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ANNUAL REVIEWS

Annual Review of Microbiology

The History of Microbiology—A Personal Interpretation

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Annu. Rev. Microbiol. 2021. 75:1–17

First published as a Review in Advance on May 11, 2021

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-033020-020648

Keywords

microbiology, ecology, evolution, genetics, biochemistry

Abstract

Microbiology began as a unified science using the principles of chemistry to understand living systems. The unified view quickly split into the subdisciplines of medical microbiology, molecular biology, and environmental microbiology. The advent of a universal phylogeny and culture-independent approaches has helped tear down the boundaries separating the subdisciplines. The vision for the future is that the study of the fundamental roles of microbes in ecology and evolution will lead to an integrated biology with no boundary between microbiology and macrobiology.

Contents

PROLOGUE

As part of the celebration of 75 years of this Annual Reviews journal, the Editorial Committee invited me to contribute a retrospective reviewing the last 75 years of microbiology. I accepted the invitation with a strange mixture of alacrity and hesitation. I very much love history, but I am by no stretch of the imagination a historian. So, I chose to write this personal interpretation of what I perceive as the most critical developments in the history of microbiology. As a consequence, there are many important advances that I do not cover, and for that I ask exculpation. Restriction enzymes, thermostable DNA polymerases, and of course CRISPR all come to mind as microbial discoveries that led to technological revolutions. Those I do not mention. My aim is to describe how thinking about microbes evolved as separate disciplines through most of the twentieth century and how these boundaries have begun to crumble in recent decades. While my emphasis is on developments since the mid-1940s, I first present the early history of microbiology as backdrop.

I would be remiss not to mention that I find myself writing this article in the midst of the coronavirus disease 2019 (COVID-19) pandemic. In less than a year, two revolutionary mRNA vaccines were approved, speaking volumes about the contribution of microbial sciences to human health. This is not the place, nor am I the appropriate author, to cover any of the many aspects of the pandemic. Nonetheless, I am convinced that the course of the history of microbiology will be dramatically altered. Never before in my lifetime have I witnessed so much general interest in science. We should see this as a special opportunity to increase our efforts to understand the workings of the microbial world and to disseminate that knowledge.

MICROBIOLOGY COMES OF AGE

When viewed through the lens of today's lightning-fast developments in science, microbiology took an eternity to come into its own. Two hundred years elapsed between Antonie van Leeuwenhoek's (1) descriptions of *dierken*—little animals or animalcules—and Louis Pasteur's (66) introduction of the term microbe, originally coined by the surgeon Charles Sédillot, into the literature in 1878. The cumulative knowledge that gave birth to microbiology, most of it gained in the second half of the nineteenth century, came from applying multidisciplinary approaches to investigate the diverse microbial activities on the planet. The result was a unified view of microbiology.

This unified view is evident when we analyze the work of three of the most influential early microbiologists: Pasteur, Martinus Beijerinck, and Sergei Winogradsky. All three trained in chemistry, which led them to investigate the underlying chemical mechanisms at work in different microbial systems. From this vantage they were instrumental in developing our early understanding of the many roles played by microbes in phenomena ranging from respiration and fermentation to the biogeochemical cycles of Earth. In addition, all three recognized the complex interplay of microbes with their environments in the settings they investigated (16, 26, 27). They were all pioneers in microbial ecology.

The ecological perspectives that guided these three led them to have remarkably broad interests. Pasteur investigated wine fermentation and its maladies, observed how environmental conditions altered the outcome of silkworm diseases, and developed vaccinations for rabies and anthrax, to mention but a few of his many accomplishments. Beijerinck was equally at home studying the mutualism of nitrogen fixation by bacteria in root nodules or the parasitism of plant infection by tobacco mosaic virus. Winogradsky explored nitrification, discovered chemosynthesis, and invented the columns that bear his name, where the interconnectedness of the diverse metabolic capacities of microbes becomes readily apparent. For all three, such wide ranges of interests came naturally, for they all had a unified worldview of microbiology and recognized the need to study microbes through multidisciplinary approaches. This broad scientific thinking is elegantly summarized in Pasteur's (66) opening sentence of his germ theory essay from 1878: "*Les sciences gagnent toutes à se pretêr un mutuel appui*" (The sciences all benefit from mutual support).

THE FIRST SCHISM—THE BIRTH OF MEDICAL MICROBIOLOGY

The unified worldview implicit in the work of Pasteur, Beijerinck, and Winogradsky, where microbial pathogenesis was very much a part of environmental microbiology, would not last long. The major driver for the coming change in worldview was the work of Robert Koch. A contemporary of Pasteur's and usually at odds with him (80), Koch was trained as a physician and his interests were very much focused on infectious diseases (10). The powerful appeal of Koch's unequivocal demonstration that bacteria were causative agents of important infectious diseases put medical aspects of microbiology at center stage. It is difficult to overstate the impact that Koch's postulates and his development of techniques to obtain pure cultures had on microbiology (10). Knowing that bacteria were causative agents of infectious diseases, long the major cause of human mortality, meant that if you could kill the bacteria responsible, you could cure the disease. By the turn of the twentieth century, microbiology was largely focused on efforts to identify the microbes behind human infectious diseases. The horrendous death toll resulting from the influenza pandemic of 1918 only accelerated those efforts (6). Because identification of the influenza virus would not happen until the 1930s, before which the culprit was thought to be a bacterium, many pathogenic bacteria were identified and intensively studied in the years following the pandemic (81). In that context, studies on the beneficial aspects of microbes were largely cast aside.

Alongside the efforts to identify pathogenic microbes, many microbiologists turned their attention to the discovery of compounds capable of specifically killing those microbes, resulting in the most dramatic revolution in the history of human medicine of the twentieth century. Early efforts by Sahachiro Hata and Paul Ehrlich resulted in the first organic antimicrobial, the rather toxic arsenical arsphenamine (Salvarsan), used to treat syphilis as early as 1910 (83). Protracted efforts to use dyes as antibacterial magic bullets led to the somewhat serendipitous discovery of the sulfa drugs, starting with sulfamidochrysoidine (Prontosil), that went into clinical use during the 1930s (31). But without a doubt, the most influential of the early antimicrobials was penicillin (14). From Alexander Fleming's accidental discovery of penicillin in 1928 to its purification and

effective use by injection in 1942 through the pioneering work of Howard Florey, Ernst Chain, Margaret Jennings, and others at Oxford University, the development of this antibiotic remains one of humanity's greatest accomplishments.

The discovery of a clinically useful microbial product that killed other microbes had a lasting effect on the history of microbiology, of medicine, and of the entire pharmaceutical industry (14). There was an enormous and very successful rush to discover additional antibiotics. The clinical success of antibiotics led to their increased production. This was initially aided by the world war mentality of the early 1940s. Because penicillin was seen as the miracle drug of the war, its production increased dramatically in a very short time. The worldwide stock was a few milligrams in 1941, and by 1945 nearly 4,000 kg were being produced per month (14). Once such a tonnage of antibiotics was available for sale, it had to be sold and widely used. Because of the schism away from environmental microbiology, medical microbiology paid little attention to the ecological consequences of producing and using such vast amounts of antimicrobials. There was precious little conversation between those interested in the means to eradicate pathogenic bacteria and those interested in microbial ecology.

THE SECOND SCHISM—THE BIRTH OF MOLECULAR BIOLOGY

Research on a bacterial pathogen provided a key finding that served as a founding pillar for the development of molecular biology. In 1928, Frederick Griffith (34) reported that avirulent strains of the pneumococcus, now known as *Streptococcus pneumoniae*, could be transformed to become stably virulent strains when coinjected with heat-killed virulent strains into mice. Oswald Avery, Colin MacLeod, and Maclyn McCarty, still coming from the perspective of wanting to understand virulence, set out to purify the so-called transforming principle. Their extensive purification and characterization—the fundamental approaches of biochemistry—led them to the surprising conclusion, published in 1944 (2, p. 156), that "the evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of Pneumococcus Type III." In today's parlance, the evidence pointed to DNA as the genetic material. Perhaps because this work's conclusion was so unexpected—genes were widely believed to be made of protein—and perhaps because the conclusion was so mildly stated, the work was received with a mixture of enthusiasm by some and disbelief by others. As a consequence, the results did not receive the widespread and immediate recognition one might have expected (18). It is particularly surprising that a key player in the early days of molecular genetics, Max Delbrück, did not immediately begin to study the role of DNA in genetics upon learning of Avery et al.'s results. This was likely due, at least in part, to Delbrück's expressed dislike of biochemistry (74).

During the years that Avery and colleagues were purifying the transforming principle, Delbrück became the driving force that led to the birth of microbial genetics. Initially trained as a physicist in Germany, Delbrück had become interested in genes and mutations in the early 1930s (77). Delbrück left Germany for Caltech in 1938, before the onset of World War II, to work on *Drosophila* genetics with Thomas Morgan. To a physicist interested in finding a simple system in which to study how life begets life, the fruit fly proved way too complicated. He turned instead to microbiology. He had already developed an outsider's interest in viruses while in Germany, so it was a perfect match when he met Emory Ellis, who was studying bacteriophages that killed *Escherichia coli* in hopes of understanding cancer. Ellis's choice of *E. coli* appears to have been serendipitous; the bacterium grew fast, it was not fastidious, and, importantly, it was available from one of Morgan's students (76). For a physicist interested in unraveling the mysteries of biological replication, a system where an individual gave rise to a progeny of hundreds in a matter of minutes had to have been a dream come true.

By the early 1940s, Delbrück had formed collaborations with two other phage workers, Salvador Luria and Alfred Hershey. Together they started the famed Phage Group (21). The 1943 Luria & Delbrück (52) paper on the random and spontaneous nature of phage-resistant mutants marks the beginning of microbial genetics. In 1944, Delbrück put forth the Phage Treaty, in decreeing that all phage workers should focus their work on the T phages of *E. coli* so that all experiments could be compared (21). To further ensure standardization of phage experiments, Delbrück started the summer Phage Course at Cold Spring Harbor, New York, in 1945 (20). This very focused phage work proved extremely productive and led to many important insights into the workings of living systems.

By the end of World War II, the stage was set for the work that over the next three decades would lead to a deep understanding of genes and their functions. Molecular biology was born. The key tactic in molecular biology was to mix genetics and biochemistry, the perfect meld of the separate approaches to study genes used by the Phage Group on the one hand and Avery and colleagues on the other. The strong allure of working on the simple and elegant system of *E. coli* and its phages to understand the molecular basis of gene function meant that studies on microbial diversity, ecology, and evolution, took a back seat for a few years.

WHERE ART THOU, GENERAL MICROBIOLOGY?

Taking a back seat did not mean, by any stretch of the imagination, a disappearance. The Delft School of Microbiology, which started with Beijerinck, provided the starting point for much of the general microbiology that was done during the first half of the twentieth century. When Beijerinck retired, in 1921, his position was filled by Albert Jan Kluyver, also a chemist. Kluyver continued the tradition of working with many different microbes. Underlying their metabolic diversity, Kluyver discovered that the reactions of central metabolism were always the same. To emphasize this unity in biochemistry, he coined the phrase "from elephant to butyric acid bacterium—it is all the same" (48, p. 20; 70). This unity in central metabolism served as a springboard from which to determine and compare the metabolic capacities of different microbes. The legacy of Kluyver's work thus also includes the vast knowledge we have of microbial metabolic pathways, a knowledge about which many microbiologists are, sadly, largely ignorant to this day (25).

Kluyver trained many individuals at Delft but none more notable than Cornelis van Niel (4). After spending seven years with Kluyver, Van Niel moved to the Hopkins Marine Station in the Monterey Peninsula in California in 1928. While Van Niel's major contributions surround the topic of bacterial photosynthesis, he was very much a generalist, investigating innumerable areas of microbial physiology. Importantly, his interests in the diversity he observed among microbes generated in him a deep interest in understanding microbial evolution. A necessary step in this was to establish a bacterial phylogeny, a task he stuck with but was not able to complete. His passion for microbiology, along with what he saw as a great ignorance in the United States of the contributions of Beijerinck and Kluyver, prompted Van Niel to organize a general microbiology course, starting in 1930. The course became legendary, every summer attracting a few individuals—among them Roger Stanier, in 1938—who would go on to keep the tradition of general microbiology alive for decades (82). Because of its emphasis on the metabolic diversity of the microbial world, Van Niel's course stands in stark contrast to Delbrück's Phage Course. Both turned out to be highly influential in the history of microbiology, but they could not have been more different.

Thus stood microbiology at the midpoint of the twentieth century. What had started as a unified discipline had separated into the subdisciplines of medical microbiology, molecular microbiology, and environmental microbiology. Sadly, they had little cross communication, a

separation that would remain for at least 25 years. In some ways, this separation can still be felt today. But in many other ways, a reunification that started nearly 50 years ago has been steadily growing.

THE GOLDEN AGE (AND THE DARK SIDE) OF ANTIBIOTICS

The revolution that was triggered by the development of penicillin was greatly amplified by the development of many other antibiotics. The discovery of streptomycin from *Streptomyces griseus*, by Albert Schatz and Selman Waksman in 1943 (85), had two important consequences. First, it generated a worldwide explosion of very productive searches for new natural product antibiotics made by soil bacteria, many of them members of the genus *Streptomyces*. For at least 20 years these searches yielded many new antimicrobials and, importantly, new structural classes of clinically useful antibiotics. This period has rightly been named the golden era of antibiotic discovery. Along with the discovery came the rapid growth of the pharmaceutical industry, as these new compounds went through the required phases to take them from discovery to drug development to widespread clinical applications. Second, streptomycin was shown effective in the treatment of pulmonary tuberculosis through what is recognized to have been the first successful randomized clinical trial (59). While the trial was not double-blind and placebo controlled, it helped define how the efficacy of newly developed drugs would be tested going forward.

In part because medical microbiology had lost contact with environmental microbiology, the ecological consequences of widespread antibiotic use were not taken seriously by the pharmaceutical industry and the medical profession. Since most of the successful antibiotics used have a broad spectrum of activity, once administered they wreak havoc on the microbial communities of the patient (50). This amounts to a scorched-earth attack on microbes. Not surprisingly, soon after new antibiotics are introduced, antibiotic-resistant strains appear (17). Despite claims to the contrary, it is a reality that if an antibiotic is broadly used resistance will be observed. Regardless of the knowledge that antibiotic resistance eventually renders antibiotics useless, antibiotic production and use have increased continually since the early days of the antibiotic era to this day (15).

To make matters worse, antibiotics were not only used, they were quickly abused. In 1945, scientists at Lederle Laboratories (American Cyanamid) characterized a bacterium that produced gold-colored colonies. They named it *Streptomyces aureofaciens* (gold maker), perhaps hoping it would bring in real gold. From *S. aureofaciens* they obtained the first tetracycline, aureomycin (47). At the same time at Lederle, Thomas Jukes and Robert Stokstad assayed the waste product from aureomycin production to determine whether it contained vitamin B_{12}, using recovery of starved chickens as their assay. Amazingly, when chickens were fed the waste product (which contained trace amounts of aureomycin) their growth was greatly accelerated. In 1950, they reported that the inexpensive wonder drug aureomycin added in very small amounts to animal feed increased the growth of farmed animals by 50% (51). Soon thereafter, the practice of using antibiotics as growth promoters became widespread and grew exponentially (15). Unfortunately, despite many efforts to end it, the practice continues to grow (94).

The overuse and abuse of antibiotics constitute the very dark side of these astonishing drugs. Precise numbers on the amounts of antibiotics produced worldwide are hard to come by, as companies need not make those numbers public. Current estimates suggest that 100,000 tons are produced per year, with more than half used for growth promotion in animal husbandry (15). Since the majority of the antibiotics pass through human or animal, it is no surprise that they end up in soil and water and become what amounts to an ecological catastrophe whose global effects we are only beginning to appreciate (67). What is clear is that the spread of antibiotic resistance has reached critical levels (15). So much so that many are already forecasting a post-antibiotic era (68).

MOLECULAR BIOLOGY REVEALS THE INNER WORKINGS OF THE (*E. COLI*) CELL

A quick look at the progress made in molecular biology from 1947 to 1976 leaves one in absolute wonderment. Science went from a state of uncertainty as to the nature of the gene to a rather complete understanding of the molecular underpinnings of replication, transcription, translation and the basics of how these processes are regulated. Most of the advances were the result of a coming together of bacterial genetics (the legacy of Delbrück and the Phage Group) and biochemistry (exemplified by the purify-and-characterize approach of Avery), with critical contributions from biophysics. At the center of it all was *E. coli* and its phages.

The resistance of many scientists to accept Avery's results slowly began to erode. Many budding biophysicists were attracted to the idea of the genes being DNA, not least because William Astbury, who had done early X-ray crystallographic analyses on DNA in the 1930s, had been positively impressed (35). By 1950 even several members of the Phage Group began to toy with the idea that phage injected only their DNA into the cell to generate new phage (18). In 1952, Hershey and Chase reported on an experiment designed to test this idea by differentially labeling phage proteins with ^{35}S and DNA with ^{32}P. The results of the now classic Hershey-Chase experiment suggested that the DNA entered the cell while the protein stayed outside (37). The results quickly gained acceptance as evidence that DNA was the substance of genes.

By the time the Hershey-Chase paper was published in 1952, two groups in the United Kingdom were hard at work on the structure of DNA, one at King's College in London and one at Cambridge University. The events that transpired between 1951 and early 1953, when the structure of the double helix was published, are the stuff of legends and have been written about and analyzed in extensive detail (46, 53, 86). They remain an excellent case study in the conduct of science and are worth studying by scientists of all ages. Head of the Medical Research Council Biophysics Unit at King's College, John Randall exercised poor management form by miscommunicating with Rosalind Franklin and Maurice Wilkins as to how and by whom X-ray crystallographic studies were to be conducted, creating a terrible working environment (53). Despite this bad situation, Franklin obtained superb diffraction images of DNA fibers (32). One of them, the now famous Photo 51, was given to Francis Crick and James Watson, at Cambridge University, without Franklin's knowledge. The photograph, along with a site visit report that contained many of Franklin's calculations (also obtained surreptitiously), allowed Crick and Watson to put the finishing touches on their model for the structure of DNA (87). Franklin died in 1958 without knowledge of how her results had been used in building the model. What was perhaps the most important discovery in the life sciences of the twentieth century is thus tainted, though by no means diminished in its importance.

In hindsight, it is easy to imagine that a single look at DNA structure would reveal the mechanisms of DNA replication and protein synthesis. Nothing could be further from the truth. But the structure certainly gave all the incentive necessary to tackle those problems with all the tools available. In less than a decade, the general mechanisms of replication and protein synthesis were worked out.

"It has not escaped our notice that the specific pairing we have postulated suggests a possible copying mechanism for the genetic material" (87, p. 737). That is probably the best-known single sentence in the double-helix paper. The proof that the mechanism of DNA replication is indeed semiconservative stands as one of the most elegant experiments of that era (58). Matthew Meselson, a graduate student, and Frank Stahl, a postdoc, both at Caltech, showed this by labeling the DNA with a heavy, stable isotope of nitrogen by growing *E. coli* with $^{15}NH_4Cl$. Subsequently, they shifted the cells to a medium containing the light isotope ^{14}N. They separated DNAs of different densities using equilibrium density gradient centrifugation. Both DNA strands from the starting

culture were heavy. After one cycle of replication, all the DNA had shifted to an intermediate density, consistent with each new strand having been made with the light isotope. Importantly, after the second round of replication, half of the DNA had both strands made from the light isotope while the other half of the DNA was still of intermediate density. These results were most consistent with a semiconservative mode of replication. Their results, published in 1958, were dubbed "the most beautiful experiment in biology" (40). In a wonderful testament of the lasting friendship of Meselson and Stahl, their recollections were recorded in a 2020 interview that is a must-watch for all (57).

After the proof of the semiconservative nature of the replication process came the long quest to identify the enzymes involved. Two decades went by before the subunits of the replicase were all identified and then placed in the context of an even more complex replisome (3). A key take-home message of how this was accomplished is that the most productive approaches made use of conditional mutants that were temperature sensitive for replication (38). Cell-free extracts of these mutants were used to develop in vitro replication systems with single-stranded phage DNA as a template. These systems could be complemented by extracts from wild-type cells as a way to purify the missing component. In the following decades, such powerful melding of genetics and biochemistry proved extremely useful in characterizations of other basic cellular processes, e.g., building of the cell envelope, protein secretion, and protein turnover.

Understanding how the information contained in DNA is processed to yield proteins became a critical question soon after the DNA structure was proposed. In 1954, the cosmologist George Gamow proposed a process whereby protein synthesis occurred on the surface of the DNA through a key-and-lock mechanism where each amino acid would act as a key that specifically fit into one of the 20 holes or locks possible from the base pairs in the double helix (33). Crick dismissed the idea based on prior evidence from eukaryotic cells that protein synthesis occurred in the cytoplasm while the DNA was in the nucleus. Still, Gamow's concept that the sequence of bases in the DNA contained the protein sequence information was readily accepted.

Most of the basics of how DNA is transcribed into mRNA by RNA polymerase and how the mRNA is translated into protein at the ribosome, plus deciphering of the genetic code, were published in 1961, a remarkable year for molecular biology. Ribosomes were discovered and described as the sites of protein synthesis in eukaryotic cells in 1955 (64), and the existence of tRNA was reported in 1958 (39). But by the end of 1960, almost no more details had been published. Then, in a short spurt of advances, all the pieces of the puzzle fell into place. Based on the genetic analyses of phage mutants, Crick along with Leslie Barnett, Sydney Brenner, and Richard Watts-Tobin (23) showed that the genetic code is a triplet code, the triplets do not overlap, the code contains no commas, and each gene sequence is read from a specific starting point. Independently, Marshall Nirenberg and Heinrich Matthaei showed that ribosomes translated poly-U into poly-phenylalanine (62). Within a very short time, the Nirenberg group, as well as Gobind Khorana's group, determined virtually all of the genetic code (46, 61). Concurrently, insights gained from genetic experiments suggested to Brenner, François Jacob, and Meselson the existence of and ways to identify mRNA (11). They were not alone in discovering mRNA; several other groups working independently arrived at similar results through completely different routes (19). In addition, three groups working independently purified and characterized the DNA-dependent RNA polymerase (43).

The advances made on the subject of regulation of gene activity crowned all of the other achievements published in 1961. While many systems were investigated, there is little doubt that the most influential work on the subject came from François Jacob and Jacques Monod. By applying genetic analyses to study the induction of β-galactosidase activity in *E. coli*, they opened up a whole new world of how genes are turned off and on (65). In particular, by 1961, they published their landmark paper presenting their accumulated genetic evidence on how the lactose-utilization

genes were regulated in *E. coli* (45). Therein, they proposed their operon model, wherein multiple genes encoding enzymes were regulated by a repressor gene acting on an operator gene. Their ideas proved to have a long-lasting and extremely strong influence on how molecular biologists approached the studies of gene regulation.

Fittingly, Jacob and Monod were asked to write the closing comments on the collection of papers compiled in the 1961 volume of the *Cold Spring Harbor Symposium on Quantitative Biology* (60). A quick look at the table of contents of that volume makes it clear why 1961 can be designated as an annus mirabilis for molecular biology (89). Still, Jacob and Monod's work stood out as particularly special. In their closing comments, when addressing the possibility of the universality of the mechanisms of gene expression and its regulation, they wrote a phrase that is often attributed to Monod: "anything found to be true of *E. coli* must also be true of Elephants" (60, p. 393). While they do not cite him, this was definitely an elaboration on Kluyver's statement on the unity of biochemistry. Now, Jacob and Monod proclaimed the unity of molecular genetics.

Throughout the following 60 years, to the present day, focused studies on model organisms have led to remarkable advances in our understanding of the molecular biology of bacteria. These successes with model organisms helped attract many who were studying bacterial pathogens to take on molecular genetic approaches. Greatly due to the influence of the studies of Stanley Falkow and many of his trainees, microbial pathogenicity is now largely viewed through the lens of molecular biology to understand the roles that virulence factors play in infection and disease (29, 30). Thus were medical and molecular microbiology unified.

The wealth of new molecular knowledge is most certainly awe inspiring. Yet, standing alone, these approaches can easily lead one to lose sight of three critical and inseparable aspects of all of life: the metabolism that maintains the living state, the ecological context in which an organism lives, and how evolution plays out in that context. It is very easy, in the midst of enthusiastically pursuing molecular mechanistic studies, to lose sight of the concepts so beautifully summarized in the titles of three important writings seldom read by molecular biologists: *Bacterial Metabolism* (1969), by Horst W. Doelle (25), *The Ecological Theater and the Evolutionary Play* (1965), by G. Evelyn Hutchinson (44), and "Nothing in Biology Makes Sense Except in the Light of Evolution" (1973), by Theodosius Dobzhansky (24).

BACK FROM THE ASHES, MICROBIAL ECOLOGY RISES AGAIN

At the time that work on *E. coli* garnered more and more attention for its contributions to molecular biology, those interested in the broader question of the evolutionary relationships among microbes were in a state of crisis. From Kluyver's work, two important principles emerged: not only the unity of biochemistry but also the concept of comparative biochemistry, which led to the unraveling of the metabolic pathways underlying much of microbial life (48). That had led him and Van Niel, in 1936, down the path of establishing a "natural system of classification" of bacteria, that is, one that reflected their evolutionary relatedness, their phylogenies (49). But by the 1950s, frustrated by the seeming impossibility of establishing evolutionary relationships for bacteria, Van Niel disavowed it and stated that, in his opinion, such attempts were a waste of time (69). In its stead, Stanier and Van Niel (72) proposed—in their landmark 1962 paper, "The Concept of a Bacterium"—the prokaryote-eukaryote distinction. While it was based on cellular organization, it quickly was adopted as a phylogenetic distinction (69). Prokaryota (or Monera) was a kingdom defined by the lack of a nuclear membrane, chromosomes, mitosis, meiosis, mitochondria, etc., as if these traits would evolve later along a single time line. It should not surprise us that for those steeped in molecular biology in the 1960s and early 1970s there could "be little doubt that the simpler prokaryotes are the evolutionary antecedents of the more complex eukaryotes," as

Gunther Stent wrote in his 1971 text *Molecular Genetics* (73, p. 43). Evolutionarily, present-day prokaryotes were seen as living fossils, the primitive precursors of protists, fungi, plants, and animals. Evolution was seen as going from less complex and less diverse prokaryotes to the much more complex and diverse eukaryotes. Importantly, the prokaryote-eukaryote dichotomy offered no path to quantitate the evolutionary relatedness between organisms. But a powerful new approach to determine such relationships was already emerging, sequence comparisons.

The idea of using sequence comparisons to establish phylogenies was around before there were sequences to compare. In 1958, Crick presciently alluded to how comparing protein sequences would reveal "vast amounts of evolutionary information" (22, p. 142). Within a decade, molecular evolution got its start by comparing sequences of cytochromes (54) and hemoglobin (95) to derive phylogenies. Realizing the power of this approach, Stanier changed his perspective and saw the possibility of using it to establish a bacterial phylogeny (69). However, it would not be Stanier but a relative outsider who would first establish a molecular phylogeny of bacteria and, in so doing, turn the prevalent worldview of the natural history of life on Earth on its head and establish a universal phylogeny.

Carl Woese was one of the many physical scientists who became deeply immersed in studying protein synthesis in the early 1960s. Unlike many of his contemporary colleagues, he was primarily interested in the evolution of translation and the genetic code. His approach was to arrive at a molecular phylogeny of the translation machinery by comparing sequences of a component of the ribosome. Recall, however, that at the time there were no sequences for ribosomal proteins or RNAs. He brilliantly chose to compare sequences of the small ribosomal subunit RNA (16S rRNA in bacteria) to arrive at phylogenetic distances. There was no DNA sequencing at the time, and RNA sequencing was an arduous task. Taking on the job of obtaining sequence information for numerous rRNA molecules of 1,500 bases was a Herculean effort. In fact, the determination of the complete 16S rRNA sequence was not achievable at the time; only catalogs of oligonucleotides of known sequence could be obtained and then compared. Despite all the difficulties, by the mid-1970s Woese and colleagues (91) obtained and compared oligonucleotide catalogs from more than two dozen rRNAs. The results proved to be earth-shattering. In their landmark 1977 paper, Woese and George Fox (90) reached the conclusion that methanogens, long believed to be bacteria, were as different from bacteria as they were from eukaryotes. Some immediately recognized the significance of the results, but others, particularly molecular biologists and evolutionary biologists, did not (69). Woese and Goldenfeld (92) make this evident in their essay recollecting the entire process, "How the Microbial World Saved Evolution from the Scylla of Molecular Biology and the Charybdis of the Modern Synthesis."

Woese and colleagues continued their efforts with newfound determination over the next 13 years. Improvements in sequencing and gene isolation greatly accelerated the process of sequence gathering and comparisons. In a 1990 paper with Otto Kandler and Mark Wheelis, Woese presented the universal phylogenetic tree, with three domains: Bacteria, Archaea, and Eukarya (93). While it took some years for this new worldview to sink in across all of biology, its implications for our understanding of the evolutionary process were monumental. The universal phylogeny lent strong support to a common origin of all life and confirmed Lynn Margulis's endosymbiont hypothesis (63). The evolution of members of all three domains of life continued; no longer were prokaryotes viewed as evolutionary antecedents of eukaryotes. And, of particular interest for microbial ecology, the vast majority of the sequence diversity on Earth was in the microbial world.

The universal tree of life beckoned microbiologists to come explore its mysteries. Soon, there was an added incentive that made that exploration even more attractive. Vigdis Torsvik, Jostein Goksøyr, and Frida Daae took an approach to assess microbial diversity that was radically different

from what had been done before. Rather than cultivating bacteria present in soil, they extracted the DNA and assessed its complexity. Their results were astounding. The soil DNA they analyzed contained at least 4,000 different genomes per gram of soil (78), a previously unimagined diversity.

The technique Torsvik and colleagues used, cot analysis, relied on reannealing kinetics of denatured DNA and was developed in the 1960s by Roy Britten and David Kohne to analyze repeated sequences in eukaryotic genomes (12). One has to wonder why it took more than 20 years for someone to consider analyzing the complexity of environmental DNA directly, instead of assessing diversity only through cultivation. The question is very relevant because for decades investigators had noted the "great plate count anomaly" (71, p. 327), the orders-of-magnitude difference between the numbers of cells observed through the microscope and the numbers of colony-forming units in environmental samples. The lack of communication across disciplines certainly had to play a role in this decades-long delay.

Norman Pace and his colleagues were among the first of a new breed of intrepid explorers. They chose Yellowstone National Park as the site for their explorations. They used PCR to amplify small-subunit rRNA gene sequences from DNA extracted directly from hot spring sediment. They then cloned the products and sequenced them. Remarkably, in a single hot spring they found more archaeal diversity than had been found in all the previously cultivated archaea (5).

Once environmental microbiologists became aware of the riches waiting to be discovered through culture-independent approaches, there was a massive rush to explore every corner of the biosphere. The new approach generated great enthusiasm even among those previously not involved in microbiology. Witness the closing words of Edward O. Wilson (88, p. 364), preeminent ant ecologist, in his autobiography, *Naturalist*: "If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist. Ten billion bacteria live in a gram of ordinary soil, a mere pinch held between thumb and forefinger. They represent thousands of species, almost none of which are known to science. Into that world I would go with the aid of modern microscopy and molecular analysis. I would cut my way through clonal forests sprawled across grains of sand, travel in an imagined submarine through drops of water proportionately the size of lakes, and track predators and prey in order to discover new life ways and alien food webs."

By 1997, when Pace published his landmark review "A Molecular View of Microbial Diversity and the Biosphere," the culture-independent exploration of the planet was in full force, yielding novel insights regarding the roles of microbes in myriad environments (63). However, one environment remained largely unexplored, the human body. Except for a few limited studies, the human body was not surveyed by culture-independent methods during the 1990s. Twenty years after Woese discovered Archaea and nearly ten years after the inception of culture-independent molecular phylogenetic approaches, the schism between medical and environmental microbiology remained as strong as ever.

REUNIFICATION: IT'S ALL ENVIRONMENTAL MICROBIOLOGY

Where were all those investigators interested in studying the human host and its interactions with microbes during the last twenty years of the twentieth century? Were they not reading the papers from the budding field of microbiomes? Maybe, maybe not. No doubt, the very successful approach of studying host-pathogen interactions with model systems and pure cultures kept many of them focused on their ongoing research. One of the earliest reports describing the high diversity of the human gut microbiota using culture-independent methods was published in 1999 (75). Perhaps because its senior author, Joel Doré, had trained as an environmental microbiologist, this pioneering work went relatively unnoticed for several years. All of this changed—surprisingly late, but change it did. Better late than never.

Fifteen years after Torsvik described high bacterial diversity in soil, a widely read paper describing high microbial diversity in the human gut by Paul Eckburg, David Relman, and colleagues appeared, in 2005 (28). The work was an eye-opener to many, and it triggered an explosion of research on the human microbiota. Soon several laboratories that had been either working on model microbes in pure culture or using them in animal models shifted to work on different aspects of the human microbiota, using culture-independent approaches. This shift coincided with dramatic improvements in sequencing methodologies and increases in computational capacity. The topic gathered much interest from scientists and the public in general such that in 2007 the US National Institutes of Health started the US$170-million, ten-year-long Human Microbiome Project (79). The pendulum swung completely, and much has been learned in the process. Despite the abundance of hyperbole and widespread inappropriate interpretations of causality (36), there is a lot of wonderful work linking particular microbial community composition to functionality (7, 9). A radical departure from the Kochian view of microbes, this new work fully embraces ecology.

This increased interest in understanding the interactions between humans and their associated microbial communities has had a profound, and certainly very positive, impact in microbiology as whole. Ecologists, evolutionary biologists, chemists, physicists, computational biologists, and others have come into the field as their expertise has become essential. There is now a renewed excitement around the study of microbes and their vast diversity of metabolic pathways with the recognition that all ecosystems have microbes at their most foundational level. Ecological and evolutionary principles now guide the study of microbes. Whether one is studying the human gut or frozen lakes in Antarctica, it is all environmental microbiology.

There have been so many exciting new developments in this unified New Microbiology during the last 15 years that it is impossible to present them all here. Recent history is still in the making, and it is the most difficult to assess; others will cover those developments much better than I. I've chosen to close this retrospective with brief mentions of three events that I think provide a guide to the future of this unified microbiology and that have stimulated me greatly.

First, Laura Hug, Christopher Brown, Jillian Banfield, and collaborators provided us with a new view of the tree of life (13, 42). They discovered the Candidate Phyla Radiation, 15% of the domain Bacteria with precious few cultured representatives. That there is so much out there that we still don't know much about is both mind-boggling and utterly exciting: "If I could do it all over again. . . I would be a microbial ecologist." (88, p. 364).

Second, in 2014 Martin Blaser published *Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues* (8). As we get nearer the possibility of entering a post-antibiotic era and we continue to produce obscenely huge quantities of antibiotics, Blaser brings forth an ecological perspective not so much on the problem of resistance but on the pervasive damage that producing and abusing antibiotics is having on human health. It is inspiring to have witnessed the dramatic change in the author. As someone who for decades contributed beautifully to our understanding of pathogens, he grew seamlessly into an avid defender of microbial diversity.

In a similar vein, the influence that Margaret McFall-Ngai is having on microbiology is awe-inspiring (84). McFall-Ngai points out that she was trained as an animal physiologist. From that perspective, she has explored the *Vibrio*-squid symbiosis with Ned Ruby (55). That system, however, has served as a beginning of very broad vistas, and for at least the last 15 years, she has elaborated on her views on the unity of biology, where all animal (and I would add all plant) life occurs in a microbially dominant Earth (56). As a consequence, McFall-Ngai is pushing for what I consider is the key aspect of the future of education in the life sciences, a curriculum based on the ecology and evolution of the biosphere that integrates microbiology and macrobiology into a unified systems biology (41).

It is all biology!

EPILOGUE

Doubtless some readers will find the foregoing historical narrative deficient in that it neglects countless discoveries of importance to microbiology. Yes, I admit to such lacunae. But I suggest we all need to gain some perspective on which events lead to major changes in thinking. For that I found the words from Matt Meselson, paraphrased from his conversation with Frank Stahl, a most useful guide: "The way I think of it is that there is a river, which is a period of time when there are fundamental questions to be solved. . . . When these problems are solved, there are lots of little rivulets. The river divides into thousands of branches, using these fundamental insights into how life works and applying them to specific questions" (57). I have described what I see as rivers, not rivulets.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I am deeply indebted to Mechas Zambrano for our extensive discussions on the subject (usually during our evening forest walks with the dogs) and her critical reading of the manuscript.

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