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# MINIREVIEW

# The Winds of (Evolutionary) Change: Breathing New Life into Microbiology

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Of all the spectacular changes that have transformed biology over the last several decades, the least touted, but ultimately one of the most profound is that now under way in microbiology. It is not simply that we are coming to see microbiology per se in a new light but that we are coming to appreciate the central roles microorganisms play in shaping the past and present environments of Earth and the nature of all life on this planet. Because each organism is the product of its history, a knowledge of phylogenetic relationships—of common evolutionary histories—is essential to understanding the nature of any organism. Thus, it is unavoidable that evolution (a field much neglected in this molecular era) must be the conceptual heart of biology; all extant life traces back to common ancestors, and the earliest ancestors were microorganisms.

Despite considerable effort, microbiologists were never able to determine the phylogenetic relationships among prokaryotes (29). Not only did they ultimately give up on this problem, but some went so far as to declare it unsolvable (27, 28). By the 1960s most microbiologists concerned themselves little and cared even less about relationships among microorganisms. As a consequence, microbiology was not structured about a "natural," phylogenetically valid system of classification. Lacking this essential evolutionary touchstone, microbiology developed in an incomplete, if not distorted, way.

Having no natural center, microbiology fissioned into separate disciplines pursuing (seemingly) divergent goals. The traditional pursuits of organism isolation and determinative classification (i.e., naming) continued, as did the applied side of microbiology (medical, industrial, etc.). However, the disciplines that came to dominate and define the field reflected the new molecular outlook: i.e., the detailed genetics of particular organisms, the comprehensive molecular biology of a "representative" prokaryote, and the detailed biochemistry of this or that metabolic pathway. Microbial ecology was a weak, in a sense immature, discipline, stymied not only by the lack of a natural system but also by the requirement that a species be cultured and characterized before its role in a microbial community could be explored.

The most profound symptom of microbiology's unfortunate condition was its reliance on the prokaryote-eukaryote dichotomy as a phylogenetic crutch, something that replaced any useful understanding of microbial relationships: it represented microbiology's "... only hope of ... formulating a 'concept of a bacterium'" (28). The problem with and the pernicious nature of this dichotomy lay in the fact that the prokaryote was initially defined negatively, in cytological terms. In other words, prokaryotes lacked this or that feature characteristic of the eukaryotic cell: even oil drops, or coacervates, could fit such a negative definition. Any virtue in the prokaryote-eukaryote dichotomy lay in what it could contribute to an understanding of the eukaryote, which might have evolved through "prokaryotic" stages. With repetition (as catechism) the prokaryote-eukaryote dichotomy served only to make microbiologists easily accept their near total ignorance of the relationships among prokaryotes; they were even dulled to the fact—one of the great challenges of today—that they did not in the slightest understand the relationship between the prokaryote and the eukaryote. The matter of relationships among bacteria had boiled down to "if it isn't a eukaryote, it's a prokaryote," and to understand prokaryotes, we had only to determine how *Escherichia coli* differs from the eukaryotes. This was no invitation to creative thought, no unifying biological principle. This eukaryote-prokaryote dichotomy was a barrier that separated prokaryotic microbiology from eukaryotic microbiology.

This myopic view of microbiology failed to appreciate not only how important the problem of microbial relationships was but that an intractable problem today may not be so tomorrow. Molecular sequences had been used to determine evolutionary relationships since the 1950s, and Zuckerkandl and Pauling's seminal article "Molecules as documents of evolutionary history" made the case most compellingly in 1965 (36). Yet the record shows that microbiology—the biological science most in need—was effectively blind to the significance and potential of these approaches.

At the end of the 1970s however, the situation changed dramatically. rRNA sequences had been shown to provide a key to prokaryote phylogeny (e.g., 8). No matter that on the cellular and physiological levels the prokaryotes did not provide characteristics that permitted their reliable phylogenetic ordering; their rRNAs were more than sufficient to do so. By the early 1980s, as the rRNA-based phylogeny of prokaryotes began to emerge, microbiologists began (albeit extremely slowly) reawakening to the importance of knowing microbial phylogenies.

The folly of having taken all prokaryotes to be of a kind was dramatically revealed by the totally unanticipated discovery of the *Archaea* (originally called archaebacteria), a group of prokaryotes that, if anything, is more closely related to *Eucarya* (eukaryotes) than to the other prokaryotes, the (true) *Bacteria* (11, 13, 32, 34). Even then, the power of the eukaryote-

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prokaryote dichotomy as phylogenetic dogma was strikingly demonstrated by the fact that the majority of microbiologists (and biologists) were initially unable to accept that there could be two types of prokaryotes that are not specifically related to one another. (An amazing aspect of the history is that [at the time] a biologist would most likely have agreed that the nuclear genome came from a "prokaryote-like" ancestor, i.e., the most recent common ancestor of eukaryotes and bacteria. After all, this is inherent in the prokaryote-eukaryote dichotomy. Yet the idea that specific prokaryotic relatives of the eukaryotes have persisted to the present was anathema. As best as we can tell, these noneukaryote descendants do exist; we call them *Archaea*. However, the nature of biologist's objections was not monolithic. For example, Margulis and Schwartz [19] suggested that the origin of [most of] the nuclear genes was indeed a specific group of [true] bacteria, the "aphagramabacteria." This view had the merit of proposing that some prokaryotes are more closely related to eukaryotes than are others, but it ignored the available molecular evidence as to which prokaryotes these were. This confusion was furthered by a drawing of the proposed relationships in which present-day organisms are not clearly distinguished from their ancestors.)

To date, over 1,500 prokaryotes have been characterized by small-subunit rRNA sequencing. Figure 1 encapsulates prokaryotic phylogeny as it is now known. Given the purview of this journal, our discussion will be limited to the two prokaryotic domains. However, it is worthwhile to note in passing that molecular phylogeny has had an equally profound effect on our understanding of relationship among eukaryotes (eukaryotic microorganisms in particular) and that anyone exposed to the universal phylogenetic tree readily appreciates how artificial the strong distinction between eukaryotes and prokaryotes has become. In Fig. 1, the split between the *Archaea* and *Bacteria* is the primary phylogenetic division (in that the *Eucarya* have branched from the same side of the tree as the *Archaea* [11, 13]).

Both prokaryotic domains would seem to be of thermophilic origin—suggesting that life arose in a very warm environment (1, 2, 31). Among the *Archaea*, all of the *Crenarchaeota* cultured to date are thermophiles, and the deepest euryarchaeal branchings are represented exclusively by thermophiles. Among the *Bacteria*, the deepest known branchings are again represented exclusively by thermophiles, and thermophilia is widely scattered throughout the domain.

The *Archaea* comprise a small number of quite disparate phenotypes that grow in unusual (to us, inhospitable) niches. All are obligate or facultative anaerobes. As stated above, all (cultured) crenarchaeotes are thermophilic, some even growing optimally above the normal boiling temperature of water. The *Archaeoglobales* are sulfate reducers growing at high temperatures. The extreme halophiles grow only in highly saline environments (such as the Dead Sea and salt evaporation ponds). The methanogens, which are responsible for virtually all of the biologically produced methane on this planet, are confined to a variety of anaerobic niches, often thermophilic.

The *Bacteria*, on the other hand, are notable as being the source of life's photosynthetic capacity. Five kingdoms of bacteria contain photosynthetic species, and each of the five manifests a distinct type of (chlorophyll-based) photosynthesis. The cyanobacteria have also given rise to the chloroplasts, which are the basis for all photosynthesis found in eukaryotes. It would appear that photosynthetic metabolism is the ultimate evolutionary source of much of the metabolic diversity found among the bacteria, which in turn is the source of key biochemistries (e.g., photosynthesis and respiration) among the eukaryotes.

The molecular revolution in microbiology means much more than just a new taxonomy. For example, microbial ecology is being reborn. The remarkable insight of Norman Pace that organisms can be (phylogenetically) identified directly in their niches—through a combination of rRNA gene cloning and sequencing, and the design and use of rRNA-directed "phylogenetic stains"—has sparked a revolution in that discipline (5, 24, 25). This simple realization removes the obstacle of having to culture all organisms in order to infer their characteristics, and it permits all manner of new detailed characterizations of the populations in any given niche to be made. Perhaps the most dramatic demonstration of these new approaches to microbial ecology is the discovery (through cloning and sequencing of genes directly from environmental samples) of two new groups of *Archaea*—both of which have to this point defied cultivation (4, 10). One is a new family or order within the euryarchaeotes, the other almost certainly a new taxon of even higher rank (see Pacific Ocean station 49 DNA clone NH49-9, Santa Barbara Channel DNA clone SBAR5, and Woods Hole DNA clone WHARQ in Fig. 1). For the first time, it is possible to count not just flowers and beetles, but also microorganisms, in taking a census of life on this planet.

Another area in which evolutionary and comparative perspectives have been invaluable has been the inference of secondary and tertiary RNA structures. Comparisons of homologous RNA sequences from phylogenetically diverse organisms have provided our current understandings of the structures of rRNAs (9, 21, 22, 35), RNase P RNA (14), self-splicing introns (3, 20), signal recognition particle RNA (15, 18), and the small nuclear RNAs of the spliceosome (for a review, see reference 12). In several of these cases, it was rRNA-based phylogenetic information that guided the choice of organisms from which the corresponding molecules were identified and sequenced.

Although our interests are primarily biological, to many people the justification of any pursuit lies in its practical consequences. The revolution in microbial ecology and the inference of molecular structures do, of course, have direct practical consequences (witness *Applied and Environmental Microbiology* and RNA-binding antibiotics), but it is the application in clinical microbiology that most people would think of first. Many clinically important microorganisms have now been subjected to molecular phylogenetic analysis (e.g., 6, 26). These data and analyses have been used to develop molecular diagnostics (e.g., 16) and to guide research into the nature (and control) of various pathogens.

The universal phylogenetic tree brings us face to face with the great evolutionary questions and allows us to formulate them in molecular terms.

FIG. 1. Prokaryotic phylogenetic tree of 253 representative species derived by maximum likelihood analysis of small-subunit rRNA sequences. The tree is abstracted from that provided by the Ribosomal Database Project (17), which is a composite of several trees inferred by using maximum likelihood (7, 23). The suggested rooting is that inferred for the universal tree (11, 13). Organisms are consecutively numbered and are indexed in the accompanying alphabetic listing (Table 1). Names of the major groups (31, 34) are indicated. The distance scale indicates the expected number of changes per sequence position, for those positions changing at the median rate.

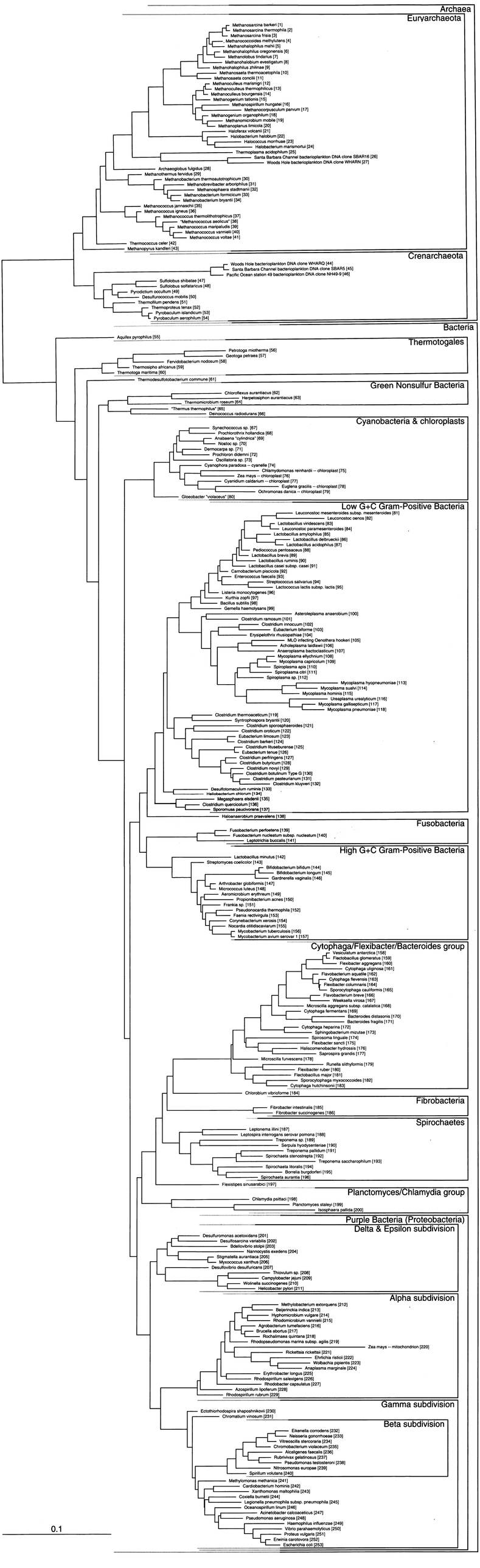
Archaea
Euryarchaeota
Methanosarcina barkeri [1]
Methanosarcina thermophila [2]
Methanosarcina frisia [3]
Methanococcoides methylutens [4]
Methanohalophilus mahii [5]
Methanohalophilus oregonensis [6]
Methanolobus tindarius [7]
Methanohalobium evestigatum [8]
Methanohalophilus zhilinae [9]
Methanosaeta thermoacetophila [10]
Methanosaeta concilii [11]
Methanoculleus marisnigri [12]
Methanoculleus thermophilicus [13]
Methanoculleus bourgensis [14]
Methanogenium tationis [15]
Methanospirillum hungatei [16]
Methanocorpusculum parvum [17]
Methanogenium organophilum [18]
Methanomicrobium mobile [19]
Methanoplanus limicola [20]
Haloferax volcanii [21]
Halobacterium halobium [22]
Halococcus morrhuae [23]
Halobacterium marismortui [24]
Thermoplasma acidophilum [25]
Santa Barbara Channel bacterioplankton DNA clone SBAR16 [26]
Woods Hole bacterioplankton DNA clone WHARN [27]
Archaeoglobus fulgidus [28]
Methanothermus fervidus [29]
Methanobacterium thermoautotrophicum [30]
Methanobrevibacter arboriphilus [31]
Methanosphaera stadtmanii [32]
Methanobacterium formicicum [33]
Methanobacterium bryantii [34]
Methanococcus jannaschii [35]
Methanococcus igneus [36]
Methanococcus thermolithotrophicus [37]
"Methanococcus aeolicus" [38]
Methanococcus maripaludis [39]
Methanococcus vannielii [40]
Methanococcus voltae [41]
Thermococcus celer [42]
Methanopyrus kandleri [43]
Crenarchaeota
Woods Hole bacterioplankton DNA clone WHARQ [44]
Santa Barbara Channel bacterioplankton DNA clone SBAR5 [45]
Pacific Ocean station 49 bacterioplankton DNA clone NH49-9 [46]
Sulfolobus shibatae [47]
Sulfolobus solfataricus [48]
Pyrodictium occultum [49]
Desulfurococcus mobilis [50]
Thermofilum pendens [51]
Thermoproteus tenax [52]
Pyrobaculum islandicum [53]
Pyrobaculum aerophilum [54]
Bacteria
Aquifex pyrophilus [55]
Thermotogales
Petrotoga miotherma [56]
Geotoga petraea [57]
Fervidobacterium nodosum [58]
Thermosipho africanus [59]
Thermotoga maritima [60]
Thermodesulfotobacterium commune [61]
Green Nonsulfur Bacteria
Chloroflexus aurantiacus [62]
Herpetosiphon aurantiacus [63]
Thermomicrobium roseum [64]
"Thermus thermophilus" [65]
Deinococcus radiodurans [66]
Cyanobacteria & chloroplasts
Synechococcus sp. [67]
Prochlorothrix hollandica [68]
Anabaena "cylindrica" [69]
Nostoc sp. [70]
Dermocarpa sp. [71]
Prochloron didemni [72]
Oscillatoria sp. [73]
Cyanophora paradoxa -- cyanelle [74]
Chlamydomonas reinhardii -- chloroplast [75]
Zea mays -- chloroplast [76]
Cyanidium caldarium -- chloroplast [77]
Euglena gracilis -- chloroplast [78]
Ochromonas danica -- chloroplast [79]
Gloeobacter "violaceus" [80]
Low G+C Gram-Positive Bacteria
Leuconostoc mesenteroides subsp. mesenteroides [81]
Leuconostoc oenos [82]
Lactobacillus viridescens [83]
Leuconostoc paramesenteroides [84]
Lactobacillus amylophilus [85]
Lactobacillus delbrueckii [86]
Lactobacillus acidophilus [87]
Pediococcus pentosaceus [88]
Lactobacillus brevis [89]
Lactobacillus ruminis [90]
Lactobacillus casei subsp. casei [91]
Carnobacterium piscicola [92]
Enterococcus faecalis [93]
Streptococcus salivarius [94]
Lactococcus lactis subsp. lactis [95]
Listeria monocytogenes [96]
Kurthia zopfii [97]
Bacillus subtilis [98]
Gemella haemolysans [99]
Asteroleplasma anaerobium [100]
Clostridium ramosum [101]
Clostridium innocuum [102]
Eubacterium biforme [103]
Erysipelothrix rhusiopathiae [104]
MLO infecting Oenothera hookeri [105]
Acholeplasma laidlawii [106]
Anaeroplasma bactoclasticum [107]
Mycoplasma ellychnium [108]
Mycoplasma capricolum [109]
Spiroplasma apis [110]
Spiroplasma citri [111]
Spiroplasma sp. [112]
Mycoplasma hyopneumoniae [113]
Mycoplasma suahi [114]
Mycoplasma hominis [115]
Ureaplasma urealyticum [116]
Mycoplasma gallisepticum [117]
Mycoplasma pneumoniae [118]
Clostridium thermoaceticum [119]
Syntrophospora bryantii [120]
Clostridium sporosphaeroides [121]
Clostridium oroticum [122]
Eubacterium limosum [123]
Clostridium barkeri [124]
Clostridium lituseburense [125]
Eubacterium tenue [126]
Clostridium perfringens [127]
Clostridium butyricum [128]
Clostridium novyi [129]
Clostridium botulinum Type G [130]
Clostridium pasteurianum [131]
Clostridium kluyveri [132]
Desulfotomaculum ruminis [133]
Heliobacterium chlorum [134]
Megasphaera elsdenii [135]
Clostridium quercicolum [136]
Sporomusa paucivorans [137]
Haloanaerobium praevalens [138]
Fusobacteria
Fusobacterium perfoetens [139]
Fusobacterium nucleatum subsp. nucleatum [140]
Leptotrichia buccalis [141]
High G+C Gram-Positive Bacteria
Lactobacillus minutus [142]
Streptomyces coelicolor [143]
Bifidobacterium bifidum [144]
Bifidobacterium longum [145]
Gardnerella vaginalis [146]
Arthrobacter globiformis [147]
Micrococcus luteus [148]
Aeromicrobium erythreum [149]
Propionibacterium acnes [150]
Frankia sp. [151]
Pseudonocardia thermophila [152]
Faenia rectivirgula [153]
Corynebacterium xerosis [154]
Nocardia otitidiscaviarum [155]
Mycobacterium tuberculosis [156]
Mycobacterium avium serovar 1 [157]
Cytophaga/Flexibacter/Bacteroides group
Vesicularia antarctica [158]
Flectobacillus glomeratus [159]
Flexibacter aggregans [160]
Cytophaga uliginosa [161]
Flavobacterium aquatile [162]
Cytophaga flevensis [163]
Flexibacter columnaris [164]
Sporocytophaga cauliformis [165]
Flavobacterium breve [166]
Weeksella virosa [167]
Microscilla aggregans subsp. catalatica [168]
Cytophaga fermentans [169]
Bacteroides distasonis [170]
Bacteroides fragilis [171]
Cytophaga heparina [172]
Sphingobacterium mizutae [173]
Spirosoma linguale [174]
Flexibacter sancti [175]
Haliscomenobacter hydrossis [176]
Saprospira grandis [177]
Microscilla furvescens [178]
Runella slithyformis [179]
Flexibacter ruber [180]
Flectobacillus major [181]
Sporocytophaga myxococcoides [182]
Cytophaga hutchinsonii [183]
Chlorobium vibrioforme [184]
Fibrobacteria
Fibrobacter intestinalis [185]
Fibrobacter succinogenes [186]
Spirochaetes
Leptonema illini [187]
Leptospira interrogans serovar pomona [188]
Treponema sp. [189]
Serpula hyodysenteriae [190]
Treponema pallidum [191]
Spirochaeta stenostrepta [192]
Treponema saccharophilum [193]
Spirochaeta litoralis [194]
Borrelia burgdorferi [195]
Spirochaeta aurantia [196]
Flexistipes sinusarabici [197]
Planctomyces/Chlamydia group
Chlamydia psittaci [198]
Planctomyces staleyi [199]
Isosphaera pallida [200]
Purple Bacteria (Proteobacteria)
Delta & Epsilon subdivision
Desulfuromonas acetoxidans [201]
Desulfosarcina variabilis [202]
Bdellovibrio stolpii [203]
Nannocystis exedens [204]
Stigmatella aurantiaca [205]
Myxococcus xanthus [206]
Desulfovibrio desulfuricans [207]
Thiovulum sp. [208]
Campylobacter jejuni [209]
Wolinella succinogenes [210]
Helicobacter pylori [211]
Alpha subdivision
Methylobacterium extorquens [212]
Beijerinckia indica [213]
Hyphomicrobium vulgare [214]
Rhodomicrobium vannielii [215]
Agrobacterium tumefaciens [216]
Brucella abortus [217]
Rochalimaea quintana [218]
Rhodopseudomonas marina subsp. agilis [219]
Zea mays -- mitochondrion [220]
Rickettsia rickettsii [221]
Ehrlichia risticii [222]
Wolbachia pipientis [223]
Anaplasma marginale [224]
Erythrobacter longus [225]
Rhodospirillum salexigens [226]
Rhodobacter capsulatus [227]
Azospirillum lipoferum [228]
Rhodospirillum rubrum [229]
Gamma subdivision
Ectothiorhodospira shaposhnikovii [230]
Chromatium vinosum [231]
Beta subdivision
Eikenella corrodens [232]
Neisseria gonorrhoeae [233]
Vitreoscilla stercoraria [234]
Chromobacterium violaceum [235]
Alcaligenes faecalis [236]
Rubrivivax gelatinosus [237]
Pseudomonas testosteroni [238]
Nitrosomonas europae [239]
Spirillum volutans [240]
Methylomonas methanica [241]
Cardiobacterium hominis [242]
Xanthomonas maltophilia [243]
Coxiella burnetii [244]
Legionella pneumophila subsp. pneumophila [245]
Oceanospirillum linum [246]
Acinetobacter calcoaceticus [247]
Pseudomonas aeruginosa [248]
Haemophilus influenzae [249]
Vibrio parahaemolyticus [250]
Proteus vulgaris [251]
Erwinia carotovora [252]
Escherichia coli [253]
0.1

TABLE 1. List of organisms from which the sequences used in Fig. 1 were obtained

| Organism | No. shown in Fig. 1 |
|---|---|
| *Acholeplasma laidlawii* JA1 | [106] |
| *Acinetobacter calcoaceticus* (ATCC 33604) | [247] |
| *Aeromicrobium erythreum* (NRRL B-3381) | [149] |
| *Agrobacterium tumefaciens* (ATCC 4720) | [216] |
| *Alcaligenes faecalis* (ATCC 8750) | [236] |
| *Anabaena "cylindrica"* (PCC 7122) | [69] |
| *Anaeroplasma bactoclasticum* JR (ATCC 27112) | [107] |
| *Anaplasma marginale* | [224] |
| *Aquifex pyrophilus* Kol5a | [55] |
| *Archaeoglobus fulgidus* VC-16 (DSM 4304) | [28] |
| *Arthrobacter globiformis* (DSM 20124) | [147] |
| *Asteroleplasma anaerobium* 161 (ATCC 27880) | [100] |
| *Azospirillum lipoferum* (ATCC 29707) | [228] |
| *Bacillus subtilis* | [98] |
| *Bacteroides distasonis* (ATCC 8503) | [170] |
| *Bacteroides fragilis* (ATCC 25285) | [171] |
| *Bdellovibrio stolpii* UKi2 (ATCC 27052) | [203] |
| *Beijerinckia indica* (ATCC 9039) | [213] |
| *Bifidobacterium bifidum* (ATCC 29521) | [144] |
| *Bifidobacterium longum* (ATCC 15707) | [145] |
| *Borrelia burgdorferi* B31 (ATCC 35210) | [195] |
| *Brucella abortus* 11/19 | [217] |
| *Campylobacter jejuni* (ATCC 33560) | [209] |
| *Cardiobacterium hominis* (ATCC 15826) | [242] |
| *Carnobacterium piscicola* (ATCC 43225) | [92] |
| *Chlamydia psittaci* 6 BC (ATCC VR 125) | [198] |
| *Chlamydomonas reinhardii* chloroplast | [75] |
| *Chlorobium vibrioforme* 6030 (DSM 260) | [184] |
| *Chloroflexus aurantiacus* J-10-fl (ATCC 29366) | [62] |
| *Chromatium vinosum* (ATCC 17899) | [231] |
| *Chromobacterium violaceum* (ATCC 12472) | [235] |
| *Clostridium barkeri* (ATCC 25849) | [124] |
| *Clostridium botulinum* type G (ATCC 27322) | [130] |
| *Clostridium butyricum* Rowett (ATCC 19398) | [128] |
| *Clostridium innocuum* B-3 (ATCC 14501) | [102] |
| *Clostridium kluyveri* (ATCC 8527) | [132] |
| *Clostridium lituseburense* (ATCC 25759) | [125] |
| *Clostridium novyi* (ATCC 17861) | [129] |
| *Clostridium oroticum* (ATCC 13619) | [122] |
| *Clostridium pasteurianum* (ATCC 6013) | [131] |
| *Clostridium perfringens* (ATCC 13124) | [127] |
| *Clostridium quercicolum* (ATCC 25974) | [136] |
| *Clostridium ramosum* 113-1 (ATCC 25582) | [101] |
| *Clostridium sporosphaeroides* (ATCC 25781) | [121] |
| *Clostridium thermoaceticum* (ATCC 39073) | [119] |
| *Corynebacterium xerosis* (ATCC 373) | [154] |
| *Coxiella burnetii* QI77 | [244] |
| *Cyanidium caldarium* 14-1-1 chloroplast | [77] |
| *Cyanophora paradoxa* cyanelle | [74] |
| *Cytophaga fermentans* (ATCC 19072) | [169] |
| *Cytophaga flevensis* (ATCC 27944) | [163] |
| *Cytophaga heparina* (ATCC 13125) | [172] |
| *Cytophaga hutchinsonii* (ATCC 33406) | [183] |
| *Cytophaga uliginosa* (ATCC 14397) | [161] |
| *Deinococcus radiodurans* (ATCC 35073) | [66] |
| *Dermocarpa* sp. (PCC 7437) | [71] |
| *Desulfovarcina variabilis* 3 be 13 (DSM 2060) | [202] |
| *Desulfotomaculum ruminis* DL (NCIB 8452) | [133] |
| *Desulfovibrio desulfuricans* (ATCC 27774) | [207] |
| *Desulfurococcus mobilis* (DSM 2161) | [50] |
| *Desulfuromonas acetoxidans* 11070 (DSM 684) | [201] |
| *Ectothiorhodospira shaposhnikovii* (DSM 243) | [230] |
| *Ehrlichia risticii* Illinois (ATCC VR 986) | [222] |
| *Eikenella corrodens* (ATCC 23834) | [232] |
| *Enterococcus faecalis* | [93] |
| *Erwinia carotovora* (ATCC 15713) | [252] |
| *Erysipelothrix rhusiopathiae* alpha-P15 (ATCC 19414) | [104] |
| *Erythrobacter longus* OCh 101 (ATCC 33941) | [225] |
| *Escherichia coli* | [253] |
| *Eubacterium biforme* (ATCC 27806) | [103] |
| *Eubacterium limosum* (ATCC 8486) | [123] |
| *Eubacterium tenue* (ATCC 25553) | [126] |
| *Euglena gracilis* chloroplast | [78] |
| *Faenia rectivirgula* (ATCC 33515) | [153] |
| *Fervidobacterium nodosum* Rt 17-B1 (ATCC 35602) | [58] |
| *Fibrobacter intestinalis* JG1 | [185] |
| *Fibrobacter succinogenes* REH9-1 (ATCC 53857) | [186] |
| *Flavobacterium aquatile* (ATCC 11947) | [162] |
| *Flavobacterium breve* (ATCC 14234) | [166] |
| *Flectobacillus glomeratus* (ATCC 43344) | [159] |
| *Flectobacillus major* (ATCC 29496) | [181] |
| *Flexibacter aggregans* (ATCC 23162) | [160] |
| *Flexibacter columnaris* (ATCC 43622) | [164] |
| *Flexibacter ruber* (ATCC 23103) | [180] |
| *Flexibacter sanci* (ATCC 23092) | [175] |
| *Flexistipes sinusarabici* MAS 10 (DSM 4947) | [197] |
| *Frankia* sp. strain 1.27 | [151] |
| *Fusobacterium nucleatum* subsp. *nucleatum* (ATCC 25586) | [140] |
| *Fusobacterium perfoetens* (ATCC 29250) | [139] |
| *Gardnerella vaginalis* (ATCC 14018) | [146] |
| *Gemella haemolysans* (ATCC 10379) | [99] |
| *Geotoga petraea* TS | [57] |
| *Gloebacter "violaceus"* (PCC 7421) | [80] |
| *Haemophilus influenzae* (ATCC 33391) | [249] |
| *Halicomenobacter hydrossis* (ATCC 27775) | [176] |
| *Haloanaerobium praevalens* (ATCC 33744) | [138] |
| *Halobacterium halobium* R1 | [22] |
| *Halobacterium maris-mortui* (gene = *rrnB*) | [24] |
| *Halococcus morrhuae* (ATCC 17082) | [23] |
| *Haloferax volcanii* DS-2 (ATCC 29605) | [21] |
| *Helicobacter pylori* (ATCC 43504) | [211] |
| *Heliobacterium chlorum* (ATCC 35205) | [134] |
| *Herpetosiphon aurantiacus* B-3 (ATCC 23779) | [63] |
| *Hyphomicrobium vulgare* MC-750 | [214] |
| *Isosphaera pallida* IS1B | [200] |
| *Kurthia zopfii* (ATCC 33403) | [97] |
| *Lactobacillus acidophilus* (ATCC 4356) | [87] |
| *Lactobacillus amylophilus* (DSM 20533) | [85] |
| *Lactobacillus brevis* (ATCC 14869) | [89] |
| *Lactobacillus casei* subsp. *casei* (ATCC 393) | [91] |
| *Lactobacillus delbrueckii* (ATCC 9649) | [86] |
| *Lactobacillus minutus* (ATCC 33267) | [142] |
| *Lactobacillus ruminis* (ATCC 27780) | [90] |
| *Lactobacillus viridescens* (ATCC 12706) | [83] |
| *Lactococcus lactis* subsp. *lactis* (ATCC 19435) | [95] |
| *Legionella pneumophila* subsp. *pneumophila* Philadelphia-1 (ATCC 33152) | [245] |
| *Leptonema illini* 3055 | [187] |
| *Leptospira interrogans* serovar pomona Kennewicki | [188] |
| *Leptotrichia buccalis* (ATCC 14201) | [141] |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* (ATCC 8293) | [81] |
| *Leuconostoc oenos* (ATCC 23279) | [82] |
| *Leuconostoc paramesenteroides* (DSM 20288) | [84] |
| *Listeria monocytogenes* (ATCC 35152) | [96] |
| *Megasphaera elsdenii* B159 (ATCC 17752) | [135] |
| *Methanobacterium bryantii* M.o.H. (DSM 863) | [34] |
| *Methanobacterium formicicum* (DSM 1312) | [33] |
| *Methanobacterium thermoautotrophicum* Marburg | [30] |
| *Methanobrevibacter arboriphilicus* (DSM 1536) | [31] |
| *Methanococcoides methylutens* TMA-10 (DSM 2657) | [4] |
| *"Methanococcus" aeolicus* A | [38] |
| *Methanococcus igneus* Kol 5 (DSM 5666) | [36] |
| *Methanococcus jannaschii* JAL-1 (DSM 2661) | [35] |
| *Methanococcus maripaludis* JJ (ATCC 43000) | [39] |
| *Methanococcus thermolithotrophicus* SN-1 (DSM 2095) | [37] |
| *Methanococcus vannielii* EY33 | [40] |
| *Methanococcus voltae* PS (ATCC 33273) | [41] |
| *Methanocorpusculum parvum* XII (ATCC 43721) | [17] |
| *Methanoculleus bourgensis* MS2 (ATCC 43281) | [14] |
| *Methanoculleus marisnigri* JR1 (ATCC 35101) | [12] |
| *Methanoculleus thermophilicus* TCI (DSM 3915) | [13] |
| *Methanogenium organophilum* CV (DSM 3596) | [18] |
| *Methanogenium tationis* (DSM 2702) | [15] |
| *Methanohalobium evestigatum* Z-7303 (DSM 3721) | [8] |
| *Methanohalophilus mahii* (ATCC 35705) | [5] |
| *Methanohalophilus oregonensis* WAL1 (DSM 5435) | [6] |
| *Methanohalophilus zhilinae* WeN5 (DSM 4017) | [9] |
| *Methanolobus tindarius* Tindari 3 (DSM 2278) | [7] |
| *Methanomicrobium mobile* BP (DSM 1539) | [19] |
| *Methanoplanus limicola* M3 (DSM 2279) | [20] |
| *Methanoprpus kandleri* av19 (DSM 6324) | [43] |
| *Methanosaeta concilii* Opfikon (DSM 2139) | [11] |
| *Methanosaeta thermoacetophila* CALS-1 (DSM 3870) | [10] |
| *Methanosarcina barkeri* 227 (DSM 1538) | [1] |
| *Methanosarcina frisia* C16 (ATCC 43330) | [3] |
| *Methanosarcina thermophila* TM-1 (DSM 1825) | [2] |
| *Methanosphaera stadtmanae* MCB-3 (DSM 3091) | [32] |
| *Methanospirillum hungatei* JF1 (DSM 864) | [16] |
| *Methanothermus fervidus* | [29] |
| *Methylobacterium extorquens* (ATCC 43645) | [212] |
| *Methylomonas methanica* (ATCC 35067) | [241] |
| *Micrococcus luteus* (ATCC 381) | [148] |
| *Microcilla aggregans* subsp. *catalatica* HI-3 (ATCC 23190) | [168] |
| *Microscilla furvescens* TV-2 (ATCC 23129) | [178] |
| MLO[a] infecting *Oenothera hookeri* | [105] |
| *Mycobacterium avium* serovar 1 | [157] |
| *Mycobacterium tuberculosis* | [156] |
| *Mycoplasma capricolum* (ATCC 27343) | [109] |
| *Mycoplasma ellychnium* ELCN-1 (ATCC 43707) | [108] |
| *Mycoplasma gallisepticum* A5969 | [117] |
| *Mycoplasma hominis* PG21 (ATCC 23114) | [115] |
| *Mycoplasma hyopneumoniae* (ATCC 27719) | [113] |
| *Mycoplasma pneumoniae* FH (ATCC 15531) | [118] |
| *Mycoplasma scalvi* Mayfield B (ATCC 33004) | [114] |
| *Myxococcus xanthus* DK1622 | [206] |
| *Nannocystis exedens* Na e1 (ATCC 25963) | [204] |
| *Neisseria gonorrhoeae* B 5025 (NCTC 8375) | [233] |
| *Nitrosomonas europaea* | [239] |
| *Nocardia otitidis-caviarum* (ATCC 14629) | [155] |
| *Nostoc* sp. (PCC 73102) | [70] |
| *Oceanospirillum linum* (ATCC 11336) | [246] |
| *Ochromonas danica* SAG 933-7 chloroplast | [79] |
| *Oscillatoria* sp. (PCC 7515) | [73] |
| Pacific Ocean station 49 500-m depth bacterioplankton DNA clone NH49-9 | [46] |
| *Pediococcus pentosaceus* (ATCC 33316) | [88] |
| *Petrotoga miotherma* 42-6 | [56][26] |
| *Planctomyces stalevi* (ATCC 27377) | [199] |
| *Prochloron didemni* | [72] |
| *Prochlorothrix hollandica* | [68] |
| *Propionibacterium acnes* (DSM 1897) | [150] |
| *Proteus vulgaris* (IFAM 1731) | [251] |
| *Pseudomonas aeruginosa* NIH 18 (ATCC 25330) | [248] |
| *Pseudomonas testosteroni* (ATCC 11996) | [238] |
| *Pseudonocardia thermophila* (ATCC 19285) | [152] |
| *Pyrobaculum aerophilum* im2 | [54] |
| *Pyrobaculum islandicum* geo3 | [53] |
| *Pyrodictium occultum* PL-19 (DSM 2709) | [49] |
| *Rhodobacter capsulatus* B10 (ATCC 33303) | [227] |
| *Rhodomicrobium vannielii* EY33 (ATCC 51194) | [215] |
| *Rhodopseudomonas marina* subsp. *agilis* GN-14 | [219] |
| *Rhodospirillum rubrum* ATH 1.1.1 (ATCC 11170) | [229] |
| *Rhodospirillum salexigens* (ATCC 35888) | [226] |
| *Rickettsia rickettsii* R (ATCC VR 891) | [221] |
| *Rochalimaea quintana* Fuller (ATCC VR 358) | [218] |
| *Rubrivivax gelatinosus* ATH 2.2.1 (ATCC 17011) | [237] |
| *Runella slithyformis* (ATCC 29530) | [179] |
| Santa Barbara Channel bacterioplankton DNA clone SBAR16 | [26] |
| Santa Barbara Channel bacterioplankton DNA clone SBAR5 | [45] |
| *Saprospira grandis* (ATCC 23119) | [177] |
| *Serpula hyodysenteriae* B204 (ATCC 31212) | [190] |
| *Sphingobacterium mizutae* (ATCC 33299) | [173] |
| *Spirillum volutans* (ATCC 19554) | [240] |
| *Spirochaeta aurantia* J1 (ATCC 25082) | [196] |
| *Spirochaeta litoralis* R1 (ATCC 27000) | [194] |
| *Spirochaeta stenostrepta* Z1 (ATCC 25083) | [192] |
| *Spiroplasma apis* B-31 (ATCC 33834) | [110] |
| *Spiroplasma citri* Maroc (ATCC 27556) | [111] |
| *Spiroplasma* sp. strain Y-32 (ATCC 33835) | [143] |
| *Spirosoma linguale* (ATCC 23276) | [174] |
| *Sporocytophaga cauliformis* (DSM 3657) | [165] |
| *Sporocytophaga myxococcoides* (ATCC 10010) | [182] |
| *Sporomusa paucivorans* X (DSM 3697) | [137] |
| *Stigmatella aurantiaca* (ATCC 25190) | [205] |
| *Streptococcus salivarius* C699 (ATCC 13419) | [94] |
| *Streptomyces coelicolor* 1147 A3(2) | [143] |
| *Sulfolobus shibatae* B12 | [47] |
| *Sulfolobus solfataricus* P1 (DSM 1616)[b] | [48] |
| *Synechococcus* sp. (PCC 6301) | [67] |
| *Syntrophospora bryantii* (DSM 3014B) | [120] |
| *Thermacetogenium phaeum* (ATCC 27380) | [42] |
| *Thermodesulfotobacterium commune* YSRA (ATCC 33708) | [61] |
| *Thermofilum pendens* (DSM 2475) | [51] |
| *Thermomicrobium roseum* (ATCC 27502) | [64] |
| *Thermoplasma acidophilum* 122-1B2 | [25] |
| *Thermoproteus tenax* | [52] |
| *Thermosipho africanus* OB7 (DSM 5309) | [59] |
| *Thermotoga maritima* MSB8 (DSM 3109) | [60] |
| *Thermus "thermophilus"* HB8 (ATCC 27634) | [65] |
| *Thiovulum* sp. | [208] |
| *Treponema pallidum* Nichols | [191] |
| *Treponema saccharophilum* PB (ATCC 43261) | [193] |
| *Treponema* sp. strain CT 11616 (ATCC 43811) | [189] |
| *Ureaplasma urealyticum* 960 (ATCC 27618) | [116] |
| *Vesiculatum antarctica* 23-P (ATCC 49675) | [158] |
| *Vibrio parahaemolyticus* (ATCC 17802) | [250] |
| *Vitreoscilla stercoraria* VT1 | [234] |
| *Weeksella virosa* (ATCC 43766) | [167] |
| *Wolbachia pipientis* | [223] |
| *Wolinella succinogenes* 602W (FDC) (ATCC 29543) | [210] |
| Woods Hole bacterioplankton DNA clone WHARN | [27] |
| Woods Hole bacterioplankton DNA clone WHARQ | [44] |
| *Xanthomonas maltophila* (ATCC 13637) | [243] |
| *Zea mays* chloroplast | [76] |
| *Zea mays* mitochondrion | [220] |

[a] MLO, *Mycoplasma*-like organism.
[b] Sequence probably corresponds to another *Sulfolobus* species contaminating early versions of this culture.

On the nature and origin of the eukaryotic cell, we can now inquire about the origins of the various parts of the eukaryotic genome: for what fraction of eukaryotic genes or gene families is the most similar homolog an archaeal gene, for what fraction is it (eu)bacterial, and for what fraction is there no detectable homolog among either *Archaea* or *Bacteria*? Conversely, what fraction of archaeal or (eu)bacterial genes find no recognizable representation in the eukaryotic genome? What nuclear genes are unique to eukaryotes with endosymbiotic organelles and which of these trace back to the expected bacterial lineages? Are there other major, undiscovered, genetic contributions from ancient symbioses, transfers, or fusions?

Considering still earlier events, we can pose questions about the nature of the most recent common ancestor of present-day life: What was its nature? Was it prokaryotic, in the sense of having a genome as complex (and well-defined) as those of extant prokaryotes, or could it have been a progenote, a hypothetical more rudimentary entity in which the link between genotype and phenotype is not yet as precise (accurate) as that seen today (33)? What were the evolutionary dynamics by which this universal ancestor spawned the three primary lineages? Was this typical evolution, or, for example, did it involve so much genetic exchange, gene duplication and divergence, and/or loss of genes, that it is not possible to conceptualize (and analyze) the ancestral state and its subsequent evolution in terms of well-defined lineages (30)?

The origins of key cellular functions can also be addressed: from what genetic roots did photosynthesis (or aerobiosis, or various other forms of metabolism) spring? What genes in other pathways are related to them? To what extent can we trace genes back to a basic aboriginal genetic complement, and what was it?

Fortunately, these questions will be to a significant extent answerable now that biology is moving into the era of genome sequencing. The answers can either be found randomly and anecdotally, or they can be found more quickly if microbiologists learn the history that links all life and use it for guidance.

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