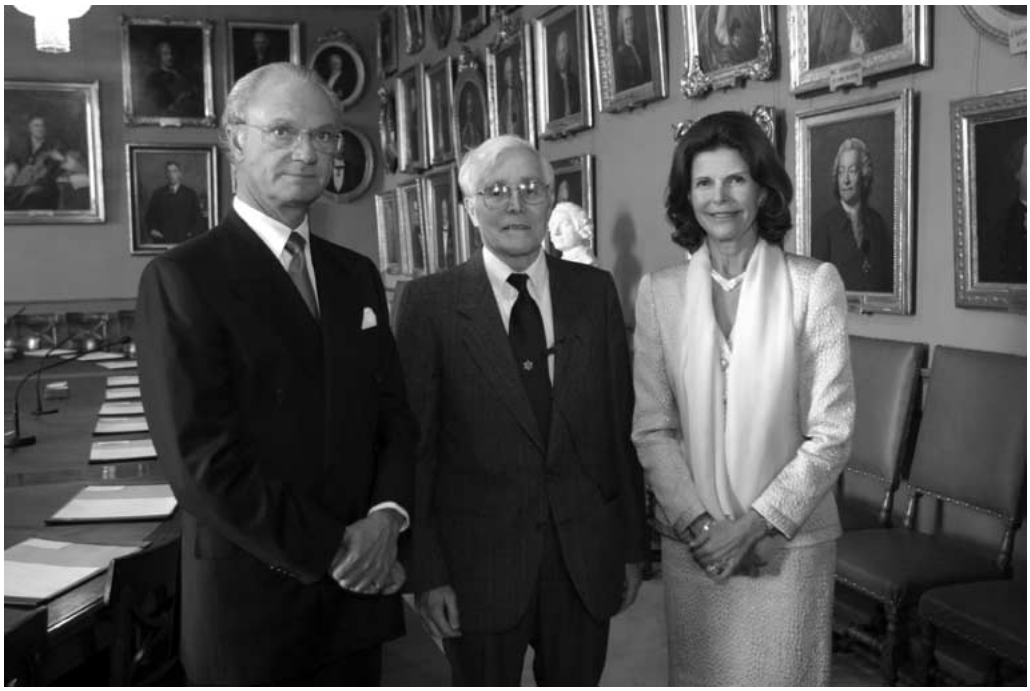

Carl R. Woese (center) with His Majesty Carl XVI Gustaf of Sweden and Queen Silvia on the occassion of his receiving the 2003 Crafoord Prize, given by the Royal Swedish Academy of Sciences. *Photo credit*: Royal Swedish Academy of Sciences.

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Personal perspective

The archaeal concept and the world it lives in: a retrospective

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Abstract

The present retrospective concerns the discovery and development of the archaea, the so-called 'third form of life' that no one anticipated and many did not, and still do not want. In its birth pangs, which the archaea had a plenty, the concept encountered biology unmasked; for it ran up against some of the key struts in the 20th century biological edifice. Consequently, the history of the development of the archaeal concept provides an excellent window on certain of the weaknesses in the 20th century biology paradigm, weaknesses that have now led that paradigm to a conceptual dead end. On the other hand, the archaeal concept has also provided us one of the pillars on which a new holistic paradigm for biology can be built. So, it would seem of value to retrace some of the twists and turns in the history of the development of the archaeal concept. Given my position *vis-à-vis* the archaea, my account will be a personal one.

Introduction

These are strange, unsettled times in biology. Biology today feels to me like a gigantic engine spinning its wheels, waiting to do something, anything. The 20th century in biology has been a time of spectacular advances, almost certainly the most productive period in biology's history. Yet it was also a time when the ways in which biologists thought about biology changed dramatically. It was a time when the world view of 19th century classical physics imposed itself on biology: reductionism and mechanism are part and parcel of both genetics and molecular biology, the dominating paradigms of the last century. Under their aegis biology was reformulated in a remarkably procrustean fashion. While one cannot deny the tremendous advances that molecular biology (and genetics) wrought, one can and should decry the price biology paid for them. A holistic perspective was effectively banished from biology. The cell was reduced merely to the sum of its parts – the cell as a whole became no more than a shadowy backdrop for the molecular drama. The fact that the cell has an evolutionary dimension, an understanding of which is essential to genuinely understand the cell, was ignored; the whole of evolution was dismissed as a collection of meaningless historical accidents. The study of development, a principal way in which biologists approached the important problem of biological form, was squeezed by 20th century genetics into a reductionist gene-centered mode (Gilbert et al. 1996).

When it was all over, almost every trace of a holistic view of living systems was gone. Yet hardly anyone paid attention – so riveting and reassuring was the mechano-reductionist view of biology. One of the few cries of alarm came from outside biology *per se*, from the physicist/philosopher David Bohm, in the 1960s:

> It does seem odd ... that just when physics is ... moving away from mechanism, biology and psychology are moving closer to it. If the trend continues ... scientists will be regarding living

> and intelligent beings as mechanical, while they suppose that inanimate matter is too complex and subtle to fit into the limited categories of mechanism (Bohm 1969).

So here biology sits, at the beginning of the 21st century with the major guiding paradigms that shaped and drove it in the last century out of steam, reduced to bodies of powerful technique, their vision of the future gone. What will now define the course of biology? Will biology become merely the servant of the society, devoted to solving practical problems? Or will a new and vibrant holistic paradigm emerge to energize the field and lead it into an even brighter future?

But first, we need to understand how biology arrived at this conundrum. In this sense, the discovery of the archaea as a 'third domain of life' and the development of the 'archaeal concept' are illuminating. I will use them as a window into the problems emanating from the 20th century biology paradigm, and as a springboard towards redefining biology for the 21st century. Of necessity, this account will be a personal one.

The story for me begins in the 1960s, when my passing interest in evolution began to intensify and congeal. At the time the genetic code was all the rage, following upon the heels of, and being invested with the panache of, James Watson and Francis Crick's solving the 'problem of the gene.' As originally conceived by George Gamow (1954) the genetic code was the cryptographic aspect of a fundamental biology problem. The codons were seen as physically templating their corresponding amino acids. Thus, to know the codon assignments was to understand the physical/chemical interactions upon which translation and its evolution were based. However, Crick and his adaptor hypothesis soon put an end to that grandiose hope (Crick 1958). Whereas Gamow saw the codon assignments as absolute, founded in interactions between nucleic acids and amino acids, the adaptor hypothesis took an opposite tack, viewing the codon assignments as arbitrary: which amino acid became associated with which adaptor (tRNA) was seen as merely a matter of historical accident: were the code to evolve all over again, the codon assignments would surely be unrelated to those that we know. Since the adaptor hypothesis gained rapid and essentially universal acceptance, the eventual determination of the codon assignments ended the matter, solved the problem. The biological significance of the genetic code lay solely in the catalog of codon assignments *per se.*

However, I did not see it that way. From the start I had been skeptical of the adaptor hypothesis. The idea never really explained or predicted anything; we were just as ignorant with as without it. What the adaptor hypothesis did do, however, was spread a veneer of complacency over our ignorance – which, of course, silenced all further questioning concerning the nature of 'sRNA' (as the tRNA molecule was originally called) (Woese 2001). While Gamow might not have been right in his speculations about the exact nature of the code, to me he was on the right track, at least a track that might lead somewhere. In any case, a molecule as complex and inherently interesting as tRNA could not be simply an adaptor for amino acids. tRNA was far more likely a central functioning part of the translation mechanism than a passive carrier of the amino acid into that mechanism (Woese 2001).

It did not feel right that the code was merely an historical accident. There was too much order in the set of codon assignments for that; order which had to be explained. The problem of the genetic code should never have been formulated in a vacuum in the first place. The code was manifest materially in the tRNAs, and to understand how the code evolved, one could not simply ignore the evolution of the tRNAs, or the rest of the translation apparatus for that matter. Right or wrong, this is the argument that convinced me that the central problem in the evolution of the cell was the evolution of translation, and from then on this has been my major, driving concern in biology.

The evolution of translation is what you can call a non-Darwinian or pre-Darwinian problem; it transcends the biological world known to Charles Darwin and his contemporaries. The problem I faced was how to approach the deep evolutionary questions that have to do with the evolution of the cell itself. At the time I did not know, and in a sense still do not. But one thing seemed certain: approaching any questions of this nature would require a comprehensive phylogeny, a universal phylogenetic tree, as a conceptual framework. Since no universal phylogeny existed at the time (our understanding of evolutionary relationships was effectively confined to plants and animals), realizing that phylogeny would 'merely' require determining the phylogenetic relationships among the bacteria and the single celled eukaryotes, and then tying all this into the phylogenies of animals and plants.

A methodology for doing this, however, was not in place at the time; but the makings of it were, with the 'oligonucleotide cataloging' approach to RNA sequencing recently developed by Frederick Sanger and

his colleagues (Sanger et al. 1965). A molecule ideally suited to both the methodology and the problem, an excellent potential indicator of phylogenetic relationship (on a global scale), seemed to be ribosomal RNA (the small subunit rRNA). Ribosomal RNA is ubiquitous, relatively easy to handle, large enough to give a reliable amount of data, and has evolved relatively slowly – hopefully slowly enough so that reasonably good organismal genealogical traces remained across the entire phylogenetic spectrum (Woese 1987). Also, rRNA is an integral part of a large molecular complex that is central to the functioning of the cell. In other words, in that weird world where bacterial genes seem to be passed around among various species, the central components of the translation apparatus would probably be the last genes you would expect to find transferred laterally (Fox et al. 1977a, b); and as we now know, this is the case. Ribosomal RNA sequence comparisons give us the entire genealogical spectrum, from the 'root' of the universal tree on down to the finer branchings.

It turned out that reworking Sanger's oligonucleotide cataloging technology to fit our needs was the least of the problems we would face. At the time I was totally unaware of the hornet's nest we were stepping into with bacterial taxonomy – something that had a long and sorry history.

To continue the story: the oligonucleotide cataloging methodology was soon ready to go, and we began (at first very slowly) to turn out rRNA 'catalogs' (sets of characteristic oligonucleotides) for a variety of microorganisms (and a few eukaryotes). In the process, one quickly gained a feel for whether a particular oligonucleotide catalog represented a 'prokaryote' or a eukaryote (Fox et al. 1980): each grouping had a very distinctive 'oligonucleotide signature' (Woese et al. 1985). Thus you can readily imagine my amazement when somewhere along the line we ran into a particular catalog (representing *Methanobacterium thermoautotrophicum*) that had neither the 'prokaryote' nor the eukaryote signature. Everyone knew that extant organisms had to be either prokaryotic or eukaryotic, did they not; so how could this be? Our first thought was experimental error; the wrong RNA molecule had somehow been picked. Repetition of the experiment ruled this out. And producing catalogs for relatives of other methanogens quickly showed that all methanogens behaved similarly; all had this weird new type of rRNA. Soon the group of methanogens had an oligonucleotide signature of its own, distinct from both the 'prokaryotic' and the eukaryotic signatures (Fox et al. 1977). The problem was that the methanogens were classically recognized as 'prokaryotes.' How to reconcile all this. Could there possibly exist organisms that were neither 'prokaryotes' nor eukaryotes? The dogma said 'no,' but the experiments suggested 'yes.' This was my first (except for the adaptor hypothesis), but definitely not last, encounter with a major biological dogma, one that had shaped how all biologists thought about life on earth, and one that, in being wrong, had caused enormous damage.

But it was not yet the time to attack the dogma publicly. First we had to see whether this strange type of rRNA occurred elsewhere, in non-methanogenic taxa. And we soon had the answer: yes, such organisms did exist. Oligonucleotide cataloging revealed that the so-called extreme halophiles were of this type, as were two strange 'thermoacidophiles,' *Thermoplasma* and *Sulfolobus* (Woese et al. 1978, 1984). At that point it became apparent that we were dealing with a major organismal grouping, a collection of highly disparate phenotypes all of which appeared to have the same pedigree (Woese 1982).

Still, caution was called for. Before going off on cloud nine about finding a whole new kingdom of organisms, make sure the organisms in the group have more in common than just a characteristic ribosomal RNA. You really cannot make a strong case on the basis of one trait alone. My main partner in crime at the time, the microbiologist Ralph Wolfe (in whose laboratory the methanogens had been grown and radioactively labeled), kept telling me that a stool with one leg would not stand; you need at least three legs! (See Figure 1 for the photograph of Wolfe with the author and Otto Kandler.) What other characteristics

Figure 1. Left to right: the author (Carl Woese), Ralph Wolfe and Otto Kandler. This photo was taken at Rofan Mountain, Austria, 1981.

did the members of this group share? It had been known that the extreme halophiles, the thermoplasmas, and *Sulfolobus* all had the same kind of very unusual lipids: the links to glycerol were ether, not ester; the lipid chains were branched (built of isoprenoid subunits) not straight; and the chirality around the central carbon of the glycerol moiety was the opposite of what one would expect of a typical lipid (Woese 1987). [This coincidence had previously been written off as convergent evolution, the result of all these organisms individually adapting to an 'extreme' environment (Brock 1978).] What was not known at the time was what sort of lipids the methanogens had. Ralph Wolfe prepared a goodly amount of a methanogen and the cell mass was shipped to T. Langworthy for lipid analysis. The answer: methanogens too have these unusual ether-linked, branched chain lipids (Tornabene and Langworthy 1979). The proverbial stool now had two legs, and confidence grew. More legs were soon to come. Members of the archaebacteria (as the archaea were initially called) showed a variety of cell wall types, but none of them were of the characteristic bacterial (peptidoglycan-containing) type (Kandler and Hippe 1977). The DNA-dependent RNA polymerases of the archaebacteria were atypical, far more like those of the eukaryotes than like (eu)bacterial RNA polymerases (Huet et al. 1983). Archaebacteria showed different antibiotic sensitivities than did normal bacteria. And the list of unique or non-eubacterial characteristics continued to grow (Woese 1987). For many of us there could no longer be any doubt that the archaebacteria were a grouping of organisms unto themselves, neither eubacterial nor eukaryotic.

That, however, is not how the majority of biologists, especially microbiologists, saw it. I was quite taken aback by the negative response the initial announcement of the existence of this 'third form of life' evoked, and the vehemence of that response! My colleague Ralph Wolfe was telephoned by Nobel laureate S.E. Luria, who scolded him: 'Ralph, you must dissociate yourself from this nonsense, or you're going to ruin your career!' (Wolfe 2003). Two of the three main weekly news magazines in the United States carried a story about the discovery of the archaebacteria; the third did not – because, it turns out, the science writer for that magazine had checked with a microbiologist, a confidante of his, who had advised him that a 'third form of life' was absurd. There was a notable amount of behind-the-scenes grumbling by microbiologists as well; but, strangely, only one biologist had the courage to challenge the archaeal concept in print at that time (Steitz 1978).

The bizarre thing about this episode in the history of the archaea is that the grouping achieved notoriety not because they represented a third type of living system *per se* (only the lay scientific public were naive enough to see it that way), but because their presumed existence violated a central dogma, the eukaryote–prokaryote dichotomy. So rather than question the dogma, most (micro)biologists were content to condemn the finding. Their failure to question that dogma is one of the black marks in microbiology's history, for the prokaryote–eukaryote is a conjecture, a theory – and scientists are supposed to *test* theories. The history behind why microbiology did not do this is an interesting and instructive one, one well worth our considering.

But I am getting ahead of myself. There is another chapter in the early development of the archaea that needs to be told before proceeding further. Prior to the public announcement of the 'archaebacteria,' I already had some inkling of trouble ahead. It had been relatively easy to convince those involved directly in the project that the archaebacteria were a group apart. They knew the data, and they knew it required explanation; it could not be dismissed. Ralph Wolfe was soon convinced and got into the habit of giving a brief 'peek' into the future at the end of his seminars – gentle, but tactful persuasion. My post-doc George Fox, who was involved in labeling and preparing the 16S rRNA for analysis, Bill Balch, Ralph's student, who actually grew the methanogens – it required a special technology Bill and Ralph had developed to do the job – and Linda Magrum, the technician who worked in the trenches to generate the Sanger two-dimensional oligonucleotide patterns; all of them became ready converts to the new perspective. But outside of this inner circle, attitudes were quite different. I remember asking a number of my colleagues what they thought something that was neither a prokaryote nor a eukaryote would be like. The responses ranged between life from Mars to something with an RNA genome. No one could imagine that there could be anything microbial that was not phylogenetically a 'prokaryote'!

Then one day (prior to our results being published) Ralph Wolfe showed up with Otto Kandler, the well-known German microbiologist and botanist, who wanted to hear our story. George Fox and I were prepared for the usual frustrating struggle to get the visitor to realize that there just might be something under the sun other than *E. coli* and company in the

bacterial world. You can imagine our surprise when shortly after beginning our sales pitch Kandler's eyes widened and he said 'Of course!,' or something like that. He had an open mind and a great feel for biology.

That, it turned out was exactly what the archaea needed to push the concept into the mainstream. Kandler returned to Germany full of enthusiasm about 'archaebacteria,' and proceeded to use his considerable prestige and power in the German microbiology establishment to help German microbiologists see the validity and great potential in the archaebacteria. It was not long before Kandler had organized the first ever symposium on 'archaebacteria,' in Munich. This was 1981, and Kandler saw to it that the manuscripts resulting from the symposium presentations were published back to back in two successive issues of the journal Systematic and Applied Microbiology (which at the time still retained its German name of Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. 1. Abt. Originale C. Allgemeine, Angewandte und ökologische Mikrobiologie) – the journal of which he was the editor. Now the archaeal concept was off and running. In large measure because of Kandler's enthusiasm and influence, Germany became the European (if not the world) leader in archaeal research.

Unbeknownst to me another German scientist, Wolfram Zillig, an Abteilung Leiter at the Max Planck Institute in Martinsried, a person who had spent most of his career on RNA polymerases, had heard of the archaebacteria, and had set about to examine their RNA polymerases. By his own admission Zillig had seen the field get bogged down in detail and his own career winding down into more of the same. To his great surprise and pleasure, Wolfram discovered archaebacterial RNA polymerases definitely not to be of the 'prokaryotic' type. In fact these enzymes were reminiscent of the eukaryotic RNA polymerases (Huet et al. 1983). So instead of going out with a whimper, his career suddenly exploded into a great new adventure, both in the lab and around the world, where he and his young colleague Karl Stetter set out to find more 'bugs' in places that could be quite dangerous, like hot springs in Iceland (where both of them almost came to catastrophic ends on a couple of occasions) (see Figure 2 for a photograph of Wolfram Zillig with Karl Stetter). Stetter later continued this new swashbuckling brand of field microbiology – taking trips into the jungles of Thailand, darkest Africa, the bottom of the ocean, and other inhospitable places, to find new and exciting thermophilic archaebacteria. Everyone could feel their excitement.

Figure 2. Wolfram Zillig (left) and Karl Stetter (right). This photograph was taken at the Archaea Gordon Research Conference, 2001.

While our program in rRNA phylogenetic reconstruction had potentially put bacteriology back on the right track to become, finally, an organismal discipline, there remained one major stumbling block to overcome in microbial ecology. Even armed with Beijerinck's enrichment culturing methodology, microbiologists had been able to detect only a small fraction of species in the bacterial world (just how tiny this fraction was would become apparent only after the fact). If there ever were to be a real bacterial ecology, niches would have to be definable in organismal (biological) terms, which meant identifying all the significant microbes therein.

Molecular phylogenetic characterization was obviously a great step in the right direction, but it did not solve microbial ecologists' problem completely – far from it. To do a 16S rRNA characterization, like any other characterization, the bacterium in question had to be under control, that is, in pure laboratory culture – or did it? While all the rest of microbiology was still fixated on the traditional need to isolate the bacterium to know anything about it, one person, Norman Pace, argued that if phylogenetic identification could be based on the sequence of one molecule (16S rRNA), then it was only that molecule that required isolation and characterization. All the crippling limitations associated with having to cultivate the bacterium in order to know anything significant about it were suddenly swept away. Genuine microbial ecology could now become a reality (Olsen et al. 1986).

Pace's methodology had enormous potential. To begin with, one could design (fluorescent) oligonuc-

leotide probes based upon particular regions in a given 16S rRNA sequence that would permit identifying under the microscope any microbe that carried that particular 16S rRNA (DeLong et al. 1989). Moreover, through proper design one could make a set of such DNA probes that not only identified an individual microorganism as to species, but as to any taxon in the overlying hierarchy – be it genus, family, order, and so on. This methodology is arguably the most powerful technology in the ecologists' armamentarium.

Bacteriology (which microbiology used to be called) is in principle an organismal science, just as are zoology and botany. An organismal science seeks to understand (naturally defined) groups of organisms in biological terms – by which is meant not merely in a structure/function context, but also in terms of the group's diversity, its ecology, and how the organisms therein are ancestrally (evolutionarily) related. In actuality, however, bacteriology never became a full-fledged organismal science, because it was never able to develop its evolutionary side – and consequently was never able to develop a real bacterial ecology, or make sense of the microbial diversity it had uncovered (Stanier and van Niel 1962). The underlying reason here is that microbiologists failed to develop a valid bacterial taxonomy. Such was not possible given the classical bacterial traits upon which it had at the time to be based (Woese 1987). Thus, microbiologists could not develop three of the four pillars of their organismal discipline.

This was a sorry state of affairs, as Roger Stanier and Cornelis B. van Niel clearly recognized when they said '... the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium' (Stanier and van Niel 1962). And that statement is as valid today as it was 40 years ago – although no one can deny that there has been great progress in microbiology over the last half century. Yet, none of it moved bacteriology in the direction of becoming a real organismal discipline. Quite the opposite. In an era defined and dominated by molecular biology (and biochemistry), it was all too easy for a conceptually floundering microbiology to shape itself in the mechano-reductionist mode; which then made it all too easy to forget about such 'theoretical' matters as developing a 'clear concept of a bacterium.'

Unfortunately microbiologists of the mid 20th century, after failing repeatedly to develop a phylogenetic system (the only way to attain a concept of a bacterium), sought to settle the problem once and for all by decree, as it were. It was simply asserted that all 'prokaryotes' were of a kind, and that by comparing the properties of 'prokaryotes' to those of eukaryotes, we could understand what a bacterium was (Stanier and van Niel 1962). Sounds good on the surface, and it made the problem go away, but it does not stand up to analysis. By the time that the archaea were discovered in 1977, microbiology had long forgotten about their old concern with a natural taxonomy. Their concept of the world now hinged on the deeply held, but flimsy, belief that all prokaryotes were of a kind. The discovery of the archaea knocked the pins out from under that world view (at long last the monophyly of the prokaryotes *had* been put to proper experimental test). No wonder microbiologists were upset when suddenly confronted with 'archaebacteria.'

There is a sad irony in all this, namely, as microbiologists were becoming more dogmatic in their belief that all bacteria are specifically related and as a consequence had lost interest in the problem of natural bacterial relationships, the groundwork for determining these relationships by using molecular measures, was being laid. In the early 1950s Sanger and coworkers had sequenced the first proteins (Sanger and Tuppy 1951; Sanger and Thompson 1953); and molecular biologists, at least, were becoming aware of how valuable protein sequence comparisons should be in determining phylogenetic relationships. In 1958 one of the great biologists of the day, Francis Crick, 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. It can be argued 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. (Crick 1958, p. 142)

But this trenchant insight fell on deafened ears within the microbiology community, though not among certain *macro*biologists. The latter saw the new molecular approach as a marvelous tool for confirming and extending the classical phylogenies of plants and animals. When microbial taxonomists finally did get into the act, they used (simple) molecular methods for essentially trivial purposes, to make minor repairs in the poor bacterial taxonomy passed down to them by classical microbiologists; which were largely confined to weeding out misclassified species from genera and properly grouping genera into families. The compelling vision of their forebears, their over-

riding concern with building a concept of bacteria on a comprehensive natural bacterial taxonomy, was simply gone.

This mix of scientific apathy, hostility, ignorance, and a reductionist perspective is what the archaea had to face in coming of age. Yet, truth has a way of triumphing (in science at least), and the archaeal notion has now found its rightful place in the pantheon of biological ideas.

A new century, a new era in microbiology

The discovery of the archaea provided the seed for microbiology's (bacteriology's) developing into a full-fledged organismal discipline, a discipline that has an active and effective interest in evolution, as well as ecology and microbial diversity. And today that seed has been planted in fertile genomic soil. The microbiology of the future will be fashioned in large measure by genomics and evolutionary considerations. The microbial world is the doorway to approaching one of the truly great biological problems, how cellular life originated. It is through the microbial world also that biology will come to understand the biosphere, the dynamics of the global environment. This is all, however, for the future. Here and now I need to discuss the impact that genomics has had on our understanding of the archaea and their implications.

The recent deluge of genomic sequence data has proven almost as confusing as it has bountiful. At least that is the impression I get from reading some of the evolutionary conclusions drawn from this wealth of data. A veritable cottage industry has arisen in trying to dismantle the archaea through genomics, and in re-rooting, uprooting, or completely destroying the basic structure of the universal phylogenetic tree based upon rRNA sequence analyses (see Pennisi 1998, 1999). Thus, it is appropriate that I summarize the evidence that genomics has brought forth in support of the archaeal grouping and the structure of the phylogenetic tree.

First let us consider a problem still posed by the eukaryote–prokaryote dichotomy. The dichotomy actually comprises two assertions, the one phylogenetic, and the other organizational. The bipartite phylogenetic division of life has, of course, now been disproven. But its organizational assertion still lingers and remains a source of confusion. The primary observation upon which the dichotomy rests is that under a light microscope one can distinguish two, and only two, types of cellular organization. The eukaryotic cell presents a very distinct, unique picture, with its membrane-bounded nucleus and various other intracellular compartments. All prokaryote cells, on the other hand, appear essentially featureless internally under the microscope. These observations are certainly justifiable reason to see eukaryotes as having a common and highly unique cellular organization. But only by a big stretch of the imagination can one then infer the same to hold for all bacteria; the evidence defining their 'common' structure being entirely negative (Chatton 1937). Yet this is what was concluded, and this is what many biologist still believe today. Now I put it to the reader: if the apparent existence of two types of cellular organization demands two corresponding primary phylogenetic categories, why is it not the case, then that the certain existence of three phylogenetic categories requires there to be three distinct types of cellular organization? Granted, archaea and eubacteria are equally featureless under the light microscope. So what? On the molecular level the two can be distinguished from one another just as easily as either can be from the eukaryote. What does the molecular level tell us about the organization of the two cell types? Nothing for certain yet, except that the two types cannot be the same. Perhaps the most tantalizing and strongest indicator that there exists a unique type of archaeal cellular organization is the existence of an archaeal genomic signature, a set of approximately 300 protein coding genes that are found in at least two major groups of archaea, but nowhere else (Graham et al. 2000). (The vast majority of these genes have yet to be assigned function, although sequence motifs in many of them do suggest general types of roles they might play.) Let us now turn to some of the specifics that distinguish the archaea from the eubacteria.

In their metabolisms the two groups differ subtly, though not dramatically. The eubacteria are far and away the most metabolically diverse and versatile organisms on this planet. While the euryarchaea show some metabolic diversity (a good deal of it imported from the eubacteria), they are most notable for two things, their unique methanogenesis, and the cofactors that are more or less unique to the group – for example, the C-1 carriers, coenzyme M, methanofuran, and methanopterin; coenzyme F420, an electron carrier analogous to NAD; F430, a nickel containing porphyrin akin to heme and the like; and methanophenazine, a membrane-bound carrier with quinone-like function. Significantly, most of the archaeal cofactors seem to be unique in their biosyn-

thesis; at least many of the enzymes involved appear to be home grown, evolved within the archaeal group (Wolfe 1992). In addition, there are the 'classically' observed biochemical uniquenesses; in archaeal membrane lipids, cell walls, and so on (mentioned above).

It is in the information processing systems that the differences between eubacteria and the archaea are most impressive. First translation: while much of the componentry of the archaeal translation mechanism is universal, homologous between the archaea and eubacteria (and eukaryotes), the archaeal-eubacterial distinction is readily apparent in their details (sequences). The distinction between the archaeal and eubacterial versions of a particular molecule is so obvious that it can usually be seen upon gross inspection of a sequence alignment (Woese et al. 2000) – where the archaeal and eubacterial versions characteristically differ by the presence or absence of rather large sequence blocks. In addition, for those columns in a sequence alignment where composition is highly conserved, one often sees that the characteristic eubacterial composition differs from that characteristic of the archaea and *vice versa*. This nearly qualitative distinction between the two versions of a sequence, this difference in 'genre,' has been designated 'canonical pattern' (Woese et al. 2000). Differences this extreme are never encountered among the various eubacterial taxa or among different archaeal taxa (although in each case there have been three billion years or so, most of this planet's history, in which such differences could have arisen). When the eukaryotic versions of these universal proteins are brought into the picture, they are almost always of the archaeal genre (Woese et al. 2000).

In addition to the universal ribosomal proteins, however, there exists a relatively small cadre that are characteristic of and found only in eubacteria, while a somewhat larger set is common and confined solely to the archaea and eukaryotes, with still a few others that are confined to eukaryotic ribosomes.

One sees a similar pattern in the archaeal and eubacterial transcription mechanisms, but with more (and more conspicuous) exceptions to universality. The two largest (the catalytic) subunits of the DNA-dependent RNA polymerase, beta and beta′ in eubacterial nomenclature, are clearly universal, and demonstrate the above canonical pattern (with, again, eukaryotic sequences being of the archaeal genre) (Langer et al. 1995). After this, however, precious little homology is seen between the eubacterial and the archaeal componentry in the mechanism. What homology there is, is confined to the third main transcriptional subunit, the so-called alpha subunit (in eubacterial nomenclature); but the homology is only partial. Two copies of alpha occur in the eubacterial holoenzyme, whereas in the archaeal case (or the eukaryotic) two distinct proteins of very different size exist, each present in single copy, with parts of each showing homology to (somewhat) different parts of eubacterial alpha (Langer et al. 1995). Over and above these differences, the archaeal transcription polymerase exhibits a number of additional (smaller) subunits, none of which are found in the eubacterial case, but all of which occur in the eukaryotic enzyme(s). When it comes to transcription initiation, homology between the archaea and eubacteria is negligible. The two mechanisms use different componentry (Bell and Jackson 2001), with the eukaryotes once again showing an augmented version of the archaeal mechanism.

The most spectacular difference between the archaea and eubacteria, of course, lies in their genome replication mechanisms. The closely related archaeal and eukaryotic systems to a first approximation share no homology with the componentry of their eubacterial counterpart (Olsen and Woese 1996). And in keeping with what we have seen above, the mechanism of initiation of chromosome replication in the eubacteria is fundamentally different from that of the archaea and eukaryotes. The genome replication mechanism appears to have evolved twice.

Many biologists deduced specific relationship between the archaea and eubacteria from their both having circular chromosome structure (which eukaryotes do not). In light of the similarity between the archaeal and eukaryotic chromosome replication and its initiation, this conclusion needs revisiting. Note also in this context that both the eukaryotic and archaeal (euryarchaeal) chromosomes exhibit nucleosome organization – the single archaeal histone being a homolog of the four (related) histones that structure the eukaryotic nucleosome (Reeve et al. 1997).

Finally I would add that a number of phylogenetic trees based upon whole genomes, confirm the original contention based upon rRNA sequence analysis that the archaea are a grouping unto themselves, neither eubacterial nor eukaryotic in nature (Fitz-Gibbon and House 1999; Snel et al. 1999).

There have been several suggestions to the effect that the archaea are not a phylogenetically coherent (i.e., a valid) organismal group, or to the effect that the archaea arose from a particular eubacterial ancestor – all based upon anecdotal evidence and particular genomic sequence analyses (Gupta and Golding 1993; Cavalier-Smith 2002; see Gupta 2003). In the light of the above (and other) evidence as to the evolutionary coherence of both the archaeal and bacterial groups and the strong, specific and detailed distinction between them (especially in key elements of their cellular designs), I do not see how these contrary claims can have any validity. The burden of proof surely rests upon those who would like things to be otherwise.

The archaea are the harbinger of the future of microbiology, a future based on the knowledge of phylogenetic relationships. Among other things this means that microbial ecology can exist in more than name only. With the great progress in microbial ecology over the last decade, which continues to escalate, we can now see first hand what having a phylogenetically based discipline can do. But bacterial ecology alone realizes only a portion of the potential in a phylogenetically constituted microbiology – one that has finally become an organismal discipline. Bacterial evolution is just beginning to come to the fore. And with it will come a fundamental change in all of biology, for biology is now on the threshold of answering the great question: 'Where did we come from?'

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