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Bacterial growth laws and their applications

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Quantitative empirical relationships between cell composition and growth rate played an important role in the early days of microbiology. Gradually, the focus of the field began to shift from growth physiology to the ever more elaborate molecular mechanisms of regulation employed by the organisms. Advances in systems biology and biotechnology have renewed interest in the physiology of the cell as a whole. Furthermore, gene expression is known to be intimately coupled to the growth state of the cell. Here, we review recent efforts in characterizing such couplings, particularly the quantitative phenomenological approaches exploiting bacterial 'growth laws.' These approaches point toward underlying design principles that can guide the predictive manipulation of cell behavior in the absence of molecular details.

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Introduction

Engineering of synthetic genetic circuits holds the promise to revolutionize medical treatment and industrial production from microbes [1–3]. Yet, progress over the last decade has been hindered by a lack of rational design principles to guide the interfacing of engineered components with the host organism [4–6]. Recent work demonstrates that even constitutive protein expression [7•,8••], and much more so genetic circuits [7•,9•], can be strongly affected by the cell's physiological state. Thus, it appears difficult to insulate synthetic circuitry from the growth state of the host. Though orthogonal expression systems [10] are designed to minimize this coupling, they nevertheless draw energy and resources away from the core processes in the cell [9•].

Despite the inherent crosstalk between synthetic and endogenous elements, however, some of the resultant global effects have been shown to obey simple mathematical relations referred to as 'growth laws' [8••]. These quantitative relations may provide a framework for the design of robust synthetic systems, opening up new directions in bioengineering and biotechnology.

Empirical growth-rate dependence

Recent advances in the phenomenological modeling of bacterial physiology are best appreciated within a historical context. Possibly the most influential early example of coarse-grained modeling was Monod's discovery of a hyperbolic relation between the growth rate of a culture and the concentration of nutrient in the growth medium [11]. Subject to some amendments over the years, Monod's relation continues to play an important role in current research [12] (Box 1).

The simplicity of Monod's relation belies the incredible complexity of physiological regulation in bacteria. In balanced exponential growth, however, all of this complexity operates in concert with clock-like regularity to ensure that every constituent in the cell doubles at the same rate [11]. The seminal work by Schaechter *et al.* [13] revealed that the consequences of that balance are remarkable; the macromolecular composition of *Salmonella* (mass of RNA, DNA, protein, and cell mass itself) is largely a function of the doubling rate alone, irrespective of the detailed composition of the growth medium. This observation has been a source of wonder and inspiration for over 50 years [14,15].

A decade after Schaechter *et al.*'s work, Helmstetter and Cooper [16] carefully analyzed synchronous cultures of *Escherichia coli* and established that during fast growth multiple origins of replication are employed simultaneously. Coupled with the exponential dependence of the cell mass on growth rate discovered by Schaechter *et al.*, Donachie [17] turned these empirical observations around and postulated that both series of measurements could be explained if new rounds of DNA replication are initiated at a constant mass per origin of replication (referred to commonly as the 'Donachie mass'). Donachie's work marks one of the first times in biology that quantitative phenomenological relations were used to infer constraints on the underlying molecular mechanisms. Elucidating the details of DNA-replication initiation remains an active area of research [18], although

Box 1 Monod's growth relation

Monod [11] empirically observed a relation connecting the growth rate of a culture, λ, with the concentration of a growth-limiting substrate $[S]$:

$$\lambda = \lambda_{max} \frac{[S]}{[S] + K_M}, \quad (1)$$

where K_M is the saturation constant for the substrate and depends upon the particular substrate and organism. Recent work on balanced exponential growth revealed a formally equivalent relation connecting the growth rate to the *quality* of a saturating substrate [8••]:

$$\lambda = \lambda_{max} \frac{\kappa_n}{\kappa_n + \kappa_t}, \quad (2)$$

where κ_n is the nutritional capacity and κ_t is the translational capacity, independent parameters that can be estimated from the composition of exponentially growing bacteria under conditions of translation and nutrient limitation, respectively (see Figure 1). The maximum growth rate, λ_{max}, is the product of the mass fraction available to protein synthesis and nutrient influx, $1 - \phi_{fixed}$, and the translational capacity,

$$\lambda_{\kappa_t^{max}} = (1 - \phi_{fixed}) \cdot \kappa_t, \quad (3)$$

where ϕ_{fixed} is the growth-rate invariant fraction of the proteome (Figure 1C, blue).

any proposed mechanism must be consistent with Donachie's observation.

In the wake of these early studies, the growth-rate dependence of a large catalogue of physiological parameters was measured in *E. coli*, largely driven by the meticulous efforts of Bremer and co-workers [19••]. That accumulated data laid the foundations for the quantitative models of bacterial physiology that began to emerge in the late 1970s and early 1980s.

Quantitative models of bacterial physiology

Growth-rate dependent physiological parameters collected over the intervening decades served as input to predictive, integrative models. Ehrenberg and Kurland [20] used a detailed model of bacterial physiology, subject to the maximization of growth rate, as a means to quantify the costs associated with accuracy in protein translation (see also Okamoto and Savageau [21]). Growth rate maximization, along with a range of other objectives [22], continues to be used in modern constraint-based reconstruction and analysis of metabolic networks [23]. In some cases, the resulting analyses have met with remarkable success in predicting changes in metabolism incurred during evolutionary adaptation [24], and in qualitatively addressing shifts to inefficient metabolic strategies during rapid growth [25]. Recently, Tadmor and Tlusty [26•] used a coarse-grained model of macromolecular synthesis in *E. coli* to predict the growth defect associated with the deletion of ribosomal RNA operons, and suggested that molecular crowding effects set the limit on the optimum copy number of the ribosomal RNA operons. Klumpp and Hwa [27], focusing on transcription, developed a model for the free RNA polymerase concentration to explain the growth-rate dependence of promoter activities, and were thereby able to constrain admissible transcriptional control functions in response to amino acid starvation.

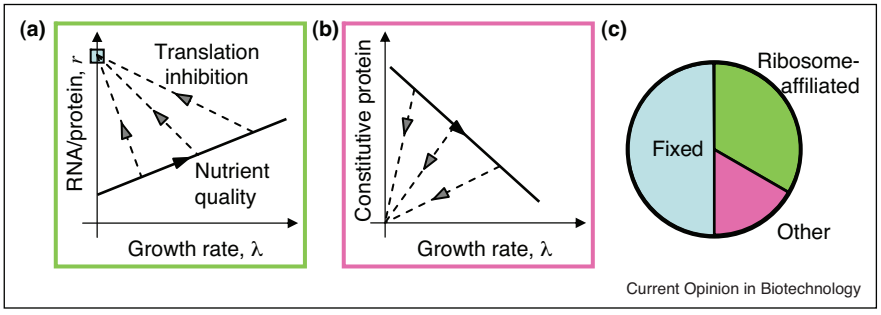
Although each of these studies has brought us closer to a more complete understanding of the complex interplay between regulation and physiology, the limitation of these approaches is that even the coarse-grained models involve a number of parameters that must be independently measured, for each growth condition of interest. As Klumpp *et al.* [7•] showed, simple constitutive gene expression in exponentially growing bacteria is strongly dependent upon the growth rate of the culture. The growth-rate dependence can be traced back to changes in global parameters such as the basal transcriptional and translational rates, gene dose, and cell volume at different growth rates. By analyzing these global parameters, the authors were able to make accurate, quantitative predictions regarding the coupling between gene expression and cell growth for a number of common genetic network motifs.

The success of these 'bottom-up' models suggests that the immense complexity of genetic and metabolic regulation under different growth conditions can be captured by a limited number of growth-rate dependent global parameters (e.g. those describing transcription and translation), at least as far as gene expression is concerned. Nevertheless, such approaches are not able to explain the origin of these growth-rate dependences. Furthermore, the growth rate can be altered in many ways: in a continuous culture, it is the quantity of the growth-limiting nutrient that is adjusted through the dilution rate. In batch culture, most often it is the quality of the saturating amount of nutrient that is changed (as in all the studies discussed above). The growth rate can, of course, be modulated in many other ways, including temperature, osmolarity, antibiotics, toxin, or other conditions. In that case, a 'bottom-up' approach requires careful wholesale measurements to be repeated for the dozen or so global parameters used in the model. A different perspective is needed to explain the origins of the growth-rate dependence of gene expression, and at the same time allow straightforward extension to other modes of growth modulation.

Bacterial growth laws

Hidden within the experimental results of Schaechter *et al.* [13] is the remarkable linear relation between RNA/total protein ratio and the growth rate over moderate to fast growth rates (faster than two hours per doubling) (Figure 1A, solid line). In *E. coli*, the total RNA is approximately 85% ribosomal RNA (a fraction that is growth-rate independent over moderate to fast growth

Figure 1



Bacterial growth laws: **(A)** When growth is modulated by changes in nutrient quality, the RNA mass fraction r (proportional to the ribosomal content) of *E. coli* increases linearly with growth rate λ (solid line): $r = r_0 + \lambda/\kappa_t$, where the parameter κ_t is related to the translation rate [8••], and r_0 is the offset. When growth is modulated by changes in translational efficiency, a conjugate relation is observed. The RNA mass fraction is inversely related to growth rate (dashed lines): $r = r_{max} - \lambda/\kappa_n$, where the parameter κ_n describes the nutrient quality of the growth medium, and r_{max} is the maximum allocation to ribosomal synthesis in the limit of complete translational inhibition. **(B)** Symmetric linear relations are observed in the mass fraction of a constitutively expressed protein, implying a linear constraint between ribosome-affiliated and constitutive proteins. **(C)** The simplest constraint is a three-component partition of the proteome: a fixed fraction that is invariant to growth-rate change (blue), ribosome and ribosome-affiliated proteins (green) and the remainder (pink), including constitutive proteins. For *E. coli* K-12 MG1655, the fixed fraction appears to occupy roughly half of the protein fraction [8••].

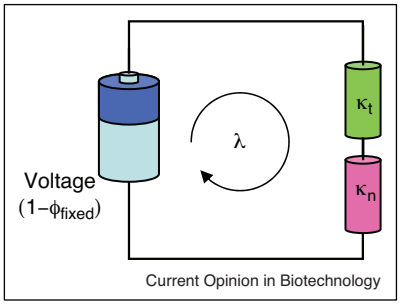
rates [19••,28]), and so the RNA/protein ratio is directly proportional to the mass fraction of ribosomes in the cell. Later experiments by Neidhardt and Magasanik [29] brought this relation between ribosome content and growth rate to center stage. The linearity can be understood as a consequence of mass balance: protein mass accumulation is generated by elongating ribosomes, and in balanced exponential growth that implies a linear relation between the ribosomal mass fraction (proportional to the RNA/protein ratio) and the growth rate. The constant of proportionality ($1/\kappa_t$) is then given by the reciprocal of the translational elongation rate [28–30]. This interpretation of the linear relationship between ribosomal content and growth rate under changes in nutrient quality suggests a conjugate relation may be revealed by changing the translation rate.

When translation is inhibited, either by subinhibitory levels of antibiotic or targeted mutations of the ribosomal proteins, a second linear relation between the RNA/protein ratio and the growth rate becomes apparent (Figure 1A, dashed lines) [8••] (see also [31–35]). In this case, the constant of proportionality ($1/\kappa_n$) is inversely related to the nutrient quality. More remarkably, the two linear relations describing the RNA mass fraction observed under changes in nutrient quality and translational ability are reflected almost perfectly in the mass fraction of a constitutively expressed protein (Figure 1B). Although these empirical relations are independent of any interpretation, the mirror-symmetry between ribosome-affiliated and constitutive proteins suggests the existence of a linear constraint. The empirical relations shown in Figure 1A and B can be unified into a phenomenological theory of bacterial growth (Box 1) by postulating a minimal three-component partitioning of the proteome (Figure 1C) that includes a growth-rate invariant fraction ϕ_{fixed}, a fraction containing ribosome and ribosome-affiliated proteins, and a third fraction containing the remainder (including constitutive proteins).

Taken together, these results suggest that the origin of the growth-rate dependence of constitutive gene expression arises from a tug-of-war between the need for protein synthesis (mediated by ribosomal proteins) and nutrient uptake/processing (mediated by other nonribosomal proteins). Qualitatively, this type of flux balance is expected in any autocatalytic (selfreproducing) reaction scheme, as was first proposed by Hinshelwood [36]. Koch [37] applied Hinshelwood's analysis to study the biophysical constraints on bacterial growth-rate regulation by considering an autocatalytic loop composed of ribosomal and nonribosomal proteins. A similar two-component model was used by Alon and coworkers [38] in their recent study of promoter activities under various modes of growth inhibition.

One limitation of a two-component proteome partition is that under favorable nutrient conditions or maximum translational inhibition, the predicted ribosomal protein fraction would be close to one. In fact, including all auxiliary proteins required for translation (initiation factors, elongation factors, etc.), that fraction is substantially less than one. The minimum partition model, then, must include at least three fractions to account for this disparity (Figure 1C). Quantitative characterization of the two distinct linear relations between the ribosomal proteins and the growth rate (Figure 1A), along with the explicit recognition of the fixed fraction, makes it possible to expand Koch's analysis into a predictive theory [8••]. As with Donachie's mass, this phenomenological model serves as a constraint for proposed molecular mechanisms of the underlying regulatory processes.

Figure 2



Analogy with Kirchoff's law: the Monod-like relation for growth, Eq. (2), is mathematically identical to the description of electric current flow through a pair of resistors connected in series to a battery with voltage $(1-\phi_{fixed})$. In this analogy, the growth rate λ is the current through the resistors. The translation-mode and nutrient-mode of growth limitation correspond to changing the conductance of one of the resistors, while the expression of unnecessary protein corresponds to reducing the applied voltage by increasing ϕ_{fixed} (see Figure 3A).

Of more direct relevance to biotechnology and synthetic biology applications, the growth theory outlined in Figure 1 provides a conceptual framework to guide the interfacing between synthetic constructs and the host organism. Combination of the two linear relations and the constraint on the total mass fraction yields a mathematical expression identical to Kirchoff's laws applied to two resistors in series (Figure 2). In this formulation, increase in translation rate is characterized by an increased conductance in the protein synthesis branch (increased κ_t), whereas an increase in nutrient quality is characterized by an increased conductance in the nutrient branch (increased κ_n).

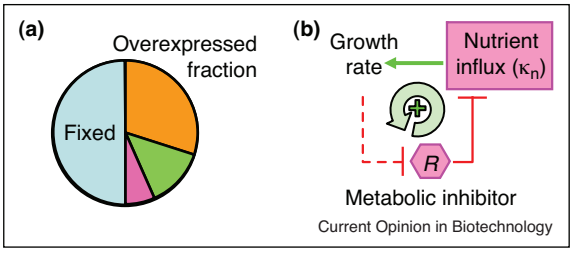
Applications of the growth laws

The analogy to Kirchoff's laws is significant in so far as it allows the growth theory to be extended to different modes of growth rate modulation, particularly to the growth defect associated with heterologous protein expression. Heterologous protein expression effectively expands the fixed protein fraction ϕ_{fixed} (Figure 1C, blue), which in the Kirchoff's law analogy corresponds to a reduction of the driving voltage. Consistent with this interpretation, overexpression of an unnecessary protein in *E. coli* results in a linear decrease in the growth rate, with the zero-growth limit occurring when the overexpressed protein occupies a mass fraction of $(1-\phi_{fixed})$ [8••], in agreement with results by others using different expression vectors and proteins [39,40]. These results disagree with those of Dekel and Alon [41], who reported a quadratic reduction in growth rate upon protein overexpression from the *lac* operon. Closer inspection shows that the protein expression level, and the concomitant growth rate reduction, reported in [41] was over a comparatively narrow range (<0.5% of the proteome), whereas the effects described in Refs. [8••,39,40] covered expression levels up to 30–40% of the proteome.

With a large part of the proteome occupied by the fixed fraction and the overexpressed protein, less of the proteome is available for the remaining fractions responsible for translation and nutrient uptake/processing (Figure 3A). The observed destruction of ribosomes upon overexpression [39] may simply reflect the native response to nutrient limitation. One immediate conclusion is that the metabolic load [42,43] associated with overexpression can be mitigated by reducing the nominal fixed protein fraction ϕ_{fixed}, which may lead to alternate methods of increasing heterologous protein yield that augment existing strategies of strain-optimization [44]. Of wider interest in evolutionary studies, the relative growth defect associated with overexpression (often called the "fitness cost") provides a basis for quantifying different strategies of gene regulation [41,45,46].

Crosstalk between endogenous and synthetic elements has long frustrated the advance of synthetic biology [5,6,47], despite the growing list of well-characterized components [48]. The phenomenological framework emerging from recent work on *E. coli* not only suggests how this crosstalk can be incorporated into network design, but also offers a strategy to exploit coupling to host physiology for rational purposes. The interdependence of gene expression and growth rate can lead to global feedback loops, as suggested by Narang and coworkers [49,50••] in a series of critical analyses of the regulation underlying carbon utilization in *E. coli*, though the existence of different modes of growth inhibition [8••] makes the feedback scenarios much richer than the dilution models considered in that work.

Figure 3



Some applications of the growth laws: **(A)** The burden of protein overexpression. Expression of an unnecessary protein (orange) effectively decreases the fraction allocable to the protein sectors responsible for protein synthesis (green) and nutrient uptake/processing (pink), leading to a decrease in the growth rate [8••]. **(B)** Growth-mediated feedback. Constitutive expression of a toxin affecting nutrient influx (*R*) could lead to bistability through positive feedback generated by the interdependence of gene expression levels and growth rate (dotted line). A decrease in growth rate under conditions of nutrient limitation results in an increase in the constitutively expressed toxin *R*, reinforcing further growth rate reduction [7•].

It should, for example, be possible to generate complicated phenotypic switching through growth-mediated feedback if the cell expresses a toxin inhibiting nutrient influx (Figure 3B) [7•]. Although selfinflicted growth-arrest seems counterintuitive to the survival of the organism, quiescent cells are resistant to many antibiotics and growth-arrest represents a bet-hedging strategy to ensure long-term viability of the colony (a phenomenon called 'bacterial persistence') [51,52]. Lou *et al.* [53] proposed a growth-mediated positive feedback mechanism to facilitate spontaneous growth transition to the dormant state using a dilution model with highly cooperative regulatory interactions controlling the expression of a toxin. Klumpp *et al.* [7•] further pointed out that growth-dependent global feedback could lead to persistence for constitutively expressed toxins [54].

In an industrial setting, bistability resulting from global feedback effects may arise unintentionally due to the action of the synthetic network used to drive heterologous expression. Regulatory motifs used in expression vectors must then be optimized to avoid bistability to prevent overgrowth of the population by the low-producing phenotype [55]. Bistability of this type has already been reported by You and coworkers [9•], where the growth defect associated with the overexpression of a foreign polymerase generates the requisite feedback. In a therapeutic setting, phenotypic bistabiltiy mediated by overexpression may underlie expression of motility and virulence factors in some bacterial pathogens [56].

Outlook

The focus of this review is on the utility of a quantitative phenomenological characterization of bacteria physiology, specifically *E. coli*. Most existing mathematical studies of biomolecular systems take a bottom-up approach, that is starting with known molecular features and including mutual interactions to predict system-level properties [57]. While the bottom-up approach can be successful in analyzing small-scale systems where most of the interactions have been characterized, it becomes more and more difficult as one moves toward larger systems, where the number of parameters ''explodes'' [47]. In contrast, phenomenological theory requires no molecular level information; it is based on the quantification and application of empirical laws. An analogy can be made to Kirchoff's and Ohm's laws which simplify the analysis and design of electrical circuits, without requiring a detailed atomic-level description. Similarly, we believe systems biology, biotechnology and synthetic biology can benefit greatly from theoretical approaches based on empirical characterizations to complement existing characterization of synthetic genetic elements [58].

Clearly empirical relationships between ribosomal content, protein expression and growth rate must be characterized under other modes of growth inhibition (including transcriptional inhibition, osmotic stress, temperature change, etc.), and much work remains to extend this approach to other organisms with high application potential, including fungi [59,60] and algae [61]. That said, there is a growing body of research suggesting that very similar phenomenological laws apply in yeast [62], including some exciting work linking gene expression and growth rate [63•,64,65]. Quantifying similar growth laws for eukaryotes may shed light on intervention strategies for diseases ranging from fungal infection to cancer proliferation [66].

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