Samurai Sword Sets Spindle Size

Simone Reber[1] and Anthony A. Hyman[1,*]
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany
*Correspondence: hyman@mpi-cbg.de
DOI 10.1016/j.cell.2011.11.039

Although the parts list is nearly complete for many cellular structures, mechanisms that control their size remain poorly understood. Loughlin and colleagues now show that phosphorylation of a single residue of katanin, a microtubule-severing protein, largely accounts for the difference in spindle length between two closely related frogs.

Two species of *Xenopus* frogs—*X. tropicalis* and *X. laevis*—are valuable systems for elucidating developmental and cell biological mechanisms. These closely related amphibians, which diverged from their common ancestor only 50 million years ago (Hellsten et al., 2007), are very similar except for their size. Adult *X. tropicalis*, at 4–5 cm, are considerably smaller than the 10 cm *X. laevis.* This difference in size is not restricted to overall body size but is observed in their eggs and somatic cells as well. Cellular structures such as meiotic spindles and nuclei are correspondingly smaller in *X. tropicalis*. Egg extracts from *X. tropicalis* or *X. laevis* enable the cell-free reconstitution of fundamental cell-cycle events and promote the assembly of subcellular structures, including the nucleus and mitotic spindle. Interestingly, spindles and nuclei assembled in extracts of *X. laevis* eggs are larger than those made in *X. tropicalis* egg extracts. The spindles are about 40% longer (Brown et al., 2007), and the nuclei have a 2-fold greater surface area (Levy and Heald, 2010). These data illustrate an important open question in cell biology: what are the molecular mechanisms that define the size of a cellular organelle? Comparing structures assembled in these two cell-free systems provides a powerful tool to study size regulation mechanisms.

In this issue, Loughlin and colleagues (Loughlin et al., 2011) show that the molecular basis of interspecies spindle length variation can be traced to increased activity of katanin (after the Japanese *katana* or "Samurai sword"; Figure 1). Katanin is a heterodimeric protein, composed of a targeting subunit (p80) and an enzymatic subunit (p60), with an ATPase activity that severs and disassembles microtubules (Hartman et al., 1998). The higher severing activity of katanin in *X. tropicalis* suggests that a decrease in microtubule stability causes the shorter spindles in *X. tropicalis* egg extract.

Strikingly, this effect of katanin on spindle size matches predictions from a previous computational model by the same groups, which quantitatively describes meiotic spindle assembly in *Xenopus* egg extracts. A key prediction of this model was that spindle length should be dependent on microtubule depolymerization velocities at the spindle poles (Loughlin et al., 2010). To test this, the authors compared microtubule stability in the two extract types and showed that taxol-stabilized microtubules are significantly less stable in *X. tropicalis* extracts. Although *Xenopus* spindle length has been reported to scale with titration of MCAK and Kif2a (Ohi et al., 2007), two microtubule depolymerases, depletion of these kinesin-13s was not responsible for the high degree of instability in *X. tropicalis* egg extracts. Consistent with increased katanin-dependent MT severing being the root cause of shorter spindles, only the depletion of the katanin p60 subunit inhibited taxol-stabilized microtubule disassembly in *X. tropicalis* egg extracts.

What is responsible for the differential katanin activity in the two extracts? Endogenous protein levels do not vary significantly between the two extract types, and both purified proteins exhibit similar affinities for microtubules and comparable intrinsic severing activities. Despite having amino acid sequences that are 95% identical, the *Xt* and *Xl* katanin p60s differ in one key residue. *Xl* katanin p60 contains a potential Aurora B phosphorylation site not present in the *X. tropicalis* version of the protein. Replacing the endogenous katanin in *X. laevis* egg extracts with a nonphosphorylatable point mutant or *Xt* p60 significantly decreases microtubule half-life, whereas a phosphomimetic mutant does not affect severing activities. These observations, together with the fact that *Xl* p60 is strongly phosphorylated in meiotic extracts, suggest that phosphorylation of p60 at this single site decreases its activity. Consequently, without this phosphorylation site, Aurora B is unable to inhibit katanin activity, resulting in a higher severing activity and smaller spindles. We have seen katanin-dependent changes in spindle length before during the meiosis-mitosis transition in *C. elegans* embryos. Here, mass degradation of katanin at the end of meiosis is critical in transitioning from the tiny meiotic spindle to the larger mitotic spindle (Pintard et al., 2003). Thus, regulation of katanin activity appears to be a widely used and presumably highly effective mechanism of adjusting spindle size.

This current study nicely combines in silico studies, egg extract experiments, and point mutations to identify a single phosphorylation site in katanin as the source of a molecular mechanism controlling spindle length. Where do we go from here? One evolutionary intriguing question is why *X. tropicalis* has a smaller spindle in the first place. In sufficiently large cells like amphibian oocytes, spindle length is uncoupled from cell size (Wühr et al., 2008). This is very different to systems, such as *C. elegans*, that show

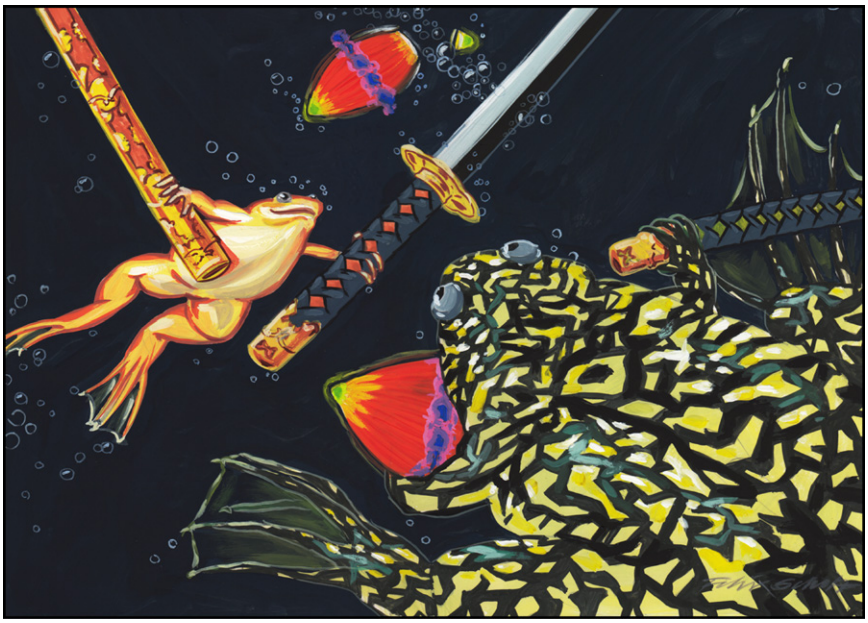

Figure 1. Spindles Sized with Samurai Swords
Xenopus tropicalis, a small relative of *Xenopus laevis*, forms a correspondingly smaller meiotic spindle. This difference in spindle size is due to higher katanin activity in *Xenopus tropicalis*. Katanin, a microtubule-severing protein named after the Japanese katana, or "Samurai sword," localizes to the spindle poles where, it restricts spindle length. Illustration by Felix Scholz.

strict coupling between cell size and spindle length. Perhaps it is due to later constraints in development that arise as cells become increasingly small, which, in *tropicalis*, may occur sooner given its smaller initial size. Ultimately, in both species, spindle and cell size become coupled, and as the authors note, it is unknown whether this katanin-dependent size-control mechanism is also used to control cell size-dependent scaling of the mitotic spindle. In this respect, it is crucial to draw a clear distinction between mechanisms that change the size of an organelle ("programmed scaling" as defined by Marshall, 2011) and mechanisms that allow the size of an organelle to be scaled in response to changes in cell size ("intrinsic scaling" [Decker et al., 2011]). In the future, it will be interesting to learn whether cell size exerts additional control on katanin activity.

REFERENCES

Brown, K.S., Blower, M.D., Maresca, T.J., Grammer, T.C., Harland, R.M., and Heald, R. (2007). J. Cell Biol. *176*, 765–770.

Decker, M., Jaensch, S., Pozniakovsky, A., Zinke, A., O'Connell, K.F., Zachariae, W., Myers, E., and Hyman, A.A. (2011). Curr. Biol. *21*, 1259–1267.

Hartman, J.J., Mahr, J., McNally, K., Okawa, K., Iwamatsu, A., Thomas, S., Cheesman, S., Heuser, J., Vale, R.D., and McNally, F.J. (1998). Cell *93*, 277–287.

Hellsten, U., Khokha, M.K., Grammer, T.C., Harland, R.M., Richardson, P., and Rokhsar, D.S. (2007). BMC Biol. *5*, 31.

Levy, D.L., and Heald, R. (2010). Cell *143*, 288–298.

Loughlin, R., Heald, R., and Nédélec, F. (2010). J. Cell Biol. *191*, 1239–1249.

Loughlin, R., Wilbur, J.D., McNally, F.J., Nédélec, F.J., and Heald, R. (2011). Cell *147*, this issue, 1397–1407.

Marshall, W.F. (2011). BMC Biol. *9*, 57.

Ohi, R., Burbank, K., Liu, Q., and Mitchison, T.J. (2007). Curr. Biol. *17*, 953–959.

Pintard, L., Kurz, T., Glaser, S., Willis, J.H., Peter, M., and Bowerman, B. (2003). Curr. Biol. *13*, 911–921.

Wühr, M., Chen, Y., Dumont, S., Groen, A.C., Needleman, D.J., Salic, A., and Mitchison, T.J. (2008). Curr. Biol. *18*, 1256–1261.