CELL BIOLOGY

Forced to Be Unequal

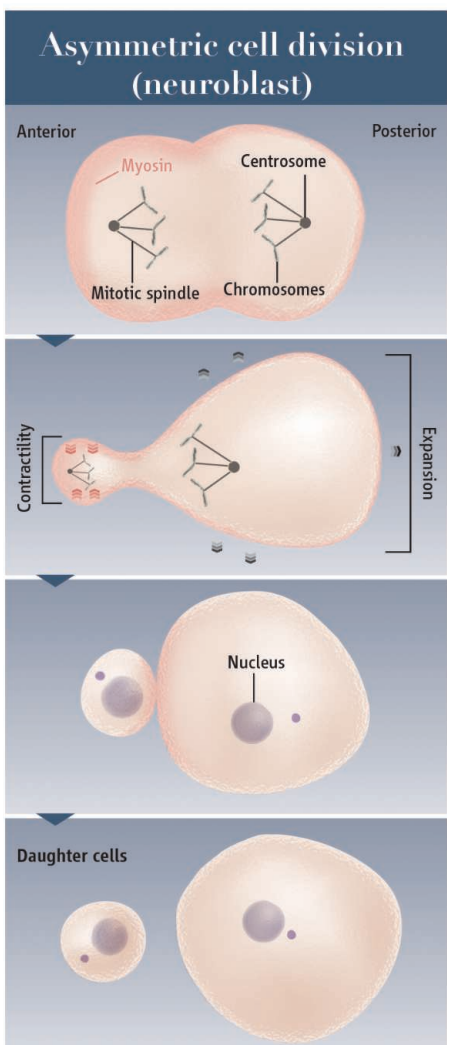
Stephan W. Grill

The distribution of a motor protein generates an unequal contractile force that controls the asymmetric division of eukaryotic cells.

A prominent way for an organism to develop many different cell types is to have cells divide unequally, generating daughter cells that differ in composition and fate, and often also in size. The importance of this process, called asymmetric cell division, for the development of multicellular organisms is undisputed (*1*). On page 677 of this issue, Ou *et al*. (*2*) describe a mechanism for generating unequal-sized daughter cells that is based on contracting one cell half by the action of the cytoskeletal motor protein myosin.

Much of our understanding about asymmetric cell division comes from studying developing embryos of the nematode *Caenorhabditis elegans* and the developing nervous system of the fruit fly *Drosophila melanogaster* (*3*, *4*). Generally, a cell divides along an axis that is determined by the orientation of the mitotic spindle to ensure that this microtubule-based structure properly segregates chromosomes. The position of the spindle can thus determine the size of the resulting two daughter cells. When the mitotic spindle is positioned off center in a dividing cell, the constriction ring (or cleavage furrow) is usually redirected to bisect the eccentrically positioned spindle. This generates daughter cells of unequal size. Lopsided positioning can occur if extra force is exerted from one side of the dividing cell, pulling the spindle toward it (*5*). Other mechanisms that direct the position of the cleavage furrow involve signaling from the pathway that controls cell polarity, as recently observed in *Drosophila* neuroblasts (*6*). This includes a polarized distribution of myosin. Now, Ou *et al*. also report that the uneven distribution of nonmuscle myosin II in directing asymmetric division. Myosins comprise a large family of motor proteins that walk along cytoskeletal actin cables. As the motor protein moves along actin, it slides filaments along each other to generate a contractile force through cycles of ATP hydrolysis and conformational changes.

Ou *et al*. discovered that a cell of the Q neuroblast lineage in *C. elegans* divides asymmetrically because of the polarized activity of cortical myosin. During division, half of the neuroblast accumulates more cortical myosin (compared to the other half) and as a result, contracts due to the forces generated by these motor proteins. Using live cell imaging, the authors observed that in one member of the Q neuroblast lineage (called the QR.a cell), the cleavage furrow does not bisect the mitotic spindle right in its center, but rather in a slightly off-center position in the spindle (but still between the paired chromosomes).



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Putting on the squeeze. Asymmetric cell division occurs by having one (future) daughter cell contract more than the other, a process driven by myosin. Because the cytosol and cellular contents have to go somewhere, contraction of the anterior half of the dividing cell causes the expansion of the posterior half. This results in an unequal division.

This is in addition to ecentric positioning of the spindle within the cell, similar to that seen in *Drosophila* neuroblast asymmetric division. Thus, a spindle-independent mechanism might control the asymmetric division in *C. elegans* neuroblasts.

Myosin is involved in several aspects of asymmetric cell division, most prominently in the establishment and maintenance of cellular polarity (*1*, *7*, *8*). Uneven distribution of myosin has been observed in asymmetric cell division (*6*, *7*, *9*), but only for extremely asymmetric meiotic divisions have they been made responsible for creating unequally sized daughter cells in a direct mechanical sense (*10*). Ou *et al*. observed that myosin is unevenly distributed in neuroblast daughter cells, with more myosin in the furrow region and within the smaller anterior daughter cell (see the figure). The authors propose that by causing the anterior half of the dividing neuroblast to contract more than the posterior half, myosin drives asymmetry in division. Indeed, the membrane of the posterior half of the dividing neuroblast appears to expand outward as the membrane of the anterior half shrinks, which is consistent with mechanical squeezing to drive asymmetry.

Perturbing the distribution and activity of myosin further confirmed that contraction at one end of the dividing cell underlies the asymmetry. This approach included chromophore-assisted laser inactivation (CALI) of myosin II (which was tagged with green fluorescent protein) to locally reduce myosin contractile activity (*11*). CALI of myosin in the anterior half of a dividing neuroblast not only diminished anterior contraction, but it resulted in a more symmetric cell division, which altered the fate of the anterior daughter cell (it avoided programmed cell death). By contrast, CALI of myosin at the posterior half of the dividing cell did not appreciably affect the ratio of daughter cell sizes that is normally observed.

Although much work on asymmetric cell division has focused on understanding how the mitotic spindle is positioned, Ou *et al*. reveal a simple mechanism that is entirely based on the differential regulation of actin-myosin (actomyosin) contractility. It will be interesting to see if asymmetric cell divisions in other cell types also use this mechanism. For example, the asymmetric distribution of

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myosin in *Drosophila* neuroblasts (*6*) might function similarly to that in *C. elegans* neuroblasts, in addition to those mechanisms already known to control asymmetric division in these cells. It will also be important to examine the precise relationship between the mitotic spindle and actomyosin network (*12*, *13*) in the neuroblast (QR.a) cells. It may be that spindle-dependent mechanisms are acting in addition to the mechanical squeezing. Might biophysical measurements confirm that actomyosin-imparted cortical tension is different in the two halves of the dividing cell (*10*)? One might also expect a flow of cytosol through the constriction zone that accompanies the shrinking of the anterior daughter cell, and this flow could affect the position of the mitotic spindle. Understanding the biophysical implications of the findings in conjunction with the cell biological observations of asymmetric cell division will be an intriguing next step in understanding what drives this process.

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STEM CELLS

Epigenome Disruptors

What can stem cells tell us about epigenetic perturbations?

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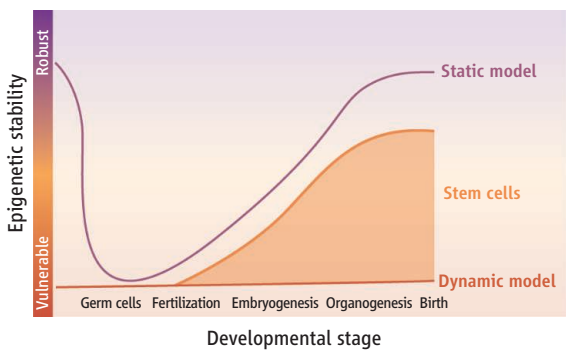
The "epigenome" comprises a range of modifications that are imposed on the genome (DNA) and ensure the stable transmission of gene expression patterns without changes to the DNA sequence. "Epigenetic disruptors" could change gene activity and in the case of stem cells, alter cell fate or number, causing, for example, an increased risk of cancer (*1*). "Epimutations" arising in this way may even pass through the germ line to the gametes, thereby affecting subsequent generations. Due to their inherent developmental plasticity, stem cells may be an ideal reporter system for epigenetic perturbations. This could be achieved by studying loci (such as imprinted genes) that undergo epigenetic alterations in normal development, and monitoring their response to potentially disruptive agents. Are such screens feasible, and would they provide a systematic and reproducible readout?



Epigenetic stability during development. In the conventional, static model, epigenetic stability is proportional to the amount of DNA methylation and histone modifications. In the dynamic model, the steady turnover of epigenetic modifications makes the epigenome persistently vulnerable. The contribution of stem cells to an organism's overall epigenetic vulnerability diminishes progressively with development as their proportion in tissues decreases. Vulnerability of individual stem cells could approach either the static or dynamic levels, depending on the actual state of their epigenome.

Early mammalian development (from fertilization) is characterized by progressive restriction of cellular plasticity and is accompanied by the acquisition of epigenetic modifications (such as DNA methylation). These impose a cellular memory that accompanies and enables stable differentiation. The unspecialized cells of the early (preimplantation) mouse embryo can give rise to "pluripotent" embryonic stem cells that exhibit the widest developmental potency and can colonize all tissues when combined with a mouse embryo to form a chimera. Epiblast stem cells derived from the postimplantation mouse embryo are also pluripotent but contribute poorly if at all to chimeras (*2*, *3*). Stem cells with progressively declining developmental plasticity can be derived from later embryonic stages or even the adult (such as neural and hematopoietic stem cells). In normal development, the epigenetically imposed restrictions to cellular plasticity are erased only in the germ line, where profound epigenetic reprogramming events lead to the formation of a new set of gametes (see the figure). However, fully differentiated cell types can be experimentally reprogrammed into induced pluripotent stem (iPS) cells by the temporary overexpression of key pluripotency factors. Human iPS cells hold therapeutic promise, as they are readily accessible from any individual (*4*).

Intense investigations of mouse and human pluripotent stem cells have established genome-wide profiles of DNA methylation, histone modifications, and DNA occupancy patterns of important chromatin-modifying enzymes (*5*). These analyses reveal fundamental epigenetic principles of pluripotency including hypomethylation of many gene promoters (versus hypermethylation in differentiated tissues) and a characteristic "bivalent" pattern of histone modifications that poise genes for activation at later stages.

However, pluripotency is inherently labile, and embryonic stem cells are epigenetically heterogeneous and dynamic. Perhaps as a consequence, they are prone to undergo epigenetic alterations during their derivation from early mouse embryos and in subsequent cell culture. Analysis of DNA methylation patterns reveals that they undergo extensive culture-induced alterations that persist throughout embryonic stem cell differentiation (*6*, *7*). DNA methylation is a key mediator of genomic imprinting, and perturbed expression of imprinted genes frequently accompanies epigenetic perturbations involving DNA methylation in mouse embryonic stem cells. However, human

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