Dispatches

Mitosis: Moesin and the Importance of Being Round

Ezrin/radixin/moesin proteins link the actin cytoskeleton to the plasma membrane. Two new reports have found that moesin phosphorylation is essential for mitotic cell rounding and identify a new role for cell rounding in spindle assembly.

Jody Rosenblatt

The muscle proteins of the cell — actin and myosin II — are usually credited for the force behind all cell movements and shape changes. However, recent work suggests that phosphorylation of moesin, an actin-binding protein, is sufficient to cause rounding of cells and efficient mitotic spindle assembly [1,2]. These studies suggest a new paradigm for a non-motor protein causing cell-shape change. Additionally, both groups find that cell rounding by moesin phosphorylation is necessary for stabilizing and aligning the metaphase spindle.

Moesin is a member of the ezrin/radixin/moesin (ERM) family of actin-binding proteins that crosslink the actin cortex to the plasma membrane. Moesin, like other ERM proteins, adopts a closed, inactive state when not phosphorylated. Upon phosphorylation by the Ste20 protein kinase Slik [3], it adopts an open conformation, allowing the carboxy-terminal tail domain to bind actin and the amino-terminal domain to bind a membrane-binding protein [4] (Figure 1 inset). Understanding the cellular function of ERM proteins has been challenging in mammals because of their functional redundancy. To circumvent this problem, researchers have recently probed ERM function using mutants or RNAi in *Drosophila,* given that flies have only one member of this family — moesin [5]. Kunda *et al.* [1] and Carreno *et al.* [2] studied the role of moesin in mitosis using RNAi in *Drosophila* S2 cells, in which live-imaging analysis of cell-shape changes is possible.

Both papers show that moesin, in its phosphorylated form, is essential for cell rounding during mitosis. Previous studies had reported that mitotic cell rounding requires Rho and its effector Rho kinase (ROCK) [6], suggesting a role for the ROCK target myosin II in cell rounding. However, inhibition of ROCK only partially disrupts mitotic cell rounding [1,6,7]. Thus, the studies on moesin suggest a missing layer to the mechanism of cell rounding. Using phospho-specific antibodies to moesin, both groups found that phosphorylated moesin accumulates at the mitotic *Drosophila* cell cortex as it rounds. As the cell lengthens during anaphase, phospho-moesin vanishes from the poles and enriches, along with myosin II, at the presumptive division site just before cytokinesis. Both groups found that loss of moesin or loss of its phosphorylation via RNAi knockdown of either moesin or Slik, respectively, disrupted cell rounding and caused cells to bleb uncontrollably. Using atomic force microscopy to probe cell stiffness, Kunda *et al.* [1] found that, while the surfaces of control cells stiffen during mitosis, cells lacking moesin remain soft. Additionally, interphase cells, which are normally flat, become round if they express a phospho-mimetic moesin (T559D) mutant, indicating that moesin phosphorylation is sufficient to induce cell rounding [1]. This rounding was not dependent on myosin II because cells expressing moesin T559D but lacking myosin II light chain (MLC) were still able to undergo rounding. Although myosin II may collaborate during mitotic cell rounding, the fact that moesin phosphorylation on its own can lead to rounding suggests that a phosphorylation-dependent conformational change in moesin enables it to crosslink the plasma membrane with the actin cortex, stiffening and rounding the cell (Figure 1).

Why do cells round up at mitosis? For as long as researchers have been studying mitosis, they have noticed that cells round during this process, yet have never understood the function of cell rounding. Although blocking ROCK or myosin II partially disrupts cell rounding, it also blocks a process called cortical flow — the directional movement of the cortex. During prometaphase, astral microtubules attach to this moving cortex to help separate the two asters of the forming spindle [7]. Therefore, it was not possible to selectively probe the function of cell rounding at mitosis until this independent role for moesin phosphorylation was identified. Both groups found that cell rounding helped stabilize spindle assembly: spindles in cells lacking moesin stalled in metaphase, vacillating dramatically with aberrant morphology. One

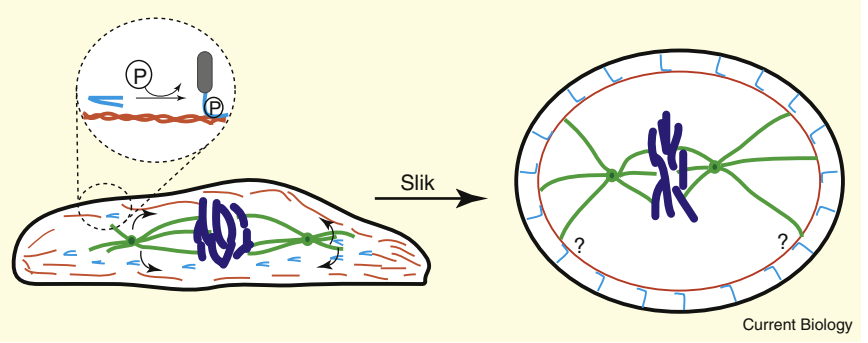

Figure 1. Moesin phosphorylation by Slik causes cell rounding during mitosis.

Moesin (pale blue) binds intramolecularly when inactive (left). Upon phosphorylation by Slik kinase, moesin changes its conformation (left, inset), so that the amino terminus binds a membrane protein (grey) and the carboxyl terminus binds actin filaments (red). By crosslinking the actin cortex to the plasma membrane, moesin helps stiffen and round the cell (right). This shape change stabilizes the attachment of astral microtubules (green) to the cortex by an unknown mechanism (denoted by ?) and thereby also stabilizes the mitotic spindle morphology (dark blue) and its position within the cell.

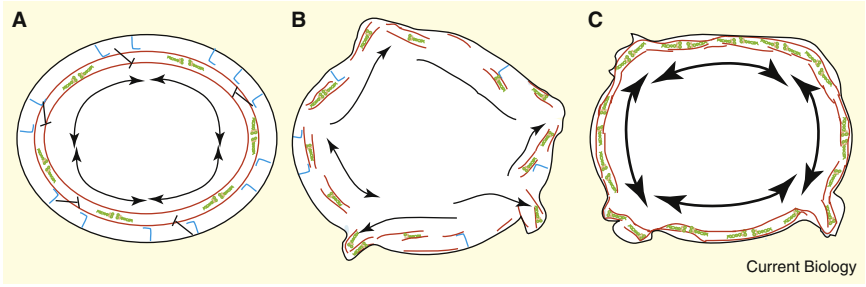

Figure 2. Models for moesin-mediated regulation of actin and myosin II contraction.

(A) Moesin (pale blue) could collaborate with myosin II (green) by either aligning actin filaments (red), enabling myosin II to contract isometrically at the cortex, or by inhibiting Rho-mediated regulation of myosin II activity, dampening contraction. Loss of moesin may alter the organization of the actin cortex, disrupting coherent myosin contraction (B) or misregulate myosin II contraction, causing over-contraction, similar to apoptotic cell blebbing (C). Further, the models are not mutually exclusive; moesin may structurally support actin and myosin II and regulate (blocking arrows) actomyosin to contract isometrically (A).

possible explanation for this spindle phenotype is that, without phosphorylated moesin, astral microtubules can no longer contact the cortex or use cortical flow to separate the two asters of the spindle. Spindles would then be rescued by self-assembly mechanisms. This rescue could account for the stall in metaphase and the apparent coalescence of extra asters to form *de novo* spindle poles, as seen by live-cell imaging. Intriguingly, spindle stability is not dependent on moesin activity per se but on the shape of the mitotic cell, since Kunda *et al.* [1] found that unstable metaphase spindles in cells lacking moesin could be rescued by artificially rounding the cells via the addition of the tetravalent lectin to the cell surface. This work suggests that a rounded cortex helps stabilize and position a mitotic spindle within the cell.

Although a stiff, rounded cortex stabilizes the spindle, it is not clear how it does so. Because artificial rounding without moesin can also stabilize spindles, it is unlikely that moesin directly stabilizes the microtubules of the spindle. Induced membrane curvature could potentially expose specific lipids on the intracellular face of the plasma membrane. For instance, phosphatidylinositol-3,4,5-triphosphate (PIP_3) has previously been found to bind and stabilize microtubules through a complex containing the microtubule-stabilizing protein CLASP and the CLASP-binding proteins LL5ß, and ELKS [8]. Thus, PIP_3 or other lipids could help stabilize the astral microtubules during mitosis. Alternatively, microtubule-stabilizing protein complexes that also associate with the cortex or plasma membrane, such as dynactin or CLASPs (see [9] for review), may prefer binding to curved surfaces.

Another key question is how actin–moesin and actin–myosin II might collaborate with each other. Neither separate knockdown of moesin or MLC completely abolishes cell rounding; moesin loss produced spiky or blebbing mitotic cells, whereas MLC loss produced cells with ruffling membranes. One possibility is that moesin helps align actin filaments into a conformation that enables myosin II to contract efficiently, like lining up tracks for a train (Figure 2B). This model is supported by the fact that moesin localizes to sites of myosin II contraction during mitosis and cytokinesis and that moesin mutations exacerbate cytokinesis defects in a MLC mutant [2]. Alternatively, moesin could limit myosin II activity. Moesin inactivates Rho activity in the *Drosophila* imaginal disc epithelium [10]. Furthermore, the uncontrolled blebbing in cells lacking moesin may be due to overactive myosin contraction, similar to apoptotic cell blebbing resulting from caspase-cleaved, constitutively active ROCK [11,12]. In this way, moesin-mediated dampening of myosin activity may fine-tune myosin contraction, reducing blebbing and encouraging isometric contraction (Figure 2C). These models, however, are not mutually exclusive, as moesin might play both a structural and a regulatory role during cell rounding (Figure 2A).

Although the studies of moesin's role in cell rounding were carried out on cells in culture, its function in mitosis is likely to be relevant to cells within tissue, because moesin mutations led to cell-division defects in fly larval testes [2]. Mitotic defects may account for the link with moesin misregulation in cancers that have poor prognosis (Oncomine data found from www.oncomine.org and [13]). Further studies will need to determine whether high-grade tumors are dependent on altered moesin and, if so, whether moesin mutations disrupt faithful mitosis, polarity, or other processes.

References

1. Kunda, P., Pelling, A.E., Liu, T., and Baum, B. (2008). Moesin controls cortical rigidity, cell rounding, and spindle morphogenesis during mitosis. Curr. Biol. *18*, 91–101.
2. Carreno, S., Kouranti, I., Glusman, E.S., Fuller, M.T., Echard, A., and Payre, F. (2008). Moesin and its activating kinase Slik are required for cortical stability and microtubule organization in mitotic cells. J. Cell Biol. *180*, 739–746.
3. Hipfner, D.R., Keller, N., and Cohen, S.M. (2004). Slik Sterile-20 kinase regulates Moesin activity to promote epithelial integrity during tissue growth. Genes Dev. *18*, 2243–2248.
4. Pearson, M.A., Reczek, D., Bretscher, A., and Karplus, P.A. (2000). Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. Cell *101*, 259–270.
5. Hughes, S.C., and Fehon, R.G. (2007). Understanding ERM proteins – the awesome power of genetics finally brought to bear. Curr. Opin. Cell Biol. *19*, 51–56.
6. Maddox, A.S., and Burridge, K. (2003). RhoA is required for cortical retraction and rigidity during mitotic cell rounding. J. Cell Biol. *160*, 255–265.
7. Rosenblatt, J., Cramer, L.P., Baum, B., and McGee, K.M. (2004). Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. Cell *117*, 361–372.
8. Lansbergen, G., Grigoriev, I., Mimori-Kiyosue, Y., Ohtsuka, T., Higa, S., Kitajima, I., Demmers, J., Galjart, N., Houtsmuller, A.B., Grosveld, F., *et al.* (2006). CLASPs attach microtubule plus ends to the cell cortex through a complex with LL5beta. Dev. Cell *11*, 21–32.
9. Mimori-Kiyosue, Y., and Tsukita, S. (2003). "Search-and-capture" of microtubules through plus-end-binding proteins (+TIPs). J. Biochem. *134*, 321–326.
10. Speck, O., Hughes, S.C., Noren, N.K., Kulikauskas, R.M., and Fehon, R.G. (2003). Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity. Nature *421*, 83–87.
11. Mills, J.C., Stone, N.L., Erhardt, J., and Pittman, R.N. (1998). Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. J. Cell Biol. *140*, 627–636.
12. Coleman, M.L., Sahai, E.A., Yeo, M., Bosch, M., Dewar, A., and Olson, M.F. (2001). Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat. Cell Biol. *3*, 339–345.
13. Charafe-Jauffret, E., Ginestier, C., Monville, F., Finetti, P., Adelaide, J., Cervera, N., Fekairi, S., Xerri, L., Jacquemier, J., Birnbaum, D., *et al.* (2006). Gene expression profiling of breast cell lines identifies potential new basal markers. Oncogene *25*, 2273–2284.

University of Utah, Department of Oncological Sciences, Huntsman Cancer Institute, 2000 Circle of Hope, Salt Lake City, Utah 84112, USA.
E-mail: jody.rosenblatt@hci.utah.edu

DOI: 10.1016/j.cub.2008.02.013