rotation is also involved and whether any other motor proteins play a role. In the developing mouse brain, the SUN–KASH complexes also interact with the kinesin complex as well as dynein [10], but a prominent role of kinesin in neuronal migration has not been clearly demonstrated.

Interkinetic nuclear migration (INM) during vertebrate neurogenesis is currently another active research area where the roles of different motor proteins need to be clarified [17]. During INM, the nucleus moves away from the centrosome during G1 phase and migrates back toward the centrosome during G2 phase after DNA synthesis. Kinesin and dynein have been proposed to drive the nuclear migration processes during G1 and G2 phases, respectively [3], and the KASH protein Syne-2/nesprin-2 has been shown to interact with both kinesin and dynein in the developing mouse brain [10]. In the zebrafish retina, myosinII and dynactin have been suggested to provide the major force for INM [18,19]. Furthermore, Syne-2/nesprin-2 has been shown to connect the nucleus to the flowing actin and thus move the nucleus after wounding in tissue culture cells [20]. Thus, it would be worthwhile to investigate the detailed mechanism of how the microtubules and actin filaments are organized in the neuronal progenitors. Which cytoskeletal component is essential for providing the driving force for nuclear migration during G1 and G2 phase? Do noncentrosomal microtubule arrays exist? Do different combinations of motors, such as dynein, kinesin and myosinII, function together to move the nucleus through cytoplasmic obstacles during INM? How does Syne-2/nesprin-2 coordinate its interactions between dynein, kinesin and actin? These questions could be addressed by combining high-resolution imaging techniques and genetic manipulation, as demonstrated in the new work by Fridolfsson and Starr [5].

References

1. Morris, N.R. (2000). Nuclear migration. From fungi to the mammalian brain. J. Cell Biol. 148, 1097–1101.
2. Wynshaw-Boris, A. (2007). Lissencephaly and LIS1: insights into the molecular mechanisms of neuronal migration and development. Clin. Genet. 72, 296–304.
3. Ayala, R., Shu, T., and Tsai, L.H. (2007). Trekking across the brain: the journey of neuronal migration. Cell 128, 29–43.
4. Starr, D.A., and Fridolfsson, H.N. (2010). Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. Annu. Rev. Cell Dev. Biol. 26, 421–444.
5. Fridolfsson, H.N., and Starr, D.A. (2010). Kinesin-1 and dynein at the nuclear envelope mediate the bidirectional migrations of nuclei. J. Cell Biol. 191, 115–128.
6. Starr, D.A., and Han, M. (2002). Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science 298, 406–409.
7. Zhou, K., and Hanna-Rose, W. (2010). Movers and shakers or anchored: Caenorhabditis elegans nuclei achieve it with KASH/SUN. Dev. Dyn. 239, 1352–1364.
8. Patterson, K., Molofsky, A.B., Robinson, C., Acosta, S., Cater, C., and Fischer, J.A. (2004). The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the Drosophila eye. Mol. Biol. Cell 15, 600–610.
9. Tsujikawa, M., Omori, Y., Biyanwila, J., and Malicki, J. (2007). Mechanism of positioning the cell nucleus in vertebrate photoreceptors. Proc. Natl. Acad. Sci. USA 104, 14819–14824.
10. Zhang, X., Lei, K., Yuan, X., Wu, X., Zhuang, Y., Xu, T., Xu, R., and Han, M. (2009). SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. Neuron 64, 173–187.
11. Malone, C.J., Misner, L., Le Bot, N., Tsai, M.C., Campbell, J.M., Ahringer, J., and White, J.G. (2003). The C. elegans hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. Cell 115, 825–836.
12. Zhou, K., Rolls, M.M., Hall, D.H., Malone, C.J., and Hanna-Rose, W. (2009). A ZYG-12-dynein interaction at the nuclear envelope defines cytoskeletal architecture in the C. elegans gonad. J. Cell Biol. 186, 229–241.
13. Meyerzon, M., Fridolfsson, H.N., Ly, N., McNally, F.J., and Starr, D.A. (2009). UNC-83 is a nuclear-specific cargo adaptor for kinesin-1-mediated nuclear migration. Development 136, 2725–2733.
14. Fridolfsson, H.N., Ly, N., Meyerzon, M., and Starr, D.A. (2010). UNC-83 coordinates kinesin-1 and dynein activities at the nuclear envelope during nuclear migration. Dev. Biol. 338, 237–250.
15. Williams-Masson, E.M., Heid, P.J., Lavin, C.A., and Hardin, J. (1998). The cellular mechanism of epithelial rearrangement during morphogenesis of the Caenorhabditis elegans dorsal hypodermis. Dev. Biol. 204, 263–276.
16. Roux, K.J., Crisp, M.L., Liu, Q., Kim, D., Kozlov, S., Stewart, C.L., and Burke, B. (2009). Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization. Proc. Natl. Acad. Sci. USA 106, 2194–2199.
17. Taverna, E., and Huttner, W.B. (2010). Neural progenitor nuclei IN motion. Neuron 67, 906–914.
18. Norden, C., Young, S., Link, B.A., and Harris, W.A. (2009). Actomyosin is the main driver of interkinetic nuclear migration in the retina. Cell 138, 1195–1208.
19. Del Bene, F., Wehman, A.M., Link, B.A., and Baier, H. (2008). Regulation of neurogenesis by interkinetic nuclear migration through an apical-basal notch gradient. Cell 134, 1055–1065.
20. Luxton, G.W., Gomes, E.R., Folker, E.S., Vintinner, E., and Gundersen, G.G. (2010). Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. Science 329, 956–959.

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Asymmetric Cell Division: A New Way to Divide Unequally

It has long been known that cells can divide unequally by shifting the mitotic spindle to one side. Two recent reports identify an alternative way to generate daughter cells of different sizes.

Christopher D. Higgins
and Bob Goldstein*

All good cell biologists know that the mitotic spindle determines the plane of cytokinesis. Ray Rappaport, the godfather of cytokinesis [1], showed that experimentally moving a spindle could change the site of cytokinesis [2], and cytokinesis can be prevented by removing the spindle from a cell at least a few minutes before the cytokinetic furrow normally forms [3,4]. Recent work has begun to outline a mechanism for the furrow-inducing activity of the mitotic spindle. Astral microtubules and midzone microtubules affect myosin distribution and actin architecture through local RhoA activation and Rac inactivation at the equatorial cortex, where the actin and myosin will form a contractile 'purse string' [5–7]. In nearly all cells, the spatial relationship between the spindle and the actomyosin-rich furrow is consistent with the above causal relationships: the spindle's position predicts accurately where furrowing will occur.
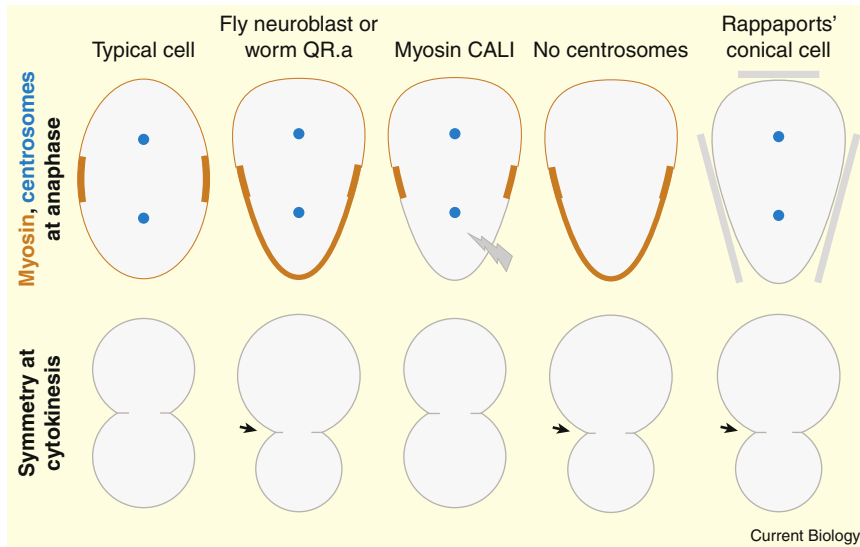
Figure 1. Asymmetric cortical myosin in mitotic cells can position the cytokinetic furrow asymmetrically.

Diagram of myosin and spindle pole (centrosome) positions at anaphase (top), and the resulting cytokinetic furrow position (bottom). Thicker regions of myosin represent cortical regions with myosin enrichment.

However, exceptions exist. In 2000, Kaltschmidt and colleagues [8] reported live imaging of microtubules in *Drosophila* neuroblasts and showed a cell division plane that did not lie midway between the two spindle poles, but instead lay closer to one of the poles, resulting in daughter cells of two different sizes. Now a new report from Cabernard and colleagues [9] provides evidence that the furrow can be positioned independently of the spindle in these neuroblasts, by a mechanism that involves an asymmetric enrichment of cortical myosin in mitotic cells. A second report from Ou and colleagues [10] reports a similar mechanism in another system, a *Caenorhabditis elegans* neuroblast, and tests directly the role of asymmetric myosin enrichment in controlling daughter cell size. The new results challenge the universality of the mitotic spindle as the primary determinant of furrow positioning, establishing an asymmetric cortical enrichment of myosin during mitosis as an alternative means to divide unequally in some cells.

Drosophila neuroblasts divide asymmetrically, producing a larger daughter that retains stem-cell characteristics and a smaller daughter that differentiates. Cabernard and colleagues [9] showed by live imaging of neuroblasts that myosin localized in an unexpected pattern during mitosis,

becoming enriched asymmetrically in the cell cortex on the side where the smaller daughter cell will form (Figure 1). Interestingly, this enrichment was established even before any mitotic spindle asymmetries were apparent, suggesting that the myosin asymmetry was not caused by any observed spindle asymmetries. Indeed, cells with spindles rotated out of their normal axis still had normal myosin enrichment on the basal side of the cell. The rotated spindle and the basal myosin each appeared to induce a furrow — a double furrow! What does it mean? In *Drosophila* neuroblasts, the myosin crescent appears to provide an independent, parallel mechanism for cleavage furrow positioning, along with canonical spindle-derived cues.

Ou and colleagues [10] investigated the asymmetric division of another cell, a *C. elegans* neuroblast. Division of a particular neuroblast, called QR.a, produces daughter cells of different sizes and fates, with the larger daughter becoming a neuron, and the smaller daughter undergoing apoptosis. Despite this asymmetry of size and fate, the mitotic spindle of this cell is aligned in the center at metaphase, just as in *Drosophila* neuroblasts [8,10]. And just as in *Drosophila* neuroblasts, the authors show that myosin becomes enriched asymmetrically in the cortex of one side

of the cell during anaphase, on the side that will form the smaller daughter cell.

Ou *et al.* [10] propose a mechanism for how asymmetric myosin might drive unequal cell division: cortical contractility driven by the myosin crescent could shrink one hemisphere of the dividing cell, driving cytoplasmic flow through the ingressing cleavage furrow and resulting in two differently-sized daughter cells (Figure 2). To test myosin's role in specific regions of the cell, they used chromophore-assisted laser inactivation (CALI), a technique that uses reactive products emitted upon fluorophore excitation to locally inactivate proteins [11–13]. They found that CALI of GFP–myosin in the region where it is enriched could prevent that side of the dividing cell from shrinking normally, leading in some cases to equal cell division (Figure 1), whereas CALI of a control GFP-tagged molecule could not. Interestingly, in some cases in which daughter cell size was affected, cell fate was also affected. The results show that asymmetric enrichment of myosin in mitosis can locally affect the size and the fate of a nascent daughter cell.

With mitotic cells constricted at one end by cortical actomyosin-derived forces, the resulting cell shape resembles one of the classic Rappaport experiments. After his
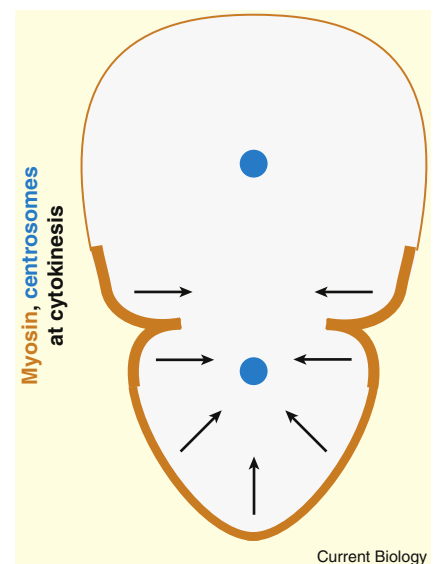

Figure 2. A proposed mechanism for asymmetric furrow positioning.

Model proposing how an asymmetric myosin crescent can affect daughter cell size (after [10]). Arrows represent actomyosin-driven contractions shrinking one end of the cell during cytokinesis.

retirement as a professor, Ray Rappaport and his wife Barbara, both in their 70s at the time, published a paper in which they reported the effect of squeezing mitotic cells into conical shapes [14]. Why squeeze cells into conical shapes? A computer model developed by Albert Harris and Sally Gewalt had predicted that cells of this shape could be used to distinguish between existing models for spindle positioning [15]. Interestingly, the result of changing cell shape was similar to that shown in worm and fly neuroblasts: the furrow formed closer to the narrow end of the cell, instead of midway between the two spindle poles (Figure 1). The authors interpreted this as resulting from a more effective interaction between the spindle and the cortex at the narrow end of the cell, as the cortex in this end of the cell lies closer to the spindle.

The Rappaports' result shows that tapering one end of a cell can result in the furrow forming closer to the spindle pole at that end of the cell. Might the asymmetric myosin observed in worm and fly neuroblasts affect furrow position in this way? Myosin is itself a key furrow component, so an indirect effect of myosin on furrow positioning through cell shape — allowing the spindle and cortex to more effectively interact at one end of the cell — might seem circuitous. Indeed, in fly neuroblasts, Cabernard *et al.* [9] were able to eliminate the spindle altogether by colcemid treatment and then genetically bypass the spindle checkpoint, and they found that the basal myosin enrichment and asymmetric cytokinesis still occurred. This result establishes the new mechanism as a truly independent mechanism, not requiring the mitotic spindle. It will be interesting to learn the extent to which this will stand as an independent mechanism in other systems.

How does myosin localize asymmetrically in mitotic cells? Temporal and spatial mechanisms must be involved. Metaphase-arrested *Drosophila* neuroblasts failed to localize myosin asymmetrically, suggesting that myosin localization must be temporally linked to mitotic progression, like asymmetric spindle positioning in certain cells [9,16]. The authors show that spatial regulation of myosin depends on familiar players, a PAR-1-like kinase called PIG-1 in *C. elegans* neuroblasts, and the asymmetric Pins protein in *Drosophila*, which has well-established roles in spindle positioning [9,10,16–18]. These molecular links are likely to serve as key steps toward dissecting the mechanisms of asymmetric myosin distribution in mitotic cells.

References
1. Canman, J.C., and Wells, W.A. (2004). Rappaport furrows on our minds: the ASCB Cytokinesis Meeting Burlington, VT July 22-25, 2004. J. Cell Biol. *166*, 943–948.
2. Rappaport, R. (1985). Repeated furrow formation from a single mitotic apparatus in cylindrical sand dollar eggs. J. Exp. Zool. *234*, 167–171.
3. Hiramoto, Y. (1956). Cell division without mitotic apparatus in sea urchin eggs. Exp. Cell Res. *11*, 630–636.
4. Rappaport, R. (1981). Cytokinesis - cleavage furrow establishment in cylindrical sand dollar eggs. J. Exp. Zool. *217*, 365–375.
5. Glotzer, M. (2005). The molecular requirements for cytokinesis. Science *307*, 1735–1739.
6. Canman, J.C. (2009). Cytokinetic astralogy. J. Cell Biol. *187*, 757–759.
7. Bement, W.M., Miller, A.L., and von Dassow, G. (2006). Rho GTPase activity zones and transient contractile arrays. Bioessays *28*, 983–993.
8. Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., and Brand, A.H. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. Nat. Cell Biol. *2*, 7–12.
9. Cabernard, C., Prehoda, K.E., and Doe, C.Q. (2010). A spindle-independent cleavage furrow positioning pathway. Nature *467*, 91–94.
10. Ou, G., Stuurman, N., D'Ambrosio, M., and Vale, R.D. (2010). Polarized myosin produces unequal-size daughters during asymmetric cell division. Science *330*, 677–680.
11. Diefenbach, T.J., Latham, V.M., Yimlamai, D., Liu, C.A., Herman, I.M., and Jay, D.G. (2002). Myosin 1c and myosin IIB serve opposing roles in lamellipodial dynamics of the neuronal growth cone. J. Cell Biol. *158*, 1207–1217.
12. Jacobson, K., Rajfur, Z., Vitriol, E., and Hahn, K. (2008). Chromophore-assisted laser inactivation in cell biology. Trends Cell Biol. *18*, 443–450.
13. Wang, F.S., Wolenski, J.S., Cheney, R.E., Mooseker, M.S., and Jay, D.G. (1996). Function of myosin-V in filopodial extension of neuronal growth cones. Science *273*, 660–663.
14. Rappaport, R., and Rappaport, B.N. (1994). Cleavage in conical sand dollar eggs. Dev. Biol. *164*, 258–266.
15. Harris, A.K., and Gewalt, S.L. (1989). Simulation testing of mechanisms for inducing the formation of the contractile ring in cytokinesis. J. Cell Biol. *109*, 2215–2223.
16. McCarthy Campbell, E.K., Werts, A.D., and Goldstein, B. (2009). A cell cycle timer for asymmetric spindle positioning. PLoS Biol. *7*, e1000088.
17. Cordes, S., Frank, C.A., and Garriga, G. (2006). The C. elegans MELK ortholog PIG-1 regulates cell size asymmetry and daughter cell fate in asymmetric neuroblast divisions. Development *133*, 2747–2756.
18. Siller, K.H., and Doe, C.Q. (2009). Spindle orientation during asymmetric cell division. Nat. Cell Biol. *11*, 365–374.

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