On Spindle Length and Shape.

Simone Reber
Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauer Strasse 108, 01307 Dresden,
www.simonereber.com

The internal organization of a cell is critical for its viability and specifically tailored to its function. Therefore, number, size, and geometry of intracellular organelles are important and must be tightly regulated according to cell cycle state and cell type. A classic example for the tight control of organelle number is the centrosome cycle. Centrosomes are the major microtubule-organizing centre of animal cells and their number is linked to the cell cycle state. In interphase, centrosome number is limited to one centrosome per cell. Before mitosis, centrosomes duplicate precisely once to become the poles of the bipolar mitotic spindle[1]. Aberrant centrosome numbers can result in the generation of abnormal mitotic spindles and thus be a source of chromosome instability, a hallmark of cancer[2]. Examples of cells adjusting their internal organization are the active size regulation of the mitochondrial meshwork in muscle cells or the over-proliferation of the ER in secretory cells. Muscle cells can respond to changes in energy demands by modifying the rates of mitochondrial biogenesis to induce compensatory changes in mitochondrial capacity[3]. B cells express immunoglobulins (Ig) on their surface but do not secrete antibodies. Upon binding of a specific antigen to the B cell receptor, B cells proliferate and differentiate into plasma cells, each of which secretes thousands of antibodies per second. This massive Ig production correlates with an expansion of the ER[4]. As basic physiological processes such as molecular transport rates across membranes are intrinsically size-dependent, their efficiency will vary with changes in organelle surface area or volume[5]. Thus to understand cell organization, it will be critical to understand how cells sense and control the number, size and shape of their organelles.

While approaching near to complete proteomic parts lists of cellular structures and organelles, mechanisms that control their defined shape and size remain poorly understood. One reason why this question has been so hard to answer is that the size of an organelle is generally not simply set by a "ruler" (Figure 1a) but is an emergent property of molecular collectives (Figure 1b). "Emergence" describes the way complex properties and patterns of a system arise by numerous elements, which interact by relatively simple rules. Examples include the generation of an infinite variety of six-sided snowflakes from frozen water in snow[6] (Figure 1c). Similarly, "flocking", the coordinated motion of animals observed in bird flocks, fish schools, or insects swarms, is

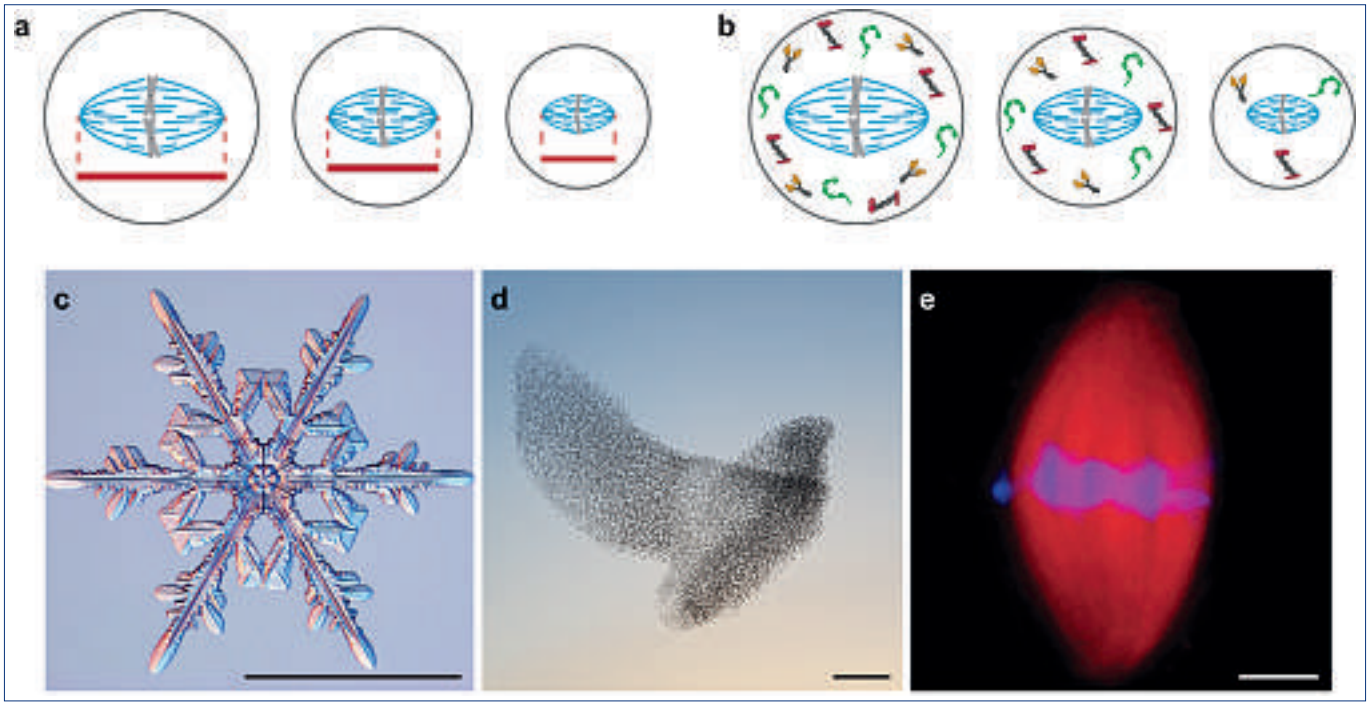

Figure 1. Emergent Properties on Different Length Scales:
a) *Xenopus* spindle length is not set by a "ruler" (a) but is a collective behaviour problem (b). (c) Snow crystal. Scale bar ~1 mm Image courtesy Kenneth G. Libbrecht (http://www.its.caltech.edu/~atomic/snowcrystals/). (d) Starling flock. Scale bar ~10 m. Image courtesy Robert Wolstenholme (http://www.robwolstenholme.co.uk/). (e) Metaphase spindle assembled in Xenopus egg extracts. Scale bar 10 µm.

considered an emergent behaviour[7] (Figure 1d). In physics emergent behaviours are commonly studied to describe complex systems. Physics thus provides a framework for relating the microscopic properties of individual molecules to the macroscopic properties of materials. This exactly is the key challenge in modern cell biology, bridging the gap between individual molecules and their collective behaviour (Figure 1e).

A Multi-Scale Model for *Xenopus* Spindles Length.

During early *Xenopus* development cell size decreases dramatically from a 1200 µm diameter fertilized egg to approximately 12 µm diameter blastomeres[8]. Consequently, the metaphase spindle needs to function in cell volumes that vary by several orders of magnitude. Because defects in spindle length result in erroneous cell division[9], robust mechanisms to set the length of a spindle and scale it according to cell type must exist. Decades of work have shown that microtubule dynamics, nucleation, and transport are critical for spindle assembly[10]. However, the combined activities of these processes, as well as their complex interplay, make it difficult to determine how each individual process contributes to the overall organization of the spindle.

We have recently shown that mass balance together with a "liquid crystal" analogy is a useful working hypothesis to account for the dynamic properties of *Xenopus* spindles and link these to its overall geometry (Figure 2a). Mass balance, which is based on the principle of mass conservation, is often used in physics and engineering to infer properties of systems that are difficult to measure directly. In the 1940s, Irvin Isenberg suggested that the spindle might be a liquid crystal[11]. Indeed, more recent observations in *Xenopus* egg extracts imply that spindles can display liquid-like properties. For example, two meiotic spindles fuse when brought close together[12]. Furthermore, micromanipulation studies on *Xenopus* spindles show that spindles recover their original shape after weak compressions and that they have anisotropic material properties[13-16]. This coarse-grained "liquid crystal" analogy has important implications, because spindle length follows from a simple book-keeping exercise based on mass balance: The length of the spindle will simply depend on the balance of material that is created (nucleation and assembly) and lost (disassembly), while the density of microtubules remains roughly constant. At steady-state, the rate of volume increase due to incorporation of tubulin into microtubules equals the rate of volume loss due to microtubule disassembly. Consequently, spindle length is ultimately related to the kinetic parameters of microtubule nucleation, assembly and disassembly[17] (Figure 2b).

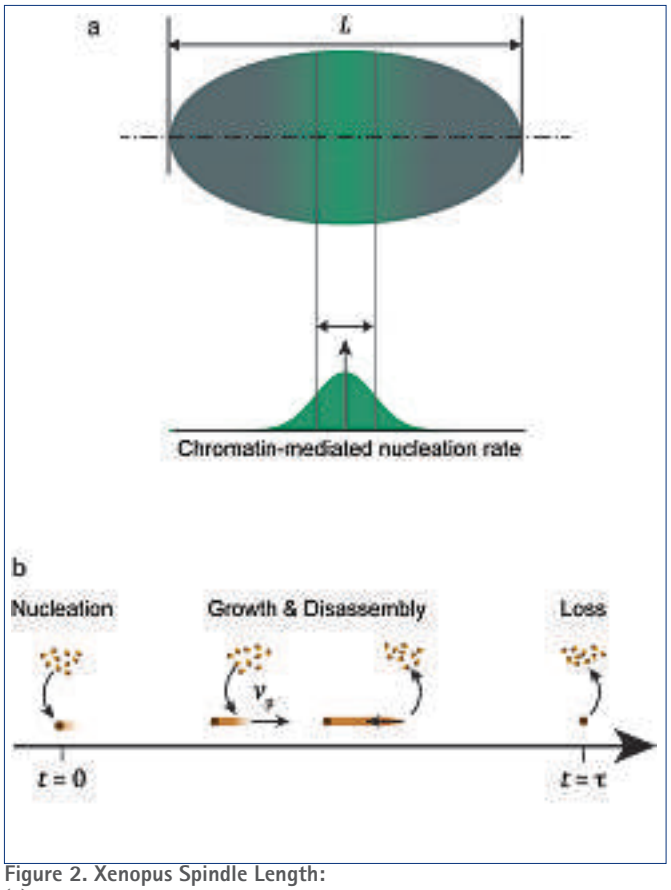

Figure 2. Xenopus Spindle Length:
(a) Mass balance together with a "liquid crystal" analogy allows us to relate kinetic parameters of microtubule nucleation, assembly and disassembly (b) and the overall geometry of Xenopus spindles.

A key prediction of our work is that spindle length scales linearly with microtubule growth velocity when other parameters of spindle organization remain constant[17]. This prediction can be tested in quantitative experiments that allow the modulation of microtubule dynamics in *Xenopus* egg extract spindles. But how can we specifically modulate microtubule growth rates? Work over the last decade established a major role for XMAP215 in microtubule growth promotion. XMAP215 was shown to act as a processive microtubule polymerase that promotes incorporation of tubulin into the growing plus end (Figure 3a). XMAP215 binds the tubulin heterodimer in a 1:1 stoichiometry, 'surfs' the growing microtubule plus end, and stays there for multiple rounds of tubulin incorporation[18]. XMAP215 was first isolated from *Xenopus* eggs[19] and subsequently found in all major kingdoms of eukaryotes, including fungi (Stu2[20] in *S. cerevisiae*, Dis1[21] and Alp14[22] in *S. pombe*), plants (Mor1[23] in *A. thaliana*) and animals (Zyg9[24] in *C. elegans*, mini spindles[25] in *D. melanogaster*, and ch-TOG[26] in humans). All members of the XMAP215 family are characterized by a varying number of TOG domains at their N-termini (Figure 3b). However, how these various domains contribute to XMAP215 activity was until recently not known. Based on mutants in TOG domains that interfere with tubulin binding[27], it has been proposed that TOG binding to tubulin is required for its catalytic activity[28]; however, there was no proof for this idea.

Recently, we have shown that the polymerase activity of XMAP215 depends on tubulin binding to multiple TOG domains. Mutation of conserved residues in different TOG domains reduces tubulin binding while concomitantly reducing the maximal growth promoting activity of the polymerase (Figure 3c). Combining mutations in different TOG domains allowed us to modulate the enzymatic activity of XMAP215 and thereby control microtubule growth velocity *in vitro* [29]. This set of well-characterized polymerase mutants served as an experimental tool kit

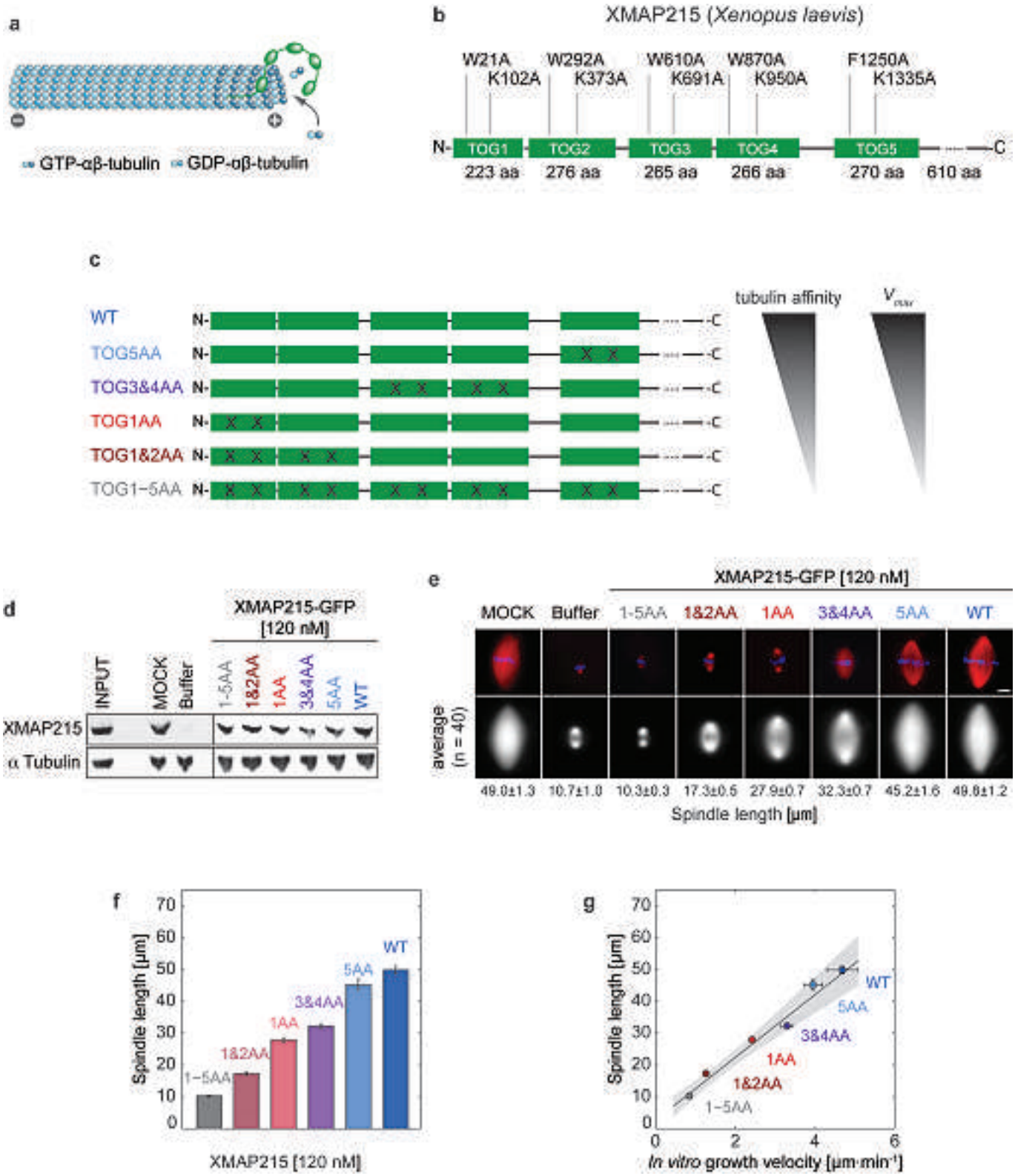

Figure 3. *Xenopus* Spindle Length Is Directly Proportional to XMAP215 Microtubule Polymerase Activity:
(a) XMAP215 acts as a processive polymerase. (b) Schematic of *Xenopus laevis* XMAP215's domain structure indicating the point mutations introduced. (c) Tubulin affinity and maximal microtubule growth promoted by XMAP215 and the mutants. (d) *Xenopus* egg extract was MOCK or XMAP215 depleted, different point mutants of XMAP215 were added back. Depletion efficiency and accuracy of add-back were controlled by Western blot analysis, tubulin served as a loading control. (e) Spindles were assembled in the presence of different XMAP215 mutants. The upper rows show a representative spindle (red: microtubules, blue: DNA), the lower rows show an average image of all spindles from three independent experiments, the average spindle length is indicated. SE, n=40. Scale bars: 10 µm. (f) Average spindle length measured in the presence of different XMAP215 point mutants. Error bars indicate SE (n=40). (g) Spindle length plotted versus the maximal *in vitro* microtubule growth velocity promoted by different XMAP215 mutants. Grey areas indicate the 95% confidence intervals of the fitted curves. Adapted from[17].

to fine-tune microtubule growth rates in *Xenopus* spindles. To implement this tool kit, we depleted the endogenous XMAP215 from *Xenopus* egg extracts (Figure 3d), added back the mutant polymerases and assembled spindles (Figure 3e). At endogenous concentration, all mutants promoted assembly of spindles at lengths proportional to their polymerase activity (Figure 3f). An alternative way to modified XMAP215 activity is adding back recombinant wildtype XMAP215 at different concentrations to XMAP215-depleted extracts. Similarly, spindle length increased with increasing wildtype XMAP215 concentrations. However, at some point adding more than XMAP215 did not result in longer spindles but spindle length plateaued, consistent with existing evidence for an upper limit to spindle length[30]. When we correlated spindle length with the maximum microtubule growth promotion, spindle length was directly proportional to the maximal growth promoted by XMAP215 (Figure 3g), exactly as predicted by our model.

Interestingly, we observed that spindle shape remains constant while spindle length changes significantly with varying XMAP215 activities[17]. This is surprising and suggests that shape is determined by distinct mechanisms that are independent from those determining length. From a developmental or scaling point of view, the separation of length and shape is appealing as it allows fine-tuning of spindle length without changing its overall morphology. We thus propose that force balances set the shape of the metaphase spindle. Nevertheless, it remains to be shown experimentally which forces shape the metaphase spindle and to characterize the molecular factors that underlie these forces.

To conclude, our molecular understanding of XMAP215 has allowed us to perform a "synthetic biology" experiment, in which we can limit the length of a spindle solely by using an appropriate, engineered mutant. Combining experiments and theory has allowed us to bridge scales from the level of single microtubule dynamics to the overall organization of the spindle by using mass balance. Many manipulations of microtubule dynamics alter the size and shape of the spindle in a variety of systems[31-36]. Our model provides a conceptual framework for understanding why perturbations in microtubule dynamics often result in spindle length changes. Any modulation that solely affects a single parameter of spindle organization will change the mass balance and thus result in changes in spindle length.

Material Properties and Organelle Scaling.

Our experiments suggest that *Xenopus* spindles join a growing number of cellular structures that can be treated as active liquids. One example are RNA/protein complexes of P granules in *C. elegans* that were shown to exhibit liquid-like behaviours, which suggest a simple physical picture for P granule localization[37]. Likewise, nucleoli in amphibian oocyte behave like liquid-like droplets[38]. Only recently, the centrosome was suggested to be an autocatalytic drop, which explains the formation of centrosomes only around centrioles and their scaling with cell size (Zwicker *et al.*, unpublished and [39]). One characteristic of all these highly dynamic structures is that they are non-membrane

bound and that their components are in constant exchange with the surrounding cytoplasm. These biophysical studies are consistent with the emerging concept that phase separation is an important physical principle for organizing the internal structures of cells[40]. In the context of our studies, we can thus think of a spindle as an active liquid crystal drop that is phase separated from the cytoplasm. In this case, the formation of the second phase is initiated by chromatin, which induces local nucleation of microtubules. While the microtubule phase is forming, other components can segregate into this phase where they can modulate spindle assembly, dynamics, and shape.

Acknowledgments

I thank all the present and past members of the Hyman lab, and my colleagues at the MPI-CBG and MPI-PKS. My work was supported by the European Commission's 7th Framework Programme grant Systems Biology of Stem Cells and Reprogramming (HEALTH-F7-2010-242129/SyBoSS).

References

1. Nigg, E. A. & Stearns, T. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. Nature Cell Biology 13, 1154–1160 (2011).
2. Bettencourt-Dias, M., Hildebrandt, F., Pellman, D., Woods, G. & Godinho, S. A. Centrosomes and cilia in human disease. Trends Genet. 27, 307–315 (2011).
3. Moyes, C. D. Controlling muscle mitochondrial content. J. Exp. Biol. 206, 4385–4391 (2003).
4. Wiest, D. L. et al. Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately. J Cell Biol 110, 1501–1511 (1990).
5. Marshall, W. F. Origins of cellular geometry. BMC Biol 9, 57 (2011).
6. Libbrecht, K. G. The physics of snow crystals. Rep. Prog. Phys. 68, 855–895 (2005).
7. Berdahl, A., Torney, C. J., Ioannou, C. C., Faria, J. J. & Couzin, I. D. Emergent sensing of complex environments by mobile animal groups. Science 339, 574–576 (2013).
8. Montorzi, M., Burgos, M. H. & Falchuk, K. H. *Xenopus* laevis embryo development: arrest of epidermal cell differentiation by the chelating agent 1,10-phenanthroline. Mol Reprod Dev 55, 75–82 (2000).
9. Dumont, J. et al. A centriole- and RanGTP-independent spindle assembly pathway in meiosis I of vertebrate oocytes. J Cell Biol 176, 295–305 (2007).
10. Helmke, K. J., Heald, R. & Wilbur, J. D. Interplay between spindle architecture and function. Int Rev Cell Mol Biol 306, 83–125 (2013).
11. Isenberg, I. On the theory of the nematic phase and its possible relation to the mitotic spindle structure. Bulletin of Mathematical Biophysics 16, 83–96 (1954).
12. Gatlin, J. C. et al. Spindle fusion requires dynein-mediated sliding of oppositely oriented microtubules. Curr Biol 19, 287–296 (2009).
13. Dumont, S. & Mitchison, T. J. Compression regulates mitotic spindle length by a mechanochemical switch at the poles. Curr Biol 19, 1086–1095 (2009).
14. Gatlin, J. C. & Bloom, K. Microtubule motors in eukaryotic spindle assembly and maintenance. Semin. Cell Dev. Biol. 21, 248–254 (2010).
15. Itabashi, T. et al. Probing the mechanical architecture of the vertebrate meiotic spindle. Nat Meth 6, 167–172 (2009).
16. Shimamoto, Y., Maeda, Y. T., Ishiwata, S., Libchaber, A. J. & Kapoor, T. M. Insights into the micromechanical properties of the metaphase spindle. Cell 145, 1062–1074 (2011).
17. Reber, S. B. et al. XMAP215 activity sets spindle length by controlling the total mass of spindle microtubules. Nature Cell Biology 9, 1116-22 (2013).
18. Brouhard, G. J. et al. XMAP215 is a processive microtubule polymerase. Cell 132, 79–88 (2008).
19. Gard, D. L. & Kirschner, M. W. A microtubule-associated protein from *Xenopus* eggs that specifically promotes assembly at the plus-end. J Cell Biol 105, 2203–2215 (1987).
20. Wang, P. J. & Huffaker, T. C. Stu2p: A microtubule-binding protein that is an essential component of the yeast spindle pole body. J Cell Biol 139, 1271–1280 (1997).
21. Nakaseko, Y., Goshima, G., Morishita, J. & Yanagida, M. M phase-specific kinetochore proteins in fission yeast: microtubule-associating Dis1 and Mtc1 display rapid separation and segregation during anaphase. Curr Biol 11, 537–549 (2001).
22. Garcia, M. A., Vardy, L., Koonrugsa, N. & Toda, T. Fission yeast ch-TOG/XMAP215 homologue Alp14 connects mitotic spindles with the kinetochore and is a component of the Mad2-dependent spindle checkpoint. EMBO J 20, 3389–3401 (2001).
23. Whittington, A. T. et al. MOR1 is essential for organizing cortical microtubules in plants. Nature 411, 610–613 (2001).
24. Matthews, L. R., Carter, P., Thierry-Mieg, D. & Kemphues, K. ZYG-9, a Caenorhabditis elegans protein required for microtubule organization and function, is a component of meiotic and mitotic spindle poles. J Cell Biol 141, 1159–1168 (1998).
25. Cullen, C. F., Deak, P., Glover, D. M. & Ohkura, H. mini spindles: A gene encoding a conserved microtubule-associated protein required for the integrity of the mitotic spindle in Drosophila. J Cell Biol 146, 1005–1018 (1999).
26. Charrasse, S. et al. The TOGp protein is a new human microtubule-associated protein homologous to the *Xenopus* XMAP215. J Cell Sci 111 (Pt 10), 1371–1383 (1998).
27. Al-Bassam, J., Larsen, N. A., Hyman, A. A. & Harrison, S. C. Crystal structure of a TOG domain: conserved features of XMAP215/Dis1-family TOG domains and implications for tubulin binding. Structure 15, 355–362 (2007).
28. Ovechkina, Y., Wagenbach, M. & Wordeman, L. K-loop insertion restores microtubule depolymerizing activity of a 'neckless' MCAK mutant. J Cell Biol 159, 557–562 (2002).
29. Widlund, P. O. et al. XMAP215 polymerase activity is built by combining multiple tubulin-binding TOG domains and a basic lattice-binding region. Proceedings of the National Academy of Sciences 108, 2741–2746 (2011).
30. Wuehr, M. et al. Evidence for an upper limit to mitotic spindle length. Current Biology 18, 1256–1261 (2008).
31. Budde, P. P., Kumagai, A., Dunphy, W. G. & Heald, R. Regulation of Op18 during spindle assembly in *Xenopus* egg extracts. J Cell Biol 153, 149–158 (2001).
32. Gaetz, J. Dynein/dynactin regulate metaphase spindle length by targeting depolymerizing activities to spindle poles. J Cell Biol 166, 465–471 (2004).
33. Goshima, G. et al. Genes required for mitotic spindle assembly in Drosophila S2 cells. Science 316, 417–421 (2007).
34. Bird, A. W. & Hyman, A. A. Building a spindle of the correct length in human cells requires the interaction between TPX2 and Aurora A. J Cell Biol 182, 289–300 (2008).
35. Brust-Mascher, I., Sommi, P., Cheerambathur, D. K. & Scholey, J. M. Kinesin-5-dependent Poleward Flux and Spindle Length Control in Drosophila Embryo Mitosis. Mol Biol Cell 20, 1749–1762 (2009).
36. Loughlin, R., Wilbur, J. D., McNally, F. J., Nédélec, F. J. & Heald, R. Katanin contributes to interspecies spindle length scaling in *Xenopus*. Cell 147, 1397–1407 (2011).
37. Brangwynne, C. P. et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324, 1729–1732 (2009).
38. Brangwynne, C. P., Mitchison, T. J. & Hyman, A. A. Active liquid-like behavior of nucleoli determines their size and shape in *Xenopus* laevis oocytes. Proc Natl Acad Sci USA 108, 4334–4339 (2011).
39. Decker, M. et al. Limiting amounts of centrosome material set centrosome size in C. elegans embryos. Curr Biol 21, 1259–1267 (2011).
40. Li, P. et al. Phase transitions in the assembly of multivalent signalling proteins. Nature 483, 336–340 (2012).

Simone Reber studied Biology at the University of Heidelberg, from where she also received her PhD. Since then she has been interested in understanding the biochemical and biophysical principles that underlie the self-organization of the mitotic spindle mainly using *Xenopus laevis* as a model system. In 2008, she joined the lab of Tony Hyman at the Max-Planck-Institute for Molecular Cell Biology and Genetics in Dresden. Together with the lab of Frank Jülicher at the Max-Planck-Institute for the Physics of Complex Systems, she started combing theory and experiment to achieve a systems understanding of spindle assembly, organization, and function.