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Applications of imaging for bacterial systems biology

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Imaging has fueled exciting advances in bacterial cell biology, which have led to exquisite understanding of mechanisms of protein localization and cell growth in select cases. Nonetheless, it remains a challenge to connect subcellular dynamics to cellular phenotypes. In this review, I explore synergies between imaging and systems approaches to bacterial physiology. I highlight how single-cell, time-lapse imaging under environmental or chemical perturbations yields insights that complement traditional observations based on population-level growth on long time-scales. Next, I discuss applications of high-throughput fluorescence imaging to dissect genetic pathways and drug targets. Finally, I describe how confocal imaging is illuminating the role of spatial organization in the structure and function of bacterial communities, from biofilms to the intestinal microbiota.

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Introduction

Spatiotemporal organization occurs over a wide range of length (from molecular assemblies to multicellular communities) and time (from milliseconds to days) scales in bacteria. To achieve a systems-level understanding of cellular growth, structure, and function, it will be necessary to elucidate how cells establish and coordinate processes over these scales. Direct observation of cell growth and intracellular protein dynamics provides powerful insight into these questions. Every such experiment addresses a system that involves a host of biochemicals: not only do cells often rely on sequential assembly of many proteins to establish subcellular structures, but localization is defined with reference to cell geometry, which in bacteria is determined by the membrane and peptidoglycan cell wall [1]. Moreover, cell shape changes as the cell grows, connecting protein localization to all of the processes essential for proliferation, such as DNA replication, transcription, and translation. Therefore, there is ample potential for synergy in integrating approaches from cell biology and systems biology.

To elucidate genotype–phenotype relationships at the genomic scale, recent chemical genomics studies [2] have exploited high-throughput measures of colony features as a measure of growth. By varying the growth environment with chemical treatments, substrates, and environmental conditions, multidimensional phenotype vectors describing a collection of mutants can be used to study gene essentiality and understand drug action [3]. Moreover, application to genomic-scale libraries empowers clustering of genes involved in a common pathway [3], thereby realizing a systems-level perspective on cellular functions. Nonetheless, a population-level growth metric such as colony size presents only a single perspective on fitness, and will not always accurately represent the effect of perturbations (be they genetic, chemical, environmental, or other) on bacterial physiology.

There is a growing consensus emerging across biological systems that physical metrics such as cell shape, which by itself provides many phenotypes such as cell width, length, body curvature, and polar morphology, are linked with fitness in meaningful ways. Hence, high-throughput analysis of these metrics across genetic libraries would complement data obtained through chemical genomics. In some bacteria, nutrient-induced increases in growth rate correlate with increased cell volume [4]. Similarly, over the course of a long-term evolution experiment, *Escherichia coli* average cell volume increased approximately twofold as fitness also increased [5]. Single point mutations in the *E. coli* actin homolog MreB have been found to increase cell size to a similar degree [6,7], with mutations at one particular residue causing a width-dependent increase in competitive fitness during growth; interestingly, this fitness increase was realized only in the presence of certain carbon sources [6], underscoring the importance of probing multiple environments to understand the relationship between cellular physiology and physical features such as cell size. Moreover, population-based measurements often conceal phenotypic heterogeneities in single-cell growth rate and morphology. In the yeast *Saccharyomyces cerevisiae*, high-throughput imaging revealed that deletion of many genes increases morphological variation [8]. *Salmonella* Typhimurium persister cells, which represent a small fraction of the population, have been associated with slow growth rates and smaller size [9•] indicating that heterogeneities

can be crucial for a community to survive fluctuating environments or stresses. Thus, cell shape and other single-cell features can provide terminal or time-dependent phenotypes in chemical screens that increase the resolution of our understanding of gene function.

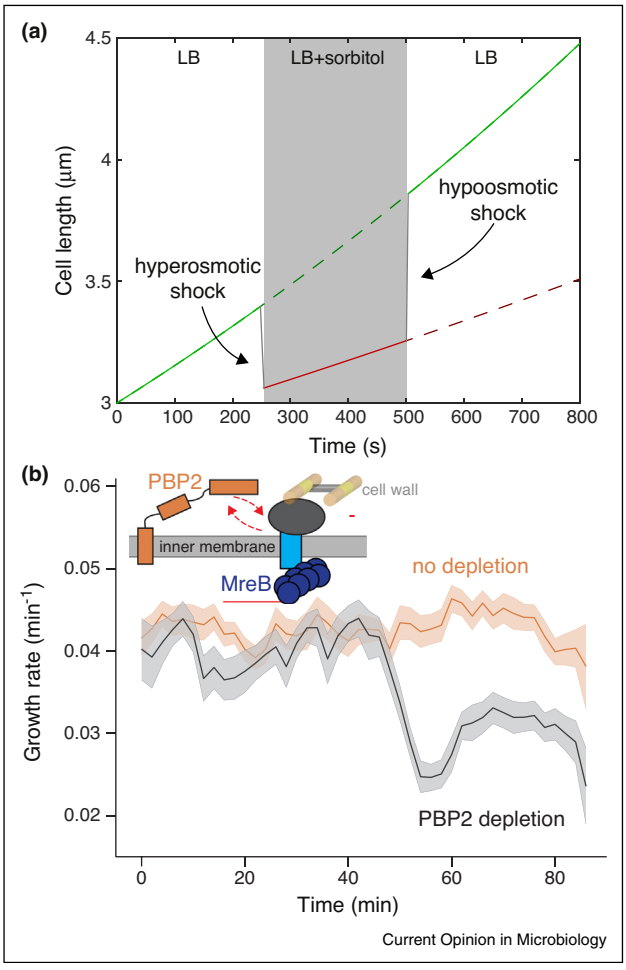
Fluorescence imaging and multiparameteric image analysis can also be used to interrogate cellular processes at the genomic scale, particularly to identify new functions for genes. An imaging-based screen of a *Drosophila* RNA interference (RNAi) library was used to identify >200 genes responsible for mitotic spindle assembly [10]. Key to this study was a high-throughput imaging and quantitative analysis platform that allowed for an unbiased screen across the entire genome, as over half the identified genes were unexpected. Automated analysis of localization dynamics and abundance of fluorescent fusions to ~3000 *S. cerevisiae* proteins was used to reveal fluxes between cellular compartments in response to chemical and genetic perturbations [11••]. Unbiased imaging screens may be similarly revelatory in bacteria. Characterization of the localization of fluorescent-protein fusions to most genes in the bacterium *Caulobacter crescentus* revealed that ~10% of the genes have nonuniform localization profiles [12] and uncovered several novel structures including filaments of the metabolic enzyme CTP synthase [13]; this aggregation may be important for efficient channeling of metabolic intermediates [14].

Here, I explore how recent imaging-based initiatives have empowered the investigation of bacterial systems biology across spatial and temporal scales. I also discuss applications of high-throughput fluorescence imaging for dissecting genetic pathways and drug actions, and for interrogating the spatial organization and function of bacterial communities.

Imaging can reveal contrasting dynamic phenomena across time scales

Many environmental stresses prompt system-wide responses that affect the rate of cell growth. Thus, assaying changes in growth after hours or days likely conflates many indirect effects and hence may motivate incorrect models of growth regulation. For instance, bacterial growth generally decreases as the osmolarity of the growth medium is increased [15], prompting the hypothesis that turgor pressure directly stimulates growth by driving the mechanical expansion of the cell wall. To distinguish between turgor-mediated effects and indirect, pressure-independent effects of osmolarity changes, a microfluidic flow cell was used to rapidly change osmolarity while quantifying the instantaneous elongation rate via single-cell imaging [16••]. On short time scales, although plasmolysis slowed cell elongation, cells nevertheless 'stored growth' whereby, upon reestablishment of turgor, they expanded to the length that they would have attained without the osmotic shocks (Figure 1a) [16••].

Figure 1



Imaging reveals qualitatively distinct phenotypes at short and long time scales. **(a)** Using a microfluidic flow cell to alternately apply hyperosmotic and hypoosmotic shocks, imaging-based quantification of cell elongation on short time-scales demonstrated that cells 'store' growth during periods of low turgor pressure; despite an apparent decrease in elongation rate, cells reached the length after turgor was restored that they would have achieved if unperturbed [16••]. This insensitivity to turgor fluctuations is by contrast to the decrease in growth on longer time-scales at higher osmolarities [15,16••]. **(b)** Using single-particle tracking photoactivated localization microscopy, PBP2 molecules exhibited diffusive motion [21••] rather than the directed motion of MreB [20]. This rapid, dynamic association with sites of cell-wall synthesis (inset) enables cells to maintain growth rate for more than two generations as PBP2 is depleted despite the loss of cell shape and eventual death [21••].

These experiments reveal a surprising robustness of cell-wall synthesis to turgor fluctuations that is concealed by the decrease in steady-state growth that occurs on longer time-scales [15,16••], and highlight the utility of time-lapse imaging of growth responses to perturbations.

Another illustrative example of the power of imaging for unraveling the systems biology of bacteria at various time