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Networking Opportunities for Bacteria

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Abstract

In this post-genomic era, our capacity to explore biological networks and predict network architectures has been greatly expanded, accelerating interest in systems biology. Here, we highlight recent systems biology studies in bacteria, consider the challenges ahead, and suggest opportunities for future studies in bacterial models.

There is currently much excitement surrounding the field of systems biology and the study of biological networks. This enthusiasm has been fueled, in part, by the results of successful forays into genomic research, most notably the completion of whole-genome sequencing projects in a diverse array of organisms. Recent technological advances, increased computational power and new cross-disciplinary approaches together with a wealth of high-throughput biological data have significantly enhanced our ability to construct and analyze the biomolecular networks of cells.

A decade ago, many rushed ahead to use systems-level approaches to study higher-order organisms, particularly mammalian systems. Along with this development came a sense of dismissiveness in the systems biology community about work on prokaryotes. Some wondered why systems biologists would want to work on *E. coli*, or other bacteria for that matter, when apparently we know all there is to know about these organisms.

This appears to have now changed. Bacteria, which have long served as model organisms for studies in genetics and molecular biology, have emerged as model organisms for systems biology. These comparatively “simple” creatures, and the relative ease with which one can conduct genetic and phenotypic experiments on them, are providing marvelous platforms for expanding our understanding of how network circuitry is able to influence and control cellular behavior. Our current understanding of prokaryotic genomic architecture, gene regulation and metabolism, however, is nowhere near complete and will continue to benefit from network-based approaches.

Accordingly, there is still a great deal to be learned about bacterial networks and many opportunities exist for biology-driven discovery based on systems-level analyses of prokaryotes. Such efforts will not only be critical in characterizing the function and dynamics of newly discovered bacterial gene circuits, but will in many cases also influence the study of biomolecular networks in higher-order organisms. Here, we provide some context for bacterial network biology, highlight some recent successes, and discuss the opportunities and challenges ahead.

Bacterial network biology: a reductionist approach

The notion that genes, proteins and other biomolecules operate in networks is far from a new idea. It can be argued that the study of network biology began several decades ago with the

landmark work of François Jacob and Jacques Monod on the *lac* operon in *Escherichia coli*, the seminal studies of Mark Ptashne on bacteriophage lamba's lytic/lysogenic switch, as well as research on the suite of stress-related genes that comprise the SOS response.

So, what is different now and why all the buzz about the study of biomolecular networks? For starters, we have a new and different cast of characters. In the late 1990s, with the genomes of several organisms sequenced and the parts list of genetic components growing, the genomics community became increasingly interested in understanding how genes and proteins interact in complex cellular networks. The community turned to physicists, engineers and computer scientists, who are trained to deal with complexity and tend to focus on creating innovative methods and models.

This development led to tremendous growth in the number and capacity of high-throughput techniques that contribute vast amounts and varied types of biological data, as well as increasingly powerful computational methods that can use such data to generate and analyze network models. Early studies of prokaryotic networks were bound by the relatively limited methods available at the time. We can now with relative ease, conduct computational-experimental studies that monitor and analyze global cellular responses to chemical, environmental and genomic perturbations, as well as track dynamic cellular processes at the genetic and physical level.

Unfortunately, these enhanced high-throughput capabilities engendered both a misperception that insightful answers will naturally arise from increasingly large datasets and reconstructed networks, and a general disregard for reductionist approaches that have dominated work in genetics and molecular biology. However, more data does not necessarily mean more meaningful biological insights, and much of our present ability to study the interactions between functional groups of biomolecules actually stems from reductionist approaches. Efforts in molecular and cell biology designed to reduce the complexity of physiological observations to the actions of individual biological components have been highly successful at elucidating the sequence, organization and regulation of specific genes, and equally the structure and functional roles of their encoded protein products. Collecting data globally (for example, on a genome-wide scale) is fine and preferable in many instances (Bonneau et al., 2007; Faith et al., 2007), but such data should be analyzed locally in the context of small-scale networks and pathways as, in most instances, that is where the interesting biology happens.

As we discuss next, recent studies in bacteria have shown that systems biology and the study of biological networks can benefit by embracing reductionist approaches and focusing on biological questions.

Recent successes, opportunities and challenges

There is a need to integrate phenotypic and genetic studies with systems analyses to put relevant biological context into network biology. Blueprints for these efforts have been provided in recent studies of asymmetric cell division in *Caulobacter crescentus* and sporulation in *Bacillus subtilis* where, over time, scientists have put together portions of the control mechanisms and networks involved in these processes.

Asymmetric cell division in *C. crescentus* and sporulation in *B. subtilis* are both examples where the timing and location of protein function dictates differential gene expression and the phenotypic outcome. In *C. crescentus*, the asymmetric division process produces a motile swarmer cell and a sessile stalked cell. Critical to this process is appropriate sequestration and activation of the master regulator protein, CtrA, where high concentrations are required in the swarmer cell and low concentrations in the stalked cell. As worked out by Shapiro and McAdams (2003), activation or repression of CtrA leads to modulation of expression of an

integrated set of pathways, including flagellar biosynthesis genes, genes required for cell division, and metabolic and ribosomal genes, which culminate in the formation of two morphologically and functionally distinct cell types.

With *C. crescentus* as well as other bacteria, two-component systems are key signal processing systems that integrate external signals via phosphorelay cascades, stimulating gene expression-based responses from the cell. Laub and colleagues used a systems approach, integrating genetic information with protein data, to show that the phosphorylation and stabilization of CtrA is influenced by two phosphorelays (Biondi et al., 2006). They derived an integrated genetic circuit model for the system, working out the connections between its known components, and importantly were able to show how the circuit's multiple feedback loops can account for cell cycle oscillations of CtrA activity.

Sporulation represents one of the most dramatic changes in cell fate that Gram-positive bacteria can undergo. The developmental decision to sporulate is regulated by both internal and external signals, which are integrated via an exquisite control system under the direction of the master regulator Spo0A (Shapiro and Losick, 1997). Losick and colleagues discovered that during the course of the decision process, *B. subtilis* uses a cannibalistic pathway involving cell-to-cell communication (Gonzalez-Pastor et al., 2003). In this pathway, a cell “thinking” about sporulating can secrete lethal factors that are taken-up by nearby cells, killing them. This enables the cells that secreted the killing factor to use the nutrients of the dead cells and delay the final commitment to sporulation.

This cannibalistic feature of sporulation highlights an important concept in the study of bacterial network biology, namely, that networks functioning in individual bacterial cells often do not operate in isolation but rather as part of a larger community. Accordingly, we need to develop a better understanding of how networks, both at the population and single-cell levels, respond to signal propagation via extracellular factors, such as short peptides and quorum sensing molecules including acylhomoserine-lactones and autoinducers. In the context of pathogenic bacteria, experimental evidence is increasingly pointing toward an expanded role for quorum sensing and cell-to-cell contact in the expression of virulence factors, the establishment of infection, and the formation of biofilms. *Staphylococcus aureus*, for example, uses a peptide quorum signaling molecule for virulence induction. It is also thought that different *S. aureus* strains use these peptides to turn on their own quorum sensing cascades and turn off those in competing strains in a mixed infection setting (Bassler and Losick, 2006). We would benefit from having the ability to predict how “rogue” cell populations emerge, and how signal transduction pathways and gene networks together coordinate the adaptive responses required by opportunistic pathogens to infect susceptible host organisms. New systems-level techniques and approaches are needed to study cell-to-cell communication and single-cell dynamics to gain context-specific insight into community-regulated network function.

The alternative to sporulation during times of environmental stress is for *B. subtilis* to enter into a competence state, in which a small percentage of cells are capable of taking up DNA from their surroundings. The key players involved in the switch to competence have been elucidated, and recent systems-level network studies have provided insight into the dynamics of its intriguing genetic circuitry. Elowitz and colleagues, for example, measured simultaneously in individual cells the activities of promoters involved in the competence decision-making circuit, and used a computational model to analyze the data (Suel et al., 2006). They found that the underlying genetic circuit exhibits excitable dynamics as a result of combined positive and negative feedback loops. Moreover, they showed that this excitable core module coupled with random fluctuations in the levels of its interacting proteins, could account for transient cellular differentiation into the competent state.

The work by Elowitz and colleagues on competence highlights growing interest in studying how heterogeneity arises in a bacterial population and the roles it plays. From a clinical perspective, it is especially important to understand how and why certain sub-populations of cells are able to survive both antibiotic treatment and attack from the host immune system, potentially leading to recurrent infection and antibiotic resistance. Along these lines, persister cells, whose presence in locales of infection have important clinical implications, are an example of a sub-population of cells where the regulatory mechanisms and networks underlying their formation and maintenance remain unknown. Persisters are a small subset of cells that are considered dormant. These dormant cells can survive many types of stressful conditions, including exposure to lethal antibiotics. Following antibiotic exposure, some of these dormant persister cells wake up and can repopulate a culture, potentially contributing to recurrent and chronic infections.

Currently, there are few genes or pathways associated with changes in persister levels. One of the major challenges with studying persisters is that they occur in very small numbers in any given culture, making it difficult to assess changes in gene expression. High-throughput gene expression measurement techniques such as microarrays are useful for studying the average behavior of networks across a population of cells, but less so for looking at the behavior of individual outlier cells. As we develop better means to interrogate single cells (for example, using microfluidic devices and enhanced imaging methods), systems-level approaches will become invaluable for uncovering the networks and pathways involved in persister formation and resuscitation.

With the alarming spread of antibiotic-resistant strains of bacteria, a better understanding of the specific sequences of events leading to cell death from the wide range of bactericidal antibiotics is needed for future antibacterial drug development. Recently, we used a systems biology approach---combining phenotypic and genetic experiments with microarray analyses--- to show that all classes of bactericidal antibiotics, regardless of their specific target, promote the generation of lethal hydroxyl radicals (Kohanski et al., 2007). We demonstrated that the mechanism of hydroxyl radical formation is the end product of a common oxidative damage cellular death pathway involving metabolism-related NADH depletion, leaching of iron from iron-sulfur clusters, and stimulation of the Fenton reaction. We also showed that all major classes of bactericidal drugs can be potentiated by disabling the DNA damage response network (that is, the SOS response), a bacterial system that remediates hydroxyl radical damage.

Much remains to be learned about how bacteria respond to antibiotics. The common mechanism of killing we identified stresses the role of metabolism at the core. It is essential that we better understand how changes in iron homeostasis and metabolic flux affect bactericidal-mediated cell death. This is particularly important given that bacterial infections often occur under unique growth conditions in the body, such as those in the urinary tract where human nitrogen waste products can affect bacterial metabolism. This area of research would benefit from the development of systems biology approaches that integrate metabolic models with transcriptional regulatory network models.

Moreover, we do not yet understand how different antibiotic drug-target interactions trigger a common mode of killing. Network approaches could help us to uncover the class-specific triggers for hydroxyl radical formation. With such knowledge in hand, we may be able to significantly increase the potency of current antibiotics. Additionally, much remains to be discovered from a systems perspective about cellular protective responses induced by antibiotics. Given that free radicals and the SOS response have mutagenic properties, it is possible that bactericidal antibiotics trigger protective, mutagenic, survival responses in treated

bacteria. If a protective-mutagenic response does indeed exist, it would have broad implications regarding current antibiotic use and the emergence of resistance.

Not all bacteria respond in the same way to antibiotic treatment, nor do they prefer the same host infection sites. Comparative network biology will be essential to understanding how species-specific differences in pathways lead to these varied responses and preferences. This could eventually result in the development of species-specific treatments, which could be useful in killing off harmful, invasive bacteria while leaving our normal bacterial flora intact.

More broadly, there is a need to develop a deeper understanding of how bacteria respond to their environment, particularly when challenged. For over 20 years, the SOS response has served as a classic example of a bacterial genomic stress response and as a model system for studies of inducible, autoregulated genetic networks (Friedberg et al., 2006). Because its connectivity is well worked out, it has also served as a model system to experimentally test and validate systems biology approaches for reverse engineering endogenous gene networks (Gardner et al., 2003; Ronen et al., 2002). At its most basic level, expression of the SOS regulon is controlled by the RecA sensory and LexA repressor proteins. LexA presides over a large set of core genes whose function is to deal with DNA damage and return the cell to working order (Friedberg et al., 2006). Various perturbations have been shown to induce the expression of over 100 genes in response to the formation of DNA lesions. Not surprisingly then, the SOS network is quite far from simple in terms of its behavior, and systems-level analyses are yielding surprising new insights. For example, single-cell analyses have shown that the SOS network generates temporally modulated pulses of activity in response to DNA damage, thereby exhibiting digital behavior (Friedman et al., 2005). Interestingly, the frequency and not the amplitude of these pulses was shown to correlate with the degree of DNA damage.

Network-based approaches could also turn out to be quite useful in the systematic characterization and annotation of the lengthy list of bacterial genes of unknown function. Here, the construction of genetic network maps, based on linking phenotypic and biochemical studies to systems-level studies, could enable the prediction of cellular roles based on inclusion in reconstructed, functional networks. The biggest challenge here will be setting up appropriate phenotypic studies to validate the predictions arising from such network studies.

In light of the increasing number of microorganisms identified and characterized as part of numerous concerted sequencing projects, the building of network diagrams will both enable and require the development of comparative network analysis methods, analogous to the comparative sequence analyses of the past decade. Eventually, one might be able to search for meaningful network homologs in the same spirit as one currently searches for gene homologs. Alon and colleagues embraced this notion in a seminal study in which they analyzed the *E. coli* transcriptional regulatory network and discovered recurrent network motifs, that is, patterns of interconnections such as feedforward loops (Shen-Orr et al., 2002). They also studied how information is processed by such motifs, as well as the relationship between evolutionary design and physiological functionality. Subsequent studies identified similar circuit motifs in higher organisms, including microRNA-mediated recurrent network motifs in mammals (Tsang et al., 2007). Work of this sort shows how studies in bacterial network biology can be used to discern design principles of relevance to both prokaryotes and eukaryotes.

Looking ahead to the near future, there are also several biotechnology applications that offer intriguing networking opportunities for bacteria. First, the integration of global physiological and genomic data in biological network reconstruction will no doubt enable microbiologists to better harness the inherent biosynthetic abilities of bacteria, creating more efficient bacterial factories. Systems biologists are well-suited to assist in current ventures that use bacteria for

bioenergy and biomaterial applications, including the large-scale production of biofuels (such as, ethanol, butanol, hydrogen) and biopolymers (such as, bioplastics). Moreover, the growing number of efforts aimed at using bacteria as a cheap, “green” labor force to clean up polluted or contaminated environments will certainly benefit from a deeper understanding of the gene regulatory and metabolic networks that allow certain bacterial species to use oil, radioactive materials or other contaminants as nutrient sources.

Clearly, we are still far from being able to say that we know all there is to know about bacteria. There is much more to be discovered and understood about organisms that exist at the microscopic level. Their story is far from complete, and they have much to offer systems biology.

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