

Cell division: Biochemically controlled mechanics

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Technical advances are providing new insights into the mechanical properties of cells. Now, by combining genetic and biochemical studies with high-resolution mechanical measurements from atomic force microscopy, the biochemical bases of mechanical processes such as cytokinesis should be discernible.

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One of the ultimate challenges for understanding cellular morphogenesis is to identify the mechanical parameters that produce a morphogenic event and to uncover the biochemical events that produce and control the required mechanics. The extension of a pseudopod and neuronal growth cone, and the transition from a squamous to columnar epithelium, are examples of such morphogenic events. Cytokinesis is an extraordinary example of a morphogenic process, because during normal mitotic divisions a mother cell reshapes itself and then cleaves into two equal-sized daughter cells. Cytokinesis is an appealing model for studies on cellular morphogenesis because of the elegant orchestration of nearly the entire cytoskeletal system and its relatively simple geometry.

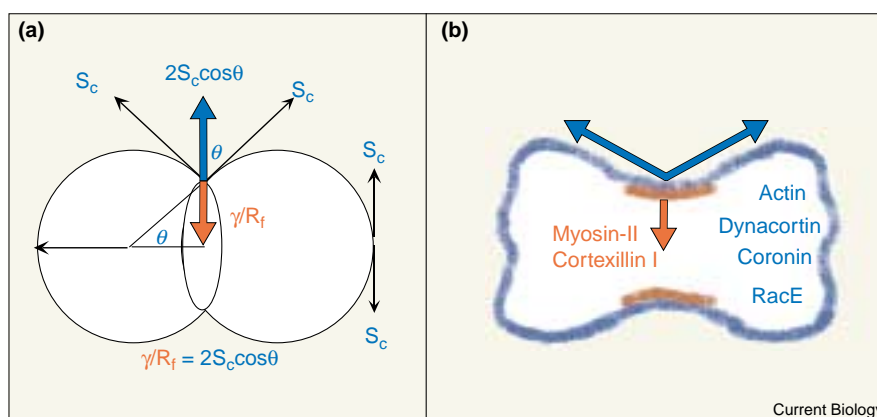
Two recent studies [1,2] have provided new data for understanding the contraction of the cleavage furrow during cytokinesis. Matzke *et al.* [1] have made the first direct high-resolution mechanical measurements of the cortex using atomic force microscopy (AFM), and shown that the cleavage furrow progressively increases its apparent stiffness as the furrow contracts. In contrast, O'Connell *et al.* [2] applied cytochalasin D to partially disrupt the actin cytoskeleton in a localized manner and thereby revealed that disruption of the global or equatorial cortex has unique and surprising effects on contraction of the cleavage furrow.

It has long been appreciated that the cleavage furrow generates force [3]. By inserting glass needles in the region of the cleavage furrow of echinoderm eggs, Rappaport showed that the furrow bent the needle and produced on the order of 20 nN of force. The molecular basis of this force generation was suggested when it was discovered that myosin-II localizes to the cleavage furrow (reviewed in [4]). Indeed, a nearly ubiquitous role for myosin-II has been revealed by genetic and immunocytochemical studies in a variety of eukaryotic cells, from yeasts and amoebae to metazoan cells (reviewed in [5]).

One of the major questions in cytokinesis is how the contractile mechanism works, particularly in the context of the whole cell. Yoneda and Dan [6] suggested a simple relationship based on Hooke's Law, which says that the amount of contractile force required to cleave a cell is proportional to the global stiffness or surface tension of the cortex (Figure 1a). This suggests that cell division is at

Figure 1

(a) A cartoon of a dividing cell (adapted from [6]). The force vectors (arrows) indicate the interaction between the equatorial contractile mechanism and the global stiffness. This simplistic mathematical relationship derived from Hooke's Law was first applied to a dividing echinoderm egg, and depicts the notion that the amount of required contractile force (γ) is proportional to the global stiffness (S_c) or surface tension of the cell. It also depends partly on the furrow radius (R_f), which reflects the extent to which the cell has constricted. While it is an oversimplification, this model helps to relate the role of the contractile ring to overcome the global cortex during division. (b) A cartoon depicting a dividing *Dictyostellium discoideum* cell [18]. The force vectors reflect the global stiffness or surface tension (blue arrows) and the equatorial



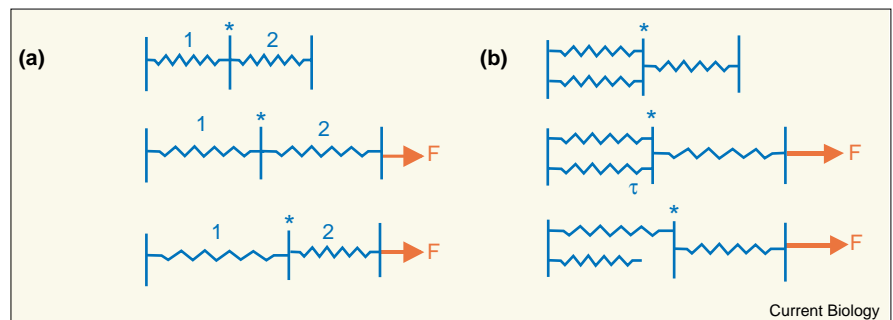
contractility (red arrow). The proteins that are thought to contribute to global shape control

and equatorial contractility are color-coded to match the appropriate vector.

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Figure 2

(a) A schematic of two springs attached in series. If force (F) is applied, both springs will stretch in a manner proportional to each spring's stiffness, allowing movement of the reference point (*). If the stiffness of one spring (spring 2) is increased, it will resist deformation even more, resulting in a greater displacement of the reference point (*). (b) A schematic of the three springs connected, one in series with a parallel pair. If a force (F) is applied, it can stretch the springs. However, if the anchors have a characteristic half-life (τ), then release of one spring can allow the other parallel spring to advance a greater distance. The asterisk (*) marks a reference site to demarcate the advancement as one anchor releases. These schemes can serve as a simple one-dimensional model for envisioning



how a cell is remodeled during morphogenesis. In the cleavage furrow, force (F) could be generated, at least in part, by myosin-II. The springs can represent the actin

filaments and their cross-linkers. By modulating the stiffness and crosslinking, the cortex can be deformed in specific ways producing the desired shape changes.

least a two-component process in which the contractile force antagonizes or works against the global stiffness to cleave the cell. This relationship provides a framework for relating the biochemistry of cell division to the potential mechanics of the process.

To formulate a view of global stiffness, the use of the word 'stiffness' — force/displacement — must be defined. Stiffness describes a mechanical parameter of a particular object made up of a particular material. It is proportional to the general material property called the modulus of elasticity (Young's Modulus) and depends on the object's particular geometry. The stiffness of the cell cortex reflects the cytoskeletal density, the degree of cross-linking between the cytoskeletal filaments and between the filaments and the membrane, the half-lives of these cross-links and the forces that are applied to it. The stiffness will have a persistent nature that reflects the half-lives of the cross-links; this contributes to the viscous quality of the cell. For example, the cell cortex might be thought of as a shell of interlocking springs, where the spring anchors have a typical half-life (Figure 2). For an elegant treatment of these physical principles, see the recent textbook by Howard [7].

Matzke *et al.* [1] used AFM to measure the modulus of elasticity of dividing mammalian cell culture lines. The amount of force required to cause an indentation of a particular size is calculated by considering the stiffness of the cantilever; by considering other material factors, the modulus of elasticity is calculated. Matzke *et al.* [1] found that, during cell division, the stiffness of the entire (global) cortex increased in metaphase relative to the interphase level. The global cortex stiffness continued to increase until just before the onset of furrowing. During anaphase, the equatorial region began to show a two-fold increase in stiffness above the background global stiffness. The

equatorial region that showed the increased stiffness was restricted spatially to the central approximately 20–30% of the pole-to-pole cell length. This equatorial rise continued until late anaphase, when the first sign of furrowing was detected. As the furrow constricted, the level of stiffness in the furrow region continued to rise up to 10-fold above the level of the global cortex during metaphase. Two trends should be noted. First, the overall global cortical stiffness rises before any regional increases become apparent. Second, the stiffness of the equator rises ahead of the appearance of the furrow, and the equatorial stiffness continues to increase until the end of cytokinesis.

An interesting aspect of these observations is that the equator shows a regional increase in stiffness. If the cell were a purely elastic body, then an increase in contractility would be expected to dissipate across the whole cortex, leading to an average rise in total stiffness. This viscous nature of the cortex may help compartmentalize the cortex so that the deformation by the contractile ring can act locally. This can help to ensure that the cleavage furrow is focused and can allow for other cortical events such as polar ruffling to proceed. The persistence of stiffness may also be required to allow surface protrusions such as pseudopodia to form. Indeed, the stiffness of the leading edge of a mammalian cell is about 60% lower than that of the trailing edge [8].

In molecular terms, the increase in stiffness of the cleavage furrow is likely due to the composite effect of myosin-II contractility, actin cross-linking and the increasing density of the cleavage-furrow cortical cytoskeleton as a result of the decrease in cleavage-furrow volume as the furrow contracts. In *Dictyostelium discoideum*, myosin-II and the actin cross-linking protein cortexillin appear to collaboratively define a midzone of contractility [9]. Both proteins

contribute to the global cortical stiffness [10,11] and during cytokinesis both become enriched in the cleavage furrow [12,13]. Mutant cells that are devoid of either of these proteins fail to have a focused equatorial contractile zone, so that the region of contractility widens and frequently slips out of the midzone. This suggests that the two proteins modulate the formation of a regional stiffness; cortexillin may increase the actin cross-linking and viscosity, while myosin-II generates contractile force.

The idea for a persistent stiffness that is defined by the viscosity of the cortex is also suggested by the pharmacological perturbation experiments of O'Connell *et al.* [2]. They found that partial disruption of the cleavage furrow cytoskeleton by localized application of cytochalasin D, which can disrupt actin filaments, actually led to an increase in the rate of furrow progression. In contrast, application of jasplakinolide, which stabilizes actin filaments, inhibited furrow progression. The increased rate of furrowing caused by cytochalasin D treatment is likely due to a reduction in the viscosity of the cytoplasm and cortex, as a result of a lowering of the density of actin filaments and hence of the cross-linked actin filament meshwork.

Direct measurements of the effects of cytochalasins on cytoplasmic viscosity and cortical stiffness have been made on other cell types and have revealed that both properties are affected [14]. Significantly, many of the actin filaments remain in the region after cytochalasin treatment, so there is still a filament network upon which the contractile apparatus can act. A role for actin disassembly is verified by the properties of cells from *Drosophila twinstar* mutants, which are devoid of the actin severing protein, cofilin [15]. These mutant cells contract their cleavage furrows, but cleavage fails because actin filaments accumulate in the furrow.

From work on *D. discoideum*, a model of global versus equatorial pathways has been suggested because proteins enriched at the equator and the global cortex are both required for cytokinesis (Figure 1b). The equatorially enriched proteins cortexillin I and myosin-II have been mentioned above. The globally distributed proteins include the small GTPase RacE [16] and the actin cross-linking protein coronin [17]. A genetic interaction between another global cortical protein, dynacortin, and equatorial cortexillin I also points towards a two-component genetic system for cytokinesis [18].

The *racE* mutant *D. discoideum* cells have an 80% reduction in cortical stiffness compared to parental control cells, and form blebs along the lateral membrane during division [19]. This failure of cortical integrity leads to failure of the contractile ring to progress. Interestingly, coronin and dynacortin are depleted from the lateral cortex of *racE* mutant cells, suggesting that the reduction of cortical stiffness is

due to a failure of cortical component assembly [18]. If the cytoplasm is an incompressible fluid and the volume of the cell is conserved during division, then the integrity of even the polar cortex may be necessary to resist the mechanical stresses of division. For example, a normal density of cross-links within the cortical cytoskeleton, and between the cytoskeleton and the plasma membrane, may prevent the plasma membrane from forming blebs as the cell divides. An additional feature of conservation of volume is that, as a cell divides, the surface area to volume ratio increases so that new cortex must be constructed, and *racE* mutant cells may have difficulty assembling the new cortex.

Another interesting result from O'Connell *et al.* [2] is that cytochalasin D treatment of the polar cortex of mammalian NRK cells leads to a failure of cytokinesis. This effect is opposite of what was observed with the equatorial treatment. It is possible that the cells fail because of a defect in global cortical integrity, similar to that resulting from loss of RacE in *D. discoideum*, or the cytochalasin D treatment may disrupt the formation of new actin filaments which are constructed in the polar cortex and then travel to the cleavage furrow [20]. In either case, this observation again points towards a two-component — global *versus* equatorial — system for the control of cytokinesis.

Future directions

To understand even the 'simple' shape changes of cytokinesis, the biochemical activities and the mechanical properties of dividing cells need to be completely defined. The cellular distribution of each biochemical factor will need to be quantified in space and time, so that its contributions to the process may be evaluated. Comparisons of the mechanical properties of wild-type cells, genetically modified cells and pharmacologically treated cells, determined using technology like AFM, will provide essential insights into the contribution of each factor to the process. Finally, rigorous mathematical modeling will help to relate the biophysical properties of cytoskeletal proteins such as the force-generating myosin-II to the three-dimensional cell undergoing these shape changes over time.

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