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Growth rate and cell size: a re-examination of the growth law

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Research into the mechanisms regulating bacterial cell size has its origins in a single paper published over 50 years ago. In it Schaechter and colleagues made the observation that the chemical composition and size of a bacterial cell is a function of growth rate, independent of the medium used to achieve that growth rate, a finding that is colloquially referred to as 'the growth law'. Recent findings hint at unforeseen complexity in the growth law, and suggest that nutrients rather than growth rate are the primary arbiter of size. The emerging picture suggests that size is a complex, multifactorial phenomenon mediated through the varied impacts of central carbon metabolism on cell cycle progression and biosynthetic capacity.

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Introduction

Our current understanding of the relationship between growth rate and bacterial cell size has its roots in a single study conducted over 50 years ago. Working in *Salmonella enterica* Typhimurium, Moselio Schaechter, Ole Maaløe and Neils Kjeldgaard observed that cell size and composition varied as a function of growth rate. Employing 22 different media to generate a wide range of mass doubling times, Schaechter and colleagues concluded that not only cell mass, but also nucleic acid and protein content are a function of growth rate rather than the composition of the medium used to achieve that growth rate [1]. Extension of these findings to *Escherichia coli*, a gamma proteobacterium and close relative of *Salmonella*, and *Bacillus subtilis* a Gram-positive bacterium and member of the highly divergent firmicutes, suggested the presence of a conserved mechanism responsible for coordinating cell composition and size with growth rate [2,3]. Significantly, in all three organisms, faster growing cells are up to three times the size of their slower growing counterparts [1,2,4]. Upon a shift to nutrient-rich conditions, *B. subtilis* elongate but maintains their width, while *E. coli* and *S. enterica* increase in both length and width [1,4,5].

Colloquially referred to as 'the growth law,' the striking correlation between growth rate, cell composition and size first observed by Schaechter and colleagues has inspired generations of scientists investigating nutrient-dependent changes in growth rate and cell size. Here we discuss research addressing the molecular basis for the growth law, focusing on the relationship between nutrient availability, growth rate and cell size.

Growth rate, nutrient availability and cellular composition

As Schaechter *et al.* originally observed, the cellular response to changes in growth rate depends upon the manner in which growth is modified. Slowing growth by reducing temperature does not significantly impact the mass or composition of cells cultured in the same medium [see Table 2 in reference [1]]. At the same time, changes in cell size are not always correlated with changes in growth rate. As we explore below, defects in UDP-glucose (UDP-glc) biosynthesis substantially reduce *E. coli* and *B. subtilis* cell size during growth in rich medium, but do not significantly affect growth rate [6••,7••]. Likewise, mutations in the actin-like protein MreB may increase the width of *E. coli* cells without impacting growth [8].

Despite connotations inherent in its name, since the inception of the growth law it has been clear that nutrient availability rather than growth rate is the primary determinant of cell size and composition. Bremer, Dennis and colleagues have not unreasonably suggested that cell composition and size be referred to as 'growth medium-dependent' phenomena, reasoning that growth rate itself is the product of nutrient availability and the ability of the bacterium to utilize those nutrients. It is for good reason then, that the molecular mechanisms underlying the growth law are typically investigated through the comparative analysis of populations of bacteria cultured at steady state in nutrient-rich or nutrient-poor medium, or shifted between the two. The results of these studies have provided a detailed, albeit incomplete, view of how changes in nutrient availability impact bacterial growth and composition [9•,10–15].

The parameter that responds first and most dramatically when cells are shifted from a nutrient-poor to a nutrient-rich medium, is the cellular pool of stable RNA, particularly ribosomal RNA (rRNA). Surges in rRNA synthesis are followed by increases in the number of ribosomes and expression of other components of the translation machinery, which together provide the biosynthetic capacity necessary to support rapid growth [3,16]. In a parallel response pathway, expression of genes encoding the four subunits of acetyl-CoA carboxylase, which catalyzes the first committed step of fatty acid synthesis, increases upon nutrient upshift, providing the raw materials necessary to sustain the rapid expansion of the cell envelope demanded by the new growth rate [17,18].

While the rate of DNA replication reaches maximum velocity in *E. coli* and *B. subtilis* at mass doubling times of ~60 min, the DNA-to-mass ratio remains constant in wild type cells with shorter doubling times, despite their increase in size [5,19]. To compensate for this limitation, cells cultured in nutrient-rich medium employ a phenomenon known as multifork replication, during which they couple the initiation of DNA replication to mass doubling time, even when they are unable to finish new rounds of replication in the allotted period of time. As a consequence, such cells can have as many as 16 replication forks proceeding simultaneously at the fastest growth rates [19,20]. Although the replication initiation protein DnaA has been implicated, the molecular mechanisms responsible for coupling replication to mass doubling time remain elusive [21–23].

(p)ppGpp: a tunable signal coordinating growth and nutrient availability

Nutrient-dependent changes in the rates of RNA, DNA, protein, and lipid synthesis are controlled if not wholly, then in large part through accumulation of guanosine pentaphosphate or tetraphosphate [(p)ppGpp], mediators of the so-called stringent response. In *E. coli*, (p)ppGpp levels are controlled by two enzymes that react to amino acid limitation as well as deficiencies in carbon, nitrogen, phosphorus, iron, or fatty acids: RelA, a (p)ppGpp synthase, and SpoT, a (p)ppGpp hydrolase [24–29]. Accumulation of (p)ppGpp under nutrient-poor conditions triggers a rapid increase in expression of amino acid biosynthesis genes, a decrease in expression of rRNA and tRNA, and inhibits translation initiation factor 2 and elongation factor G [30–33]. Repression of rRNA transcription is achieved through direct interactions between (p)ppGpp, RNAP, and the RNAP-binding protein DksA [34]. Increases in (p)ppGpp levels inhibit lipid synthesis through downregulation of the *fabHDG* operon encoding enzymes necessary for the first steps in fatty acid biosynthesis, and inhibition of PlsB, a glycerol-P acetyltransferase that catalyzes the first step of phospholipid synthesis [35,36].

While the molecule itself is present in a wide range of species, production of (p)ppGpp and its mechanism of action vary [37]. *B. subtilis* in particular controls (p)ppGpp levels through a bifunctional RelA homologue capable of synthesis and hydrolysis, and two small RelA-like synthases (YjbM and YwaC) [37–39]. *B. subtilis* lacks a DksA homologue, instead inhibiting transcription indirectly by controlling GTP levels in the cell [31]. As (p)ppGpp levels rise, the concomitant reduction in GTP reduces transcription from rRNA promoters, which utilize GTP as an initiating nucleotide in *B. subtilis* [40,41].

The nutrient-dependent regulation of cell size

Size is a multifactorial phenomenon controlled through the integration of both cell cycle-dependent and growth rate-dependent signals. Significantly, as we detail below, specific defects in central carbon metabolism can reduce cell size without impacting growth rate, while defects in early steps in fatty acid biosynthesis render cells unable to increase size in response to increases in nutrient availability. In both cases, the link between size and growth rate first observed by Schaechter and colleagues is broken.

UDP-glucose as an intracellular signal for carbon availability

Nutrient-dependent increases in *B. subtilis* and *E. coli* cell size are achieved in part through the integration of central carbon metabolism with cell division, via accumulation of the nucleotide sugar UDP-glc. Generated in two reversible steps from glucose-6-phosphate at the top of glycolysis, UDP-glc serves as a metabolic signal, activating division inhibitors that increase size in response to increases in carbon availability. Defects in UDP-glc biosynthesis reduce the size of *B. subtilis* and *E. coli* cells by ~35% and 25%, respectively, during growth in carbon-rich medium (Figure 1b). Significantly, while cell size is reduced in UDP-glc biosynthetic mutants, growth rate is not impaired indicating that size can be uncoupled from aspects of nutrient-dependent regulation.

In *B. subtilis*, nutrient-dependent changes in UDP-glc levels are sensed by the glucosyltransferase UgtP [6••,42]. In nutrient-poor medium, apo-UgtP favors self-interaction, forming higher order oligomers that sequester it from the cell division machinery at mid-cell. The abundant levels of UDP-glc that are presumably present during growth in nutrient-rich medium inhibit oligomerization, facilitating interaction between UgtP and the highly conserved cell division protein FtsZ. Under these conditions, interactions between UgtP and FtsZ delay assembly of the cytokinetic ring, inhibiting division and increasing cell size (Figure 2) [43].