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GUEST COMMENTARY

How the Microbial World Saved Evolution from the Scylla of Molecular Biology and the Charybdis of the Modern Synthesis

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There must be no barriers for freedom of inquiry. There is no place for dogma in science. The scientist is free, and must be free to ask any question, to doubt any assertion, to seek for any evidence, to correct any errors.

—J. Robert Oppenheimer, *The Open Mind*, p. 114 (1955)

This is the story of how biology of the 20th century neglected and otherwise mishandled the study of what is arguably the most important problem in all of science: the nature of the evolutionary process. This problem has suffered the indignity of being dismissed as unimportant to a basic understanding of biology by molecular biology; it went effectively unrecognized by a microbiology still in the throes of trying to find itself; and it became the private domain of a quasi-scientific movement, who secreted it away in a morass of petty scholasticism, effectively disguising the fact that their primary concern with it was ideological, not scientific. Despite this discouraging beginning, our story will end well: the study of the microbial world at the beginning of the 21st century is liberating biology from the Procrustean bed of dogma on which it has been cast for so long, and a new understanding of evolution as a process is already beginning to form, in a manner that will eventually supersede the scientifically stultifying language-culture of the 20th century.

The volume of this very journal that you are now reading—*Microbiology and Molecular Biology Reviews* (MMBR)—is one of the reasons for our optimism about the future of the science of evolution: for it is microbiology and molecular biology, so long unfairly divided from their proper evolutionary context, that are sowing the seeds of biology's next and greatest flowering. And what is to us especially exciting and meaningful is that the emerging understanding of evolution is being, to a large extent, catalyzed by microbiology's return to its roots in microbial ecology, as long ago articulated by Beijerinck (6c). In short, we stand at a juncture in science that is relatively rare: two fields of study, at an impasse and exhausted after what we regard (without disrespect to their practitioners) as a long search for purpose, find their resolution, meaning, and rejuvenation in one another.

Such an unusual convergence has happened before in science, and it is worth reflecting on the circumstances of this lest the reader, having being lulled into a sense of complacency by the marvelous achievements of 20th-century biology, is puzzled by our stance. We are referring to the famous speech delivered to the Royal Institution of Great Britain by Lord Kelvin (William Thomson) on 27 April 1900 entitled *Nineteenth-Century Clouds over the Dynamical Theory of Heat and Light* (29). At that time Kelvin could reflect with satisfaction on the numerous triumphs in the field of physics that took place in the 19th century: Dalton's atomic theory as the foundation of chemistry, providing the basis for a theory of matter, and connecting, via Maxwell and Boltzmann's statistical mechanics, to a recognition that heat is a reflection of the motion of atoms or molecules; the discovery of the conservation of energy and the brilliant deduction of the concept of entropy; the discovery of the phenomena of electromagnetism and the mathematical synthesis that yielded the prediction of radio waves and explained finally the nature of light. By the turn of the century, a feeling of "mission accomplished" infected physics, and sentiments concerning the near-completeness of the understanding of physical law had been expressed on a number of occasions (6a). All the great laws that structure the natural world were in place. From the standpoint of this panorama of transformative accomplishments, Kelvin famously told his audience that "The beauty and clearness of the dynamical theory, which asserts light and heat to be modes of motion, is at present obscured by two clouds." In choosing this analogy, Kelvin implied a contrast between the expanse, beauty, and clarity of a wide-open sky and the blemish of only two clouds, which do not detract from the big picture. The only job left for physicists was in finishing off details in the great new structure that had been erected—no small job, but one that could be accomplished without need of any more grand developments on the part of theory. The Newtonian worldview had attained its apotheosis and stood complete and triumphant astride the border of the 19th and 20th centuries.

Nevertheless, Kelvin himself clearly thought the clouds worthy of his attentions; he attempted to dismiss one of them, but of the other, after a partially successful attempt to resolve it using classical concepts and ad hoc assumptions, he remarked that it was still "very dense." Today, we know that Lord Kelvin's clouds presaged a storm and were certainly neither

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ephemeral nor easily dismissed. The first cloud was the Michelson-Morley null experiment that failed to detect the ether and led to Einstein's special theory of relativity. The second cloud, the one that Kelvin dismissed, was the apparent violation of statistical mechanical principles in the heat capacity of gases, an observation that was only explained by quantum theory. Together, these two clouds led to a complete and unimaginable transformation of the physical sciences that required almost a century to bring to fruition. Kelvin's clouds were the harbingers of physics to come, of radical fundamental developments at the base of the physical sciences, which would shake the Newtonian foundations of a world solid in its materialism, certain in its deterministic reductionism, and safe in its completeness. While all this is relatively well known, what is not so frequently emphasized is how shocking it must have been for Kelvin to learn that after a century of truly groundbreaking discoveries, there was still so much more to do. Just as a mountaineer after a steep and arduous climb is at once dismayed but exhilarated to find him- or herself not on the summit, but only on a shoulder, so it was with 20th-century physics at the time of Lord Kelvin's address.

And so it is with biology today.

Although 2009 will be marked by a plethora of celebrations on the subject of evolution, most of the attention is being bestowed on the personalities and historical circumstances surrounding the theory of natural selection, as if this and its synthesis with genetics in the first decades of the 20th century marks the culmination of the theory of evolution. It does not. The MMBR community has been at the forefront of defying the standard wisdom; and thus it is, in many ways, its story that we now wish to tell.

The microbial world: lost and found. For the first hundred years of the discipline's existence, microbiology struggled, first with establishing a technology sufficient to deal with the problems the study of the microbial world would present and second with the foundational issue any new science faces, establishing a proper axiomatic base, i.e., parsing the venue—defining the proper categories, selecting the pertinent facts and key concepts that will serve as the foundation of the science. A proper "language" in which the discipline can work must be established.

In microbiology's case, the foundational issue has persisted almost to this day. Microbiology has been a scientific itinerant throughout most of the 20th century—always an applied science in one way or another, at times liege to biochemistry or to genetics and molecular biology. It has served well as a venue within which to work out some of the major problems in these fields.

What is seldom recognized is that at the beginning of the 20th century, microbiology was on a path that, if followed, would have resolved its foundational issue and made the study of the microbial world very different from what it was, one that would have led microbiology to confront both sum-of-the-parts molecularism and the petrified form of evolution that emerged from the modern synthesis. But the wandering course it did follow meant that microbiology contributed little to the conceptual development of 20th-century biology.

The path not taken was the microbiology of Beijerinck, arguably the greatest microbiologist in the discipline's history. When asked, on the occasion of his being presented with the Leeuwenhoek Medal—microbiology's highest honor—in 1905, to describe his view and practice of microbiology, Beijerinck said

> [My] approach can be concisely stated as the study of microbial ecology, i.e., of the relation between environmental conditions and the special forms of life corresponding to them. It is my conviction that . . . this is the most necessary and fruitful direction to guide us in organizing our knowledge of that part of nature which deals with the lowest limits of the organic world, and which constantly keeps before our minds the profound problem of the origin of life itself. (Reproduced from the translation by van Niel [30].)

A century before its time, Beijerinck is outlining the microbiology of today—an integrated microbiology that synthesizes organism, ecology, and evolution. Beijerinck's was not a reductionist view of the microbial world. He realized that the organism cannot be understood apart from the ecology, the community structures in which it is found. He understood that the organism does not define the organismal community—if anything, the reverse. And he understood that the microbial world was an invitation to the study of the origin of life and its evolution.

But Beijerinck's microbiology was not to be. His successor, Jan Kluyver, perhaps overly influenced by the progress in biochemistry that was gaining momentum at the time, was to put the discipline on the reductionist track, treating microorganisms effectively as bags of interesting biochemistry. It is indeed unfortunate that Beijerinck's view did not survive him. Kluyver's microbiology would become that of a sum-of-the-parts biochemist. To Kluyver and his school the organism was a secondary consideration at best. Organisms, like pinatas, were there to be burst open in order to get at the (biochemical) goodies within—a view of microorganisms that, with justification, persists today among some subfields of microbiology.

By midcentury, microbiology's situation had come to an impasse. The discipline clearly had a foundational issue. Biochemistry was simply not sufficient to serve as the basis upon which the study of the microbial world could rest. Neither the organism nor its ecology could be satisfactorily encompassed by this reductionistic perspective—an objection that could also be raised against all the sum-of-the-parts disciplines, such as molecular biology and genetics.

The insufficiency of a biochemical foundation for microbiology was given voice by R. Y. Stanier in 1962, when he and coauthor C. B. van Niel (26) declared microbiology's foundational issue to be "an abiding scandal"—a scientifically unfortunate way to call attention to a foundational issue. The authors identified the issue as the "absence of a clear concept of a bacterium" and added ". . . the problem of defining these organisms as a group in terms of their biological organization is clearly still of great importance, and remains unsolved."

The authors then went on to solve the problem they had posed, the solution being the famous "prokaryote" hypothesis. This notion, that all bacteria shared a common cellular organization, was to stand firmly in the path of microbiology's development for the better part of half a century. Moreover, Stanier explicitly tied the assumed common cellular organiza-

tion to an implied common ancestry (1), writing that "All these organisms share the distinctive structural properties associated with the procaryotic cell . . . and we can therefore safely infer a common origin for the whole group in the remote evolutionary past."

This dogmatic approach to the essence of microbiology would shunt development away from the problem of the nature of cellular organization, which Stanier and van Niel had solved by fiat. We refer the interested reader to the detailed historical scientific account of these developments provided in this journal by Sapp (23).

Stanier and van Niel's assertion that all bacteria were prokaryotes was an eminently testable hypothesis—the only problem being that neither the authors nor microbiologists in general perceived it that way. It was "the answer," the solution to microbial biology's foundational issue. Within a decade of its announcement, Stanier could proudly say that the "prokaryote" had become a "truism" in, was an axiom of, microbiology (25).

The test of the hypothesis came about as a result of a research program that one of us (C. R. Woese) had set in motion to determine the universal phylogenetic framework. Woese's motivations for this at the time are perhaps best captured in a letter written to Francis Crick, dated June 24, 1969. In that letter, Woese writes

> If we are ever to unravel the course of events leading to the evolution of the prokaryotic (i.e., simplest) cells, I feel it will be necessary to extend our knowledge of evolution backward in time by a billion years or so—i.e., backward into the period of actual "Cellular Evolution". There is a possibility, though not a certainty, that this can be done by using the cell's "internal fossil record"—i.e., the primary structures of various genes. Therefore, what I want to do is to determine primary structures for a number of genes in a very diverse group of organisms, on the hope that by deducing rather ancient ancestor sequences for these genes, one will eventually be in the position of being able to see features of the cell's evolution—i.e., by knowing what features of the primary structures are "locked-in", what regularities (repeats, etc.) existed, and how one ancient primary relates to another ancient primary structure(s) [sic] (which gave rise to some different cellular function).

We have quoted from this letter at length for two reasons in addition to its intrinsic historical interest. First, there is the clearly articulated intention to see what major groupings of life existed beyond the eukaryotes, in an open-ended but precise way. By this we mean that the outcome of this research program was not predetermined by a prevailing set of views on the question, but the fact that there would be an actual outcome was predetermined, as long as the technology of molecular biology was adequate and the scale of the enterprise was not unachievable. Note also that Woese found it necessary to be more explicit to Crick about the objects of his investigation than the term "prokaryote" implied. Second, as we will expand upon below, Woese foresaw the unique possibilities for molecular biology, through Sanger sequencing specifically, to address questions about the evolutionary process. For Woese, molecular biology was more than just a tool to expose the workings of the cell machinery.

By the end of the 1970s, Woese's program to explore the evolutionary process had yielded results, in effect providing the first molecular test of the prokaryote hypothesis (33). The answer was unequivocal. There were indeed many fundamental molecular properties that distinguished the eukaryotes from all bacteria. But there were also many fundamental molecular properties in which the bacteria (declared to be synonomous with prokaryotes by Stanier and van Niel) differed from one another. The "archaebacteria" (*Archaea*) had finally appeared on the scene, and their molecular properties were as distinct from those of the "eubacteria" (*Bacteria*) as either one's were from those of the *Eukarya*. The prokaryote hypothesis had been proven false.

We now know, of course, that the prokaryote hypothesis was wrong not just at the molecular level: subsequent developments, especially in Germany, clearly showed that there is no common cellular organization for the organisms that Stanier and van Niel termed prokaryotes (7, 13, 15, 34). Nevertheless, this has not prevented microbiologists, by and large, from basing their thinking implicitly upon this wrong-headed hypothesis/concept. Fortunately, the "prokaryote era" is fast drawing to a close, and many microbiologists (especially the younger ones) are no longer strictured in their thinking by the prokaryote notion.

Liberated from the distraction of the foundational issues, microbiology has become enamored once again with microbial ecology, a move that was catalyzed by the recognition that the same molecular techniques that finally put to rest the phylogenetic issues could be deployed in the environment. The results have been nothing short of breathtaking. Indeed, we have undergone a revolution in biology that is fully comparable to the Copernican revolution in astronomy and the very recent and ongoing revolution in observational cosmology. We now know that what astronomers used to think of as "the Universe," the visible universe, is less than 4% of the total matter/energy density of the universe, the rest being made up of the still-mysterious dark matter and energy. Similarly, we now understand that in terms of both numbers and genetic diversity, the microbial world not only dominates the biosphere but is almost impossible to sample properly. This point is, of course, even more emphatically made if one includes in this calculation the virosphere, which we regard as an intrinsic aspect of the microbial world, not to be separated from it. The discovery and ongoing quantification of the rare biosphere has not only exacerbated the problem but leads on to the fundamentally unresolved question: is there a meaningful concept of species that can be applied to the microbial world? (11).

As in all good stories, the cliffhanger is where there is a change in narrative perspective; and so we turn now to our other spectacular success story, that of molecular biology.

Molecular biology: not even lost. It is difficult to imagine that the discipline which defined biology in the last century—that taught us so much and provided such benefit to the ambient society—is fundamentally flawed. But that is the case. Molecular biology expressly established itself within the (classical) Newtonian worldview. As such, its perspective was fundamentally reductionist. In other words, all things were explainable,

completely and solely, as the sum of their various parts—which also meant that they could (in principle) be predicted a priori.

In this Newtonian world, the study of biology becomes a highly derived subdiscipline of the basic science of physics—in effect, an engineering enterprise; there is nothing "fundamental" about it. Biology becomes a study of machines made of assemblages of parts and the interactions among them, an exercise in describing, but not explaining, things as they are.

However, it is intuitively obvious that the essence of biology lies not in things as they are, but in things coming into existence. Biology is a study, not in being, but in becoming. For us, the fascination of the subject derives from the astounding fact that life exists at all and has developed such remarkable complexity. A discipline whose perspective is that of classical 19th-century physics is inherently incapable of dealings with the problems of a nonlinear world, which is nonreductionist, nondeterministic (acausal), and works in terms of fields and emergent properties, not a static world of particles with linear relationships among them.

It is one thing to hold a perspective in principle, another to apply it in detail. Thus, in the early decades of the 20th century, molecular biology's fundamental reductionist perspective was innocuous—especially when there were many problems that could benefit from a (simple) reductionist approach. It was another thing altogether when molecular biology began reconceptualizing biology in an exclusively reductionist fashion. Then the inadequacies of reductionist metaphysics began to show. The major wrong turn in biology's course was its conceptualization and subsequent handling of the problem of the gene. It would come to a point where the discipline had to choose between the obvious biology of the situation and the tenets of reductionism. Molecularists choose the latter, thereby taking off the table a major biological question.

It began simply enough, with the problem of "biological specificity." Was this category simply an olio of unrelated phenomena each having a separate, unrelated explanation, or did "biological specificity" represent a fundamental tendency in the process that evolves biological organization? Molecular biologists saw it in the former sense, focusing on the specificity manifest by biological systems at the molecular level, for example, by antibodies or enzymes. A reductionist analysis—in terms of "complementary molecular surfaces"—came easily, and just as easily the tacit assumption that biological specificity was a (group of) problem(s) in the here and now, not an evolutionary one.

In 1940, Pauling and Delbruck (22) had generalized the molecular notion of biological specificity to include not just "pockets" of specific recognition ("hand in glove" was a common metaphor at the time) but surfaces that recognized one another, and, further, molecular surfaces that recognized and aligned monomer units in preparation for their polymerization. Under the term "templating," Pauling and Delbruck's concept became commonplace in biology and served as the framework within which to formulate the problem of the gene—its structure, replication, and expression.

The grand triumph of the templating notion came with the Watson-Crick double-stranded structure of the gene, a structure that displayed, in a most obvious fashion, the mode by which a gene becomes replicated. Thus, the hereditary aspect of the gene was nicely explained in the molecular reductionist framework—identical copying resulting from complementary copying of each DNA strand. The essence of replication lay in stereochemical interactions (i.e., base pairing) so simple that there was no need to seek a role for an evolutionary process in the mechanism of heredity.

The next major issue was the problem of translation—the essence of gene expression. After the publication of Watson and Crick's masterpiece (31a, 31b) it was naturally tempting to look for the solution again in templating. George Gamow (12) thought he could see "pockets" in the double-stranded structure of DNA that could serve somehow as the required template, specifically recognizing the amino acid(s) and aligning them for polymerization. So started what became known as the "coding problem."

For Gamow, a remarkably creative physicist whose works included the prediction of the cosmic microwave background from his theory of the Big Bang and the theory of alpha-decay by quantum tunneling, the true biological problem was probably of little concern; he was intrigued by the "coding problem" imbedded therein, i.e., explaining by means of a simple theoretical conjecture why there were 64 potential codons (the number of different trinucleotide sequences) used to encode exactly 20 different amino acids in the synthesis of the cell's proteins; if there were a simple enough mathematical rule behind the numerology of getting 64 things into 20 categories, that might provide a clue as to the nature of the mechanism that performs cellular translation—just as the Watson-Crick base-pairing rules had been the key to the mechanism of gene replication. What was a theoretician's divertissement for Gamow, however, became a major turning point in the history of 20th-century (molecular) biology.

Francis Crick took exception to Gamow's model (though not to the theoretical numbers game that was being played [9]), and proposed (F. Crick, unpublished data, and reference 8) that nucleic acids were incapable of recognizing amino acids (or anything that wasn't itself nucleic acid—which, incidentally, denied the possible existence of what we today know as the "RNA world"). In this way, Crick had effectively cut the direct link between the worlds of biology and physical chemistry provided by the templating concept. Without such a simple stereochemical ingredient, it would seem unavoidable that only a complex explanation would suffice, one in which the evolutionary process would be playing a fundamental role. However, Crick had an ingenious work-around to restore the simplicity inherent in the templating concept: the "adaptor hypothesis" (Crick, unpublished, and reference 8). Instead of direct (specific) recognition of the amino acid by the nucleic acid, each amino acid would be fitted to a corresponding adaptor, which then required a corresponding set of enzymes that could specifically attach these adaptors to their respective amino acids. It was the adapted amino acids that would be used in the templating mechanism for protein synthesis. Crick's original proposal envisaged that the adaptors would be a few nucleotides in length, although the discovery of the tRNAs showed that this was not the case: the tRNAs were large complex macromolecules. At this point it should have been evident that the entire machinery was an evolved entity, right at the heart of the gene expression process.

To summarize, we are drawing a distinction between a pure templating process, consisting of recognition and alignment,

and an evolutionary process that involves a much more complex operation. In the case of translation, this means the "tape reading" machinery in the ribosome and its cohort of molecules. The important point is that in a pure templating process, stereochemical binding suffices to explain what is going on, whereas the more complicated process of the real world operates in a way that transcends such a simple molecular picture and requires evolutionary considerations to account for the complexity. We have dwelt on this point because in templating, evolution is a peripheral consideration: it does not play a defining role, and its influences can safely be regarded as a problem that is not of overriding importance. Something as simple as templating cannot, and need not, evolve. One can understand the machine by looking at its current state. However, the actual situation is very different: the evolution of the translational machinery is a central problem and requires an evolutionary description at the level of the entire system of translation. In short, the dichotomy is between evolution as enhancement versus evolution as essence.

Almost no one seemed to notice the shift in the concept of the gene at the time. Nobody saw that the solution to the real problem of the gene did not lie in the here and now. Instead, it lay in the emergence of an incredible and complex mechanism that can extract information (pattern) from the sequence of one type of macromolecule and "express," i.e., store, most of it as the structure (sequence pattern) of another macromolecule of a different type. This process thus gives rise to a new world (space) of macromolecules and possible interactions among them. Moreover, this process of encoding can continue to higher and higher levels of organization (6b), eventually giving rise to cells as we know them. By turning its back on the evolution process (note that we do not say the "fact of evolution"), molecular biology steadfastly refused to entertain this world of emergent properties, of organization whose nature is fundamentally evolutionary and unpredictable a priori. Now the essence of the gene came to lie not in the genotype-phenotype relationship (the process linking them); it lay in the gene (the genotype) alone. Translation, now ancillary to the gene, was relegated to the catchall category of "historical accidents"—molecular biology's convenient way of dismissing the products of an evolutionary process. The translation apparatus was just one of many macromolecular "historical accidents" awaiting its turn to be described (but not explained) when molecular technology reached the point of being able to do so.

This turn in the road (of applying reductionist metaphysics to the understanding of the biological world) would become a superhighway that dead-ended before it reached molecular biology's ultimate goal, that of understanding the essence of "livingness" and directly answering the question of how molecules come to life.

Evolution: not even on the map. Throughout the last century, evolution has been, if you will, "the sick man of science." We have seen that molecular biology, the dominant biological discipline of the time, did not even recognize the evolutionary process as a scientific problem. Given its overview (axiomatic assumptions), molecular biology took evolution simply as biological epiphenomenology, "historical accident"—which means that evolutionary considerations have no bearing whatsoever on any fundamental understanding of living systems.

On the other hand, 20th-century microbiology did not have a concept of evolution. Indeed, for most of the century, the discipline didn't even have a proper understanding of itself! Microbiology was defined by those problems for which microbial systems were useful, be they practical (e.g., medical) or in the service of the better-developed scientific disciplines such as biochemistry and molecular biology or otherwise. Until relatively recently, microbiology's concern with evolution never went beyond the stage of idle speculation.

As for evolution, it had been developed from a phenomenological description centering around what was generally termed natural selection into the modern evolutionary synthesis through its union with Mendelian genetics. The modern evolutionary synthesis should have been the 20th century's evolutionary bastion, the forefront of research into the evolutionary process. No such luck!

The basic understanding of evolution, considered as a process, did not advance at all under its tutelage. The presumed fundamental explanation of the evolutionary process, "natural selection," went unchanged and unchallenged from one end of the 20th century to the other. Was this because there was nothing more to understand about the nature of the evolutionary process? Hardly! Instead, the focus was not the study of the evolutionary process so much as the care and tending of the modern synthesis. Safeguarding an old concept, protecting "truths too fragile to bear translation" is scientific anathema. (The quote here is Alfred North Whitehead's, and it continues thus: "A science which hesitates to forget its founders is lost" [32].) What makes the treatment of evolution by biologists of the last century insufferable scientifically is not the modern synthesis per se. Rather, it is the fact that molecular biology accepted the synthesis as a complete theory unquestioningly—thereby giving the impression that evolution was essentially a solved scientific problem with its roots lying only within the molecular paradigm.

There you have it. An entire century spent studying biology without seriously addressing evolution, without assigning importance to the study of the evolutionary process. Our understanding of biology, of biological organization, far from being near complete (as molecularists would have us believe), seems still in its infancy. And what about fundamental progress elsewhere? Microbiology had reached a dead end in its uninspired search for a proper, "natural" taxonomy (which it desperately needed). Molecular biology was at a dead end (but didn't know it) in its attempt to understand the gene (having failed completely with the problem of "gene expression"). Few appreciated that both microbiology's foundational issue and molecular biology's conceptual failure resulted from the inability to see that an evolutionary conceptualization was required to resolve them. Twentieth-century biology did not effectively appreciate that biological organization is an evolutionary problem—and that it cannot be fundamentally understood unless formulated accordingly, as flowing form.

Evolution: the road to recovery. Molecular biology's untenable position vis-à-vis evolution could not go unrecognized forever. The irony of it was that it was molecular biology itself, in the form of Fred Sanger's development of macromolecular sequencing technology, which exposed the flaw in its position and would eventually tip the balance.

At first, nobody seemed fully to appreciate the ramifications

of this revolutionary methodology. Molecular biologists, of course, thought they did: they would use it to sequence every macromolecule of interest and thereby complete the "parts list" of the living cell, which would, in turn allow them to put paid to the problem of the nature of the cell—or so they thought. It is doubly ironic then that it was the ingenious Francis Crick, the staunchest of molecular reductionists, who first pointed out the phylogenetic potential in Sanger's method. In 1958, Crick had dropped an incidental bombshell in a review article he wrote about the status of the problem of translation. It went as follows:

> Biologists should realize that before long we shall have a subject which might be called "protein taxonomy"—the study of amino acid sequences of proteins of an organism and the comparison of them between species . . . these sequences are the most delicate expression possible of the phenotype of an organism and . . . vast amounts of evolutionary information may be hidden away within them (8).

He may have said it, but did he appreciate the statement's full import? We shall never know. All that we do know is that Crick continued undeterred down the path of the molecular gene—looking at translation as one of those historical accidents, interesting as a machine but not fundamental to understanding biology (in the way the gene itself was).

Crick's early insight into the evolutionary power of molecular sequencing was rediscovered in the mid-1960s by Zuckerkandl and Pauling (36), and this time the message proved influential. The classical phylogenetic tree, the inference of which had a somewhat circular quality to it, could now be confirmed—indeed, both tested and extended—by data that had no part in the classical determination of the relationships between the taxa. Comparative studies of molecular sequences of molecules such as cytochrome *c* and hemoglobin came to confirm the classical phylogenetic tree, reinforcing it with steel, as it were. But the molecule that would emerge as first among equals for purposes of phylogenetic inference was rRNA. Ribosomes existed in large numbers in all self-replicating cells; the rRNA was relatively simple to extract. But best of all, as the central molecule in the translation apparatus, rRNA was also central in the organization of the cell; it was functionally constant, relatively immune to the vagaries of evolution, and its sequence changed relatively slowly over evolutionary time. It was as near perfect an "evolutionary chronometer" as one could wish for. Indeed, in the 1969 letter to Crick, Woese would write

> The obvious choice of molecules here lies in the components of the translation apparatus. What more ancient lineages are there? A priori it seems impossible to evolve any structural gene without the capacity to translate the gene—making the evolution of some rudimentary translation machine necessarily a very early happening. Hopefully that machine was a direct lineal ancestor (both functionally and structurally) of the present one. Also, I feel (and you may too) that the RNA components of the machine hold more promise than (most of the) protein components. For example, how can we get activating enzymes—as we now have them—without a good deal of evolution, and how can these evolve without translation, etc?

The rRNA was able to span and connect all the branchings in the tree of life. This was the first time biologists could begin to glimpse the large-scale structure of the evolutionary world. The discovery of the *Archaea*, the fact that there were two, not one, primary lineages of microorganisms, was now there for all to see.

And this was merely the beginning. For not only was the evolutionary world revealed for all to see, but the ecological world, the biosphere, also became accessible to molecular techniques. Norman Pace would seem to be the scientist who first recognized and experimentally developed the idea that genetic sequences relieved (molecular) evolutionists of the necessity of confining their studies to organisms in the zoo—which, in the case of microorganisms, meant organisms that existed in pure laboratory culture. Genes, he proved, could be extracted directly from the environment; the need to capture the organism in which they resided was eliminated. Through the studies that branched from Pace's original work, the biologist's world has exploded again (21). It is beyond the scope of this article to do full justice to the ramifications of these ideas for microbial ecology. Suffice it to say that with the inclusion of molecular techniques, microbiology is now fully realizing Beijerinck's original vision for the subject.

The future of evolution. Perhaps there is no clearer demonstration of the hollowness of the modern evolutionary synthesis in its claim to be a full account of the evolutionary process than the unfortunate circumstances of its encounter with microbiology. For we can trace the beginning of the end of the modern evolutionary synthesis to the moment when microbiology, molecular biology, and the evolution process first collided, in the years following the classic experiment by Avery, Macleod, and McCarty (6) on pneumococci that first provided evidence that DNA was the hereditary material of bacteria. This result was one of the direct motivations for the program that led to Lederberg and Tatum's discovery of bacterial conjugation (19) and Zinder and Lederberg's subsequent discovery of transduction (35), thus constituting the key mechanisms of what we now call horizontal gene transfer. Here was a manifestly nonclassical genetic phenomenon, and one whose evolutionary implications were soon to become apparent once this genetic mechanism was demonstrated to be capable of transferring drug resistance between different bacterial species (2, 31). However, the power exerted by the modern evolutionary synthesis meant that the implications were not generally noticed for another 30 years (albeit with some notable exceptions [3, 4, 24, 28]). This particular discovery sheds light not only on the failure of the modern evolutionary synthesis, but also on the failure of microbiology itself. Reflecting on his discoveries many years later, Lederberg (18) would write "In later years, I came to wonder why such simple experiments as came to fruition in 1946 had not been concocted, say in the wake of the rediscovery of Mendel's laws in 1900. That might have set microbiology ahead by a half century."

Lederberg was so intrigued by the way in which his discoveries had not been made earlier that he went on to analyze this failure in considerable detail, broadening the whole question into what he and Zuckerman termed "postmature discovery"

(37). They attributed part of the failure explicitly to the power of dogmatic thinking in science, writing "Thus the work by Avery et al. made the question of bacterial sex newly consequential. Dubos makes it clear that had sexual reproduction been observed, it would have been understood and appreciated. But bacteria were so widely assumed not to reproduce sexually that no one considered this problem to be important. Dogma prevailed over focused curiosity."

Dogmatic thinking has prevailed all too often in our account, with disastrous consequences for the progress of the fields of microbiology, molecular biology, and the study of the evolutionary process. It led to the stagnant and scientifically invalid notion of the prokaryote; it led to the redefinition of the problem of the gene; and through a slavish adherence to the modern evolutionary synthesis, it led to a premature declaration of victory in the struggle to understand the evolutionary process.

Today, we know that horizontal gene transfer is a powerful evolutionary force in the microbial world, well-documented in the phylogenetic record, and one whose ecological significance is only beginning to be fully understood. Spurred on by advances in genomic technology, microbial ecology is presenting new insights into the workings of the biosphere, demanding a synthesis with the evolutionary process, and forcing evolutionary biology to pay attention (11, 27). The power of horizontal gene transfer is so great that it is a major puzzle to understand why it would be that the eukaryotic world would turn its back on such a wonderful source of genetic novelty and innovation. The exciting answer, bursting through decades of dogmatic prejudice, is that it hasn't. There are now compelling documentations of horizontal gene transfer in eukaryotes (17), not only in plants, protists, and fungi, but in animals (including mammals) as well (5, 14, 16, 20). The evolutionary implications have not yet been worked out, but we are confident that a fully worked out theory of the evolutionary process is required in order to properly meet the challenges posed initially by microbiology.

And so we end this story on an uplifting note of optimism. These are exciting times to be a biologist. Genome technology, growing out of molecular biology, is providing an unprecedented microscope into organisms. The study of evolution is poised to cast off a century of dogma and to become a true science, fully integrated with discoveries that owe their roots to microbiology and molecular biology. It is time for biology to put its past behind and begin rethinking the discipline's future. It can no longer afford to keep the study of evolution within the narrow confines of the so-called modern evolutionary synthesis. If 21st-century biology is to remain a basic discipline, it must focus on the nature of biological organization, which means biology must now be put on an evolutionary base. Biological organization will never be understood except as the expression of an underlying evolutionary process. As biology becomes a science in the true meaning of the word, it must become self-conscious of the structure of the only theoretical foundation that can unify our understanding of it: the evolutionary process. Only in this way will biology ever become more than a disconnected collection of facts.

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REFERENCES

1. **Adelberg, E. A., J. L. Ingraham, and R. Y. Stanier.** 1963. The microbial world, 2nd ed., p. 409. Prentice-Hall, Englewood Cliffs, NJ.
2. **Akiba, T., K. Koyama, Y. Ishiki, S. Kimura, and T. Fukushima.** 1960. On the mechanism of the development of multiple-drug-resistant clones of *Shigella*. Jpn. J. Microbiol. **4:**219–227.
3. **Anderson, E. S.** 1966. Possible importance of transfer factors in bacterial evolution. Nature **209:**637–638.
4. **Anderson, N. G.** 1970. Evolutionary significance of virus infection. Nature **227:**1346–1347.
5. **Arnold, M. L.** 2006. Evolution through genetic exchange. Oxford University Press, New York, NY.
6. **Avery, O. T., C. M. MacLeod, and M. McCarty.** 1944. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J. Exp. Med. **79:**137–158.

6a. **Badash, L.** 1972. The completeness of nineteenth-century science. Isis **63:**48.
6b. **Barbieri, M.** 2003. The organic codes: an introduction to semantic biology. Cambridge University Press, Cambridge, United Kingdom.
6c. **Beijerinck, M. W.** 1921–1940. Versamelde Werken, vol. 1–6, p. 168–169. M. Nijhof, The Hague, The Netherlands.

7. **Cavicchioli, R. (ed.).** 2007. *Archaea*: molecular and cellular biology. ASM Press, Washington, DC.
8. **Crick, F. H. C.** 1958. On protein synthesis. Symp. Soc. Exp. Biol. **12:**138–163.
9. **Crick, F. H. C., J. S. Griffith, and L. E. Orgel.** 1957. Codes without commas. Proc. Natl. Acad. Sci. USA **43:**416–421.
10. **Fisher, M., B. G. Spratt, and J. T. Staley (ed.).** 2006. Species and speciation in micro-organisms. Philos. Trans. R. Soc. London B Biol. Sci. **365:**1897–2053.
11. **Frigaard, N. U., A. Martinez, T. J. Mincer, and E. F. DeLong.** 2006. Proteorhodopsin lateral gene transfer between marine planktonic *Bacteria* and *Archaea*. Nature **439:**847–850.
12. **Gamow, G.** 1954. Possible relation between deoxyribonucleic acid and protein structures. Nature **173:**318.
13. **Garrett, R. A., and H. P. Klenk.** 2007. *Archaea*: evolution, physiology, and molecular biology. Blackwell Publishing, Malden, MA.
14. **Gladyshev, E. A., M. Meselson, and I. R. Arkhipova.** 2008. Massive horizontal gene transfer in bdelloid rotifers. Science **320:**1210.
15. **Graham, D. E., R. Overbeek, G. J. Olsen, and C. R. Woese.** 2000. An archaeal genomic signature. Proc. Natl. Acad. Sci. USA **97:**3304.
16. **Hotopp, J. C. D., M. E. Clark, D. Oliveira, J. M. Foster, P. Fischer, M. C. M. Torres, J. D. Giebel, N. Kumar, N. Ishmael, and S. Wang.** 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science **317:**1753.
17. **Keeling, P. J., and J. D. Palmer.** 2008. Horizontal gene transfer in eukaryotic evolution. Nat. Rev. Genet. **9:**605.
18. **Lederberg, J.** 1996. Genetic recombination in *Escherichia coli*: disputation at Cold Spring Harbor, 1946-1996. Genetics **144:**439–443.
19. **Lederberg, J., and E. L. Tatum.** 1946. Gene recombination in *Escherichia coli*. Nature **158:**558.
20. **Pace, J. K., C. Gilbert, M. S. Clark, and C. Feschotte.** 2008. Repeated horizontal transfer of a DNA transposon in mammals and other tetrapods. Proc. Natl. Acad. Sci. USA **105:**17023.
21. **Pace, N. R.** 1997. A molecular view of microbial diversity and the biosphere. Science **276:**734.
22. **Pauling, L., and M. Delbruck.** 1940. The nature of the intermolecular forces operative in biological processes. Science **92:**77–79.
23. **Sapp, J.** 2005. The prokaryote-eukaryote dichotomy: meanings and mythology. Microbiol. Mol. Biol. Rev. **69:**292.
24. **Sonea, S.** 1988. A bacterial way of life. Nature **331:**216.
25. **Stanier, R. Y.** 1970. Some aspects of the biology of cells and their possible evolutionary significance, p. 1–38. *In* H. P. Charles and B. C. J. G. Knight (ed.), Organization and control in prokaryotic and eukaryotic cells, 20th ed. Cambridge University Press, Cambridge, England.
26. **Stanier, R. Y., and C. B. van Niel.** 1962. The concept of a bacterium. Arch. Mikrobiol. **42:**17–35.
27. **Sullivan, M. B., D. Lindell, J. A. Lee, L. R. Thompson, J. P. Bielawski, and S. W. Chisholm.** 2006. Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol. **4:**e234.
28. **Syvanen, M.** 1985. Cross-species gene transfer; implications for a new theory of evolution. J. Theor. Biol. **112:**333–343.
29. **Thomson, W. (Lord Kelvin).** 1901. 19th century clouds over the dynamical theory of heat and light. Philosophical Magazine **2:**1–39.
30. **van Niel, C. B.** 1949. The "Delft School" and the rise of general microbiology. Microbiol. Mol. Biol. Rev. **13:**161–174.
31. **Watanabe, T.** 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27:**87–115.

31a. **Watson, J. D., and F. H. C. Crick.** 1953. A structure for deoxyribonucleic acid. Nature **171:**737–738.
31b. **Watson, J. D., and F. H. C. Crick.** 1953. General implications of the structure of deoxyribonucleic acid. Nature **171:**964–967.
32. **Whitehead, A. N.** 1967. The aims of education and other essays. Free Press, New York, NY.
33. **Woese, C. R., and G. E. Fox.** 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. USA **74:**5088–5090.
34. **Woese, C. R., and R. S. Wolfe (ed.).** 1985. The bacteria, vol. 8. Archaebacteria. Academic Press, Inc., New York, NY.
35. **Zinder, N. D., and J. Lederberg.** 1952. Genetic exchange in salmonella. J. Bacteriol. **64:**679–699.
36. **Zuckerkandl, E., and L. Pauling.** 1965. Molecules as documents of evolutionary history. J. Theor. Biol. **8:**357–366.
37. **Zuckerman, H., and J. Lederberg.** 1986. Postmature scientific discovery? Nature **324:**629–631.