

for future experiments will be to integrate the physical processes of cellular mechanics, cell motility, adhesion, and tissue architecture with the detailed workings of cellular biochemistry. The paper by Ninomiya and Winklbauer provides an excellent starting point by complementing molecular approaches with the powerful microsurgical techniques that brought amphibian model systems to the forefront of embryology.

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FERMing Up the Plasma Membrane

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As cells enter mitosis, shape changes occur that involve rearrangements of the actin cytoskeleton and an increase in cortical stiffness. In a recent article in *Current Biology*, Kunda et al. describe a new role for ERM proteins in regulating rearrangements of the cortical cytoskeleton during mitosis.

The precise regulation of cortical tension is essential in dividing cells to allow the complex cell shape changes that accompany cytokinesis. Interphase cells in culture lie flat against the substrate and often have quite irregular shapes, but during mitosis, cells round up and become almost spherical by retracting the cell margin and increasing cortical stiffness (Cramer and Mitchison, 1997). The actin cytoskeleton is crucial to this process, as is myosin II, at least in some cells (Maddox and Burridge, 2003). During interphase, F-actin is found in stress fibers, but as cells enter mitosis, the actin cytoskeleton rearranges to the cell cortex to form a continuous layer of actin filaments positioned beneath the plasma membrane.

While these aspects of mitosis are well known, the underlying mechanisms are poorly understood. Using cultured *Drosophila* S2 cells and RNA interference, Kunda et al. (2008) now demonstrate that Moesin, the only ERM (Ezrin, Radixin, Moesin) protein in *Drosophila*, plays a crucial role in this process. ERM proteins are generally thought to link the plasma membrane to the underlying actin

cytoskeleton by interacting with transmembrane proteins via an N-terminal FERM domain (Chishti et al., 1998) and the cytoskeleton via a C-terminal actin-binding domain (Bretscher et al., 2002; Figure 1). Activation of ERM proteins occurs upon phosphorylation of a conserved threonine residue near the C terminus, which unfolds the protein by disrupting interactions between these two domains. In the fly, phosphorylation of this residue is dependent upon Slik, a member of the Sterile-20 family of serine/threonine kinases (Hipfner et al., 2004; Hughes and Fehon, 2006).

To ask if Moesin may be involved in cell-shape changes associated with mitosis, Kunda et al. first examined the activation state of Moesin in interphase and mitotic cells using antibodies specific for activated or phosphorylated Moesin (P-Moesin). P-Moesin is strongly upregulated at the onset of mitosis, initially at the retracting margins then spreading around the entire cortex, and ultimately becomes restricted to the region of the cleavage furrow by telophase. Depletion of either Moesin or the Slik kinase by RNAi has

no effect on the morphology of interphase cells but does block retraction of the cell margin and cell rounding in mitotic cells. Conversely, reducing Myosin II activity does not affect either retraction or cell rounding, though these cells exhibit aberrant cortical morphologies. Taken together, these results suggest that both Myosin II and Moesin are necessary for cortical regulation during mitosis, but Moesin alone controls the initial rearrangements of the actin cytoskeleton.

Live imaging of Moesin-depleted cells revealed dynamic defects during mitosis including abnormal contractile waves at the cell cortex and abnormal membrane blebbing, suggesting that some aspect of cortical rigidity may be disrupted. Consistent with this idea, atomic force microscopy showed that although in control cells cortical stiffness increases during the transition from interphase to mitosis, it does not in Moesin-depleted cells. Expression of a phosphomimetic Moesin mutant mimics the increase in cortical rigidity typical of mitotic cells and induces cell rounding, even in the absence of Myosin II function.

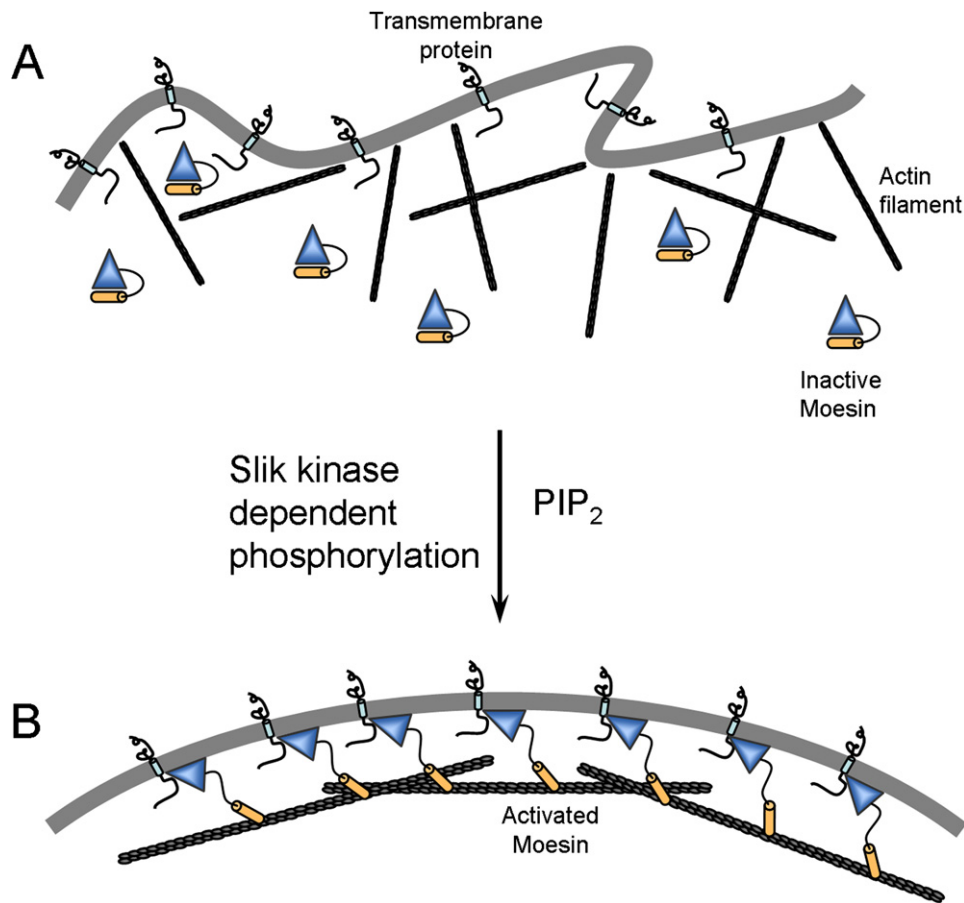


Figure 1. Simplified Model of Moesin Function in Cell Rounding during Mitosis

(A) In interphase cells, the cortex contains many actin filaments that are not oriented parallel to the plasma membrane. Moesin protein is largely in an inactive state due to intramolecular interaction between the head and tail domains. In this state, binding sites with transmembrane proteins and filamentous actin are completely masked.

(B) In response to phosphorylation by the Slik kinase, probably in combination with phospholipid binding, Moesin intramolecular binding is released, allowing interactions with both transmembrane proteins and filamentous actin. This binding in turn may tend to orient actin filaments parallel to the plasma membrane, thereby increasing cortical stiffness and facilitating cell rounding that occurs at the onset of mitosis.

These results are novel and at the same time fit well with our current understanding of ERM protein function in organizing the cortical cytoskeleton. Less expected is the authors' observation that in addition to cortical defects, the mitotic spindle is often irregular in shape, orientation, and position in Moesin RNAi-treated cells. In addition, these cells display slow mitotic progression, suggesting that a mitotic checkpoint may be triggered. To elucidate whether these spindle defects are due only to the softness of the cell cortex or if Moesin has an instructive role in regulating the mitotic spindle, cortical stiffness was rescued in Moesin RNAi cells using Concanavalin A to crosslink glycoproteins in the plasma membrane. Remarkably, this treatment results in a dramatic reduction of spindle defects,

suggesting that the primary role of Moesin in mitosis is to regulate cortical stiffness.

Taken together, these results suggest a simple model to explain how Moesin regulates cell shape and spindle morphology in mitotic cells (Figure 1). By linking filamentous actin to the cytoplasmic face of the plasma membrane, activation of Moesin at the onset of mitosis may cause alignment of microfilaments parallel to the plasma membrane, thereby providing a stiff cortical meshwork. According to their model, this in turn would tend to promote rounding of the cell cortex in much the same way that a fishing rod will form smooth curves rather than kinks when bent. The rigid, regular cortex in turn serves to position and shape the mitotic spindle by interactions with astral microtubules, allowing smooth progres-

sion through the spindle assembly checkpoint.

Although satisfying in its simplicity, this model leaves several important questions unanswered. The results suggest that cortical retraction and rounding are not dependent on Myosin II, yet contractile forces seem likely to be important in this process. Because the RNAi technique depletes rather than abolishes gene function, it is possible that low levels of Myosin II function provide sufficient contractile force to round cells at they enter mitosis. Alternatively, other unknown motors may play a role.

A related question left unanswered by this study is how the functions of Moesin in generating cortical stiffness are coordinated with those of Myosin II in mediating cleavage furrow contractions during

mitosis. Myosin II function and cleavage furrow progression are known to be regulated by activity of the small GTPase RhoA. Interestingly, previous studies in flies and mammalian cells have provided evidence for both positive and negative regulatory interactions between ERM proteins and RhoA (Speck et al., 2003; Takahashi et al., 1997). In particular, genetic studies in flies show that many phenotypes associated with loss-of-function mutations in Moesin are strongly suppressed by reduction in RhoA activity, suggesting that Moesin negatively regulates RhoA. Thus, in addition to the structural role identified by Kunda et al. in organizing cortical actin, Moesin may also regulate Myosin II via its effects on RhoA activity. Unfortunately, this possible link between Moesin and RhoA was not investigated in this study, so further work will need to be done.

Finally, while this study was carried out in cultured cells, ERM proteins are best known in the context of polarized epithelial cells. It is possible that in interphase cells, ERM proteins also function to stiffen

the cortex to maintain the structural integrity of these cells. Indeed, genetic studies in the mouse gut epithelium suggest that ERM function is necessary for proper tensioning of the apical membrane during villar morphogenesis (Saotome et al., 2004). Whether ERM functions in epithelial tissues are similar to those identified by Kunda et al. in mitotic cells remains to be determined. Similarly, it will be interesting to learn how ERM proteins impact mitotic events in epithelial tissues. This study has opened the door to these questions, but much remains to be answered.

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Note Added in Proof

In a parallel study, Carreno et al. confirm that Moesin contributes to cortical stability and spindle positioning in a Slik kinase-dependent manner. Interestingly, they also demonstrate that RhoA is mislocalized in Moesin-depleted cells and that the effects of Moesin depletion on cortical deformation and spindle function can be suppressed partially by reducing RhoA activity, suggesting possible regulatory crosstalk between Moesin and RhoA. Carreno, S., Kouranti, I., Szafer Glusman, E., Fuller, M.T., Echard, A., Payre, F. 2008. Moesin and its activating kinase Slik are required for cortical stability and microtubule organization in mitotic cells. *J. Cell. Biol.* 180, in press. 10.1083/jcb.200709161.

Running Rings around Chromosomes to Trim Axons and Target Dendrites

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The cohesin protein complex holds sister chromatids together to ensure proper chromosome segregation at mitosis in dividing cells. New experiments by two laboratories (reviewed in this issue of *Developmental Cell*) using different techniques reveal that cohesin also plays critical roles in morphogenesis of nondividing neurons. Other recent studies argue that these roles involve regulation of gene transcription.

The cohesin complex plays a crucial role in chromosome segregation in dividing cells from yeast to man. Extensive evidence argues that the ring-like structure formed by the Smc1, Smc3, Rad21, and Stromalin (SA) cohesin subunits encircles sister chromatids to hold them together, and that at mitosis, Rad21 is proteolytically cleaved by separase to

permit sister segregation and cell division (Figure 1A; reviewed by Losada, 2007).

Two groups using different innovative techniques now provide compelling evidence that cohesin is required for proper morphogenesis of nondividing neurons in *Drosophila*. During the development of the nervous system, neurons often extend

excess axons, and later prune away the inappropriate connections (reviewed by Luo and O'Leary, 2005). In this issue of *Developmental Cell*, Pauli et al. (2008) and Schuldiner et al. (2008) show that cohesin is required for axon pruning in the *Drosophila* mushroom body, a brain structure involved in olfactory learning and memory.