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The early evolution of cellular life

J. Peter Gogarten

Two main sources have been used to study early evolution: the fossil and the molecular records. Although the early Precambrian fossil record remains sparse, significant progress has been made in identifying and characterizing microfossils from this era¹. Microfossils have been described in sedimentary rocks dating back to around 3500 million years BP. Some of these sediments also contain stromatolite-like structures². Modern stromatolites are the result of interactions of complex microbial communities, including oxygen-producing photosynthetic cyanobacteria. The morphologies of some of the early microfossils are similar to extant *Oscillatoria*. This morphological resemblance and the presence of stromatolite-like structures were interpreted as proof, or at least as strongly suggestive, of cyanobacteria being already present about 3500 million years BP. While some of these findings have been questioned (summarized in Ref. 1), the resemblance between filamentous microfossils and present-day cyanobacteria is impressive. However, other

Recent progress in data collection and analysis has changed the study of origin of life from an area dominated by speculation into a field abundant with testable hypotheses. This review discusses advances in the following areas: the fossil records; the 'retrodiction' of biochemical pathways; and contradictions between different molecular phylogenies. The latter indicates a limited number of horizontal gene transfers during the early evolution. However, these cases of horizontal gene transfer are so infrequent that they can be detected as exceptions in an otherwise coherent picture. Cases of horizontal gene transfer can be recognized within the background of the majority consensus of molecular markers. The fusion of separate lineages to form new species is revealed by the simultaneous horizontal transfer of several independent genes.

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bacterial groups (e.g. the green non-sulfur bacteria, *Chloroflexus*, *Heliothrix* and *Oscillochloris*, and the non-photosynthetic Beggiatoales) contain morphologically similar multicellular filamentous forms as well; some of these also resemble *Oscillatoria*. The archaeal fossil record is too sparse to provide continuity sufficient to trace different evolutionary lineages. It appears premature to conclude that the members of these archaeal microbial communities represent extant groups of bacteria, and they have therefore been described formally as prokaryotes *incertae sedis*¹.

The fossil record does demonstrate that complex microbial communities already existed 3500 million years BP. It appears that life had established itself on early Earth not long after or even during the time of the early heavy meteorite bombardment. This early bombardment is mainly inferred from cratering records of the Moon³. The energy of the impacts forming these craters decreased over time. During the very early history of our planet, impacts were likely to have had an energy sufficient to completely

evaporate the oceans. The early heavy meteorite bombardment is likely to have prevented life from persisting uninterrupted before 3900–3700 million years BP. Even at these later stages of Earth's history the available biotopes seem unfriendly towards life⁴. Today's extremely hot environments (e.g. the deep-sea vents of the mid-ocean rifts) may have provided the first stable biotopes, while the upper layers of the oceans were still drastically altered by meteorite impacts. Therefore, these extreme habitats are good candidates for the origins of life⁵, or at least they provided refuge for early cellular life to survive global catastrophic environmental changes⁶.

The evolution of biochemical pathways

The molecular records are mainly preserved in extant organisms. Two types of molecular records are used to study the early evolution of life on this planet: the structure of macromolecules and the biochemical pathways.

One way by which biochemical pathways can evolve is the addition of new reactions to already existing pathways⁷. Grannik⁸ summarized this concept in the slogan 'biosynthesis recapitulates biogenesis', which leads to the assumption that the central core of present-day biochemical pathways was the one that evolved first. Combining this approach with pyrite formation as an energy-providing reaction (the formation of FeS₂ from FeS and H₂S provides the reducing agent), Wächtershäuser⁹ devised a scenario for an autotrophic origin

of biochemistry. The reactions in this primordial biochemistry are homologous to the reductive citric acid cycle. The reactants in these cycles remain bound to the pyrite surface.

More recently, Wächtershäuser¹⁰ presented a more-detailed set of rules to unravel the evolution of biochemical pathways. In addition to pathway extension, it considers other mechanisms by which biochemical pathways might have changed in the past. This approach to predict early metabolic pathways from present-day biochemistry was termed retrodiction. At present, many of the steps leading from surface-bound autotrophic metabolism to the formation of cells and cellular biochemical pathways remain hypothetical. However, retrodiction allows predictions to be made; it reduces the amount of chance encounters; and it provides a framework of conditions in which one might try laboratory experiments simulating prebiotic chemistry. Some of the experimental findings that support Wächtershäuser's theory are summarized in Ref. 11.

Molecular evolution – 16S rRNAs

Amino acid and nucleotide sequences of biological macromolecules provide information that can be used to decipher phylogenetic relationships. However, at best, the comparative analysis of sequences reveals information about the phylogeny of the molecules under study. The step from gene to species tree involves the additional assumption that genetic material has not been exchanged between organisms belonging to different lines of descent. The study of resistance genes that are passed between different bacteria, and the verification of the endosymbiotic origins of mitochondria and chloroplasts demonstrate that horizontal transfer of genes, or even the merger of formerly independent lineages, does indeed occur. To date, the best-studied molecular markers are the 16S-like ribosomal RNAs. At present, more than 2600 different 16S rRNA sequences are available through the RNA databank project¹². The compilation and analysis of these sequences constitute a major breakthrough in bacterial systematics¹³.

An advantage of 16S rRNA is that ribosomes play a central role in cellular information processing. The ribosome interacts specifically with many cellular components. Therefore, it is unlikely that this molecule could have been transferred between only distantly related organisms.

Most of the eubacterial and archaeobacterial groups (see Fig. 1) are well defined by their 16S rRNA sequences as well as by additional biochemical characters. However, the relationships between these groups remain questionable. The tree depicted in Fig. 1 reflects the topology of the phylogenetic tree contained in release 4.0 of the ribosomal database project¹². In this release, the relationship between several of the eubacterial groups differs from previous analyses. For example, the cyanobacteria group together with the cytophaga–flexibacter–bacteroides group, whereas earlier 16S phylogenies depicted the cyanobacteria as branching off before¹³ or together with the Gram-positive bacteria¹⁴.

Several other molecular phylogenies confirm the 16S rRNA-based groupings¹⁵. However, again some of the relationships between the major eubacterial groups remain ambiguous. For example, the F-ATPases suggest a closer association of *Propionigenium* with the cyanobacteria¹⁵ (this is not reflected in the 16S rRNAs). Also, *Thermotoga* represents an early branch in the 16S rRNA and ATPase phylogenies^{14,16}, whereas RNA polymerases¹⁷ and glutamine synthetases¹⁸ suggest otherwise.

In contrast to the discrepancies discussed below, it appears possible that the ambiguities mentioned above result from insufficient data and/or convergent evolution.

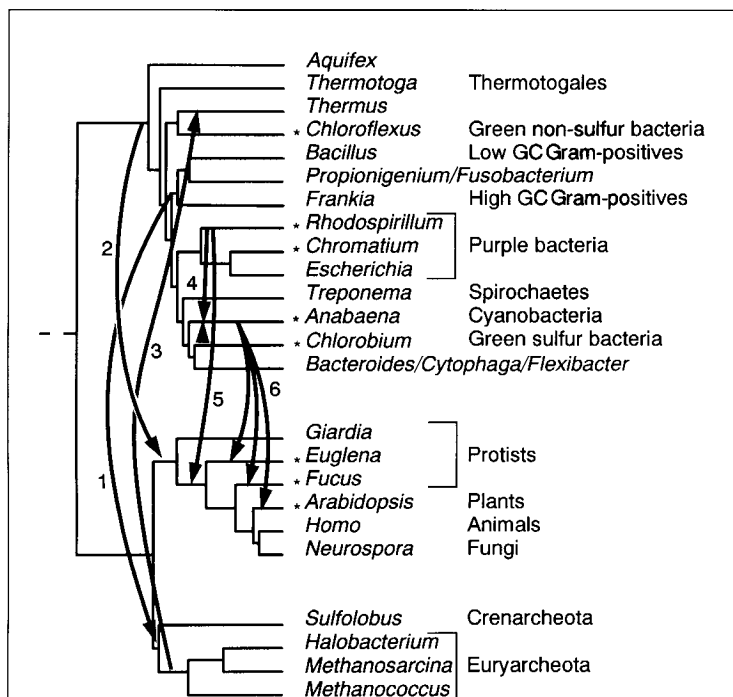
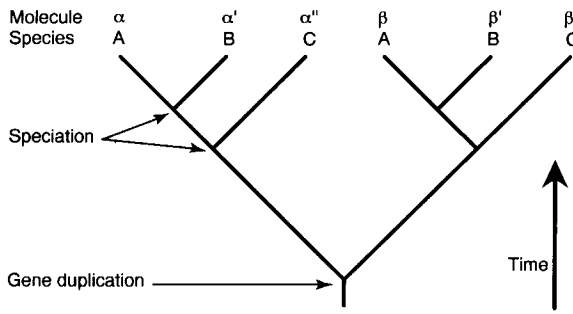


Fig. 1. Phylogenetic tree depicting the evolution of main groups of eubacteria, archaeobacteria and eukaryotes. The topology corresponds to the 16S rRNA phylogeny reported in the rRNA databank project release 4.0 (Refs 12 and 38). The branch lengths approximate the similarities between the 16S rRNAs, except that the branch lengths for the eukaryotes are greatly shortened. The root is placed using ancient gene duplications of ATPases²³, elongation factors and dehydrogenases²⁴. Groups containing organisms that use chlorophyll-based photosynthesis are indicated by an asterisk. Potential cases of horizontal gene transfer are indicated by arrows: (1) genes that show a close association between archaeobacteria and Gram-positive bacteria (see Fig. 2); (2) early eubacterial contributions to the eukaryotic nucleocytoplasm^{28,35}; (3) the transfer of an archaeobacterial proton-pumping ATPase/ATP synthase¹⁶; (4) the acquisition of the two photosystems used in oxygenic photosynthesis³³; (5) the endosymbionts that evolved into mitochondria³⁹; and (6) endosymbionts that evolved into the different types of plastids³⁹.

Box 1. Rooting molecular phylogenies

Molecular phylogenies are calculated as unrooted trees, if one does not assume a molecular clock running with the same speed in the different lineages. A reliable method to root molecular phylogenies is to include an outgroup in the analysis. The outgroup is provided by organisms that are closely related to, but not part of, the group of organisms whose phylogeny is studied. For example, if one is studying the evolution of birds, the outgroup could be provided by crocodiles. By definition, there is no living species that can be used as an outgroup in the universal tree of life. However, in the case of molecular phylogenies, sequences can be used that evolved independently of each other after an ancient gene duplication³⁷. In the example depicted below, proteins of type β could be used as an outgroup, and proteins of type α could be used as markers for the organisms, or *vice versa*.



The latter can be caused by chance, or it can be because of codon, nucleotide or amino acid biases. The debate on the origin of chloroplasts provides ample illustrations of these problems. Most molecular markers suggest that plastids evolved from cyanobacteria (one exception is the RuBP carboxylase in red algae, which might represent another case of horizontal gene transfer¹⁹). Furthermore, several sequence analyses had suggested that all chloroplasts evolved from a single endosymbiotic event (see Refs 19 and 20 for reviews). In contrast, the distribution of light-harvesting pigments indicates that the plastids from green algae and higher plants evolved from a *Prochloron*-like cyanobacterium (chlorophyll b, no phycobilins), whereas the plastids of red algae are more similar to other cyanobacteria that use phycobilisomes for light harvesting. This conclusion is also supported by a deletion in the gene encoding the light-harvesting complex. This deletion is present in a subgroup of the prochlorophytes and in the chloroplasts of green algae and higher plants, but it is absent in the other cyanobacteria and in the plastids of red and brown algae²¹.

Organelles that evolved from endosymbionts have a high A/T nucleotide content in their DNA. It was shown that the currently available nucleotide-based algorithms are insufficient to decide between a mono- or polyphyletic origin of the plastids; the appearance of a monophyletic origin of plastids might result from convergent evolution²². So far, it has not been shown that convergent evolution influences amino acid-based analyses to the same extent.

Ancient gene duplications and the root of the tree of life

Sequence comparisons can reveal the phylogeny of the compared molecules. The study of the evolutionary history of molecules that evolved from ancient gene duplications can yield information about a time from which only the descendants of a single species have survived. One application of this approach was to place the last common ancestor in the universal tree of life²³⁻²⁵ (see Fig. 1 and Box 1). Several ancient duplicated genes that reflect the fundamental division of the prokaryotes into two groups place the root of

the universal tree of life between the eubacteria on one side and the archaeobacteria and the eukaryotes on the other. The three Ur-kingdoms (Eubacteria, Archaeobacteria and Eukaryotia) were recently renamed into the three domains Bacteria, Archaea and Eucarya²⁶. The analyses of ancient duplicated genes (ATPases, elongation factors, dehydrogenases) indicate that an archaeobacterium or an ancestor of the archaeobacteria gave rise to a major component of the eukaryotic nucleocytoplasm^{23,24}. The debate continues as to whether the archaeobacteria as a whole form the sistergroup to the eukaryotic nucleocytoplasm, or whether a subgroup of the archaeobacteria (i.e. the crenarcheota¹⁴ or eocytes²⁷) groups closer to the eukaryotes than the other archaeobacteria.

Horizontal gene transfer

An increasing number of disagreements between molecular phylogenies has been found that cannot be attributed to ill-resolved bifurcations or biases influencing the branching pattern. The most striking of these concern the relationships between archaeobacteria, eubacteria and eukaryotes. One central conclusion from the analysis of rRNAs is that the prokaryotes can be divided into two distinct groups, the archaeobacteria and the eubacteria. This fundamental separation of the prokaryotes is supported by several other traits²⁸, including some molecules that had undergone an ancient gene duplication.

However, several molecular phylogenies were recently published in which the archaeobacteria group with, or even within, the eubacteria. The first analyses had low confidence intervals attached to this eubacterial-archaeobacterial grouping^{16,29}; however, the analyses of glutamine synthetases^{18,30} and heat shock proteins³¹ also group some of the archaeobacteria (i.e. the Euryarcheota, see Fig. 1) together with Gram-positive bacteria. Figure 2 depicts the evolution of heat shock protein (HSP70) homologs. The grouping of the two archaeobacterial sequences together with the Gram positives was found in 100% of bootstrapped samples analyzed with protein parsimony (J.P. Gogarten, unpublished). A similar result was obtained for the glutamine synthetases: the group comprising Euryarcheota and Gram positives was recovered in 98% of the bootstrapped samples¹⁸. Both of these molecules underwent an ancient gene duplication. In the case of HSP70, this duplication gave rise to the mreB proteins (rod shape-determining proteins) found in *Escherichia* and *Bacillus*³¹; in the case of glutamine synthetase the duplication led to the glutamine synthetase I and II (Ref. 32).

The situation in both of these cases is more complicated than in other ancient duplicated genes^{23,24}. The genes evolving from the duplication have not been found in all three Ur-kingdoms (Archaeobacteria, Eubacteria, Eukaryotia). Therefore, it

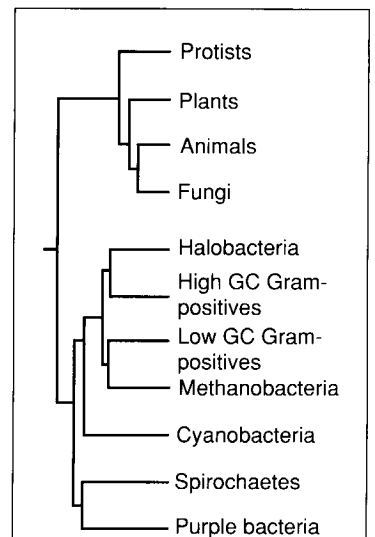


Fig. 2. Example of a molecular phylogeny that results in a closer association between Gram positive- and archaeobacteria. The topology was calculated using sequences for heat shock protein homologs³¹ and protein parsimony analysis⁴⁰. The branch lengths approximate the similarities between the protein sequences. The dotted line represents the position where the mreB proteins from *E. coli*, *Bacillus subtilis* and *B. cereus* join the tree of the heat shock proteins.

is possible that the duplication occurred only after the last common ancestor. However, in both cases the duplication preceded the separation of Gram positives from purple bacteria; therefore, the grouping of the archaeobacteria with the Gram positives is incompatible with the molecular phylogenies that find a clear separation between archae- and eubacteria.

The assumption of horizontal gene transfer between a eubacterium and an archaeobacterium can reconcile the different molecular phylogenies. An increasing number of molecular markers indicates a closer association between Gram positives and archaeobacteria^{18,31}, indicating that a substantial portion of the genome might have been involved in this transfer. This event resembles the merger of two formerly independent lineages rather than the transfer of a single gene or operon from one species to another.

A few other documented cases of likely horizontal gene transfer during early evolution do exist:

- Photosynthesis using (bacterio)chlorophyll as the reaction-center pigment occurs in different eubacterial lineages (see Fig. 1). The proteins forming the reaction centers are homologous to each other³³. Oxygen-releasing photosynthesis in cyanobacteria, eukaryotic algae and higher plants uses two different reaction centers or photosystems operating in series. One of these photosystems is more similar to the reaction center found in green sulfur bacteria³⁴; the other is more similar to the reaction center found in purple bacteria and in *Chloroflexus*³³. One either has to conclude that both reaction centers were already present in the ancestor of purple, green sulfur and cyanobacteria (and one or the other reaction center got lost in the purple and green sulfur bacteria), or else the cyanobacterial ancestor obtained at least one of these reaction center proteins by means of horizontal gene transfer³³.
- Archaeobacterial type ATPases were found as proton-pumping ATPases in *Thermus* and as sodium-pumping ATPase in *Enterococcus hirae*. Phylogenetic analysis and comparison to other molecular phylogenies suggest that these ATPases were obtained by means of horizontal gene transfer and that these archaeobacterial type ATPases had not been present in the eubacterial ancestor¹⁶.
- Eubacterial genes were found in protists, which are considered to have branched off the main eukaryotic lineage before the evolution of mitochondria³⁵. The presence of eubacterial proteins in these eukaryotes suggests at least one other early eubacterial contribution (besides mitochondria and plastids) to the eukaryotic cell²⁸.

The last common ancestor

Molecular biology reveals the fundamental unity of modern life. Extant organisms are cellular, the genetic information is stored in DNA, transcribed into RNA and translated into proteins. All organisms use the same (or a very similar) genetic code and the same amino acids in their proteins. Although there are differences in the transcription and translation machinery, the process is very similar in all cells. All cells use lipid membranes to separate their protoplasm from the environment or from the cell wall; they use the same energy-rich metabolites; and all living organisms use homologous enzymes to generate ion gradients across their cell membranes.

However, because of the possibility of horizontal gene transfer, one cannot conclude that characteristics that are shared by all present-day organisms were already present in the last common ancestor. To assess whether a character was already present in the last common ancestor, one also needs to know its molecular phylogeny and compare this phylogeny to the organismal phylogeny. Under the assumption

that Fig. 1 approximates the organismal phylogeny, the study of ancient duplicated genes suggests that the last common ancestor was already a prokaryotic cell with ribosomes and energy-conserving membranes. This organism possessed ion-translocating ATPases that were already multi-subunit enzymes. It had different elongation factors, two types of methionin-tRNAs, and different dehydrogenases^{23,24,36}. The last common ancestor does not seem to have been fundamentally different from present-day prokaryotes^{25,36}.

Outlook

16S-like rRNAs provide a solid backbone for future phylogenetic analyses concerning early evolution. The different genome projects currently under way hopefully will provide more-detailed information as to how these genomes evolved; in particular, they might shed light on the question whether only single genes or large chunks of genomes were transferred between organisms. However, at present these genome projects cover only a few of the relevant groups; furthermore, many of the sequenced genes have evolved too fast to allow a deep phylogenetic analysis. To pinpoint events of horizontal gene transfer on the tree of life, and to accurately judge their extent, a more extensive sampling of species for slowly evolving molecular markers is needed.

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Prenatal influences on reproductive life history strategies

Mertice M. Clark and Bennett G. Galef, Jr

Exposure to hormones before birth can profoundly influence the life history strategies of many vertebrates. Such prenatal effects on reproductive phenotype can have far-reaching consequences and may help to explain the physiological bases of biases in sex ratios, differences in adult competitive abilities and alternative reproductive tactics that are of interest to evolutionary and behavioural ecologists^{1–3}.

The rodent model

In many litter-bearing rodent species, such as the house mouse (*Mus musculus*), the intra-uterine position (IUP) that a male or female foetus occupies relative to siblings of the same or the opposite sex influences the hormonal milieu in which it matures. For example, the testosterone level within a male or female foetus is affected by adjacent foetuses. Male foetuses occupying IUPs between two males (2M males) have greater blood concentrations of testosterone than do their brothers in IUPs between two females (2F males). Similarly, female foetuses located between male foetuses (2M females) have higher testosterone titres than do their sisters (2F females) located between two female foetuses. The 50% or more of foetal rodents situated in IUPs adjacent to a single male foetus have levels of testosterone that lie between those of foetuses from 2M and 2F IUPs^{4–6}. Thus, IUP during gestation has powerful effects on the hormone levels to which a foetus is exposed during development.

Although there has been considerable controversy as to how steroids travel from one foetus to another^{7–9}, recent studies involving the transport of dye within the uterus of pregnant rats and of radioactively labelled testosterone between foetuses indicate that androgens secreted by male foetuses late in gestation diffuse through the amniotic fluid and across foetal membranes to adjacent foetuses^{10,11}. Such diffusion of testosterone causes a foetus located between male foetuses to receive greatest exposure to exogenous testosterone.

Over the past two decades, evolutionary and behavioural ecologists have become increasingly interested in the adaptive consequences of intraspecific variability in life history and behavioural strategies. Recently, behavioural endocrinologists have begun to uncover surprising relationships between levels of prenatal exposure to gonadal hormones and variation in reproductive behaviour in adulthood. Such relationships may provide a causal explanation for many variations in adult phenotype that are of interest to behavioural and evolutionary ecologists.

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The reproductive behaviours of adult rodents from different IUPs are greatly affected by the different levels of exposure to androgen that they experienced prenatally (Table 1). For example, female mice from 2M IUPs have higher plasma testosterone levels during foetal life, are more aggressive as adults, have longer oestrus cycles, are less sexually attractive to males, and have a shorter reproductive life than do 2F females. In virtually all characters measured, the phenotypes of 2M females are more masculine than are those of 2F females^{4,5}. Foetal IUP affects a similar suite of traits in Mongolian gerbils (*Meriones unguiculatus*)^{12–14}, though the direction of

effects of IUP on adult phenotype sometimes appears to differ between gerbils and mice. For instance, 2M male Mongolian gerbils are more successful than are 2F male gerbils in impregnating females they encounter¹⁵, while 2F male mice are more sexually active than are their 2M brothers⁵.

Effects of IUP on primary sex ratio in rodents

Intra-uterine position affects not only the phenotypes of rodent offspring but also the sex ratios of the litters in which they are born. 2M female house mice and Mongolian gerbils give birth to male-biased litters (>57% males), while 2F females of both species deliver female-biased litters (<43% males)^{16,17}. Although the process producing such variance is presently unknown, it is unlikely to be the result of differential mortality either during or shortly following vaginal delivery¹⁸. The percentage of males (61.9%) in litters delivered by caesarian section from 2M gerbils on the last day of their gestation is significantly greater than the percentage of males found in caesarian-delivered litters of 2F females (43.4% males)¹⁹ on the last day of their gestation.

The difference in sex ratios of litters of 2M and 2F females may result from differences in their timing of copulation with respect to ovulation. Because the sex ratios of rodent litters change with the temporal relationship between insemination and ovulation^{20,21}, females from different IUPs