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Growth Meets Virulence: Confluence of Two Paths of Microbiology

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With 2 Tables

Abstract

Microbiologists have always been fascinated by two distinct aspects of microbial life: the growth of microbes, and their diverse activities. Microbes have emerged from 3 to 4 billion years of evolution as champions of self-reproduction, the defining property of life; furthermore, microbes are uniquely amenable to biochemical and genetic analysis of growth. Hence microbial growth has attracted the attention of many biologists interested in this central aspect of life. But microbes have also developed during their evolution an astounding array of functional capabilities which are largely independent of growth. For the century-and-a-half that microbiology has been a discipline within biology, microbiologists have generally fallen into two camps based on these two different interests. Today these two threads of microbiology cannot be studied separate from each other, as the theme of this symposium illustrates.

Zusammenfassung

Mikrobiologen waren immer begeistert von zwei unterschiedlichen Aspekten des mikrobiellen Lebens: vom Wachstum bzw. von der Vermehrung der Mikroorganismen sowie ihren unterschiedlichen Aktivitäten und Lebensweisen. Bakterien sind vor etwa drei bis vier Milliarden Jahren als die Meister der Selbstreproduktion entstanden und haben damit die wesentliche Eigenschaft von Leben definiert und auch den Zugang zur genetischen und biochemischen Analyse von Wachstum/Vermehrung ermöglicht. Dieser zentrale Aspekt des Lebens war schon immer der große Anziehungspunkt für das wissenschaftliche Interesse von Biologen. Mikroorganismen haben in ihrer Evolution auch eine erstaunliche Vielzahl von Fähigkeiten entwickelt, die nichts mit Wachstum zu tun haben. Als sich die Mikrobiologie vor ungefähr 150 Jahren als eigenständige Disziplin aus der Biologie entwickelte, waren die Mikrobiologen in zwei Interessenlager gespalten: mikrobieller Stoffwechsel und mikrobielle Pathogenität. Heute können diese zwei Forschungsgebiete nicht mehr getrennt bearbeitet werden, wie dieses Symposium zeigt.

1. Microbial Growth

Those of us interested in growth have been especially influenced by the ideas that flowed in the last half-century from the Paris and Copenhagen schools of microbiology. To Jacques Monod (1949), "The study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: it is the basic method of Microbiology […] (T)he growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws […]"

The first part of this statement is certainly outdated in this era in which metagenomics and pangenomics have permitted one to study microbes without cultivating them at all. But the second part has been completely vindicated. A half-century of intensive exploration of bacte-

rial growth has yielded a number of principles, or "laws", of growth that are both "simple" and satisfying in their elegance. MONOD's view has been born out by the results of the subsequent 60 years of research on bacterial growth. Intensive exploration by dozens of researchers has yielded a number of principles of microbial growth (cf. NEIDHARDT et al. 1990). Most of these principles have been learned using *E. coli* or *Salmonella*, but are believed to be reasonably universal, that is, applicable directly to prokaryotic cells (Bacteria and Archaea), and perhaps to others as well (Tab. 1).

Tab. 1 Some Principles of Microbial Growth

– At constant temperature, with surplus nutrient, bacteria synthesize all their constituents at near-constant differential rates and divide at a particular cell size, a state called balanced growth.
– Certain major phenotypic characteristics of cells in balanced growth (their size, and macromolecular composition) are coordinated with the absolute growth rate, almost independent of the chemical nature of the medium.
– Over a wide range of growth rates at a given temperature, the rates of chain elongation of proteins, RNA, and DNA vary little.
– Over a wide range of growth rates at a given temperature, the time between replication termination and cell division varies little.
– When environmental conditions change, reducing or increasing the growth rate, the pattern of macromolecule synthesis responds in a consistent pattern: first RNA, then protein, and finally DNA synthesis, achieves the new rate.

MONOD's prediction that relatively simple laws would be discovered about growth was correct. These growth studies, along with the near complete elucidation of metabolic pathways and the components of the genome, encourage the attractive view that whole-cell modeling with predictive capabilities is possible. In fact, modeling is not just *possible*, it is *necessary* to advance to the next phase of understanding cell growth.

Without system modeling, one cannot know if the current understanding of any one regulatory circuit is complete or lacking. Thus, one must ask not just how something works, but how it works within the complete cellular network.

Currently there are (at least) two new considerations related to the goal of modeling cell growth. One is the choice of the cell to be modeled. Not too long ago an issue for microbial physiologists interested in growth and its mechanisms was: which K-12 strain or B/r strain of *E. coli* should be chosen as the paradigm. This choice has not been made easier by the embarrassing fact that we can no longer be certain what *E. coli* is.

Pangenomic studies astonish us with the fact that the creature we call *E. coli* does not have a repertoire of 4,500 genes with which to live its life, rather it has available perhaps 15,000 counting the cousins with which it can interact genetically in nature (USSERY 2008, unpublished).

Bacterial growth (and stress) studies have in large measure inferred the nature and behavior of an individual cell in the population from measurements of populations (BREMER et al. 1996, NEIDHARDT et al. 1996). This average cell is a useful (and necessary) construct, but it is a fiction. It is a virtual cell, and within a population there may be no single cell that fits the calculated dimensions and composition of the average cell. This fact takes on special significance when one realizes that clonal populations of cells show phenotypic heterogeneity under homogeneous and invariant conditions.

Initially, identical cells in a population can become significantly different as a result of bistability (= a dynamic system resting in either of two stable states) brought about by such factors as noise, positive feedback, and hysteresis operating on cellular regulatory networks (Veening et al. 2008). Noise (stochastic variation) is significant because the number of molecules of critical proteins involved in transcription and translation is small. This is especially true of transcription factors when not activated or induced. Noise amplified by positive feedback can result in bistability. A striking example of bistability is the clinically relevant phenomenon called persistence. Persistence (Bigger 1944, Moyed and Broderich 1983, Veening et al. 2008) is a striking example of bet-hedging. Persister cells are those few cells in a population that have the ability to survive antibiotic treatment. Persister cells, it is now understood, are impeded in growth, and though inherently sensitive to an antibiotic, are protected by their slow or non-growth. Persister cells arise from normal cells by stochastic processes that are epigenetic.

Summary: Studies focused on modeling the growth of individual cells must acknowledge that the basis of much of the data about growth is derived from population measurements under laboratory conditions, not individual cells in a changing environment.

2. Microbial Activities

Most bacteriologists are concerned with microbial processes and activities that are independent of growth. The variety of activities is staggering. Table 2 lists merely those that come to mind from current studies.

Tab. 2 Some non-growth activities of bacteria

Motility/taxes	Biofilm formation	Quorum sensing
Secretion	Export	Survival
Adhesion	Sporulation	Commensalism
Virulence	Community	Stress responses
Resistance	Persistence	Germination
Stationary phase	Luminescence	Colonization

Given the significance of these varied manifestations of microbial effects on the environment and on human beings, the intensity with which molecular microbiologists have focused on growth would seem to be disproportionate to its importance in microbe/human interaction. Some critics have maintained that exponential growth is an artifact of the laboratory and is largely irrelevant in the real world. Even the fact that bacteria respond to stochastic changes in gene expression caused by environmental changes through bistable switches may make most growth studies misleading, if not irrelevant.

This view that growth physiology is not central to understanding virulence demands examination.

Among the largest regulatory networks in the bacterial cell are carbon catabolite repression and the stringent response. Even a cursory perusal of the current research into molecular pathogenesis reveals the central role of these two systems.

2.1 Carbon Catabolite Repression

Carbon catabolite repression refers to the growth-related regulation by bacteria of their utilization of carbon sources when presented with an environment that offers a choice. Well studied in *Escherichia coli*, this regulatory behavior is widespread in the microbial world (reviewed in Görke and Stülke 2008). In the firmicutes (Gram-positives with low G-C DNA content) work on *Bacillus subtilis* has shown that a global regulatory protein, CcpA, contributes to catabolite repression by inhibiting the induction of catabolic operons for other carbon sources when glucose is present in the environment. This behavior helps result in the economy of preserving secondary substrates for later use rather than squandering them needlessly while the favored glucose is available. The regulation is achieved, in part, by the ability of Ccp, when complexed with a phosphorylated form of the protein HPr, to act as a transcriptional repressor. Phosphorylation of HPr from fructose-1,6-bisposphate and glucose-6-phosphate is a key signal to the cell that glucose is being utilized by the cell (reviewed in Görke and Stülke 2008).

In the human pathogen, *Streptococcus pyogenes*, the analogous CcpA protein is central to virulence. In addition to activating the expression of several genes important to virulence, CcpA directly activates the gene producing the protein Mga, a master regulator of virulence in this organism. Mga governs genes for adhesion, internalization, and immune evasion. Likewise, in both *Staphylococcus aureus* and *Streptococcus gondii*, CcpA has been shown to contribute to antibiotic resistance (by, as yet, undiscovered mechanisms). In enteric bacteria, one gene involved in catabolite repression (*crp*) is essential for the expression of virulence genes (reviewed in Görke and Stülke 2008).

2.2 The Stringent Response and Magic Spot

When growing bacterial cells become restricted for either a required amino acid or for their carbon and energy source, they synthesize the nucleotides pppGpp (guanosine 5'triphosphate, 3'diphosphate) and ppGpp (guanosine 5'diphosphate, 3'diphosphate) from ATP and GTP. In enteric bacteria these "magic spot" nucleotides, collectively termed (p)ppGpp, are made on ribosome-bound RelA protein in the case of amino acid restriction, and from the cytosolic protein SpoT in the case of stress and energy starvation in *E. coli*. Magic spot accumulation is rapid, and likewise, its effects are also rapid; binding to RNA polymerase, these nucleotides alter the transcriptional pattern of the cell to a massive extent, activating genes for fatty acid oxidation, glycogen synthesis, nucleotide catabolism, amino acid synthesis and cell division machinery, while diminishing transcription of genes for DNA replication, ribosome synthesis, nucleotide biosynthesis, and phospholipid biosynthesis. Indirectly, by activating the general stress response, central metabolism is accelerated and cell morphology is altered. Moreover, there is evidence that during balanced growth the activity of ppGpp is the major controlling factor in matching ribosome synthesis to the cellular growth rate. A recent review summarizes the current understanding of this important system (Potrykus and Cashel 2008).

Clearly this is a gigantic regulatory network, and its elucidation and function have occupied bacterial growth physiologists for almost half a century. Current studies on molecular pathogenesis are revealing that the operation of this network is crucial for the virulence of many pathogens. The list currently includes *Salmonella typhimurium*, *Legionella pneumophila*, *Escherichia coli* (EHEC), *Pseudomonas aeruginosa, Campylobacter jejuni*, *Brucella*

abortus, *Listeria monocytogenes*, *Borrelia burgdorferi*, *Vibrio cholera*, and *Mycobacterium tuberculosis* (POTRYKUS and CASHEL 2008).

A particularly well studied example is that of *L. pneumophila*, a ubiquitous bacterium residing largely in biofilms or within freshwater protozoa. When inhaled by humans in aerosols of contaminated water, *L. pneumophila* can invade alveolar macrophages, producing a potentially fatal pneumonia. This bacterium alternates between a replicative and a transmissive state, both in broth and in its host. The two phenotypes differ in many ways, including the cell's rates of protein synthesis and DNA replication, stress resistance, ability to evade lysosomes, flagella-mediated motility, Na^+ sensitivity, contact cytotoxicity, beta-hydroxybutyrate storage granules, and the ability to recruit the endoplasmic reticulum (see reviews: MOLOFSKY and SWANSON 2004, HILBI et al. 2007).

Within the host's macrophages, the replicative state results in multiplication of the bacteria until the host cell nutrients are exhausted. At this point the bacteria differentiate into the transmissive state enabling them to survive the dearth of nutrients and transmit themselves into a new macrophage. It turns out that the classical stringent response system that is an essential component of bacterial growth, is also an essential component of the differentiation process. In brief, the current working models indicate that SpoT might monitor the macrophage's metabolic state through an alteration of the bacterium's acyl-carrier protein (ACP), which interacts with SpoT, causing the latter to produce (p)ppGpp. Elevated (p)ppGpp then activates the regulatory cascade responsible for transcriptional regulation of the gene set that produces the differentiation from the replicative into the transmissive state of the bacterium (DALEBROUX et al. 2009, EDWARDS et al. 2009). A central feature of bacterial balanced exponential growth is thus also a key part of virulence.

3. Summary

Studies of bacterial virulence are a productive way to study bacteria in the natural settings that have shaped their evolution. Whether one's interest is focused on growth mechanisms or on processes unrelated to growth, neither aspect can be understood independent from the other. The contribution of this symposium is to highlight the new frontier: the intersection of microbial physiology and microbial virulence, which is a paradigm for future studies that will integrate molecular approaches in the laboratory and in natural environments.

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