Trends in Genetics

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Spotlight

A Fundamental Unit of Cell Size in Bacteria

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A new study clarifies a relationship between growth, gene expression, and cell size in cyanobacteria. Quite unexpectedly, cyanobacteria and *Escherichia coli* appear to share an invariance principle to coordinate growth and chromosome replication. This principle allows quantitative predictions of cell size across a range of growth conditions in both organisms.

Physics has a long history of discovering invariance principles that are intimately connected to conservation laws. In classical physics, examples of such laws include conservation of energy, momentum, electric charge, and mass. These laws are important because they help us understand the inner workings of physical systems so that we can predict their behavior. One may say that our ability to predict directly reflects our understanding of the system.

At first glance, biology seems different. The power of mathematical representations of physical laws appears to stem from the fundamental simplicity of physical interactions. However, every measurement in biology involves a huge underlying complexity of molecular detail. Yet, the search for mathematical regularities in biological data has been surprisingly fruitful, because, in part, reducing a large data set to a simple mathematical rule sharpens our thinking. It compels us to ask for an explanation of the formula, and it draws our attention to anomalous mutants or conditions that break the mathematical rule.

In a recent study, Zheng and O'Shea took an elegant, minimalist approach to understand the relationship between gene expression, chromosome copy number, and cell size in cyanobacteria [1]. They expressed yellow fluorescent proteins from a constitutive promoter as a readout of global regulation of protein levels, simultaneously measuring the chromosome copy number and cell size using microscopy.

They noticed that the protein concentration was constant from cell to cell despite variation in chromosome copy number and, thus, in gene dosage. For a stable protein, the average rate at which the number of protein copies in the cell increases should be proportional to the product of the average transcription rate, the average translation rate, and the gene dosage. Therefore, for the concentration of the protein to remain constant during growth, this total protein synthesis rate should be the same as the rate growth.

Zheng and O'Shea saw a gratifying resolution when they realized that the number of genome copies increased linearly with cell volume in individual cells. Thus, increased gene dosage supports the higher rate of protein production in a longer cell. This is an elegant way to keep protein concentration independent of size, because it means that the cytoplasm of long cells and short cells has approximately the same capacity for transcription and translation and that all copies of the genome are transcriptionally active. This linear relationship is in agreement with previous findings [2,3].

However, there is more to the story. Cyanobacteria are photosynthetic prokaryotes and their growth rate depends on the intensity of illumination. Surprisingly, close examination of Zheng and O'Shea's data reveals that neither the cell size distributions nor the chromosome copy number distributions are affected by the illumination-imposed growth rate in their experimental conditions. Most newborn cells contain, on average, three chromosomes, and double their number by the time they divide, consistent with previous results [2,3]. The average newborn size is independent of the growth rate. Furthermore, previous work suggests that replication initiation is asynchronous and, at any given time, only one of the chromosome copies undergoes DNA replication [2–4]. Taken together, current findings show that growth and the chromosome replication cycle are coupled such that the amount of protein produced during the replication cycle of one chromosome is invariant (Figure 1A).

These results suggest a common principle of cell size control between cyanobacteria and *E. coli*, which was once thought unlikely. One of the major lessons from the studies of *E. coli* physiology can be summarized as the '(nutrient) growth law', which relates cell size to growth rate [5]. Based on this foundational work, later studies showed that the increase in the average cell size is directly proportional to the average number of replication origins present during multifork replication [6,7] (Figure 1B). In fact, the average cell size per replication origin is invariant even when the biosynthetic capacity of the cell is severely perturbed [8]. Therefore, both cyanobacteria and *E. coli* appear to follow the 'general growth law' that cell size is the sum of all invariant 'unit cells', where the number of unit cells is determined by the number of replication origins simultaneously present in the cells (Figure 1B) [8].

An obvious and important biological question is what mechanism underlies the observed invariance of the unit cell in both cyanobacteria and *E. coli*. In *E. coli*, a longstanding idea is based on the accumulation of a fixed critical amount of replication initiators (e.g., DnaA) at the origin. Following initiation, these initiators are thought to be titrated away by binding sites in the newly replicated DNA [8]. Initiator expression is known to be autoregulated so that their concentration is maintained constant independent of cell

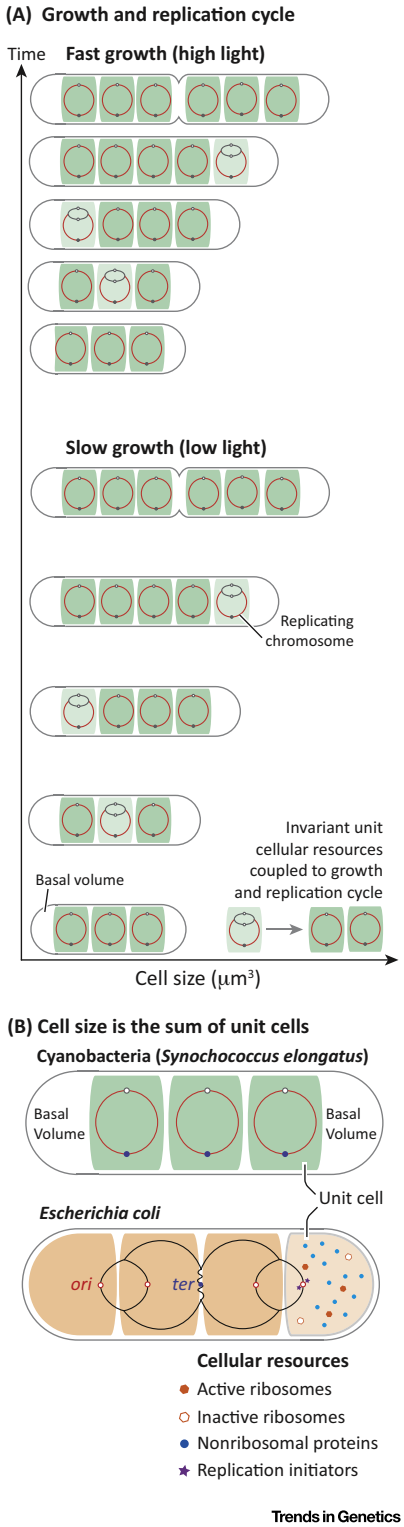

Figure 1. The General Growth Law for Cyanobacteria and E.scherichia coli [1,8]. (A) In cyanobacteria, growth and chromosome replication are coupled so that the amount of protein produced and, thus, cell size added during the replication cycle of one chromosome is invariant regardless of the growth rate. The average cell volume (V) increases linearly with respect to the number (N) of unit volume (V_0) from the basal

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size and growth rate [9]. In principle, the same mechanism could apply to cyanobacteria, so that a fixed amount of initiators accumulate per chromosome cycle, implying a constant increase in cell volume. Highly cooperative binding of initiators [10] might also provide a clue to the mechanism that selects only a single cyanobacterial chromosome copy for replication at a time; once a particular origin is selected stochastically by the binding of a pioneer initiator protein, cooperative interactions might ensure that initiators continue to accumulate predominantly at that site. The invariance of the unit cell under growth inhibition is consistent with the 'initiator threshold' idea [8], and would be a straightforward hypothesis to test in cyanobacteria.

Cyanobacteria such as *Synechococcus elongatus* have a different lifestyle from well-studied bacteria, such as *E. coli*. *S. elongatus* has a rhythmic growth environment controlled by the light–dark cycle, it maintains multiple copies of its chromosomes, which replicate asynchronously. The relationship between chromosome copy number and the initiation of cytokinesis is flexible, depending on both illumination and time of day. There are many questions about the molecular mechanisms in cyanobacteria that underlie these phenomena. Despite these differences, the simple mathematical rules that both *E. coli* and *S. elongatus* appear to follow (the invariance of the unit cell) allow us to predict the cell size of either organism by simply counting the average number of chromosomes in a given condition. This is reminiscent of how physicists can make predictions based on a conservation law without knowing all of the details of a system, made even more remarkable that it applies to bacteria from widely divergent phyla. We believe that it likely points to a fundamental coordination principle of the bacterial cell.

Acknowledgment

We thank David Savage for helpful discussions.

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http://dx.doi.org/10.1016/j.tig.2017.05.001

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volume (V_{basal}), that is, $V = N^* V_0 + V_{basal}$. (B) The general growth law states that cell size is the sum of all invariant unit cells, where the unit cell is the average cell size per replication origins at initiation [8]. Both cyanobacteria and *E. coli* appear to follow this principle, with additional basal volume (V_{basal}) for cyanobacteria (white space in the illustrated cyanobacteria cell). This basal term may reflect specialized structures associated with the cell poles that do not scale with the number of chromosomes