



Published in final edited form as:

Curr Opin Microbiol. 2015 April ; 24: 96–103. doi:10.1016/j.mib.2015.01.011.

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

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Abstract

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.

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

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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 [9–15].

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 minutes, 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 midcell. 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].

In *E. coli*, an unrelated integral membrane protein, the glucosyltransferase OpgH, transmits information about intracellular levels of UDP-glc to the division machinery [7]. Genetic and biochemical experiments support a model in which UDP-glc binding stimulates a conformational change that reveals a binding site for FtsZ monomers on the cytoplasmic N-terminal domain of OpgH. During growth in nutrient-rich medium, UDP-glc-dependent interactions between the OpgH-N domain and FtsZ at midcell effectively reduce the amount of FtsZ available for assembly, delaying division and increasing cell size (Figure 2).

In a striking example of convergent evolution, UgtP and OpgH are both moonlighting proteins with additional functions in cell envelope biogenesis. UgtP is responsible for transferring glucosyl residues from UDP-glc to diacylglycerol, generating the diglucosyldiacylglycerol anchor for lipoteichoic acid, a major anionic component of the Gram-positive cell wall [6,44]. OpgH is responsible for synthesizing the osmoregulated periplasmic glucan moieties (the OPGs) that are thought to protect Gram-negative cells from osmotic stress [45]. Lipoteichoic acid and OPG synthesis are the largest sources of free diacylglycerol in *B. subtilis* and *E. coli* respectively, raising the intriguing possibility of a connection between cell envelope biogenesis, lipid homeostasis and cell size.

FtsZ, a tubulin homolog that assembles in a concentration-dependent manner to form the scaffold for the cytokinetic ring [46], provides an ideal target for a division inhibitor. Even modest (~20%) reductions in FtsZ levels have a large impact on bacterial size during exponential growth under steady state conditions [22,47]. Given its proximity to glycolysis and central carbon metabolism, UDP-glc activation of UgtP/OpgH thus provides a straightforward means of fine-tuning the pool of FtsZ available for assembly and coordinating cell size with growth rate. (For a more detailed explanation of this model see Chien et al. 2012 [42,43]). While the preponderance of evidence suggests that UgtP and OpgH delay FtsZ assembly upon a shift to nutrient-rich medium, this delay is undoubtedly transient, and is eliminated once cells reach their new, larger size. The timing of FtsZ assembly is identical and precisely coordinated with mass doubling time in both wild type and UDP-glc-deficient cells [6,7].

Analysis of chromosome segregation in UDP-glc-deficient *B. subtilis* and *E. coli* mutants suggests that nutrient-dependent increases in size help cells adapt to the large amounts of DNA generated through multifork replication [6,7]. Deleting *pgcA*, a phosphoglucomutase,

in combination with secondary mutations in *spoIIIE*, a DNA translocase that pumps chromosomal material away from the invaginating septum, leads to a high frequency of “guillotined” chromosomes and anucleate cells during *B. subtilis* growth in nutrient-rich medium [6]. In *E. coli*, loss of phosphoglucomutase correlates with an increase in the frequency of cytokinetic ring assembly over unsegregated chromosomes under conditions supporting rapid growth [7].

Central carbon metabolism as a conduit in the regulatory circuit coordinating size with nutrient availability

Significantly, UDP-glc biosynthesis accounts for only 25–35% of the difference in size between cells grown in nutrient-rich versus nutrient-poor media (Figure 1b), strongly suggesting the existence of additional nutrient-dependent signals and cognate effector proteins. Given the intimate relationship between metabolic flux and nutrient availability, we speculate that a subset of these signals are metabolically derived small molecules. Cognate effector proteins, which could target not just division but also DNA replication, are themselves likely to have roles in metabolism.

In support of this idea, several enzymes involved in central carbon metabolism, including phosphoglyceromutase, enolase and the E1 α subunit of pyruvate dehydrogenase, have been implicated in DNA replication and division site selection, supporting an integral role for central carbon metabolism in coordinating cell cycle progression and cell growth [48–51]. Recent work has extended some of these findings and uncovered additional links between DNA replication control, central carbon metabolism, respiration, protein synthesis and lipid synthesis [51].

Fatty acid biosynthesis: a growth rate-dependent mediator of cell size

Perturbations in fatty acid biosynthesis in *E. coli* point to an additional link between central carbon metabolism and cell size. Disruption of early steps in fatty acid biosynthesis, either through deletion of *fabH*, encoding a component of β -ketoacyl-acyl carrier protein (ACP) synthase III, or by targeting additional β -ketoacyl-ACP synthases with the chemical inhibitor cerulenin, reduced the volume of *E. coli* cells by as much as 70% during growth in nutrient-rich medium [52].

More striking than the reduction in size, is the observation that perturbations in *fabH* uncouple size from growth rate, effectively breaking the growth law. *fabH* mutant size is essentially constant regardless of growth rate (See table 4 in reference [52]). *fabH* mutants cultured in rich medium (Luria broth) or in minimal glucose medium, are approximately the same size, despite a nearly 1.6-fold difference in growth rate [*fabH* mutants exhibit a twofold reduction in growth rate relative to wild type cells under all conditions]. The inability to increase size in response to increases in growth rate contrasts starkly with cells defective in UDP-glc biosynthesis, which retain the ability to increase size in response to increases in nutrient availability, albeit to a lesser degree than their wild type counterparts (Figure 1b) [6,7]. Importantly, slow growth does not necessarily translate into reductions in size. As Yao et al. reported, mutants carrying a defective *rrmJ* allele, encoding a 23S rRNA

methyltransferase, grow slowly but exhibit no significant defects in cell size [52]. The impact of defects in *fabH* on cell cycle progression have yet to be determined.

Size as a function of cell cycle progression and cell expansion

Cell size, at its most basic, is a product of growth and division. At constant growth rates, delays in division translate into increases in cell size. Conversely, maintaining interdivision time but reducing the rate of cell expansion, reduces cell size. Based on this idea and in light of recent empirical data, we propose that cell size is coordinated with growth rate through nutrient-dependent changes in both cell cycle progression and biosynthetic capacity (Figure 3). Under nutrient-rich conditions, accumulation of small molecules generated through central carbon metabolism serve to activate effector proteins that delay cycle progression and division relative to cell expansion, thereby increasing cell size. Under nutrient-poor conditions, low levels of these metabolites permit the cell cycle to proceed unimpeded, reducing cell size. UDP-glc mediated activation of UgtP/OpgH is an example of this type of regulation.

Biosynthetic capacity, itself a product of nutrient availability, modulates size through its impact on cell expansion rates. Under nutrient-rich conditions, peak rates of biosynthesis increase growth rate, transiently outpacing cell cycle progression and leading to an increase in cell size. In contrast, curtailing cell expansion rates through nutrient limitation or other means, reduces size at division in the absence of compensatory changes in cell cycle progression. Importantly, feedback between growth rate and cell cycle progression through the mass-dependent accumulation of cell cycle proteins including FtsZ and the dose-dependent regulator of DNA replication initiation, DnaA, allows cells to maintain size under steady state conditions [53,54].

Recent reports from several laboratories indicate that instead of doubling in size each generation, individual bacteria maintain size under steady state conditions through the addition of constant volume, the value of which increases with growth rate [55–59]. We propose that the value of this “constant volume” is a function of both metabolic signals that alter progression through the cell cycle, particularly at the stage of cell division, and overall biosynthetic capacity. Consistent with this idea, defects in UDP-glc biosynthesis reduce the value of this “constant volume” over a wide range of growth rates.

Conclusion

The linear relationship between size and growth rate first observed by Schaechter, Maaløe and Kjeldgaard over 50 years ago, remains an area of intense investigation. While recent work sheds light on the relationship between cell size, growth rate and nutrient availability, the molecular mechanisms modulating size are far from clear. The actions of the carbon-dependent effectors, UgtP and OpgH, account for only a fraction of the size difference between cells cultured in nutrient-rich and nutrient-poor conditions (Figure 1b). The precise nature of other nutrient-dependent effectors and their cognate metabolic signals remains to be seen. The contribution of (p)ppGpp, a master regulator of many growth rate-dependent phenomena, to cell size is also an open question.

Until recently, our understanding of cell size control has depended on careful observation of the behavior of wild type cells cultured under different nutrient conditions, or the all too rare identification of a cell size defective mutant. The advent of systems level approaches for the analysis of cellular morphology and composition now make it possible to conduct phenotypic analyses on a genomic scale. Such technologies provide a means to identify the entirety of regulatory mechanisms governing cell size and ultimately, determine the molecular basis for the growth law itself.

Acknowledgements

The authors thank, Jenny Chien and Norbert Hill for fluorescence images of *E. coli* and *B. subtilis*, and KC Huang, Suckjoon Jun, Corey Westfall, Heidi Arjes, and other members of the Levin laboratory for insightful comments and discussion. Work in the Levin laboratory is supported in part by Public Health Services grant (GM64671) from the US National Institutes of Health.

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- Bacterial size and chemical composition are thought to be growth rate-dependent
- The correlation between growth rate and size is affected by nutrient availability
- Nutrient-dependent regulators target the cytokinetic machinery
- Disruption of biosynthetic pathways may uncouple size from growth rate
- Cell size is a function of the rates of cell expansion and cell cycle progression

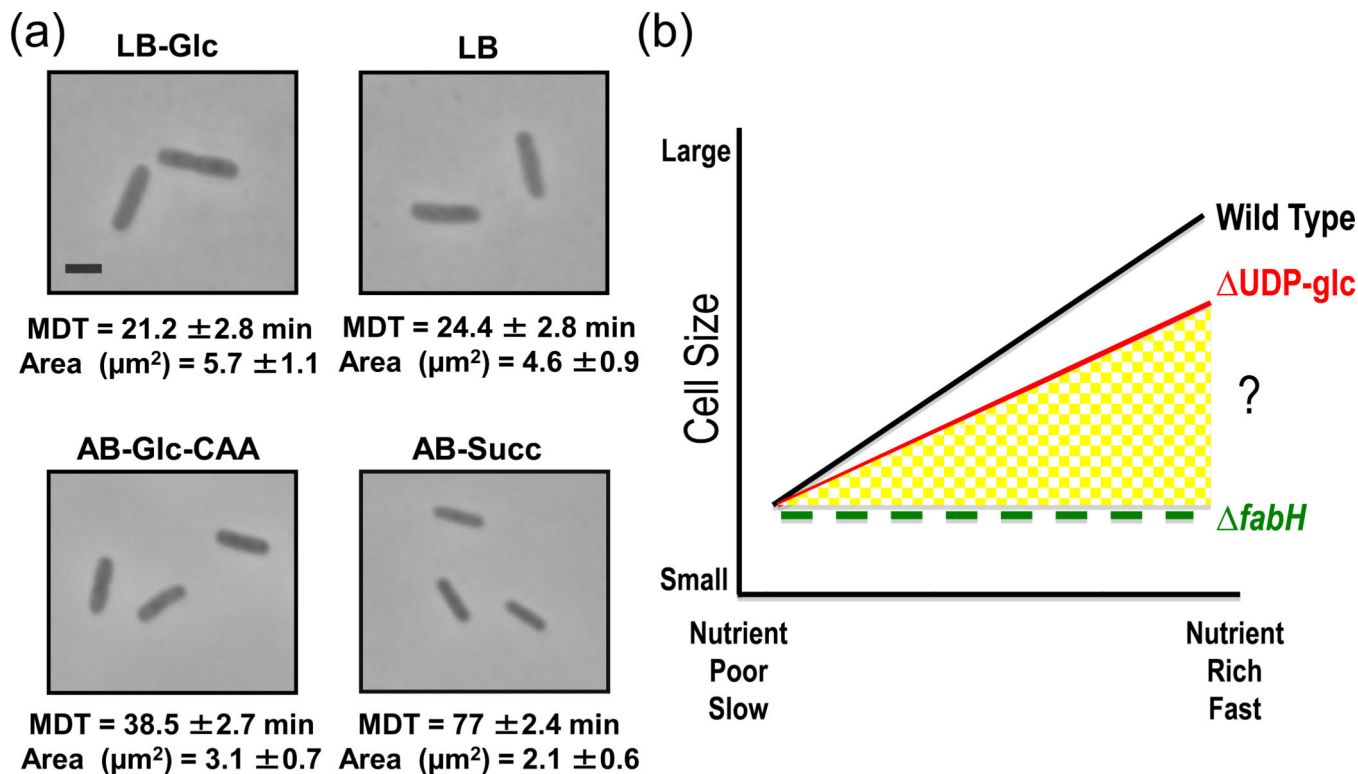


Figure 1. Cell size is a multifactorial phenomenon

(A) Size as a function of nutrient availability. *E. coli* cultured in LB or AB minimal media [60] supplemented with 0.2% glucose (glc), 0.4% succinate (succ), and 0.5% casamino acids (CAA) exhibit a more than twofold difference in surface area. MDT = mass doubling time. Scale bar = 2 μm (B) Graphic depiction of the growth law as it relates to cell size [See Fig. 1 in [1]]. (Black line) Cell size is a linear function of nutrient availability and growth rate. (Red line) UDP-glc mediated division inhibition accounts for ~30% of the increase in size exhibited by bacteria cultured under nutrient rich conditions. (Dashed green line) Defects in the early steps in fatty acid biosynthesis, as in a *fabH* mutant, uncouple size from growth rate. Yellow stipples represent the contribution of unknown factors to nutrient-dependent increases in cell size.

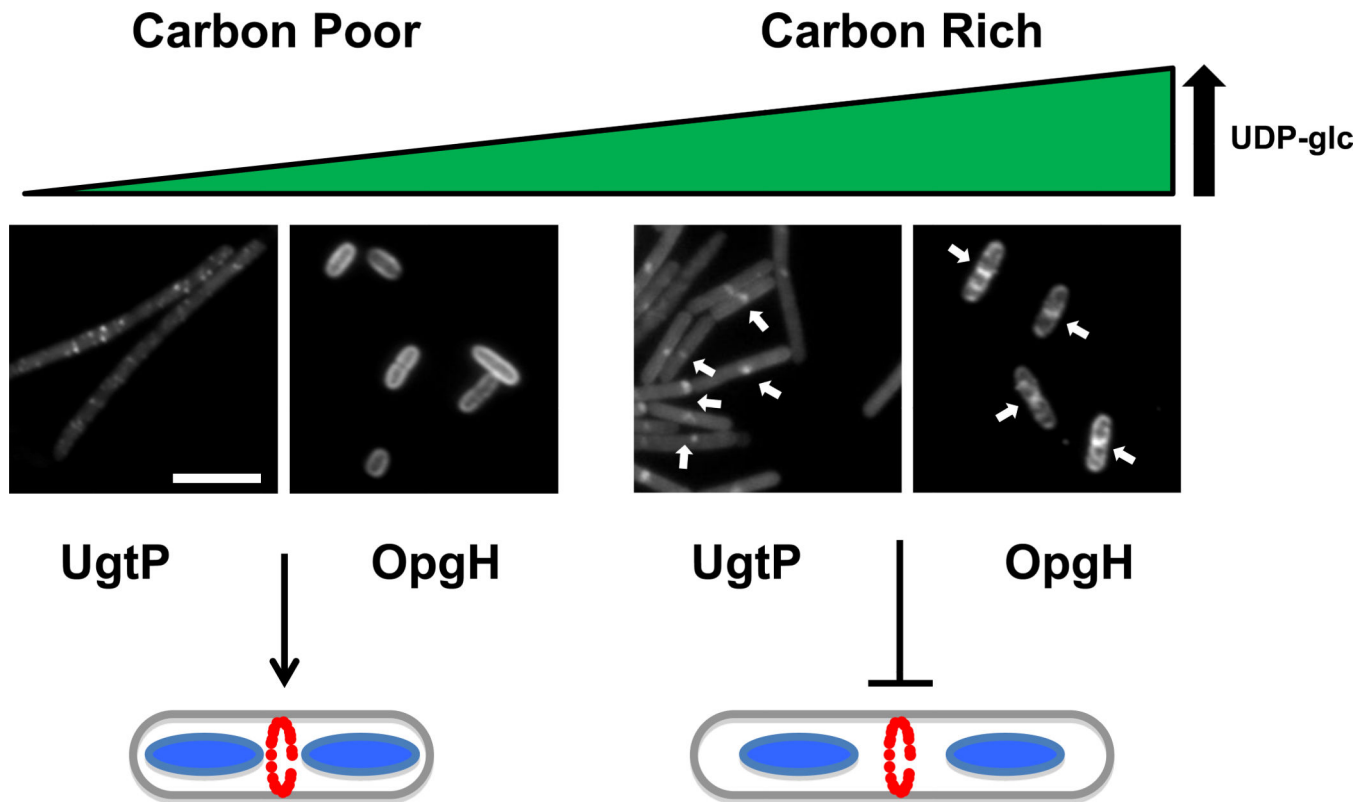


Figure 2. UDP-glucose-dependent changes in the localization of the division inhibitors UgtP and OpgH coordinate size with nutrient availability
 The nucleotide sugar UDP-glc acts as a proxy for carbon availability in the regulatory circuit coordinating cell size with growth rate. In nutrient-poor environments, cell division inhibitors self-oligomerize into punctate foci (UgtP in *B. subtilis*) or remain evenly distributed along the cell periphery (OpgH in *E. coli*), allowing FtsZ assembly and cytokinesis. In nutrient-rich environments, both UgtP and OpgH localize strongly to midcell where they interfere with FtsZ assembly to delay division, resulting in an increase in cell size. Images show chains of *B. subtilis* expressing YFP-UgtP or immunofluorescence labeling of OpgH in *E. coli*. Arrows indicate UgtP or OpgH at midcell. Scale bar = 5 μm .

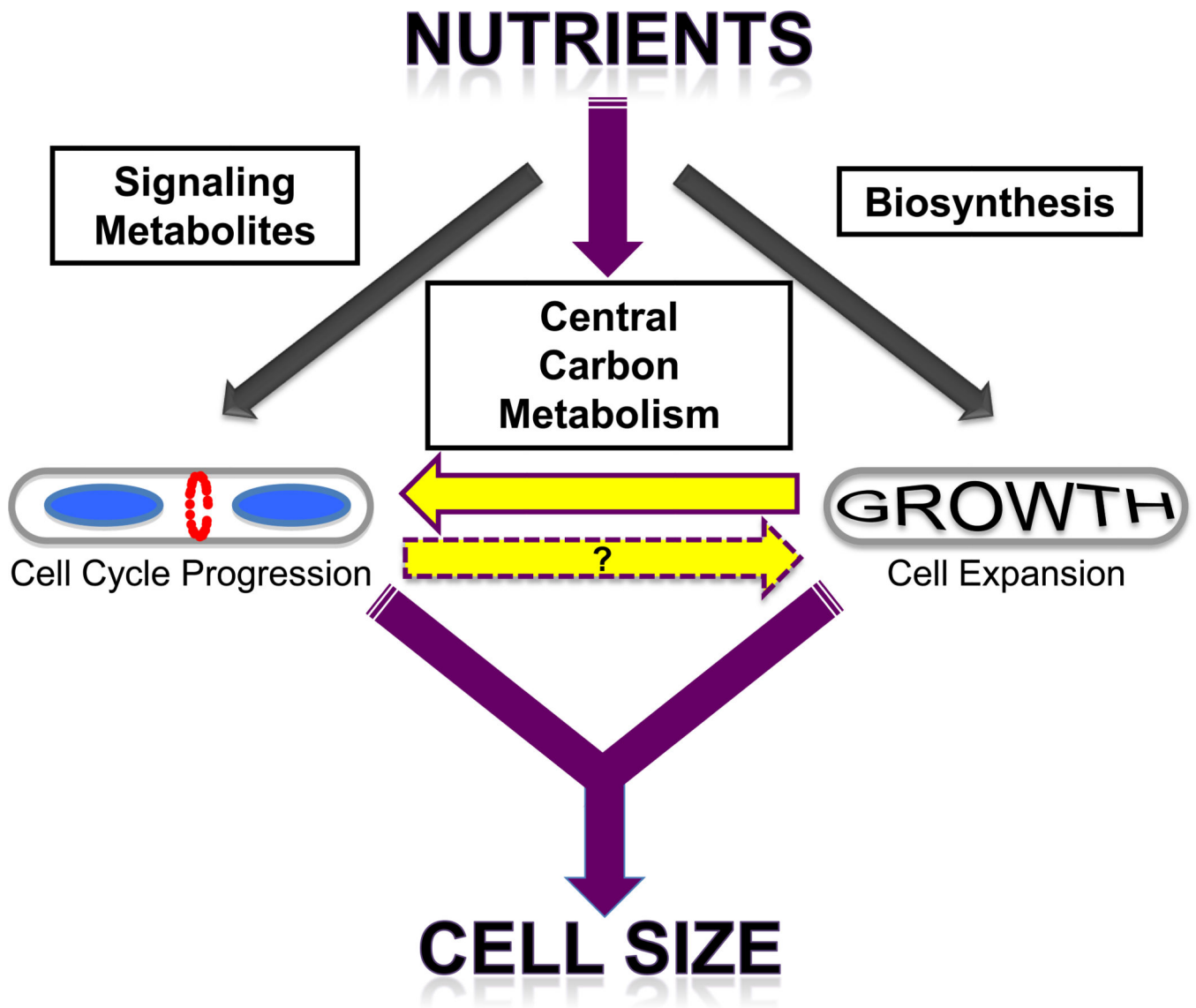


Figure 3. Cell size is a function of cell cycle progression and cell expansion

Nutrients are catabolized, releasing energy and generating building blocks for biosynthetic reactions. Metabolites generated through central carbon metabolism serve as intracellular signals for nutrient availability, activating effector proteins that modulate cell cycle progression. Cell size increases when the rate of cell expansion outpaces the rate of cell cycle progression upon a shift to nutrient-rich medium. Conversely, size is reduced when the rate of cell expansion decreases relative to the rate of cell cycle progression following a shift to nutrient-poor medium. Mass-dependent accumulation of cell cycle proteins including DnaA and FtsZ ensure that cell size is maintained during steady state growth (solid yellow arrow). Cell cycle signals mediating cell expansion rates are inferred in the absence of direct experimental data (dashed yellow arrow). Under constant conditions, cells maintain size regularity by adding a constant volume between birth and division, the value of which increases with nutrient availability. Empirical evidence suggests that part of the increase in

added volume is mediated through UDP-glc-dependent inhibition of FtsZ assembly, although additional targets are likely.

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Table 1Metabolic genes implicated in cell size control and cell cycle progression in *E. coli* and/or *B. subtilis*.

Gene	Function	Product	Organism	Ref.
<i>pgcA; pgm</i>	Synthesis of UDP-glc	α -phosphoglucomutase	<i>B. subtilis</i> ; <i>E. coli</i>	[6**,7**]
<i>gtaB; galU</i>	Synthesis of UDP-glc	UTP-glucose-1-phosphate uridylyltransferase	<i>B. subtilis</i> ; <i>E. coli</i>	[6**,7**]
<i>ugtP</i>	Synthesis of membrane anchor for lipoteichoic acid	UDP-glucose-dependent diacylglycerol glucosyltransferase	<i>B. subtilis</i>	[6**]
<i>opgH</i>	Synthesis of osmoregulated periplasmic glucans	UDP-glucose-dependent glucosyltransferase	<i>E. coli</i>	[7**]
¹ <i>ltaS</i>	Lipoteichoic acid synthesis	Lipoteichoic acid synthase	<i>B. subtilis</i> ; <i>S. aureus</i>	[51**,60]
² <i>plsC</i>	Phospholipid synthesis	Acyl-ACP:1-acylglycerolphosphate acyltransferase	<i>B. subtilis</i>	[51**]
² <i>pgsA</i>	Phospholipid synthesis	Phosphatidylglycerophosphate synthase	<i>B. subtilis</i>	[51**]
² <i>rpsU</i>	Translation	Ribosomal protein S21	<i>B. subtilis</i>	[51**]
² <i>rplA</i>	Translation	Ribosomal protein L1	<i>B. subtilis</i>	[51**]
² <i>rplW</i>	Translation	Ribosomal protein L23	<i>B. subtilis</i>	[51**]
² <i>rplM</i>	Translation	Ribosomal protein L36	<i>B. subtilis</i>	[51**]
² <i>ndh</i>	Respiration	NADH dehydrogenase	<i>B. subtilis</i>	[51**]
² <i>pgi</i>	Glycolysis/Gluconeogenesis	Glucose-6-phosphate isomerase	<i>E. coli</i>	[50]
² <i>gapA</i>	Glycolysis/Gluconeogenesis	Glyceraldehyde-3-phosphate dehydrogenase	<i>B. subtilis</i>	[49,51**]
² <i>pgk</i>	Glycolysis/Gluconeogenesis	Phosphoglycerate kinase	<i>B. subtilis</i>	[49]
² <i>gpmA; pgm</i>	Glycolysis/Gluconeogenesis	Phosphoglyceromutase	<i>B. subtilis</i> ; <i>E. coli</i>	[49,50]
² <i>eno</i>	Glycolysis/Gluconeogenesis	Enolase	<i>B. subtilis</i>	[49]
¹ <i>pyk</i>	Glycolysis	Pyruvate kinase	<i>B. subtilis</i>	[48*,49,51**]
² <i>pdhB</i>	Glycolysis/TCA cycle	Pyruvate dehydrogenase (E1 β subunit)	<i>B. subtilis</i>	[51**]
<i>accD</i>	Fatty acid biosynthesis	Subunit of Acetyl-CoA carboxylase	<i>E. coli</i>	[52**]
¹ <i>fabH</i>	Fatty acid biosynthesis	β -ketoacyl-ACP synthase III	<i>B. subtilis</i> ; <i>E. coli</i>	[51**,52**]
³ <i>fabB</i>	Fatty acid biosynthesis	β -ketoacyl-ACP synthase III	<i>E. coli</i>	[52**]
² <i>tktB</i>	Pentose phosphate pathway	Transketolase II	<i>E. coli</i>	[50]
² <i>ackA</i>	Overflow pathway	Acetate kinase	<i>E. coli</i>	[50]