for the development of microfluidic LOCs what it has done for medical diagnostics, by providing a generally applicable, powerful, and flexible tool to study a complex chemical and biological system without perturbing its operation.

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MICROBIOLOGY

Topping Off a Multiscale Balancing Act

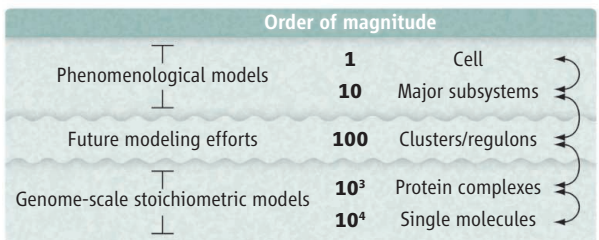
Quantitative predictions of the relationship between cell growth and gene expression have been made and validated in *Escherichia coli*.

Joshua Lerman and Bernhard O. Palsson

The genotype-phenotype relationship is fundamental to biology. Finding general, underlying rules that govern the complex relationship between gene expression and cell growth, however, has proven a challenge. On page 1099 of this issue, Scott *et al*. (*1*) offer empirical "growth laws" that correlate the growth rates of bacteria with how they allocate resources to protein synthesis and metabolic functions.

The genotype-phenotype relationship in microbes can be conceptualized as a five-layer hierarchical model (see the figure). A cell faces myriad constraints on its function at all layers (*2*, *3*). At the whole-cell level, it may be difficult to determine the constraints that govern cellular functions on a mechanistic basis, but they can be identified from empirical observation. Microbiologists pursued this approach in the 1950s and 1960s, resulting in empirical parameters such as the growth and nongrowth maintenance coefficients (*4*) and yield coefficients that are widely used in the bioprocessing literature (*5*).

Scott *et al*. expand on the whole-cell empirical approach by means of an insightful combination of targeted experimentation and mathematical analysis. Using *Escherichia coli* cells grown under a variety of conditions, they first confirmed a previously established correlation between growth rate and the ribosomal content of the cell (ribosomes assemble proteins from amino acids). Next, they used mutant strains to show that the proportionality constant between ribosomal content and growth rate depends on the overall rate at which the cell incorporates amino acids into protein. To achieve a small increase in growth rate, mutant strains that incorporate amino acids slowly must dedicate more resources to ribosome production than their more rapidly synthesizing counterparts.



The microbial genotype-phenotype relationship. Bacterial cell growth and gene expression are linked through a hierarchy that extends from tens of thousands of molecules to a single cell. Each layer in the hierarchy imposes constraints on adjacent layers (arrows, right). At the top, empirical models can predict the relative levels of proteins belonging to major subsystems within a cell (e.g., metabolism, macromolecular synthesis). At the bottom, genome-scale models can make predictions by accounting for all single molecules and protein complexes. A future modeling challenge is to characterize the functionality of the ~100 coordinately expressed clusters of protein complexes and to determine the evolutionary pressures leading to regulon formation (middle layer).

The authors then subjected cells growing in different nutritional environments to increasing concentrations of an antibiotic that disables ribosomal function. Another striking correlation emerged: As the concentration of the antibiotic increased, translation capacity and growth dropped linearly toward zero, and there was a corresponding linear increase in the fraction of the proteome dedicated to ribosomes. Finally, chemical composition data taken from cells close to the no-growth point suggested that there is a hard upper bound on the fraction of the proteome that can be dedicated to ribosomes and related proteins; it is capped at around 0.55, independent of growth conditions.

Given these findings, and given that the cell's regulatory efforts enforce a balance between metabolism and macromolecular synthesis, the authors reasoned that they could mathematically describe proteome resource allocation using only three variables: R, P, and Q, where R represents the growth rate–dependent fraction of the proteome that is dedicated to macromolecular synthesis, P represents the growth rate–dependent fraction of the proteome dedicated to everything else, and Q represents the growth rate–independent (or housekeeping) fraction. This grouping of proteins into three categories is able to relate R, P, and Q in quantitative terms without any adjustable parameters, and without requiring detailed knowledge of the underlying regulatory circuits. Surprisingly, Q-class proteins occupy a substantial fraction of the proteome (50%).

The authors evaluated the predictive potential of these empirical equations through a series of experiments. They exploited the empirical relationships between proteome fractions to (i) validate the predicted constrained positive linear relationship between the abundance of P-class proteins and growth rate in a fixed nutritional environment, and (ii) mathematically characterize cells forced to express unnecessary (or useless) protein.

This work comes on the heels of parallel developments at lower levels of the hierarchy shown in the figure. The availability of

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full genome sequences in the mid-1990s led to the construction of genome-scale metabolic models that were able to recapitulate optimal growth phenotypes (*6*). The reconstruction of the entire protein synthesis machinery (*7*) moved us up a notch in the hierarchy, enabling the description of the optimal ribosomal content as a function of growth rate (the starting point for Scott *et al*.). At the systems biology level, "omics" data sets have led to an understanding of how optimal network properties form (*8*, *9*). At this level, a combination of inference methods (*10*) and bottom-up reconstructions has proved productive (*11*). Decomposing network functionality into coordinately expressed gene clusters, and determining the degree of flexibility within and among these clusters (in terms of expression levels), could complete our understanding of the hierarchy, now that we have the top-level relationships developed by Scott *et al*.

Taken together, these developments lead to a multiscale understanding of the genotype-phenotype relationships underlying metabolism and growth in microbes. At all levels, model structures must be developed in order to adequately capture constraints and allow for optimization subject to these constraints (*12*). Cementing these levels into a coherent multiscale framework is a challenge facing the field. Experiments that enable bacteria to rapidly evolve in controlled laboratory settings are a way to interrogate this relationship further, as they produce optimal growth phenotypes (*13*, *14*). The genetic basis for such changes in phenotype can now be determined through whole-genome resequencing, followed by allelic replacement to identify causal mutations (*15*). Clearly, an exciting era is ahead of us, in which a combination of in silico and experimental approaches promises to continue the development of mechanistic and principled genotype-phenotype relationships that are akin to the development of fundamental physical laws a century ago. If successful, such development will move microbiology into a fundamentally new realm.

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NEUROSCIENCE

π = Visual Cortex

Three distantly-related mammals share a brain architecture characterized by a density of π

Kenneth D. Miller

Archimedes, the great scientist of ancient Greece, performed the first systematic calculation of the value of π, the ratio of a circle's circumference to its diameter. Twenty-three centuries later, scientists continue to be delighted by π's appearance in new and unexpected areas of science. The latest is perhaps the most surprising: On page 1113 of this issue, Kaschube *et al*. (*1*) show that three distantly-related mammals share a common organizing scheme for neurons in the brain's visual cortex characterized by a density closely approaching 3.14 (π). The result offers insight into the development and evolution of the visual cortex, and strongly suggests that key architectural features are self-organized rather than genetically hard-wired.

The cerebral cortex is a thin, six-layer sheet of neurons. A long-standing model system for cortical studies is the primary visual cortex (V1), the first piece of cortex to receive visual input (*2*). Neurons in V1 are highly selective for the spatial orientation of a light/dark edge; some prefer (respond best to) vertical edges, whereas others prefer horizontal or diagonal lines. Preferred orientation exhibits what is called "columnar" organization: The neurons beneath any given spot on the cortical sheet, across the layers, prefer the same orientation. Imaging techniques allow researchers to visualize the arrangement, or "map," of preferred orientations across the cortical sheet (see the figure). These orientation maps have a quasi-periodic structure: Preferred orientations change continuously across the sheet, repeating every millimeter or so. The local distance between repeats is the local "map period" (λ). The maps also contain "pinwheels"— points at which all preferred orientations converge. There has long been debate over the degree to which these features reflect detailed genetic programming or self-organization based on general rules that guide the growth and decay of synapses (*3*, *4*).

To explore this question, Kaschube *et al*. compared, with unprecedented quantitative precision, the density and arrangement of pinwheels in three mammals: the galago, a primate; a tree shrew, a close primate relative; and a distantly related carnivore, the ferret. This precise measurement of pinwheel distribution required considerable advances. Measurement "noise" corrupts maps, and existing methods to "smooth" the noise also smooth away, or hide, real pinwheels. The authors found filters that solve this problem. They also used wavelet-based methods that they had previously developed to precisely measure the local map period (*5*), and they gained precision by analyzing an unprecedented number of maps (more than 100) and pinwheels (roughly 10,000).

They found strong evidence of a common design. Most strikingly, the mean density of pinwheels per λ^2 was within 1% of π for all three species. The grand average was 3.14, with a 95% confidence interval of 3.08 to 3.20 ($\pi \pm 2\%$). Does a density of π just follow from the periodic map structure? To test this, they "phase-randomized" measured maps, creating maps that precisely retained the measured periodic structure but were otherwise random (see the figure). These maps had much higher pinwheel densities (mean 3.50), which suggests that π is a special property of the maps found in brains.

Why should the pinwheel density be π? Kaschube *et al*. have a beautiful theoretical answer. For many years, the senior author, Fred Wolf, and his group have been constructing a theory of orientation map development that builds on mathematical methods devel-

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