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14. I gratefully acknowledge support from the NSF (award 0547394).

CELL BIOLOGY

Sizing Up the Cell

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Control over cell division depends on coordinately functioning sensors of cell size and age.

The coordination of cell growth and division is responsible for fundamental characteristics of cells such as their size: Fast growth with slow division makes big cells, whereas slow growth with fast division makes small cells. Yet despite decades of effort, the kinetics of cell growth and its influence on cell division have remained elusive topics, at least for animal cells. Is cell growth linear (constant) or exponential (proportional to cell size)? Does cell division occur after cells have grown beyond a minimum size, or is there rather some "age of consent" for division, or both? A report by Tzur *et al.* on page 167 of this issue (*1*) combines a new experimental method with careful mathematical analysis to answer these questions for cultured mammalian lymphoblasts.

In principle, one could measure size-dependent growth rates by following individual cells through time, but current technology does not allow this on a large scale. Instead, Tzur *et al.* used a method originally proposed by Collins and Richmond (*2*) to infer growth rates from the distribution of cell sizes in a population of asynchronously dividing cells. At steady state, the size distribution of cells in a population is constant because growth (which increases cell size) is balanced by division (which produces small cells). Collins and Richmond derived a relation that equates the size-dependent growth rate to the distribution of all cell sizes and the size distributions of dividing and newborn cells. Intuitively, if one observes very few cells with a volume of, say, 1 picoliter, this might be because (i) cells of this size grow very quickly (and spend very little time at this volume), (ii) most newborn cells are larger than this, or (iii) most cells divide before reaching this size. By having the three size distributions in the Collins-Richmond relation, the effects of (ii) and
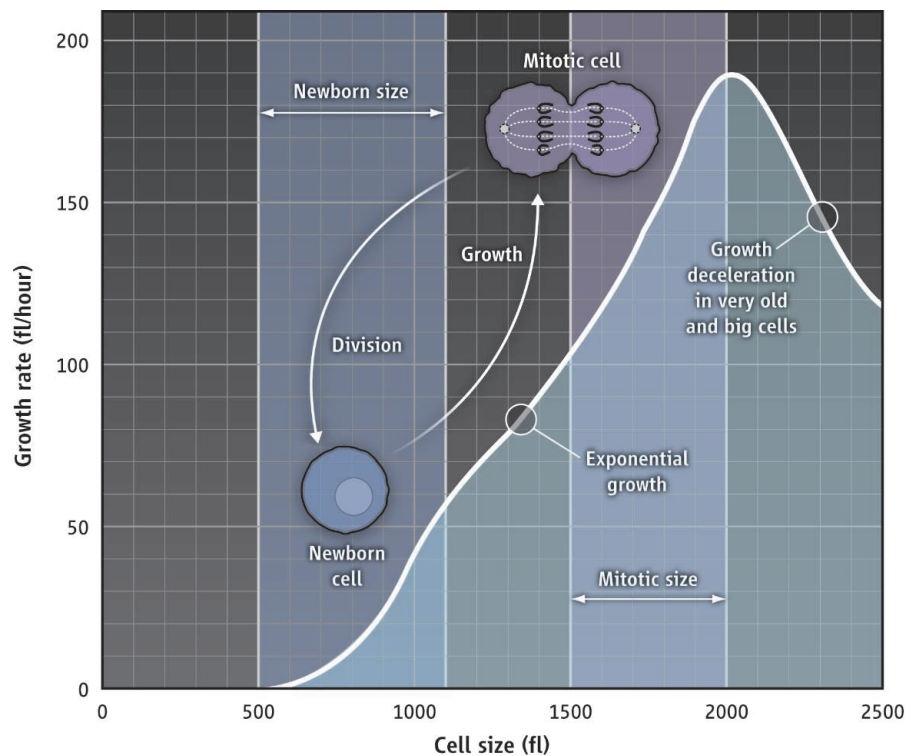
(iii) can be estimated, and one can infer the cell growth rate for cells of any size.

The most difficult aspect of applying the Collins-Richmond relation is measuring the sizes of newborn and dividing cells. To do this, Tzur *et al.* grew the lymphoblasts loosely attached to a membrane, such that after division, one of the daughter cells was released from the membrane for sizing. The size distribution of dividing cells was inferred under the assumption that the volume of the cell just before division was the same as the total volume of the two resulting newborns. Plugging their cell volume distributions into the Collins-Richmond equation revealed that lymphoblast growth is exponential after early G_1 phase of the cell division cycle (the phase

just prior to DNA replication), followed by a growth deceleration for the very largest cells (see the figure).

The finding that growth is exponential during most of the cell cycle suggests that lymphoblasts must have a robust mechanism to maintain their size during each cycle. Otherwise, small differences in the size of cells at division would be amplified each generation. The conclusions of Tzur *et al.* differ sharply from an earlier study of rat Schwann cells (*3*), which concluded that those cells grew rather linearly and probably had no special mechanism for maintaining a constant size. For Schwann cells, size at division depended on the relative concentrations of a circulating growth factor (insulin-like growth factor) that affected size but not division, and a mitogen (glial growth factor) that promoted both. Yet the two studies are difficult to compare because the cells and methods are very different, leaving the meaning of the discrepancy unclear (*4*). Regardless, the implication of a cell-sizing mechanism in at least one animal cell type offers the opportunity to pinpoint the underlying mechanism.

Tzur *et al.* were also able to track the sizes of a synchronized population of newborn cells (just released from the membrane) over several cycles, something rarely done with animal cells. They found that for cells of equal age, the larger is more likely to divide. Similarly, for two cells of equal size, the older cell is more likely to divide. This implies that

Grow and divide. Animal cell growth is exponential during most of the cell division cycle; fl, femtoliters.

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lymphoblast division is regulated by both cell size and age—that both a "sizer" and a "timer" are involved.

Numerous molecular mechanisms that might constitute cell sizers or timers have been proposed. Perhaps the best-informed ideas come from studies of yeasts, where the kinetics of cell growth and division and the molecules controlling them are relatively well understood. A bona fide sizer was recently proposed for *Schizosaccharomyces pombe*, a fission yeast that grows as a rod and divides at a constant length (*5*). In this case, a factor (Pom1) tethered at the cell ends was proposed to inhibit activators of division in the cell's center. As the cell elongates, it eventually passes a critical length at which Pom1's influence no longer reaches to the cell's center, triggering division. This is an attractive mechanism for a rod-shaped cell but seems unlikely to apply generally, especially for cells with different shapes. In the budding yeast *Saccharomyces cerevisiae*, and probably also in many mammalian cells, a growth or size threshold must be surpassed to initiate S phase of the cell cycle (when DNA replication occurs), and division into two daughter cells (mitosis, or M phase) follows automatically in a stereotyped sequence of events suggestive of a timer. In these cases, the various sizer and timer mechanisms proposed are potentially quite general.

One popular model invokes the rate of production of a limiting cell cycle activator as a critical parameter. This activator is envisioned to be produced at a constant rate per unit of cytoplasm and progressively concentrated in an organelle of fixed volume (e.g., the nucleus) or on target binding sites of fixed number (e.g., chromosomes). As the cell grows and the activator accumulates at its targets, it eventually reaches a threshold necessary to trigger cell cycle progression (normally, $G_1 \rightarrow$ S phase progression). With this arrangement, the sizer senses a metabolic index rather than size, and so a cell's size at division will be affected by its nutrient status and growth factor milieu, as well as the status of genes involved in cell growth and metabolism. Indeed, this is the rule from yeast to human cells (*6*).

There are many cell cycle activators that could fill the role of a limiting regulator in such a system. Likely candidates include the G_1 cyclins and cyclin-dependent kinases that promote S phase, origin-licensing factors (such as Cdc6 and Cdt1), and transcription factors that activate cell cycle genes (such as E2F). If the size-sensing cell cycle activator were stable and accumulated in one cell cycle phase (e.g., G_1) but was periodically degraded in another (e.g., S or at the M $\rightarrow$ G_1 phase transition), as many of these factors are, then the sizing mechanism could also function effectively as a timer. Cell cycle suppressors might also act as size sensors if they were sequestered or degraded in a growing compartment, or as timers if their activity were periodically gated. Indeed, many of the core cell cycle regulators are able to affect cell size in dose dependency experiments, and so it has been expected for many years that some of these must function naturally as size sensors and timers. Precisely which factor fills this role in any particular biological context still needs to be determined, but given the marvelous diversity of cell types, stereotypical sizes, and proliferation styles found in nature, we are likely to find many different flavors of cell sizers and timers.

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MATERIALS SCIENCE

Oriented Assembly of Metamaterials

The creation of complex materials may be aided by advanced colloidal assembly methods involving anisotropically shaped particles.

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Regular assemblies of colloidal particles have many potential uses from self-assembled electronics to biosensors. Recent advances in particle self-assembly suggest that such assemblies may also provide a simple route to metamaterials at infrared and visible length scales. Such metamaterials may, for example, be used to create cloaking devices or light-based circuits based on manipulations of local optical electric fields rather than on the flow of electrons (*1*).

Metamaterials are periodically structured composites with unit cells smaller than the wavelength used to interrogate them (*2*). By tailoring the unit cells to create a wide range of responses, technologies that were once the realm of fantasy—from computing with light to invisibility cloaks and superlenses—become reality. Such materials are relatively easy to create for use at radio frequencies, where the subunits need only be a few millimeters in size. However, use at optical and infrared wavelengths requires the assembly of three-dimensional micrometer- and nanometer-scale structures. This task is extremely challenging, but recent studies of particle self-assembly point the way to metamaterials at relevant length scales.

Metamaterials contain inclusions deliberately embedded in host media. The size, shape, and electromagnetic properties of the inclusions, along with inclusion density, arrangement, and alignment, determine their effective properties in a given host. Negative index of refraction metamaterials have been demonstrated in the optical range, made by serial approaches based on lithography (*3*, *4*). If, instead, metamaterials were designed to incorporate anisotropic nanoparticles through self-assembly, cumbersome lithographic approaches could be avoided.

An extensive library of anisotropic microparticles and nanoparticles now exists (*5*), allowing inclusion size and shape to be readily selected. Particles can be synthesized from many different materials, which can be selected for their electromagnetic properties. However, incorporating the particles as inclusions in self-assembled metamaterials requires techniques for assembly with control over particle orientation and spatial arrangement in periodic structures.

Convective assembly is a promising technique for creating close-packed assemblies of particles. The method is easy, inexpensive, and amenable to creating relatively large, defect-free periodically ordered structures of spherical particles. New assembly methods are also being developed to promote oriented assembly of anisotropically shaped particles in close-packed structures and to control deposition of particles in prescribed spatial locations on substrates, with potential for creating non–close-packed structures. We review

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