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Editorial overview: Bacterial cell regulation: from genes to complex environments

Jan-Willem Veening and Rita Tamayo

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Scientists, funding agencies, policy makers, and the general public have come to appreciate and embrace the societal and environmental relevance of the microbial world. For instance, in 2014, the first museum dedicated entirely to microbes was opened (<https://www.micropia.nl/>). Microbiology has become a truly interdisciplinary field, and this, combined with recent technological advances in areas such as DNA sequencing, gene editing, and fluorescence microscopy, is revolutionizing our knowledge of microbial life. Recent work on how bacterial cells regulate basic processes such as DNA replication and division and central metabolism reveal remarkable elegance and sophistication in the control mechanisms involved. In addition to these core functions, bacteria have evolved numerous strategies to make the best of their environment. Consider chemotaxis and bacterial exploration towards new nutrient-rich niches, production of nanotubes to transmit information with others, immune evasion of the host they are feeding off, or killing competitors in their environment. In this issue of Current Opinion in Microbiology, we highlight new insights in bacterial regulation in its broadest sense. New developments in understanding bacterial chromosomal organization to regulation of anti-bacteriophage CRISPR systems are discussed. The latest technologies for studying bacterial life that have made these advances possible, such as single cell (dual) RNA-seq, are also discussed. The overall picture emerging from this collection of reviews is that tremendous progress is being made towards our understanding of the molecular basis of bacterial cell regulation, demonstrating the versatility, flexibility, and exquisite regulation that bacteria employ.

Control of fundamental physiological processes: bacterial chromosome organization and transcription

We begin our introduction on the reviews in this ‘Cell regulation’ issue with an update on the current state-of-the-art concerning the organization and segregation of the bacterial chromosome. Structural Maintenance of Chromosomes (SMC) complexes are highly conserved and have fundamental roles in chromosome organization and dynamics. The development of techniques such as chromosome conformation capture (Hi-C) has made it possible to build three dimensional maps of bacterial chromosomes and begin to determine the molecular basis of SMC complex function. The review by [Stephan Gruber](#) [1] highlights recent findings regarding two major classes of SMC complexes in bacteria, SMC-ScpAB and MukBEF. Features that distinguish these SMC complexes, potential models by which they interact with DNA and the roles of protein cofactors, and how they circumvent problems from head-on collisions with other DNA machinery are also discussed.

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Rita Tamayo is an associate professor at the University of North Carolina at Chapel Hill School of Medicine. Her lab studies the mechanisms underlying the ability of facultative pathogenic bacteria to adapt to changing environmental stimuli. Her research primarily focuses on the regulation of virulence genes by nucleotide second messengers in the intestinal pathogens *Clostridium difficile* and *Vibrio cholerae*.

The first regulated step of gene regulation is transcription. *In vivo* transcriptional errors have been estimated to be in the range of 10^{-4} – 10^{-5} [2] while recent studies using nascent elongating transcript sequencing (NET-seq) have shown that up to 3% of all elongation complexes are stalled at one point due to nucleotide misincorporation events in *Escherichia coli* [3]. There are several mechanisms at play to ensure high fidelity of transcription by RNA polymerase (RNAP) and for restarting transcription elongation. [Gamba and Zenkin](#) review recent progress made in our understanding of how transcription fidelity is established [4]. Not only was a ribozyme-like mechanism shown to be involved in intrinsic proofreading activity, a new catalytic domain, the Trigger loop, was also recently discovered to help RNA polymerase guide its use of (correct) substrate and hence enhance fidelity [4].

Advances in understanding regulation of gene expression

Another level of gene regulation involves small RNAs (sRNAs) that target mRNAs to post-transcriptionally regulate their translation or stability. The review by [Kavita et al.](#) describes recent progress in the identification of new Hfq-dependent RNAs, where Hfq binds on those RNAs, and, in some cases, suggesting sRNA-mRNA pairs [5]. These discoveries have been greatly facilitated by the application of modern sequencing techniques such as RNA-seq, CLIP-seq, RIL-seq, and GRIL-seq [6–8]. Interestingly, these approaches have revealed that many sRNAs are encoded within open reading frames, which complicates the ability to distinguish a functional sRNA from an overlapping or parent mRNA. Further, sRNAs can have more than one regulatory function [9] and Hfq itself can be modulated through the action of sRNA ‘decoys’ or ‘sponges’ [10,11], can promote or interfere with Rho-dependent termination events, and may regulate translation of mRNAs independent of an sRNA. These findings underscore that we have only scratched the surface of the potential regulatory schemes involving Hfq, sRNAs, and mRNAs in controlling gene expression.

A very new notion of how cells control gene expression is not by sensing and responding to changes in the environment, but rather by sensing and responding to changes in intracellular metabolic fluxes. This emerging field, stimulated by advances in quantitative metabolomics, is covered by [Litsios et al.](#) [12]. Recent work has shown that key metabolites, such as fructose-1,6-biphosphate, can act as metabolic flux sensors [13] which for instance can drive the entry into bacterial persistence. By interacting with other cellular components such as transcriptional regulators, the concentration of flux-signaling metabolites can relay and control gene regulation, protein activity, or growth [12]. The beauty of using metabolite fluxes as a control mechanism is that it allows cells to respond to the actual metabolic situation inside the cell as opposed to measuring which substrates are available externally. [Litsios et al.](#) argue that using metabolic flux sensing might also be an excellent way to buffer against noise in gene expression and make regulation more robust.

[Rojas and Huang](#) discuss the recent insights that have been made in the century-old concept of growth by osmosis-dependent cell swelling [14]. It turns out that many bacteria make use of the turgor pressure of the membrane against the cell wall to regulate growth and division. For instance, in *Staphylococcus aureus*, cell separation occurs very rapidly, within milliseconds. This process presumably initiates by partial degradation of the cell wall then leading to rapid, turgor-pressure driven splitting of the daughter cells [15,16]. Furthermore, turgor pressure can also drive cell growth and constriction, and [Rojas and Huang](#) speculate that there is interplay between

turgor and septal cell wall synthesis [14]. Future work will shed light on how important turgor is in general for bacterial growth and regulation.

Controlling type IV pili and CRISPR

Looking outside of the bacterial cell, a major area of intense research is the type IV pilus. Type IV pili are appendages on the outside of bacteria that act as nanomotors to generate motile forces. Such pili are involved in twitching motility in for instance *Myxococcus xanthus* but also in DNA uptake in naturally competent bacteria such as *Neisseria meningitidis* [17]. Mignolet *et al.* discuss how the Tad/Cpa system, a type IV pilus machine in *Caulobacter crescentus* important for biofilm formation, pathogenesis, and adhesion, is controlled [18]. This system is a secretion system that is strictly localized at newborn cell poles and is involved in various processes. Recent work has elucidated how the components are regulated such that the pilus is formed only once per cell cycle, at the cell pole of the newborn cell. It turns out that the Tad/Cpa system is cell-cycle controlled at multiple levels by various factors including transcription factors CtrA, CcrM, and GcrA [18].

Although certain bacteria are infamous for causing infection, bacteria themselves are under heavy predation as well from bacteriophages. To counter bacteriophage infection, bacteria have evolved several defense mechanisms including restriction-modification systems and CRISPR (clustered regularly interspaced short palindromic repeats). A diverse set of CRISPR systems has been described, and they all work by binding and cutting non-host DNA or RNA. Leon *et al.* review recent insights gained on how bacteria obtain immunity against bacteriophages and, just as important, prevent auto-immunity due to cutting of their own DNA or RNA [19]. A special focus is given on the post-translational processes that control CRISPR enzyme activity.

Surface and social behaviors

Bacteria can employ multiple distinct mechanisms to migrate along surfaces. Some *Streptomyces* species can escape the well defined cycle between vegetative mycelium formation and aerial hyphae development to undergo a novel process of surface ‘exploration’ [20,21]. Jones and Elliot review recent evidence that exploration behavior is independent of the Bld and Whi regulators that control the classical developmental pathways [22]. They speculate that exploration provides a bet-hedging strategy to safeguard survival if the classical pathways are blocked, for example, by mutations in *bld* or *whi* genes. Interestingly, environmental cues such as glucose limitation and alkaline pH promote exploration. The interaction of Streptomyces with other species, such as yeast that sequester glucose, thus impacts their ability to initiate exploration. In fact, exploration was first observed in

recent work in which *Streptomyces* was co-cultured with yeast [21].

That new properties of bacteria arise during growth in a multi-species community is becoming increasingly appreciated. Madsen *et al.* emphasize the importance of studying bacterial behaviors and phenomena in the appropriate social context, as this will allow ‘community-intrinsic properties’ to emerge (<https://doi.org/10.1016/j.mib.2017.11.018>). The more complex a system/community, the more difficult it becomes to predict the ultimate composition and functions based on the properties of the individual components, or even the interactions of pairs of bacterial species. Examples of community-intrinsic properties are discussed and framed in the context of recent studies of multispecies biofilms. For example, biofilm biomass, architecture, and stress tolerance can vary depending on the combination of species that are incorporated. The impact of community-intrinsic properties is thus relevant to applied, environmental, and clinical microbiology. As interest in the contribution of the microbiome to health and disease increases, it will be important to consider the impact of properties that arise only in the context of the complex microbial (and host) community.

Bacteria in complex communities influence each other’s behavior through the production and depletion of diffusible resources as well as toxic compounds. Yet new mechanisms of bacterial cooperation and competition involving direct interbacterial interactions are coming to light. For instance, Baidya *et al.* review the state-of-the-art concerning a very recently demonstrated mode of intercellular connection, namely bacterial nanotubes [23]. Bacterial nanotubes are membranous channels of varying length that have been observed in both Gram-positive and Gram-negative bacteria. What their exact biological functions are as yet unknown, but Baidya *et al.* speculate that they serve as bridges to exchange cytoplasmic molecules to scavenge for nutrients or even help in communication to build multicellular communities. Key players in nanotube formation, such as the recently described YmdB protein of *B. subtilis*, are discussed.

In recent years, multiple mechanisms of inter-species and intra-species antagonism have been uncovered. Garcia highlights two mechanisms for delivery of a toxic protein effector into neighboring cells upon contact, contact-dependent growth inhibition (CDI) and Type VI secretion [24–27]. Recent work suggests that these processes have functions beyond antagonism. For example, bacteria targeted by a CDI toxin but producing the cognate immunity protein, rather than succumb to the toxin, respond with transcriptional changes as a result of ‘contact-dependent signaling’ (CDS) [28]. The consequent phenotypic changes promote cooperative behaviors among self cells, such as autoaggregation and biofilm

formation. The emphasis on understanding these contact-dependent toxin delivery systems outside the laboratory, in the context of natural biological processes, will surely bring new functions to light.

The importance of examining microbial behaviors in the correct context is further emphasized by Radlinski and Conlon [29]. Not surprisingly, bacteria exhibit different phenotypes in a complex environment (e.g., a host) than in broth culture *in vitro*, and changes in susceptibility to antibiotics are a prime example of this. Indeed, the host environment, including the immune system and the presence of other microbial species, greatly influences the effectiveness of antibiotics — in some cases potentiating and in others inhibiting antibiotic killing. Metabolic determinants such as oxygen and nutrient availability in host microenvironments are also major drivers of antibiotic efficacy. Persister cell populations that survive antibiotic exposure despite lack of resistance appear largely due to metabolic heterogeneity. Radlinski and Conlon underscore the need to consider a pathogen's environment and metabolic state when developing and implementing antimicrobials as therapies.

Of course, the human microbiome and its role in health and disease have been of immense interest to the biomedical research community. Advances in sequencing and bioinformatics analysis strategies, as well as improved culturing methods, have led to the identification of many new species and the correlation of the presence (or absence) of a bacterial species with a particular host status. Neville and Lawley propose a variant of Koch's Postulates that would allow investigators to test hypotheses inferred from microbiome studies, and specifically aimed at attributing health-promoting functions to particular beneficial microbial species [30]. The proposed postulates integrate genomic and metagenomics analyses, culturing and taxonomic identification of candidate species, experimental linkage of the species with health outcome, and recovery of the species from the host with restored health. Ultimately, the implementation of these cross-disciplinary strategies may allow the targeted manipulation of the microbiota to effect the desired improvements in health.

New technologies, new possibilities

An upcoming technique used to interrogate host-microbe interaction is so-called dual RNA-seq [31]. In dual RNA-seq the transcriptome of both host and microbe is examined simultaneously, without the need to physically separate the interacting partners. Wolf and coworkers describe the typical workflow for such an experiment and highlight recent biological insights made using this technique [32]. Notably, Wolf *et al.* argue for better standards in data sharing and urge scientists to not only make sequencing data available but also searchable and reusable.

A recent development and a very hot field at the moment is that of single-cell RNA-seq, and in fact even the first single-cell dual-RNA seq papers are being published [33]. Hen-Avivi and Avraham describe how single-cell RNA-seq can be applied to study host-microbe interactions, and how it can address the issue of variation in infection outcome [34]. Why do some people get sick from infection and others not? Single-cell RNA-seq studies have revealed large variation in the number of different immune cells between individual people, and Hen-Avivi and Avraham argue that this unique immune cell type 'fingerprint' may determine infection outcome. In their review they also provide a guideline on how to setup a single-cell RNA-seq experiment and point to potential caveats in this type of research.

Concluding remarks

This issue features topics in bacterial cell regulation ranging from basic physiologic properties such as chromosome maintenance, to interspecies and intraspecies competitive and cooperative behaviors, to the complexity that arises in multispecies interactions among microbes and also with their hosts. One theme that arises is that much of the progress made in recent years has been facilitated by significant advances in sequencing technologies, which have been adapted in myriad ways to interrogate the genomes and transcriptomes of bacteria in diverse circumstances. It is clear that these and future approaches will offer strategies for the in depth exploration of microbial behaviors and interactions.

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