Concentrating on the mitotic spindle

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In eukaryotes, the microtubule-based spindle drives chromosome segregation. In this issue, Schweizer et al. (2015; *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506107) find that the spindle area is demarcated by a semipermeable organelle barrier. Molecular crowding, which is microtubule independent, causes the enrichment and/or retention of crucial factors in the spindle region. Their results add an important new feature to the models of how this structure assembles and is regulated.

Mitosis is marked by the assembly of the mitotic spindle, a microtubule-based structure that facilitates accurate chromosome segregation. Many biochemical reactions are coupled to spindle assembly, from tubulin polymerization itself to the mitotic checkpoint, which inhibits chromosome disjunction until all the chromosomes are properly attached and aligned (Cleveland et al., 2003). Interestingly, these reactions are virtually unaltered over a broad morphological range; large spindles and small spindles follow roughly the same biochemical rules despite quite distinct geometries (Brown et al., 2007; Wühr et al., 2008). In this issue, Schweizer et al. report that the mitotic spindle area is delineated by membrane-bound organelles, generating a "spindle envelope" with unique molecular constituents compared with the surrounding cytoplasm. Spindle envelope–based molecular crowding provides an enticing hypothetical solution to the broad problem of confining mitotic biochemistry to a specific cellular space irrespective of cell size.

Schweizer et al. (2015) used FRAP and fluorescence correlation spectroscopy (FCS) to measure the mobility of specific proteins. The authors found that tubulin and the Mad2 spindle assembly checkpoint protein were enriched in the spindle area in a microtubule polymer-independent manner. Given that the mobility of these proteins outside and within the spindle area was the same, changes in local concentration were likely a result of a barrier effect. In support of this hypothesis, modeling their FCS data with a fenestrated barrier separating the spindle area from the cytoplasm reproduced the measured FCS results. To test if a barrier surrounding the spindle area was important for spindle function, the authors disrupted the envelope area by laser microsurgery and found chromosome segregation errors consistent with defects in spindle assembly and kinetochore attachment monitoring. Thus, spindle envelope–based concentration of basal components in two critical spindle reactions, spindle assembly and mitotic checkpoint signaling, could mechanistically catalyze cell division.

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Molecular crowding can catalyze reactions and stabilize proteins by altering the local concentration of one or more rate-limiting components and is best characterized by membrane-bound organelles. Crowding by aggregation can greatly increase biochemical reactions without the need for a contiguous membrane (Weber and Brangwynne, 2012; Brangwynne, 2013) and this phenomenon can regulate cell cycle states (Lee et al., 2013). Here, Schweizer et al. (2015) propose that by creating a membrane-bound organelle exclusion zone, a spindle envelope could cause the molecular crowding of important spindle proteins and thereby their enrichment in the spindle area.

The mitotic spindle is known to scale with cell size: smaller cells have smaller spindles (Levy and Heald, 2012). Spindle size scaling is prominent during development when repeated cell division without embryonic growth results in cells that can be several orders of magnitude smaller than that of the zygote. Recently, cytoplasm volume and tubulin concentration was shown to be an important factor in spindle size scaling; however, a curious exception to the size scaling rule is that there seems to be an upper limit to spindle size, resulting in stable spindle size when a threshold cell size is reached (Wühr et al., 2008; Good et al., 2013; Hazel et al., 2013). A spindle envelope would provide mechanisms to maintain increased local tubulin concentration independent of the absolute amount available in the cell. The net effect would be that spindle size scales in very large cells to the spindle envelope size rather than cell size in a manner analogous to chromosome size scaling to nuclear size independently of cell size (Fig. 1 A). Clearly this is a more complex problem and factors such as tubulin protein production and polymerization cofactors (such as the Tog family of proteins) clearly play an important role (Slep, 2009). However, spindle envelope–based molecular crowding could provide an elegant solution to a biochemical problem.

A spindle envelope could also provide a cell biological solution to another developmental problem—independent cell cycle control of separate nuclei within a single cytoplasm (syncytia). For example, the mitotic region of the *Caenorhabditis elegans* germline contains germ cell precursors that divide independently of one another in a common cytoplasm. In some cases, two neighboring dividing nuclei can have different biochemistry, one arrested in metaphase because of a kinetochore microtubule attachment defect while the other is progressing into anaphase (Gerhold et al., 2015). By restricting the diffusive radius of signaling molecules like Mad2, a steep threshold of checkpoint activity can be maintained, allowing independent cell cycle control even in a common cytoplasm (Fig. 1 B).

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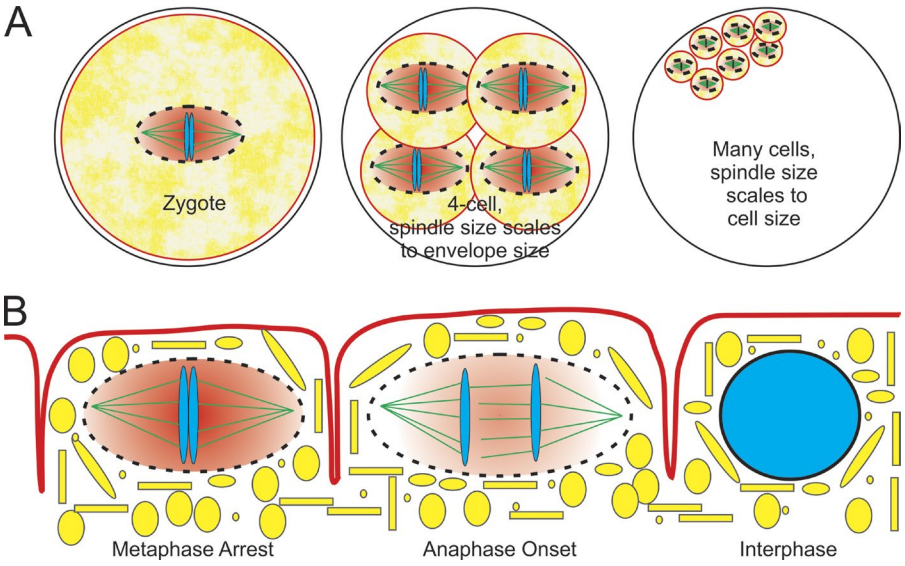

Figure 1. **Molecular crowding in the mitotic spindle.** Schematic view of how a spindle envelope could mitigate spindle size scaling during development (A) or cell cycle control (B) in a common cytoplasm. (A) A spindle envelope (black dotted line) that excludes large membrane-bound organelles (yellow) could locally increase the concentration of spindle proteins (depicted as a red background) such as tubulin to control spindle size independent of cell size during early development. (B) Neighboring nuclei in a common cytoplasm (e.g., the syncytial mitotic gonad in *C. elegans*) could have differing mitotic states by restricting the diffusion of important regulatory proteins such as Mad2 (similar coloring as in A).

The mitotic spindle has long been known to exclude large membrane-bound organelles, even in the absence of microtubule polymer, leading to a hypothesized nontubulin-based "spindle matrix." A spindle matrix would be an excellent candidate to underlie the spindle envelope. The molecular nature of a spindle matrix, however, has never been agreed upon with candidate mechanisms ranging from nonprotein macromolecules to actin (Pickett-Heaps et al., 1984; Chang et al., 2004). A convincing argument can be made that the Skeletor/Megator/Chromator proteins first identified in *Drosophila melanogaster* constitute a spindle matrix (Walker et al., 2000; Qi et al., 2004; Rath et al., 2004; Schweizer et al., 2014). These proteins are large and are found in the nucleus in interphase and as microtubule-independent fibrous structures in and around the spindle in mitosis. Depletion of these proteins results in mitotic errors; however, these may or may not be caused by a role as the spindle matrix.

Schweizer et al. (2015) evaluated Megator (as a representative member of the complex) as a possible basis for generating the spindle envelope. FRAP and FCS showed that, like tubulin and Mad2, Megator is concentrated in the spindle envelope region independent of microtubules. However, Megator in the spindle region had slower diffusive properties compared with that around the cell periphery. Thus, unlike tubulin and Mad2, the mobility of Megator within the spindle was altered, indicating that Megator likely forms a high molecular weight complex with its binding partners Skeletor and Chromator in the spindle area, which may help form the spindle envelope.

The sum of these results lead to a possible model whereby the Skeletor/Megator/Chromator proteins complex together and subsequently support a spindle envelope independent of microtubules. The spindle envelope excludes large membrane-bound organelles, leading to increased concentration of mitotic reaction constituents and thus ultimately catalyzing cell division. It will be exciting in the future to determine if the spindle area is indeed subject to molecular crowding in the purest of forms (solvent exclusion) and how this effect drives cell division.

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References

Brangwynne, C.P. 2013. Phase transitions and size scaling of membrane-less organelles. *J. Cell Biol.* 203:875–881. http://dx.doi.org/10.1083/jcb.201308087

Brown, K.S., M.D. Blower, T.J. Maresca, T.C. Grammer, R.M. Harland, and R. Heald. 2007. *Xenopus tropicalis* egg extracts provide insight into scaling of the mitotic spindle. *J. Cell Biol.* 176:765–770. http://dx.doi.org/10.1083/jcb.200610043

Chang, P., M.K. Jacobson, and T.J. Mitchison. 2004. Poly(ADP-ribose) is required for spindle assembly and structure. *Nature.* 432:645–649. http://dx.doi.org/10.1038/nature03061

Cleveland, D.W., Y. Mao, and K.F. Sullivan. 2003. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell.* 112:407–421. http://dx.doi.org/10.1016/S0092-8674(03)00115-6

Gerhold, A.R., J. Ryan, J.N. Vallée-Trudeau, J.F. Dorn, J.C. Labbé, and P.S. Maddox. 2015. Investigating the regulation of stem and progenitor cell mitotic progression by in situ imaging. *Curr. Biol.* 25:1123–1134. http://dx.doi.org/10.1016/j.cub.2015.02.054

Good, M.C., M.D. Vahey, A. Skandarajah, D.A. Fletcher, and R. Heald. 2013. Cytoplasmic volume modulates spindle size during embryogenesis. *Science.* 342:856–860. http://dx.doi.org/10.1126/science.1243147

Hazel, J., K. Krutkramelis, P. Mooney, M. Tomschik, K. Gerow, J. Oakey, and J.C. Gatlin. 2013. Changes in cytoplasmic volume are sufficient to drive spindle scaling. *Science.* 342:853–856. http://dx.doi.org/10.1126/science.1243110

Lee, C., H. Zhang, A.E. Baker, P. Occhipinti, M.E. Borsuk, and A.S. Gladfelter. 2013. Protein aggregation behavior regulates cyclin transcript localization and cell-cycle control. *Dev. Cell.* 25:572–584. http://dx.doi.org/10.1016/j.devcel.2013.05.007

Levy, D.L., and R. Heald. 2012. Mechanisms of intracellular scaling. *Annu. Rev. Cell Dev. Biol.* 28:113–135. http://dx.doi.org/10.1146/annurev-cellbio-092910-154158

Pickett-Heaps, J., T. Spurck, and D. Tippit. 1984. Chromosome motion and the spindle matrix. *J. Cell Biol.* 99:137s–143s. http://dx.doi.org/10.1083/jcb.99.1.137s

Qi, H., U. Rath, D. Wang, Y.Z. Xu, Y. Ding, W. Zhang, M.J. Blacketer, M.R. Paddy, J. Girton, J. Johansen, and K.M. Johansen. 2004. Megator, an essential coiled-coil protein that localizes to the putative spindle matrix during mitosis in *Drosophila*. *Mol. Biol. Cell.* 15:4854–4865. http://dx.doi.org/10.1091/mbc.E04-07-0579

Rath, U., D. Wang, Y. Ding, Y.Z. Xu, H. Qi, M.J. Blacketer, J. Girton, J. Johansen, and K.M. Johansen. 2004. Chromator, a novel and essential chromodomain protein interacts directly with the putative spindle matrix protein skeletor. *J. Cell. Biochem.* 93:1033–1047. http://dx.doi.org/10.1002/jcb.20243

Schweizer, N., M. Weiss, and H. Maiato. 2014. The dynamic spindle matrix. *Curr. Opin. Cell Biol.* 28:1–7. http://dx.doi.org/10.1016/j.ceb.2014.01.002

Schweizer, N., N. Pawar, M. Weiss, and H. Maiato. 2015. An organelle-exclusion envelope assists mitosis and underlies distinct molecular crowding in the spindle region. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506107http://dx.doi.org/10.1016/j.ceb.2014.01.002

Slep, K.C. 2009. The role of TOG domains in microtubule plus end dynamics. *Biochem. Soc. Trans.* 37:1002–1006. http://dx.doi.org/10.1042/BST0371002

Walker, D.L., D. Wang, Y. Jin, U. Rath, Y. Wang, J. Johansen, and K.M. Johansen. 2000. Skeletor, a novel chromosomal protein that redistributes during mitosis provides evidence for the formation of a spindle matrix. *J. Cell Biol.* 151:1401–1412. http://dx.doi.org/10.1083/jcb.151.7.1401

Weber, S.C., and C.P. Brangwynne. 2012. Getting RNA and protein in phase. *Cell.* 149:1188–1191. http://dx.doi.org/10.1016/j.cell.2012.05.022

Wühr, M., Y. Chen, S. Dumont, A.C. Groen, D.J. Needleman, A. Salic, and T.J. Mitchison. 2008. Evidence for an upper limit to mitotic spindle length. *Curr. Biol.* 18:1256–1261. http://dx.doi.org/10.1016/j.cub.2008.07.092