Big Questions in Microbiology

Putting a Stop to Persisters

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The spread of drug-resistant microbes and spectre of a "post-antibiotic" world have made the fight against antimicrobial resistance (AMR) one of the most important scientific challenges of our time. The threat of AMR has raised critical questions that are profoundly influencing the research agenda in all spheres of microbiology. For instance, the development of new intervention strategies against AMR demands a deeper understanding of host-pathogen relationships and how they evolve during the course of transmission, infection, and disease, as well as insight into the interplay between and among pathogens and other microbes within their host and environmental ecosystems. Likewise, paving the way toward sterilizing therapies that are effective and fast-acting requires that we come to grips with the microbial factors that mitigate antibiotic efficacy, which include, but are not restricted to, persistence and drug metabolism, permeation, and efflux. In the case of bacterial persistence, we need to move from an essentially operational definition of this phenomenon toward mechanistic understanding of the genesis, physiology, and metabolic state of persister cells that can survive prolonged exposure to bactericidal antibiotics and of the role, if any, that drugs might play in this phenomenon. At this time of spectacular technological advancement and revolutionary change in our view of the microbial world, I believe that microbiologists are poised to tackle these questions head on.

The Chemistry of Symbiosis

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Symbiotic bacteria secrete soluble factors, including small molecules, which influence host survival. These represent the language of bacteria as they communicate with each other and with hosts. In recent years, interest has increased as it becomes clear that bacterial small molecules influence human health and disease. However, despite some excellent results, chemical interactions in human and other animal microbiota can be very challenging to accurately assess. How can we robustly move beyond correlation to causation? How can we more readily discover the small molecules that profoundly influence biology, including health and disease? I suggest that a large part of the problem stems from the very aspect that gives the field its strength, its interdisciplinary nature. Because researchers have borrowed from microbiology, medicine, organic chemistry, analytical chemistry, genomics, and others, there is inconsistency in the language and methods used by different labs. It is very difficult for a single investigator to deeply understand all of these fields. One solution is for the field of chemical symbiosis to come together and to set its own standards independent of the patchwork of disciplines on which it relies. Achieving consistency will push the field beyond misleading results that are well known to specialists but that bog down broader understanding of the central importance of chemistry in the microbiota.

V Is for Virosphere

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How large is the virus universe, the virosphere? What proportion of this vast array of viruses cause disease in their hosts? Although these may seem like timeless questions, they have taken on a new importance with the advent of bulk sequencing (metagenomic) surveys of animal and plant populations. Metagenomics also raises new questions about the diversity of genomic structures in viruses, and the evolutionary processes that create them, and may provide a fresh perspective on the factors responsible for the restricted genome sizes that characterize RNA viruses. The vogue for large-scale metagenomics surveys has already revealed that mixed infections may be the norm, although the key question now is whether and how these interactions impact disease ecology and evolution. Yet metagenomics may be less informative in perhaps the highest stakes topic in infectious disease evolution—predicting the next pandemic. Given the vastness and darkness of the virosphere, metagenomic surveys in animal populations will likely tell us little about which viruses may eventually emerge in humans, and we still need a nuanced understanding of the factors, both ecological and genetic, that help or hinder viruses to successfully invade new hosts. A more fruitful approach may be regular genomic surveillance at the frontline of disease emergence, such as live animal markets or other localities that capture the human-animal interface.

Back to the Disease Triangle

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One of the most significant challenges of the 21st century is to discover innovative ways of increasing global crop production to meet the demands for food from the growing human population. A major roadblock to global food sufficiency is persistent loss of staple crops to pathogen infections. Greater efforts are urgently needed to accelerate the buildup of a comprehensive knowledge base that explains how plant diseases occur; how plants defend against microbial pathogens; and how dynamic climate conditions impact plants, microbes, and their interactions.

In 1960, RB Stevens, writing in *Plant Pathology: An Advanced Treatise*, formulated the famous "disease triangle" concept, proposing that plant disease outbreaks require not only a susceptible plant and a virulent pathogen, but also conducive environmental conditions. For practical reasons, however, most contemporary investigations into plant-microbe interactions at the molecular level devote little effort to understanding why climatic conditions, such as humidity and temperature, have a profound effect on pathogen virulence and host susceptibility. Moreover, these studies often ignore the potentially pervasive effect a plant's endogenous microbiome may have on basic plant health and host-pathogen interactions. The classically accepted paradigms used to approach plant-pathogen interactions must now change. Future studies of plant-pathogen interactions (and research funding) should increasingly consider the multi-dimensional nature of "disease-environment-microbiome" interactions that are more reflective of what occur in crop fields and natural ecosystems.

High Tide for Marine Microbes

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There is no better sign that the golden age of marine microbiology is here than the request of this journal to write a short comment about them. Marine microbiology was long considered marginal compared to heavy hitters such as clinical and pathogenic microbiology. But without sea microbes, you would not be able to read these lines. Minute algae in the vastness of the world's oceans produce the oxygen for every second breath you take. Marine microbes make up a staggering 98% of the ocean's biomass, process most of our planet's greenhouse gases, and are rich sources of antimicrobials and pharmaceuticals.

Why did it take so long for us to understand how important marine microorganisms are? We weren't even able to reliably count and identify them until a few decades ago. Now, thanks to advances in imaging and sequencing methods, combined with instruments that enable us to collect samples from the ocean's surface to the deep-sea, we are discovering an unprecedented diversity of microbial life with novel metabolic functions.

What lies ahead? Until recently, we focused largely on interactions between microorganisms and their abiotic environment, but recent research has demonstrated the fundamental importance of the marine microbiome. We are just beginning to recognize that interactions between microorganisms, including viruses, and their symbioses with marine animals and plants, are critical to the ecology and health of all marine ecosystems and our biosphere.

Host and Pathogen Up Close

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New technologies always bring the opportunity to tackle new scientific questions. In this sense, this is an exciting time to study microbial pathogens, given the myriad of technological developments that are almost tailor-made for the study of host-pathogen interactions. The timing could not be more appropriate, as the dwindling pipeline of new antibiotics coupled to the relentless emergence of antibiotic resistance make the study of microbial pathogens more relevant than ever. Host pathogen interactions occur at all scales, from populations and whole body to cells, nanomachines, and individual proteins. Recent advances in imaging technologies are already transforming the study of microbial pathogens at all resolution scales. For example, fluorescence imaging allowing the examination of host-pathogen interactions at the single-cell level, reveals unique biology that studies of cell populations previously missed. Single-particle cryoelectron microscopy is profoundly transforming structural biology, while cryoelectron tomography is providing a detailed view of protein complexes and molecular machines at an unprecedented resolution. Finally, whole-body imaging technologies catch pathogens in action. Although animal models have been invaluable for the study of microbial pathogens, controlled human infections studies are increasingly gaining center stage. It is expected that this type of studies combined with new developments in non-invasive imaging technologies such as MRI and PET will bring a new dawn in infectious disease research.

Harnessing Bacterial Predators

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The recent renaissance in bacteriology has been fueled largely by our fear of antibiotic-resistant strains as well as by our growing appreciation that our microbial companions—our microbiome—are essential to our health. While the threat of antibiotic resistance has challenged us to develop new approaches for killing bacteria, we have also learned that broad spectrum antibiotics, previously thought to be the ideal treatments, may cause severe collateral damage to our microbiota, potentially leading to long-term deleterious effects on the immune system, gut health, and other yet to be discovered processes. Thus, the big question is: how can we target anti-bacterial therapies so that they will kill only the noxious strains without affecting the health of our microbiome? While this is a challenging problem, it has already been solved by bacteriophages, nature's most prevalent bacterial predators. Phages are highly specific in their choice of bacterial host, and the receptor proteins that mediate this specificity are generally identifiable. Coupling these host-recognition machineries to appropriate bactericidal platforms is an approach with great promise for the treatment of bacterial diseases. The ability to specifically target any bacterial strain will also allow us to address fundamental "cause and effect" questions relating to the impact of specific species in the microbiome, something that is currently impossible.

Exploring Microbial Ecosystems

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In 1683, Antonin van Leeuwenhoek observed that "animalcules" from his mouth "were in such enormous numbers, that all the water…seemed to be alive." This appreciation of microbes naturally living in multi-species ecosystems lost favor with the clinical focus on single-agent microbial etiologies of pathogenesis, driven by the pioneering work of Pasteur and Koch in the 1850s. Recent democratization of multi-omics technologies has enabled a renaissance in studying culture-unbiased, real-world microbial ecosystems. In particular, metagenomic sequencing continues to illuminate their vast, unexplored taxonomic and functional diversity. A major bottleneck with interpreting multi-omics data are its analysis, based on principles gleaned from a tiny set of axenically cultured microbes. Remarkably, the functions of nearly a third of the *E. coli* proteome is unannotated, despite this being the most extensively studied microbe. Accordingly, microbiology needs truly disruptive technological innovations to reproduce and manipulate complete, diverse, natural microbial communities in artificial environmental such as soil mesocosms, and living host habitats, for instance experimental mice. We need to enable high-throughput functional characterization of novel predicted genes and pathways, and computationally predict novel functions of genes, pathways, and microbes with sparse prior knowledge from remote homologs. This will enable both derivation of new fundamental basic science insights from multi-omics microbial community data and translation of these into novel microbial therapeutic and biotechnological modalities.

Targeting Bacterial Effectors

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According to an old Chinese proverb, "if you know the enemy and know yourself, you need not fear the results of a hundred battles." This may be a helpful perspective when we pursue new strategies to combat pathogens or develop new types of antimicrobial drugs or vaccines. Gut bacterial pathogens possess sophisticated secretion machinery, including the type III and type IV secretion systems, by which they deliver a diverse array of proteins, called effectors, into the gut mucosa. Thus, the pathogens can subvert host defenses and manipulate cellular signaling for their own benefit. Our gut mucosa has multiple layers of defense against microbial intrusion, but the mucosal barriers are often compromised during bacterial infection, allowing bacterial colonization, and in some cases dissemination into deeper tissues, leading to severe diseases. These bacterial effectors, as well as their host cellular targets, have attracted a great deal of attention, because they are essential for bacterial infection and often share functional similarities in their enzymatic activities. The discovery of small compounds that can specifically inhibit them is a promising approach for creation of new types of antimicrobial drugs. Recent studies have also revealed numerous bacterial effectors that interfere with host immune responses during infection, an important tactic to ensure bacterial survival within the host. Attacking these effectors could help improve live vaccines by eliminating the bacterial arsenal.