lower half. The cycle then repeats and oscillations are established. Pecreaux *et al.* [7] explain the build-up and die-down by a gradual decrease in k_{off}, meaning that the motors interact more persistently (processively) as mitosis proceeds. Eventually, nearly all the motors are engaged nearly all the time, and oscillations cease.

decreases. Now, motor-generated forces pulling towards the lower half of the embryo will oppose motor forces in the upper half of the embryo, decreasing the amplitude of oscillations. In the limit, all of the motors in the two halves are engaged all the time, and the resulting stalemate ensures that the spindle pole no longer oscillates. In this way, oscillations will begin to decrease in amplitude, and, once a sufficient number of motors remain attached to both halves of the embryo, forces will be balanced, and the oscillations will die out.

The only continuous parameter value adjustment in the model of Pecreaux *et al.* [7], which accounted simultaneously for both axial spindle pole displacement and transverse spindle pole oscillations during mitosis, was a monotonic decrease in the motor off-rate constant. This means that motors become increasingly persistent in their interaction with microtubules, effectively increasing their activity (they are more 'processive'). One might suspect that motor activity could be increased by increasing the on-rate constant just as easily as by decreasing the off-rate constant. How could these be distinguished? Pecreaux *et al.* [7] cleverly argued that, if it were the on-rate constant that increased, then the frequency of the oscillations should increase during build-up and die-down, whereas if the off-rate constant decreased, then the frequency should decrease. By careful measurement of the oscillation frequency, it was found that the frequency decreases slightly during mitosis, confirming that it is indeed the off-rate constant that is controlling the build-up and die-down of oscillations.

A key prediction of this model is that the observed build-up of oscillations requires a minimum threshold in force generation, whereas the die-down in oscillations results from the ultimate saturation of opposing force generators at a higher threshold. Consistent with the mechanical model, in which a minimum threshold of motor activity was required for onset of transverse spindle oscillations, either a modest depletion of G-proteins that regulate cortical force generators [4,8] or the partial inactivation of cytoplasmic dynein function [9,10] resulted in a complete loss of spindle oscillations. Thus, the threshold requirement for active motors is yet another hallmark of the model that is predicted and observed experimentally.

What are the key elements of the model? By our count, the model has only a few key parameters: the number of motors, the motor stall force, the unloaded velocity, the characteristic detachment force, the on-rate constant, the off-rate constant and the centering spring constant. Fortunately, single molecule *in vitro* measurements have yielded accurate measurements of the stall force and the unloaded velocity that can be used as starting points for modeling motors in *vivo*. The detachment force must be fairly similar to the stall force (~picoNewtons), else detachment will occur too frequently (before motors stall) or not at all (motors will always stall). Because the elements of the model are very basic, it will be interesting to see where oscillations might arise in other systems where motors work against elastic loads with load-dependent detachment from the filaments that they are working against.

It should be pointed out that Pecreaux *et al.* [7] considered completely alternative models that hinge on an oscillating signal that instructs motors to turn on and off in a spatial-temporal oscillatory manner. Precedent exists for such oscillators in the form of the Min system in bacteria, which defines the center of the dividing bacterium [11,12]. In the *C. elegans* embryo, however, such a system based on diffusion-reaction is essentially impossible, as the period of the oscillations is shorter than the time required for a globular protein to diffuse across the relatively large

embryo. Thus, these physical arguments lend considerable credence to the view that elastic mechanical force, which travels at the speed of sound, is highly efficient at transmitting information rapidly across the spindle.

One aspect of the oscillations that remains unclear is whether they are really transverse in a single plane. What determines this plane of transverse oscillation? Alternatively, one might expect that, because of symmetry about the spindle axis, the oscillations might actually manifest themselves as a precession about the spindle axis, perhaps like a spinning top that precesses about its spinning axis. These more detailed three-dimensional aspects will provide further challenges to the model.

Nevertheless, Pecreaux *et al.* [7] have developed a relatively simple model that explains a variety of aspects of a very complex system. Is this the 'correct' mathematical model? One could always, in principle, posit alternatives that might also explain all the data at hand, and so there is never any 'unique' solution to the problem. Nevertheless, the hallmarks of a good model are that it holds up to repeated testing, that it makes surprising predictions that turn out to be true, and that it explains a lot with a little. The results of Pecreaux *et al.* [7] meet these subjective criteria, and provide both a relatively simple explanation for spindle movements in *C. elegans*, and a fundamental theoretical framework for further investigation of asymmetric cell division.

In summary, the antagonistic motor model has been subjected to a number of experimental tests, some of which would have been difficult to conceive without the modeling — the slight change of the oscillation frequency during build-up and die-down — and the same explanation emerges consistently: motor persistence builds monotonically during the first mitosis of *C. elegans.*

References

1. Horvitz, H.R., and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237–255.
2. Knoblich, J.A. (2001). Asymmetric cell division during animal development. Nat. Rev. Mol. Cell Biol. *2*, 11–20.
3. Labbe, J.C., McCarthy, E.K., and Goldstein, B. (2004). The forces that position a mitotic spindle asymmetrically are tethered until after the time of spindle assembly. J. Cell Biol. *167*, 245–256.
4. Tsou, M.F., Ku, W., Hayashi, A., and Rose, L.S. (2003). PAR-dependent and geometry-dependent mechanisms of spindle positioning. J. Cell Biol. *160*, 845–855.
5. Grill, S.W., Howard, J., Schaffer, E., Stelzer, E.H., and Hyman, A.A. (2003). The distribution of active force generators controls mitotic spindle position. Science *301*, 518–521.
6. Grill, S.W., Kruse, K., and Julicher, F. (2005). Theory of mitotic spindle oscillations. Phys. Rev. Lett. *94*, 108104.
7. Pecreaux, J., Röper, J., Kruse, K., Julicher, F., Hyman, A., Grill, S.W., and Howard, J. (2006). Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators. Curr. Biol. *16*, 2111–2122.
8. Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S. (2003). A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C. elegans*. Genes Dev. *17*, 1225–1239.
9. Gonczy, P., Pichler, S., Kirkham, M., and Hyman, A.A. (1999). Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage *Caenorhabditis elegans* embryo. J. Cell Biol. *147*, 135–150.
10. Schmidt, D.J., Rose, D.J., Saxton, W.M., and Strome, S. (2005). Functional analysis of cytoplasmic dynein heavy chain in *Caenorhabditis elegans* with fast-acting temperature-sensitive mutations. Mol. Biol. Cell *16*, 1200–1212.
11. Kruse, K. (2002). A dynamic model for determining the middle of Escherichia coli. Biophys. J. *82*, 618–627.
12. Howard, M. (2004). A mechanism for polar protein localization in bacteria. J. Mol. Biol. *335*, 655–663.

Department of Biomedical Engineering, University of Minnesota, 7-132 Hasselmo Hall, 312 Church Street S.E., Minneapolis, Minnesota 55455, USA.
E-mail: oddex002@umn.edu

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Social Evolution: Early Production of Deadly Males by Competing Queens

Males usually have little involvement in the dramas of social insect societies, but a newly identified *Cardiocondyla* ant species has been found to produce long-lived, murderous males, even before the first workers, in a new form of queen–queen competition.

Joan E. Strassmann

Understanding conflict and its resolution has been the goal of much social insect research ever since Hamilton taught us how to think about selection in groups of relatives [1,2]. Conflict arises in ant, bee and wasp societies because colony members are not genetically identical. Evolutionary strife can occur over sex ratios because workers and queens are not equally related to the next generation of males and females. [3–6]. For example, this difference in interests causes nearly complete specialization in male or female production in tropical wasp colonies [7]. Workers and queens may differ over who should produce males — virgin workers can produce males which are haploid. This conflict results in the methodical policing by honeybee workers on the lookout for rogue worker-laid eggs [2]. Interests can also differ over whether a given female should become a worker or a queen [2]. In *Melipona* stingless bees, many females become queens that are executed by the workers [8].

Workers are the most numerous party in colonies, and therefore often have the power to enforce their interests, but conflict need not involve workers [9]. Multiple-queen colonies are widespread in ants, bees and wasps, and competition between queens in the same colony is common [6,10]. For example, a newly emerged honeybee queen stings sister queens to death before they get