The Birth of the Archaea: a Personal Retrospective

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Dedicated to Wolfram Zillig:
A founder of the archaeal revolution.

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Let There Be Light

For me the moment was, I believe, the afternoon of June 11, 1976. I had just taped the film of a primary "Sanger pattern" to a back-lit translucent "wall" in the lab and had begun to "interpret" the pattern in terms of the "secondary cuts" taken from it, the corresponding films of which were lying on a huge light table directly beneath the "primary"; the object being to infer the sequences of all the oligonucleotides (of significant length) in the primary pattern. Except for this eerie lighting arrangement, the room was fairly dark, with the only prominent features being the pattern of back-lit black spots on the "primary" film and the corresponding transluminated lines of black "sub-cut" spots on the "secondary" film lying below.

The spots on the "primary" film represented specific oligonucleotide fragments into which a (radio-labeled) 16S rRNA (ribosomal RNA) had been cut by T1 ribonuclease, then subjected to a two dimensional paper electrophoretic separation, with the resulting oligonucleotide "spots" detected by means of X-ray film (Uchida et al., 1974). The "isopleth" pattern on the film of the "Sanger pattern" (Sanger et al, 1965) already revealed a great deal about the sequence(s) of the oligonucleotide(s) in the individual spots; for instance, the length of oligo, number of uracil residues it contained (a primary determinant of the overall structure of the isopleth pattern), and the C (cytosine) *vs*. A (adenine) contents of the individual spots in each in an isopleth (Sanger et al, 1965). [Each oligonucleotide had but one G residue, at its 3' end, the cut site of ribonuclease T1 (Sanger et al, 1965)].

My job was to determine the complete sequence of every oligonucleotide of significant length (five or more nucleotides) in the primary pattern, which required the aforementioned "secondary" patterns. These in turn were created by removing little snippets of paper at the appropriate places in the corresponding original electrophoretogram and further digesting the oligonucleotide(s) therein (*in situ*) with one or a few ribonucleases of different cutting specificities than that of T1 RNAse (thereby creating sub-fragments). These enzymatically treated snippets were then individually reinserted (mashed) into a very large sheet of (DEAE cellulose) paper (about 30 of them in a line near the "bottom" of such a sheet). Each large "secondary" sheet was then subject to one dimensional electrophoresis to resolve the sub-fragments in each of the thirty-odd secondary digestions from one another. From the one or several "secondary" cuts taken from a primary spot, the exact sequence of the oligonucleotide(s) in the corresponding primary spot could (almost always) be deduced (Uchida et al, 1974).

"Reading" a Sanger pattern in this fashion was painstaking work, requiring a good fraction of the day to work up a single "primary", something I at the time had been doing for several days a week off and on for a long time. It was routine work, boring, but demanding full concentration. [There were days when I'd walk home from work saying to myself: "Woese, you have destroyed your mind again today"]. But this day was special: I and Biology were in for a surprise! First, however, more background.

Starting Down the Yellow Brick Road

I had had an abiding interest in the translation process since the latter part of the 1950s; first with the ribosome and its subunits, then, starting in 1960, with the genetic code — *the* hot topic of molecular biology at the time. The code had come into prominence on the heels of Watson and Crick's two world-shaking 1953 publications. The physicist George Gamow thought he could see "pockets" in the double stranded structure of DNA, pockets of just the right size and spacing to hold and discriminate among amino acids, suggesting the basis for a direct templating mechanism upon which translation could be based (Watson & Crick, 1953; Gamow, 1954).

Then came a thrilling but brief period when a clique of physicists and molecular biologists worked together and competed to see who would be first to derive the "code" from "first principles". The prospect of theoretically solving the genetic code, the "language of life", was so seductive that cameo appearances on the coding stage were made by Feynmann and Teller (no doubt prompted by the charismatic Gamow). The decoders soon split into two camps, however, those who, like Gamow, believed that the basis of the code lay in specific recognition of amino acids by nucleic acids, and those who, like Francis Crick, believed it impossible that nucleic acids could recognize anything except other nucleic acids/nucleotides, which they did through base pairing (F. H. C. Crick, unpublished "Letter to the RNA Tie Club; see Judson, 1996). When I belatedly entered the area, my intuition sided with Gamow.

However, I differed from the whole lot of them in perceiving the nature of the code as inseparable from the problem of the nature and origin of the decoding mechanism. Thus, translation to me was *the* central biological concern. It represented one of a new class of major evolutionary problems that molecular probings of the cell were bringing to light. Now was the time to start thinking about the evolution of the cell and its macromolecular componentry. How this evolution occurred is almost as much a mystery today as it was four decades ago. But one thing was certain from the start: approaching these sorts of "deep" universal evolutionary problems would require a universal phylogenetic framework within which to work effectively. Since no universal phylogeny then existed — our understanding of evolutionary relationships being effectively confined to plants and animals — this meant taking on the rather large task of determining genealogical relationships for the microbial world, the bacteria and single-celled eukaryotes, which, as it turned out, meant determining the missing 95% or more of the "tree of life". A slight diversion in my research program would be necessary — a diversion that lasted a good two decades!

A method for my madness. In 1965, on his way to developing nucleic acid sequencing technology, Fred Sanger had spun off an "oligonucleotide cataloging" methodology (Sanger et al., 1965). This procedure, applied to ribosomal RNA (the small subunit rRNA it turned out), was exactly what we needed to determine genealogical relationships across the entire breadth of the phylogenetic spectrum. It was already apparent from DNA-rRNA hybridization studies that the sequence of a ribosomal RNA tended to be highly conserved, probably to the point that recognizable sequence similarity would

extend across the full taxonomic spectrum (Yankofsky & Spiegelman, 1962). Ribosomal RNAs are obviously ubiquitous; they occur in the cell in thousands of copies; and they can be radio-labeled and isolated with relative ease. In addition, they are functionally about as constant as one could wish for — they are *not* adaptive characters. And last but not least, rRNAs are integral parts of a complex, integrated molecular aggregate (genetically dispersed within the genome), which would make them as insensitive as can be to the vicissitudes of reticulate evolution (Fox et al, 1977a). Only technological problems seemed to stand in our way: growing the various organisms and doing so in a low phosphate, radioactive medium; tweaking the Sanger method to fit our needs; finding needed help; and so on. Scientists do not want to, or often cannot, create all the things they need in their work. In our case the chief problem was the organisms required for the project. Half of them at least would be too fastidious for anyone but an expert to grow. Striking up collaborations with experts in the culture of particular organisms was essential.

Learning our way around. Coming into the game as a biophysicist/molecular biologist my knowledge of bacteria and bacteriologists didn't extend far beyond *E. coli*, *Bacillus*, and Louis Pasteur; and I didn't have the foggiest notion of how bacteria were related to one another. It was time to ask real microbiologists for help in choosing the right organisms. Each, of course, had a different opinion (the bacteria they themselves worked with, that is). At that stage, I didn't know that actually there *were* no experts on bacterial relationships (those above the level, say, of genus and occasionally family, that is). And I was completely unaware of the bizarre state that the microbiologist's search for these relationships had gotten itself into.

The best advice I'd solicited regarding organism choice came from my colleague in the Microbiology Department at Illinois, Ralph Wolfe. By now I'd gotten used to microbiologists suggesting that we work on their favorite bugs, and in this respect, Wolfe was no different. But what he had to say was; his advice was more compelling than any other I had received! I can almost remember his words: he told me the methanogens were united as a group by a unique biochemistry that involved a set of unusual coenzymes. Yet, the organisms showed no uniformity in their morphologies, which latter fact had caused taxonomists initially to scatter them throughout the various taxa in the 7th edition of Bergey's Manual (Breed et al, 1957). [In Bergey's 8th, however, they had all been grouped on the basis of their common biochemistry (Murray et al., 1974)]. Finally! here was the kind of phylogenetic challenge I was hoping for. I longed to characterize a methanogen rRNA as soon as possible. But it wasn't possible — at least not yet. Wolfe and I had spoken in early 1974 (if I recall correctly), and the technology needed for growing and radio-labeling the methanogens safely was not at that time in place. Now, back to the main thread.

Epiphany!

By the beginning of 1976 my lab had "cataloged" (generated T1 oligonucleotide lists) for roughly 30 organisms, mainly "procaryotes" and a smattering of eukaryotes. It had become obvious that the two groups could be readily distinguished from each other on the basis of "oligonucleotide signatures", which were lists of

oligonucleotides characteristic of one of the two groups to the exclusion of the other. The two apposing oligonucleotide signatures were remarkably distinct. [In addition, a set of "universal" oligonucleotides existed, those found in all the rRNA catalogs we had so far generated]. In working up a Sanger pattern for an organism, one had only to "read" a small number of oligos into it before being able to smile and say: "Oh, that's a procaryote", or "that a euk". There were two spots on the primary films of all procaryotic rRNAs that easily caught one's eye, for they contained modified nucleotides and, so, were located at places in the Sanger pattern where normally there would be no oligonucleotides. These "odd oligos" allowed one to declare "procaryote" at first glance: after that, it was just a matter of detailing the rest of the pattern to figure out the relationship of the new procaryote to ones already cataloged.

By 1976 Wolfe and his student Bill Balch had developed a technique for growing methanogens (in pressurized serum bottles) that was sterile, fast, and (most important from our point of view), safe enough to permit cells to be radio-labeled (Balch & Wolfe, 1976). George Fox, then my post-doc, had known Bill from a course they'd taken together at Woods Hole the summer before George arrived at Illinois. Their acquaintance made it easy for George to approach Bill about a collaboration to work on methangens — which George did on his own initiative — the year the Balch-Wolfe method was being published. It was on that aforementioned day in June 1976 that I began to read the Sanger pattern produced (by my technician Linda Magrum) from George and Bill's first successful methanogen rRNA prep. The formal name of the organism was *Methanobacterium thermoautotrophicum*, a fourteen syllable monstrosity that was always shortened to "ΔH", the organism's strain designation (Zeikus & Wolfe, 1972).

From the get-go ΔH's Sanger pattern was strange. First of all the two small "odd" oligos on the primary pattern that screamed out "procaryote" were absent. Intrigued by this appetizer, but afraid to make too much of it, I quickly jumped into the "G isopleth" (oligonucleotides that lack a uracil residue), hoping to find the first of the procaryotic signature oligos, which would certainly set things back on the procaryote track! Imagine my surprise when that "signature" oligo was missing as well. Not only that, but the G-isopleth contained the rather large 3' terminal oligonucleotide of this 16S rRNA, which *did not* belong there! What was going on? This methanogen rRNA was not feeling procaryotic. The more oligos I sequenced, the less procaryotic it felt, as signature oligo after procaryotic signature oligo failed to turn up. However, a number of them were still there, as, surprisingly, were some oligos from the eukaryotic signature, and, thankfully, quite a few of the oligos we'd considered universal in distribution. What was this RNA? It was not that of a procaryote. It was not eukaryotic. Nor was it from Mars (because of the "universals"). Then it dawned on me. Was there something out there other than procaryotes and eucaryotes — perhaps a distant relative of their's that no one had realized was there? Why not? But the idea surely wasn't in keeping with conventional wisdom!

I rushed to share my out-of-biology experience with George, a skeptical George Fox to be sure. George was always skeptical. That's what made him a good scientist; and because of that, whatever skepticism he initially evinced

quickly dissipated. "Yes", he agreed, there probably was something else out there: it wasn't just procaryotes and eukaryotes all the way down. That was a heady thought, novel enough that we sensed trouble in trying to convince other biologists of the idea. Little did we know how *much* trouble there would be!

A finding like this you do not immediately go out and shout about. You had better have all your ducks in a row and have firmer evidence than would otherwise be needed. We went into fast-forward mode: by the end of the year (1976) we had five additional methanogen catalogs in hand, with more on the way. They would cover all the disparate morphologies associated with the known methanogens. And sure enough, none of the new catalogs was "procaryotic" (or eukaryotic): and they were all of a kind! The methanogens represented a new highest level taxonomic grouping, which could be defined by a characteristic oligonucleotide signature. [And tellingly, that signature was no more extensive than were those of the "procaryotes" or the eukaryotes, implying that within whatever new taxon they represented, the methanogens were quite a highly diverged group]. Darwin had long ago said that there would come a day when there would be "very fairly true genealogical trees of each great kingdom of nature." Perhaps that day was at hand! In any case, there was lots of work still to do.

Build It and They Will Come.

A new "urkingdom" (as we were beginning to call our new highest level phylogenetic group) would be a major evolutionary find. It afforded a rare opportunity to put the theory of evolution to serious predictive test. As I've said, ribosomal RNA is a non-adaptive, universal character. That's what makes its sequence so good for tracing organismal genealogies. It is also what makes it completely uninformative as regards the *phenotypes* of the organisms whose genealogies it traces. Darwin had defined evolution as descent with variation. In our case there had been a long trail of descent, and, therefore, we should find a comparably huge amount of variation, not only quantitative but qualitative. In other words, there had to be important features characteristic of our new urkingdom that distinguished it sharply from the rest of the living world, and there should be impressive variation among the species in the urkingdom, as well. Testing these two main evolutionary predictions drove our work from that point on.

There had already been a promising sign in the biochemistry of the methanogens. We now knew for sure that methanogenesis was indeed confined to a particular phylogenetically-defined group of organisms and that the process utilized a set of co-enzymes that apparently were found no where else (Balch & Wolfe, 1979). Doubly good! But with their highly specific, restricted distribution these coenzymes were going to be of no use to us in searching for non-methanogenic members of our new urkingdom.

The big question was then: where are the predicted other phenotypes? How could they be found? [As it turned out, a couple of the sought-for non-methanogenic archaeal phenotypes lay unknown on our "to-do" list. *Thermoplasma* (which had been described as a mycoplasma growing freely under hot and acid conditions) was among the mycoplasmas tabbed for

rRNA characterization in a collaboration with Jack Maniloff (University of Rochester), a collaboration that had begun in earnest in the spring of 1975 (with *Mycoplasma gallisepticum*). And there were the extreme halophiles, whose unusual obligate high salt growth conditions made them obvious candidates for the phylogeny project in any case. I have often mused in recent times about how the history of the archaea would have played out had their entrance into our world been through one of these other portals. No doubt that things would have gone very differently!

Fortunately, the job of hunting for other phenotypes that might belong to the new urkingdom turned out to be easier than I imagined. Unbeknownst to George and me, our collaborator Ralph Wolfe had invited the well-known German microbiologist Otto Kandler to Urbana for a visit. [Being a molecular biologist, I had never heard of this Kandler fellow — or almost any other well-known microbiologist, for that matter — until the day that Ralph marched him into my office to hear the official word from George and myself about the new urkingdom]. According to his records, Otto (as I would soon come to call him) visited Urbana in January of 1977, well before publication of our finding.

The German Smiled. Amazingly, Kandler — unlike the others we'd tried to convince about what our findings meant — wasn't incredulous or disbelieving or anything. I think he smiled. A "third form of life" was fine with him; he had almost expected it. For some time Kandler had, from his own work and that of others, known that the walls of certain procaryotes were highly atypical. The walls of the bacteria so far characterized had (almost) all contained peptidoglycan; and possession of peptidoglycan-containing cell walls had come to be considered one (of the few positive) unifying characteristics of procaryotes (Stanier et al, 1963). There had been no systematic examination of bacterial walls before about 1967, although a fair number of them had been characterized on a hit-or-miss basis. What Kandler had known (which we didn't) was that the walls of at least one methanogen and those of some extreme halophiles did *not* contain peptidoglycan (Kandler & Hippe, 1977).

What did these atypical walls imply? On the basis of the cell wall studies alone (the *lack* of peptidoglycan), one could not reliably infer much, especially infer that the peptidoglycan-less organisms constituted a monophyletic grouping unto themselves. [Bacteria atypical in one way or another were often encountered, and sometimes they lacked some property that microbiologists had come to believe was typical of "procaryotes". But in all such cases, the idiosyncrasy in question had been passed off as adaptation to some unusual environment. It would require much stronger evidence than one or two out-of-the-blue idiosyncrasies to make a microbiologist question a bacterium's procaryotic pedigree. And as we were soon to find out, there were some among them who would not question that pedigree even when confronted with *strong* evidence!]

Kandler had fully realized the potential significance of the cell wall studies in the light of our rRNA molecular phylogenetic evidence, and had immediately gone back to Germany intent upon fleshing out the comparative study of cell walls — and to spread the word. But his visit had left us with a critical clue in our hunt for novel archaebacterial phenotypes: if

unusual cell walls meant anything, perhaps the extreme halophiles would turn out to be members of our new "far out" group. We were desperate to get our hands on cultures of extreme halophiles.

With the help of Jane Gibson (Cornell) we obtained some halophile cultures from the Woods Hole collection. I was not about to wait for a student or a collaboration to come along to grow the organisms. I donned my acid-eaten lab coat (which had hung on the back of my office door for over a decade) and went back to the bench. I grew the cultures myself, turning them over to one of my students, Kenneth Leuhrsen, for the more exacting extraction and isolation procedures for a radio-labeled 16S rRNA; and Ken's prep would as always be given to our trusty Linda for Sanger pattern production. By late spring of 1977, a year after we'd seen our first methanogen catalog, we were gazing at a 16S rRNA catalog from the first extreme halophile. It didn't disappoint. Here was the first non-methanogenic phenotype to join the group — and a novel phenotype it indeed was. But I'm getting ahead of myself. It's time to see how the public — and the biology establishment — reacted when this strange archaeal chimera was loosed upon their world.

Confrontation and Heresy

The press release concerning the "third form of life" was set to coincide with the publication of the first of our two papers in the Proceedings of the U. S. National Academy of Sciences, November 3rd 1977. A telling coincidence was to occur. November 3rd just so happened to be the date chosen by the then president of the U.S. National Academy of Sciences, Philip Handler, to release an official statement heralding the dawn of the cloning era (signaled by the recent cloning in bacteria of the gene for the growth hormone somatotrophin). At the time, no one could see that this fortuitous coincidence foretold the coming battle over biology's future, and the coincident press releases were tantamount to the first skirmish in the ideological struggle that would pit the forces of what would become the biomedical-industrial complex against those representing a resurgent Darwinism. Our "third form of life", which touched upon one of the deepest chords in human nature (*i.e.,* where we came from), completely wiped the press release announcing the era of "Man-the-medical-miracle" off the front pages of the papers. I was overjoyed at the public's appreciation of our work (Fox et al, 1977b; Woese & Fox, 1977b)!

But there were already rumblings from the scientific heights. On the day the front page of The New York Times announced our discovery of a "third form of life", my colleague Ralph Wolfe received a telephone call from his friend, the Nobel Laureate Salvador Luria (whom he did not initially name), an upset Salvador Luria. According to Wolfe, Luria told him in no uncertain terms to publicly dissociate himself from this scientific fakery or face the ruination of his career. In a recent recounting of the episode (Wolfe, 2001) Ralph said he was so humiliated he "wanted to crawl under something and hide"; but he managed to tell Luria that supporting evidence for the claim had just been published in the Proceedings of the National Academy of Sciences (a fact that had appeared in the New York Times account). Luria begrudged that he hadn't known about any publication, but that the journal happened to be there on his desk. Fortunately, Ralph left for a planned family gathering out of

town the next day (Wolfe, 2001) and thereby escaped further humiliation.

As you might expect, I saw the episode and its overall significance differently. How could this Luria fellow have the temerity to excoriate his friend and my colleague like that? What pedestal was he standing on? It appears that he had blustered at Ralph something to the effect that: "Everybody knows that all bacteria are procaryotes; there can't be any such thing as a 'third form of life'"! Irony of ironies! As time (and the diligence of a particular scientific historian) have shown, the fakery lay not in *our* work, but in the prokaryote concept itself (Sapp, 2005): it is now clear that the "procaryote" was mere guesswork. [More on this below]. But in the hey-day of the procaryote, which this was, the true believers were out to pillory us for our heresy: how dare we proclaim that the mighty *PROCARYOTIC EMPEROR WASN'T WEARING ANY CLOTHES!*

Expanding the Urkingdom

With the halophiles came another clue — lipids, highly unusual ether-linked, branched chain lipids. In my whole career I'd never paid attention to lipids, and here we were with lipids on the brain! The fact that extreme halophiles possessed ether-linked lipids had been published in the mid-1960s (Kates et al, 1965; Kates, 1972), but nothing much had been made of it: these strange lipids seemed to be just one more unexplainable biological idiosyncrasy. Microbiologists and biochemists didn't view things evolutionarily. From a genuinely biological perspective, however, these lipids were significant — *evolutionarily* significant.

It was not simply the extreme halophiles that had the strange lipids, either: Two other recently isolated bacteria, *Thermoplasma acidophilum* (Darland & Brock, 1970) and *Sulfolobus acidocaldarius* (Brock et al, 1972) did as well (Langworthy et al, 1972; 1974). Thomas Brock, whose laboratory had first isolated and characterized both organisms, had noted the lipid coincidence. And he later would say that the unusual lipids the two shared was a clear case of *convergent* evolution. In support of that assertion Brock would invoke the ether-linked lipids of the extreme halophiles: "This hypothesis is strengthened by the fact that *Halobacterium*, another quite different organism, also has lipids similar to those of the two acidophilic thermophiles" (Brock, 1978).

Brock's argument doesn't make sense; at least *today* it doesn't. In its time, however, it was reasonable. Like all biologists of that period, Brock firmly believed all bacteria to be "procaryotes". If both of the thermophiles were procaryotes and they were not specifically related (as was believed), then their common idiosyncrasy has to represent convergence, independent adaptations to their respective extreme niches. From this perspective the case for convergent evolution is *indeed* strengthened by pointing to a third prokaryotic species (the extreme halophiles), from a still different extreme niche, that also had similar strange lipids. Brock's conventional mind set here is a wonderful example of what comes of unquestioned acceptance of the assumption that all bacteria are "procaryotes" — all idiosyncratic characteristics then become *adaptive* changes.

We didn't know what kind of lipids the methanogens had, and that was critical, for the lipids were a good candidate for a property shared by all of the organisms in the new urkingdom. Determining the lipid type of the methanogens was essential. I frequently discussed with Wolfe whom we could enlist to find the answer. Although Brock wasn't the one to approach, one of his colleagues, Tom Langworthy at the University of South Dakota (an expert in lipid analysis), seemed to me a good bet; and it was decided (I thought) to send cells to Langworthy.

Ralph then threw me a curve ball. He had come up with a different candidate, a young Professor at Yale who was about to join our Microbiology faculty, who was also an expert lipid chemist, name of John Cronan. Ralph felt (rightly, I had to admit) that it would be more collegial for us to work with Cronan. So Ralph ordered a pellet of methanogen cells sent off to John for lipid analysis. Days went by; weeks went by; it seemed like more; and no word from Yale. Ralph finally agreed to call Cronan up and find out what had happened. It turns out that John's lab had done a quick, but definitive, initial screen of the methanogen for lipid type upon receiving the prep and then dropped the project as uninteresting: there was nothing new there; the methanogen lipids were just like the halophile lipids! John had perceived the whole thing from a strictly biochemical perspective. Hadn't Ralph made clear to him how critical methanogen lipids were to our case?

It was back to plan A. Bill Balch was asked to prepare methanogens again, and these were sent to Tom Langworthy and another lipid biochemist, Thomas Tornabene (a colleague and oft-time collaborator of Langworthy's). And in short order there the answer was: methanogens have ether-linked lipids (Tornabene & Langworthy, 1979)! The circle had been closed. The first archaebacterial-universal phenotypic property had been found.

Recently Tom L. recalled to me in touching detail our first contact (he has a photographic kind of memory). It was warm that day in mid-November 1977. He had been eating lunch and reading an article in the latest issue of Time magazine about a "third form of life". Since there had been no mention of our unpublished halophile work in the Time article, Tom was unaware that we knew about any lipid connection. So, Tom had his own "eureka moment", for some of the bacteria he was working with, *Thermoplasma* and *Sulfolobus*, were unusual enough in their properties that they might even represent a *fourth* urkingdom; *c.f.* Brock quote above. He was thinking of contacting Time magazine about the possibility, when the phone rang. It was Woese calling to ask him for help working with just these organisms. We had decided to grow them ourselves and were seeking cultures from Tom, which he provided on the spot. I was not willing to wait for *Thermoplasma* rRNA to come in under the mycoplasma collaboration; it was at the bottom of Maniloff's list,

The work on *Thermoplasma* went smoothly, and by the beginning of 1978 there existed a catalog defining the 2nd major non-methanogenic archaeal phenotype. I wanted to publish this one in Nature, to give wide scientific coverage to the fact that archaebacteria were now a *real* group (comprising at least *two* diverse organismal phenotypes). A manuscript had been

prepared accordingly and submitted. In early June of that year a letter from Nature's editor arrived, which I opened expectantly. Rejection! "Dear Professor Woese ... I realize that there is considerable interest in your Archaebacterial group; nevertheless the basic idea has been so well exposed that I am not convinced that the assignation of T. acidophilum to the group really demands a place in Nature". Perhaps I should have expected it. Nature was a mouth-piece for molecular biology, a biology in which the organism's evolutionary history and the organism in its own right don't really count. An evolutionary finding rates only a "so what!" with these people — be it world shaking or not. Fortunately, our work on the halophiles, moving toward publication on a separate track *was* accepted (quite rapidly, I might add) — the only drawback being that it had been submitted to a journal having much less coverage than Nature had (Magrum et al., 1978). So at least some of the biology community would be aware that the archaebacteria comprised more than one organismal phenotype. The *Thermoplasma* rRNA catalog was never published in its own right.

The other organism Langworthy had sent us, *Sulfolobus acidocaldarius*, was not being so cooperative. It would turn out to be the first one of what we now call the Crenarchaeota, the second of the two archaeal kingdoms (the first now goes under the name Euryarchaeota) (Woese et al, 1990). We had actually begun the *Sulfolobus* catalog a month before starting in on *Thermoplasma*, but it took seven months in all to bring it to fruition. The primary obstacle here was the hard-to-characterize modified oligonucleotides. Instead of having half a dozen or so modified oligonucleotides (as had the eubacteria and all archaebacteria we'd so far encountered), the 16S rRNA from *Sulfolobus*, the first crenarchaeon, had near an order of magnitude more of them. The high levels of modified nucleotides had proven true of their tRNAs as well (Gupta & Woese, unpublished). [A high degree of modification in rRNA and tRNA, of course, was reminiscent of eukaryotes. Strange coincidence! It was tucked away for future consideration].

It was clear from the start that *Sulfolobus* was indeed one of the archaebacteria: it had many of the by-now familiar archaeabacterial signature oligonucleotides. Yet, its catalog did not fall *within* the grouping defined by the archaebacteria we'd already characterized. Our new urkingdom not only contained several disparate phenotypes, but was deeply divided into two major subgroups within itself. [This latter fact would become more apparent when the less informative oligonucleotide cataloging method was replaced by direct sequencing of whole rRNA molecules — and we started to present the data primarily in the form of phylogenetic trees rather than oligonucleotide signatures (Woese & Olsen, 1986)].

The Archaeal family grows. There began to grow — in good measure thanks to the prosylatizing and other efforts of Otto Kandler in Germany — an increasingly large coterie of "archaeo-philes", each of whom approached the archaebacteria from their own perspective. Thus by 1980 a fair amount was known about the specific and general characteristics of the archaebacteria. Notable among the new faces in the movement were Wolfram Zillig and his associate Karl Stetter, then a sort-of post-doc. Wolfram, one of the Abteilung Leiters at

the Martinsried Max Planck Institute, had built his career around molecular characterizations of the DNA-dependent RNA polymerases. By the late '70s he (by his own admission) had settled into "the rut of refinement" with bacterial RNA polymerases. Somehow the word had filtered down to him about the "third form"; likely via Kandler. Immediately, Wolfram and Karl turned their attentions to the RNA polymerases of the archaebacteria, publishing first on the novel RNA polymerase of the halobacteria — in the same year we publicly announced our own findings on their rRNAs (Zillig et al., 1978). This was followed by a mid-1979 description of a similar atypical RNA polymerase from *Sulfolobus acidocaldarius* — on gross inspection whose subunit composition appeared rather eukaryote-like (Zillig et al, 1979). We were still finishing up the 16S rRNA catalog of *Sulfolobus acidocaldarius* at the time, and hadn't begun to think about publication, which actually occurred several years later in collaboration with Wolfram, a publication now based upon a total of three different [crenarchaeal] extreme thermophiles (Woese et al., 1984).

Wolfram and Karl had figured out the ether-linked lipid connection for themselves (if I'm not mistaken). Indeed it is to these two that we owe any strong emphasis that there was on the crenarchaeal branch of the archaeal tree in the beginning. Wolfram had also deduced that when Tom Brock isolated and characterized his thermoacidophiles, he had probably missed a whole world of anaerobic thermoacidophiles. And here would be the start of his and Karl's colorful (and sometimes dangerous) adventures hunting for hyperthermophilic archaea around the globe (later carried on by Karl alone). [Given that he frequented the vicinity of boiling sulfurous hot springs so often, Karl came to refer to crenarchaeal hyperthermophiles as "the organisms from hell", even naming one genus *Stygioglobus* and at least one species *infernus* accordingly].

It was only a matter of time before there would be a formal scientific conference — the field's initiation rite. Thanks to strenuous efforts on the parts of Wolfram and Otto, it happened sooner rather than later. Planning began in 1980, with furious exchanges of letters and phone calls; they also had to obtain financial support (not an easy matter). The meeting got scheduled for 1981 at the Max Planck Institute at Martinsried-bei-München. The Martinsried meeting fulfilled everyone's hopes; it covered the full spectrum of workers and work on the archaebacteria. Don't think this means that the number of participants was large; there were relatively few of us in those days. A wonderful sharing of knowledge and developing or expanding of scientific relationships occurred at the meeting. Best of all, it gave a feeling of group identity, a camaraderie, to many who attended. The archaebacteria had arrived!

Starting in 1977 (if I'm correct) George and I had begun planning to write a comprehensive publication about all the phylogeny work that had gone on in the phylogeny project — one that would give biology a little surprise! I nicknamed the project "Big Tree". There would be many authors on Big Tree, reflecting the many people who had worked on the project in my lab and all the collaborators who had contributed the radio-labeled rRNA starting materials. The actual writing, however, involved mainly George and myself. That proved difficult enough!

With the hard part over, I submitted Big Tree to Science on January 31, 1980 (eschewing Nature this time around). With Science things went smoothly, and the paper appeared in July of that year: "The phylogeny of procaryotes", by Fox *et al*, (1980). It was a heady experience, publishing a phylogenetic tree that (in outline) covered all of life, and said to boot that there were three, not two, primary lineages of organisms on this planet. Before genealogical analysis had been transposed to the molecular level, the best that biologists had been able to do was make trees for animals and/or plants. Here, for the first time, was the skeleton of the full Tree of Life. Monumental! Reviewers' reactions to the paper had been good. The reaction of the *scientific* public this time was good as well.

The dedication unwanted and recognition ungiven. I had dedicated "Big Tree" to C. B. van Niel (long retired), who was one of the great microbiologists of his era. He had won microbiology's highest honor, the Leeuwenhoek Medal 1970 — an award given once a decade by the Netherlands Royal Academy of Sciences to "the scientist who has made outstanding contributions to the advancement of microbiology during the preceding ten years". I had thought that van Niel would be pleased and honored to see the final solution to the problem of a natural bacterial classification dedicated to him; for it was a problem that he, Kluyver, and Stanier (among others) had struggled with for a very long time without having the (molecular) technology to cope with it. [A former student and friend of van Niel's, Robert Hungate, had offered to send him the paper personally].

When dedicating our paper to him I had read only van Niel's early work on natural classification of bacteria, collaborations, first with Kluyver and then with his student Stanier. Little did I know at the time what van Niel's final judgment on bacterial phylogenetics had been: it was "a waste of time to attempt a natural system of classification for bacteria" (see Stanier et al., 1957). Moreover, in 1962 he and Stanier had even renounced their earlier efforts in bacterial classification as something "neither of us cares any longer to defend". Our paper apparently didn't change van Niel's mind, for I never heard from him. But what I've just said is based upon what I discovered later, from a fairly comprehensive study of van Niel and his cohort's writings on bacterial phylogeny.

Given the nature of our Science paper and the reception it received, it should have been nominated for "Paper of the Year" in Science, which, indeed, it was. But the "Tree of Life" came in 2nd (as I was told by "sources"). I also found out what may have been the reason: one of the judges had, apparently innocently, praised the work as a contribution "almost as important as the eucaryote-procaryote dichotomy". "Hmm", I fumed; "how can our work be almost as important as something that is totally wrong-headed, something that has influenced the course of microbiology so adversely! Who is this stupid judge?"

Procaryote by Any Other Name ...

The more things progressed, the more procaryote loomed large, like a current against which no one could swim. I'd often reflected on why that day in June 1976 it seemed so incredible to discover a procaryote that wasn't a procaryote. The reason was obvious once I thought

about it, which I — and it turns out every other biologist — had never felt a need to do previously. *That's* why we encountered such difficulty in convincing others of what we'd discovered. *That's* why the Nobel Laureate Luria had so scornfully rejected the three urkingdom notion. Ralph Wolfe, who frequented microbiology meetings would tell me at the time about the "talk in the corridors" — *i.e.,* the behind-the-scenes, clubby dismissal of the three urkingdom concept. I was itching for them to come after me in print. But none of them would! It was so important to get the "procaryote" matter out in the open. Something was strange about this "procaryote"!

Whatever the reason, "procaryote" had special significance for microbiologists. Yet, the reason couldn't be historical usage of the term. "Monera" was the preferred term for bacteria in the first half of the 20th century; *Schizomycetes* also being used. And I couldn't find the term "procaryote" anywhere in the literature before 1962 — and neither (more recently) could the historian Jan Sapp (2005). “Procaryote” seemed to appear out of nowhere at that point in time (Stanier & van Niel, 1962). So why were microbiologists so wedded to the "procaryote" that they rejected any suggestion of a third urkingdom without even thinking about it?

By the 1970s a generation of microbiologists and biologists in general had been raised believing that "procaryote" (term and concept) had originally been the brain child of the protozoologist Edouard Chatton (1938; Stanier & van Niel, 1962; Sapp, 2005). And, the recent historical analysis also found no evidence for Chatton's even *presenting* the concept (Sapp, 2005). In any case, how could any biologist working in the early decades of the 20th century infer anything about the organization of a bacterial cell, much less what features were common among the organizations of *all* bacterial cells: bacteria were a morphologically diverse group, and all that one had to go on as regards their internal organizations was negative evidence: they were not (what we now call) "eucaryotic"! None of it made sense!

Although we were unaware of it at the time (or for some time thereafter), we were not faced with an ordinary scientific situation here; it was no simple case of a new, more detailed and factually supported hypothesis displacing an entrenched older one. The strength of that older idea, its dominating, dogmatic hold on microbiology, implied rather more than that. But what? Answering this question has taken me on a somewhat bizarre journey that has lasted over two decades, and only recently shows signs of coming to its end. My understanding has had to pass through three distinct stages, each broader in scope than the previous.

First attempt: apply scientific reason. When we initially realized that the procaryote concept was the immovable object in the road to microbiology's development, I thought the matter would resolve scientifically: tell biologists what their "procaryote" really is and they will understand. Accordingly, George and I prepared a paper entitled "The concept of cellular evolution" (Woese & Fox, 1977a). In it we denied the two defining tenets of "procaryote", but *kept* the term itself, hoping thereby to ameliorate the rather revolutionary change we were proposing. The prefixes "pro-" and "eu-" had a familiar ring to them; the notion

that bacteria were separate from and older than — some perhaps even ancestral to — eukaryotes was traditional.

Our argument went: "yes", the terms "procaryote" and "eukaryote" do obviously recognize distinctly different kinds of cellular organization, but "no", in the case of the prokaryote a common general organization *does not* necessarily denote common ancestry for all! Procaryote should be looked at only as a *level* of organization, a level distinct from that of the (higher, more complex) eucaryotic cellular organization. Therefore, archaebacteria and eubacteria can have the same *general* level of organization, but have arrived at it *independently*. In other words, the procaryotic *level of organization has evolved at least twice!*

The general principle here is that biology is a study in *emergent levels of increasingly complex organization*. This idea, of course, was anathema to 20th century reductionist biology; but it had currency in the 18th and 19th centuries (Burkhardt, 1977). We were simply resurrecting the notion and recasting it in modern scientific terms.

In keeping with this general notion we then postulated a primitive level of organization even simpler than the procaryotic and eucaryotic, one that had preceded the other two. We named it the "progenote" to signify that the genotype-phenotypic link at that early stage had yet to reach the eventual perfection, precision, and sophistication that characterizes modern cells (Woese & Fox, 1977a).

It may have sounded absurd at the time that archaea and eubacteria had evolved the same cellular organization independently. But the absurdity actually lies not in our proposal but in the original assertion that *all* bacterial cells were of a kind, had the *same* (procaryotic) cellular organization — from which monophyly had to follow (Stanier & van Niel, 1962; Stanier et al., 1963): as I've said, there was no way *anyone* in the early, middle, or even late decades of the 20th century could have known *anything* about the nature of the organization of any but the eucaryotic cell (and that was only a superficial description).

In spite of what I thought was sound pleading, our argument had no takers; I don't think that the vast majority of (micro)biologists even bothered to consider it. We would have to dig deeper to get at the root of the problem.

Second attempt: follow the leader. After this, I essentially went off on my own to try to solve the problem — which meant acquiring the perspective needed to see it in historical terms. Where had "procaryote" come from in the first place? Microbiologists had traditionally made a strong distinction between bacteria and the cells of "higher forms" (see above). But none of it was taken as certain or factually supported. It was all just necessary speculation on the road to developing an understanding of bacteria. All that these early speculations really accomplished was to define bacteria negatively: they were not as large as eucaryotic cells; and they lacked this, that, and the other microscopically visible features so characteristic of eucaryotic cells — in addition to their showing no (common) microscopically discernible intracellular structures of their own.

Where, then, did this strongly asserted, definitive, inflexible "procaryote" come from? Focusing on the "procaryote's" origin brought me directly to one, and apparently only one, source, R. Y. Stanier and his cohort. As I said above, I couldn't find "procaryote" in the literature before 1962, when it appeared in the classic paper of Stanier and van Niel (1962; Sapp, 2005). It was also noteworthy that, although the term "procaryote" was featured in the 2nd edition of "The Microbial World" (Stanier et al., 1963), it was nowhere to be found in the text's 1st edition (Stanier et al., 1957)!

No terminology before 1962! Was it the same for the underlying concept? Yes; 1962 was the first time the commonality of all bacterial cellular organization was asserted (rather than just mused about). There had been the older, questioning, more speculative attitude about the nature of bacteria for some time, but nothing like this, the self-assured, dogmatic "procaryote" we had encountered in 1977.

It is particularly important to know how "procaryote" was defined in 1962, for there is a tendency today to adjust the term at will (Judson, 1996), which is counterproductive in that it is tantamount to superficial "surgery" that only further conceals a deeper chronic condition. "Procaryote" rests on two definitional pillars: 1) the assertion that it represents one of "...two different *organizational patterns* of cells ...", (the other being that of the eucaryotic), from which it must follow: 2) that "... we can therefore safely infer a *common origin* for [all "procaryotes"] in the remote evolutionary past ..." (Stanier & van Niel, 1962; Stanier et al., 1963) [italics added]. The procaryote, moreover, was seen to provide "... our only hope of more clearly formulating a `concept of a bacterium'." (Stanier & van Niel, 1962), because "... *the ultimate scientific goal of biological classification [i. e.,* a natural, or phylogenetic classification] *cannot be achieved in the case of bacteria ..."* [italics original] (Stanier et al., 1963). Only in 1962-63 does one begin to see such a definitive, strongly worded and inflexible position taken on the nature of bacteria. There was no sound scientific reason for it either, for the “prokaryote” was founded solely upon rhetoric, with *no* intent or attempt to give it a firm foundation subsequently. The whole thing was only scientific "guesswork" (*sensu* Schroedinger, 1954).

What sank matters further into strangeness was the fact that the microbiology community accepted this guesswork (both alien term and out-of-the-blue concept) immediately and overwhelmingly! No criticism, no serious discussion greeted the procaryote's debut.

[An obvious weakness in the authors' presentation had been the lack of adequate comparative evidence upon which to base the assertion of properties common to *all* bacteria — too few properties and too narrow a sampling of bacterial taxa (Murray, 1962), which could have been remedied easily by doing more work, sampling a wider range of taxa. Yet *no one* did this or apparently saw the *need* to! It was as though microbiologists simply wanted to make the issue go away: it was time to close the door and move on (Sapp, 2005).

It is revealing to compare the ready and enthusiastic reception of the procaryote with the irate reaction that the three-

urkingdom archaebacterial concept engendered a decade and a half later — even though the three urkingdom concept *had* solid factual support, with more facts obviously to come. I clearly needed to get to the bottom of this, to understand the diametrically opposed responses the microbiology community had given to the two points of view.

What I had now learned was that individuals could not be held responsible for what had happened to microbiology in the middle decades of the 20th century. They were among the few who had *seen* the problem, and they had tried to resolve it — unfortunately in the wrong way! Those "responsible" were just the lead birds in a migrating flock: shooting them down does not effect the flock's course. There was a deeper dynamic at work here, one that would be far more difficult to change.

Third attempt: understand the mythology. This last insight proved critical. An intellectual tide was the key to the problem. Molecular biology, in its insistent reductionism, lay at the heart of the issue (Woese, 2004). The intellectual landscape was indeed being restructured! Two factors were going to shape 20th century microbiology, the power of molecular biology's vision and the weakness of microbiology's.

Bacteria are *organisms*, not simply bags of biochemistry. Microbiology is *a fortiori* an organismal discipline. Organisms in their fullness must be its study. The organism's parts are surely important, but important in what they contribute to an *overall* understanding; the same being true of the higher level interactions that structure microbial communities, their ecologies. These, together with an appreciation of microbial variety and long- and short-term evolutionary dynamics, make for a "tetrahedral" synthesis called "organism", quintessential biological organization.

Within the context of his time the great microbiologist Martinus Beijerinck appreciated bacteria in just this full sense. When in 1905 he was asked (on the occasion of being awarded the Leeuwenhoek Medal) to articulate his view of the microbial world and its study, he had replied that the most effective approach to understanding microorganisms was "... the study of microbial ecology..."; adding that microbes represent the "... lowest limits of the organic world, and ... constantly keep before our minds the profound problem of the origin of life itself." (van Iterson Jr. et al., 1946; van Niel, 1949). Here in the making was a rich, genuinely organismal picture of the microbial world.

Unfortunately, Beijerinck's successors did not, nor did they care to, realize his vision. Initially it was not technologically feasible to do so, but doing so in any case would have meant going against the new reductionist tide, which microbiology was conceptually unequipped to do. Therefore, outside influences, not innate tendencies, would shape the discipline.

To Beijerinck's successor A.J. Kluyver (and his "Delft School") the organism in its own right (*i.e.,* as a biological organization) meant nothing: biochemistry was the essence (Kamp et al., 1959). The emphasis would be on uncovering the main biochemical themes among the bacteria (Kluyver & Donker, 1926) — and *all* of the nuanced variations thereupon (Kluyver, 1931)!

Yet, how many of the organic compounds we know do you think would exist on this planet if some kind of biological *organization* weren't around to produce them (Kaufmann, 1995). [Most organic compounds we see in nature are like the new elements at the high end of the periodic table in that they require "organismal intervention" to exist at appreciable levels]. After all, bacterial metabolic diversity in the last analysis is not a biochemical problem; it is an *evolutionary* one — a question of why and how such great biochemical (metabolic) diversity arose in the first place, and why certain biochemical pathways were evolutionarily singled out for (biological) amplification. By the middle of the 20th century our conception of bacteria had moved from Beijerinck's multifaceted organismal one to the Delft School's disassembled one (see van Niel, 1949).

The Reason for Roots

Every scientific discipline rests upon an axiomatic foundation, a scientific mythology, which informs it and the world as to what it is and in the process charts its course. Microbiology's mythology, non-organismal and almost non-existent, reflected the Delft School perspective. And that is why in 1962, in the heyday of microbial biochemistry, when all manner of new biochemicals and metabolic pathways were being uncovered, R. Y. Stanier had said (in introducing the "procaryote" concept): "... the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium ... the problem of defining these organisms as a group in terms of their biological organization is clearly still of great importance, and remains unsolved" (Stanier & van Niel, 1962). It was obvious that microbiology was in a state of disarray; it did not understand itself; it had no real conceptual base (mythology) from which to draw support. And this is why the procaryote concept (or something equivalent) was needed in order for the discipline to have an "organismal" sense of itself.

The "procaryote" was only scientific guesswork (as said above). *But* it did seem to give microbiology the badly needed keystone in its conceptual foundation (its mythology). Procaryote provided an overarching, authoritative. and authenticating framework within which now to work. Yet, note that the concept had *precluded an evolutionary* (phylogenetic) *definition of bacteria* — in my opinion, the only concept possible.

Missing the train. One needs to recall here that the "procaryote" concept developed while molecular biology was providing the scientific world its first glimpse of the power that lay in molecular sequencing (Sanger & Tuppy; 1951; Sanger & Thompson; 1953). Zuckerkandl and Pauling (1965) were trumpeting the molecular approach to evolution; and F. H. C. Crick (1958) had said: "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." Adding that "... these sequences are the most delicate expression possible of the phenotype of an organism and that vast amounts of evolutionary information may be hidden away within them."

It seems highly unlikely that the vast majority of microbiologists were totally ignorant of the new molecular approach to organismal relationships, especially given the exquisite need the discipline

had for evolutionary underpinnings. What is more likely (as discussed) is that a conceptually unsettled microbiology wanted to rid itself of a perspective that was not in keeping with the reductionist tenor of the times (Woese, 2004). The procaryote accomplished that: it papered over "... the problem of defining [bacteria] as a group in terms of their biological organization ..." (Stanier & van Niel, 1962). But it had cut microbiology away from its organismal, evolutionary roots in the process. [While a considerable amount of nucleic acid hybridization work was subsequently done in microbial taxonomic structure, the work's intent was simply to improve existing bacterial taxonomy. The grand challenge of a natural classification, a universal phylogeny for bacteria (Stanier & van Niel, 1941) had disappeared from the scene!]

There is actually nothing surprising about this strange "procaryote period" in microbiology's history. The dynamic at work here is encountered in many different fields: "the past needs to be forgotten because it reveals the confusion and lack of cohesion of the present!" It's just that in this case the whole thing was so patently unscientific.

Where Are We Going?

A biology that does not concern itself with evolution is not biology! Contradicting the ill-framed reductionist view of biology is precisely why I had established the program of (molecular) phylogenetic reconstruction in the first place. The program's *raison d'etre* was to revive the evolutionary spirit that underlies biology and had been nearly squeezed out of existence by reductionism. Thus, the archaea were indeed a splendid surprise to me: they were a resonating thunderous clap that would awaken the Sleeping Giant of evolution! As I saw it, the discovery of the archaea had turned over the reductionist rock, and the weakness in that paradigm now lay there for all to see! But things didn't turn out that way.

The evolutionary message inherent in the discovery of the archaea faded! Consequently, microbiology has yet to resolve its foundational issues. The discipline goes on today rootless as ever, unconcerned with microbial evolution, living in the scientific dream world of the procaryote. The evidence is everywhere: "procaryote" remains imprinted across the discipline; structuring its modes of thought; defining its curriculum, its scope; shaping its future. One glance at how the discipline of microbiology responded to the challenge of the archaebacteria and later to the advent of genomics will show you a discipline lost in its past, blown about by the capricious winds of a society. Microbiology did not meet the challenge of the archaebacteria, nor has it yet met that of genomics.

Microbial genome sequencing began only in 1995 (Fleischmann et al., 1995). I know from personal experience that had microbiologists (and biologists in general) perceived its significance, microbial genomics could have begun by the mid 1980s (Woese, 1993). Archaea are so unlike eubacteria, and we didn't (and still don't) know enough about them. Genome sequencing was the only way to bring the "third urkingdom" up to scientific speed (Woese, 1993).

Sadly, when microbiologists finally took a genomic approach, it was only because genomics could be used (and

funds obtained for that) to attack a host of practical problems (medical, agricultural, environmental), problems that they had been dealing with for decades. To the extent that microbial genomics affected the intellectual climate in microbiology at all, it did so adversely; each microbial genome rationalized and sequenced for a different reason. No concerted program in, no organismal rationale for, microbial genome sequencing even *existed* until quite recently.

Moving horizontally.

Many microbiologists have developed interests in horizontal gene transfer (HGT) over the past decade. In that HGT provides an important, if not the most important clue to the dynamic of cellular evolution there would seem to be hope in this. With a few important exceptions, however, the microbiologist has not seen the fundamental evolutionary significance of HGT: most of the concern centers about health related matters, and a little also with the particulars, cellular mechanisms involved in HGT. Nevertheless, there are very promising studies involving metagenomic approaches to microbial community structure and dynamics beginning, in which the role of HGT (and viruses) is absolutely central (Rachel et al., 2002; DeLong, 2005; DeLong & Karl, 2005).

The Defining Problems of 21st Century Microbiology (and Biology).

The future of microbiology does not lie on the field's current path. If the *status quo* in microbiology persists, the discipline is on course to become a complete and total service discipline, simply bioengineering! What is needed, what is *essential,* is that microbiology become far more of a *basic* biological discipline than it has ever been — some sort of modern realization of Beijerinck's holistic vision. Throughout the 20th century, microbiology's course was charted by the dictates of molecular reductionism and the practical concerns of a biomedically oriented society. Biology itself was the low man on the scientific totem pole; and microbiology the low man on its.

Now things must reverse. Not only must microbiology become the leading discipline, the guide, in biology; but biology itself should become the basal discipline of the sciences. Microbiology's lack of concern with evolution has been the discipline's downfall in the past and could remain its (and Biology's) nemesis in the future. Microbiology departments today are products of historical accident, held together in essence by institutional inertia. It is not a good sign that the basic research at microbiology's forefront is increasingly being done outside of the context of formal microbiology departments.

The future of biology lies in understanding the nature of biological organization. Microorganisms are a central concern here, for they are biology's primary window on the problem. Twentieth century biology (especially microbiology) was structure oriented, reductionist, and temporal in its view of life. The biology of today must be evolutionary, holistic, and process oriented.

We meet 21st century biology right now in terms of two grand problems: 1) the evolution of the cell; and 2) an understanding of the global environment. While these two may seem quite unrelated, the one as

fundamental as biology now gets, the other essentially applied (and of pressing concern), this is not so. At base both represent problems in biological organization. And the two will become closely joined when biology comes to study the early stages in the evolution of the cell, when horizontal gene transfer dominated the evolutionary dynamic, leading to an evolution that was essentially communal, not individual (involving distinct lineages) (Woese, 1982; 2002). Only a microbiology that embodies the spirit of biological organization, organism and evolution, will be fit to lead biology in the 21st century.

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