The Very Early Stages of Biological Evolution and the Nature of the Last Common Ancestor of the Three Major Cell Domains

Arturo Becerra, Luis Delaye, Sara Islas, and Antonio Lazcano

Facultad de Ciencias, Universidad Nacional Autónoma de México, 04510 México, D.F., Mexico; email: alar@correo.unam.mx

Annu. Rev. Ecol. Evol. Syst. 2007. 38:361–79

The *Annual Review of Ecology, Evolution, and Systematics* is online at http://ecolsys.annualreviews.org

This article's doi: 10.1146/annurev.ecolsys.38.091206.095825

1543-592X/07/1201-0361$20.00

Key Words

cenancestor, early cell evolution, LCA, LCA gene complement, LUCA, RNA/protein world

Abstract

Quantitative estimates of the gene complement of the last common ancestor of all extant organisms, that is, the cenancestor, may be hindered by ancient horizontal gene transfer events and polyphyletic gene losses, as well as by biases in genome databases and methodological artifacts. Nevertheless, most reports agree that the last common ancestor resembled extant prokaryotes. A significant number of the highly conserved genes are sequences involved in the synthesis, degradation, and binding of RNA, including transcription and translation. Although the gene complement of the cenancestor includes sequences that may have originated in different epochs, the extraordinary conservation of RNA-related sequences supports the hypothesis that the last common ancestor was an evolutionary outcome of the so-called RNA/protein world. The available evidence suggests that the cenancestor was not a hyperthermophile, but it is currently not possible to assess its ecological niche or its mode of energy acquisition and carbon sources.

INTRODUCTION

LCA: last common ancestor

RNA world: an evolutionary stage prior to proteins and DNA genomes during which life was based on catalytic and replicative RNAs

"All the organic beings which have ever lived on this Earth," wrote Charles Darwin in the *Origin of Species*, "may be descended from some one primordial form." Everything in contemporary biology, including molecular cladistics and comparative genomics, has confirmed Darwin's extraordinary insight. As shown by the construction of a trifurcated, unrooted tree based on comparisons of 16S/18S rRNA sequences, all organisms can be grouped into one of three major monophyletic cell lineages, that is, the domains Bacteria, Archaea, and Eucarya (Woese et al. 1990). These are all derived from a common ancestral form, the last common ancestor (LCA). All organisms share the same genetic code, the same essential features of genome replication and gene expression, basic anabolic reactions, and membrane-associated ATPase-mediated energy production. Minor variations can easily be explained as the outcome of divergent processes from an ancestral life form that predated the separation of the three major biological domains.

It is unlikely that such traits were already present in the first living systems. The discovery of catalytically active RNA molecules has given considerable credibility to suggestions of an evolutionary stage prior to the development of proteins and DNA genomes during which early life forms based largely on ribozymes may have existed (**Figure 1**). The difficulty involved with the prebiotic synthesis and accumulation of ribonucleotides and RNA molecules has led to the suggestion that the RNA world itself was the evolutionary outcome of some earlier primordial living systems, referred to as pre-RNA worlds (Joyce 2002). However, the chemical nature of the first genetic polymers and the catalytic agents that may have formed the hypothetical pre-RNA worlds can only be surmised and cannot be deduced from comparative genomics or deep phylogenetics.

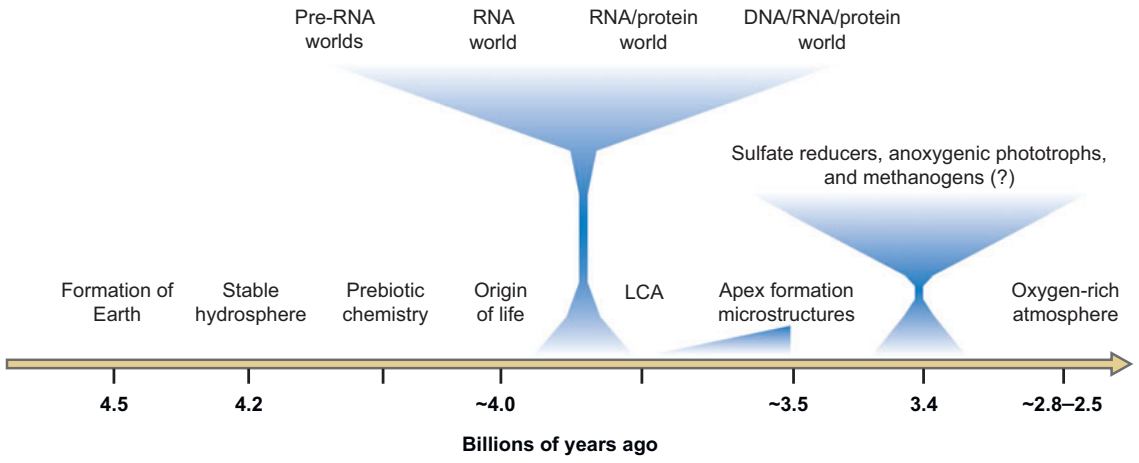

Figure 1

Timeline of the events leading to the origin and early evolution of life. LCA, last common ancestor. Figure adapted with permission from Joyce 2002.

There is little or no geological evidence for the environmental conditions on early Earth at the time of the origin and early evolution of life. It is not possible to assign a precise chronology to the origin and earliest evolution of cells (**Figure 1**), and identification of the oldest paleontological traces of life remains a contentious issue. The early Archean geological record is scarce and controversial, and most of the sediments preserved from such times have been metamorphosed to a considerable extent. Although the biological origin of the microstructures present in the 3.5×10^9 years Apex cherts of the Australian Warrawoona formation (Schopf 1993) has been disputed, currently the weight of evidence favors the idea that life existed 3.5 billion years ago (Altermann & Kazmierczak 2003).

Although no paleontological remains exist for the LCA, insight into the nature of the Archean biosphere is possible. Isotopic fractionation data and other biomarkers support the possibility of a metabolically diverse Archean microbial biosphere, which may have included members of the archaeal kingdom. The proposed timing of the onset of microbial methanogenesis, based on the low ^{13}C values in methane inclusions found in hydrothermally precipitated quartz in the 3.5-billion-year-old Dresser Formation in Australia (Ueno et al. 2006), has been challenged (Lollar & McCollom 2006). However, sulfur isotope investigations of the same site indicate biological sulfate-reducing activity (Shen et al. 2001), and analyses of 3.4×10^9-million-year-old South African cherts suggest that they were inhabited by anaerobic photosynthetic prokaryotes in a marine environment (Tice & Lowe 2004). These results support the idea that the early Archean Earth was teeming with prokaryotes, which included anoxygenic phototrophs, sulfate reducers, and, perhaps, methanogenic archaea (Canfield 2006).

Progenote: a hypothetical biological entity in which phenotype and genotype had an imprecise, rudimentary linkage relationship

Cenancestor: the organism ancestral to domains Bacteria, Archaea, and Eucarya, i.e., the last common ancestor

THE SEARCH FOR THE LAST COMMON ANCESTOR: WHAT'S IN A NAME?

Recognition of the significant differences that exist between the transcriptional and translational machineries of the Bacteria, Archaea, and Eucarya were assumed to be the result of independent evolutionary refinements. This led to the conclusion that the primary branches were the descendants of a progenote, a hypothetical biological entity in which phenotype and genotype still had an imprecise, rudimentary linkage relationship (Woese & Fox 1977). However, the analysis of homologous traits found among some of its descendants suggested that it was not a direct, immediate descendant of the RNA world, a protocell, or any other prelife progenitor system, but that it was a complex organism, much like extant bacteria (Lazcano et al. 1992).

This conclusion has been disputed. The availability of an increasingly large number of completely sequenced cellular genomes has sparked new debates, rekindling the discussion on the nature of the ancestral entity. This is shown, for instance, in the diversity of names that have been coined to describe the LCA: progenote (Woese & Fox 1977), cenancestor (Fitch & Upper 1987), last universal common ancestor (LUCA) (Kyrpides et al. 1999), or, later on, last universal cellular ancestor (Philippe & Forterre 1999), universal ancestor (Doolittle 2000), last common community, (Line 2002), and most recent common ancestor (Zhaxybayeva & Gogarten 2004), among

others. These terms are not truly synonymous, and they reflect the current controversies on the nature of the universal ancestor and the evolutionary processes that shaped it.

HGT: horizontal gene transfer

From a cladistic viewpoint, the LCA is merely an inferred inventory of features shared among extant organisms, all of which are located at the tip of the branches of molecular phylogenies. Accordingly, it could be argued that the most parsimonious characterization of the cenancestor could be achieved by summarizing the features of the oldest recognizable nodes of universal cladograms. However, large-scale studies based on the availability of genomic data have revealed major discrepancies with the topology of rRNA trees (Doolittle 2000). Very often these differences have been interpreted as evidence of horizontal gene transfer (HGT) events between different species, questioning the feasibility of the reconstruction and proper understanding of early biological history.

Reticulate phylogenies greatly complicate the inference of ancestral traits and can lead to overestimates of the LCA gene content. Recognition of HGT has also led to proposals suggesting that populations of precellular ancestral entities occupied, as a whole, the node located at the bottom of universal trees (Kandler 1994, Koonin & Martin 2005), including the suggestion that the LCA was not a single organismic entity but rather a highly diverse population of metabolically complementary, cellular progenotes endowed with multiple small, linear chromosome-like genomes that benefited from massive multidirectional horizontal transfer events (Woese 1998).

Such entities may have existed, but universally distributed features such as the genetic code and the gene expression machinery are indications of their ultimate monophyletic origin. Even if such communal progenote ancestors diverged sharply into the three domains soon after the appearance of the code and the establishment of translation, the origin of the sequences ancestral to those found in extant organisms and the separation of the Bacteria, Archaea, and Eucarya are different events. In other words, the divergence of the primary domains took place later, perhaps even much later, than the appearance of the genetic components of their LCA.

Universal gene-based phylogenies ultimately reach a single ancestor, but the LCA must have been part of a population of entities similar to it that existed throughout the same period. Its siblings may have not survived, but some of their genes did if they became integrated via lateral transfer into the LCA genome (Zhaxybayeva & Gogarten 2004). Accordingly, the cenancestor should be considered one of the last evolutionary outcomes of a tree trunk of unknown length, during which the history of a long, but not necessarily slow, series of ancestral events including lateral gene transfer, gene losses, and paralogous duplications probably played a significant role in the accretion of complex genomes (Castresana 2001, Glansdorff 2000, Lazcano et al. 1992).

AN EARLY EXPANSION OF GENE FAMILIES

The extraordinary similarities at very basic biochemical and genetic levels among all known life forms can be interpreted as propinquity of descent, that is, all organisms

are of monophyletic origin. The molecular details of these universal processes not only provide direct evidence of the monophyletic origin of all extant forms of life, but also imply that the sets of genes encoding the components of these complex traits were frozen a long time ago; that is, major changes in them are strongly selected against. Although these complex, multigenic traits must have evolved through a series of simpler states, no evolutionary intermediate stages or ancient simplified versions of ATP production, DNA replication, or ribosome-mediated protein synthesis have been discovered in extant organisms.

PDO: protein disulfide oxidoreductase

Protein sequence comparisons have confirmed the role that many ancient gene duplications have played in the evolution of genomes (Becerra & Lazcano 1998). Clues to the genetic organization and biochemical complexity of primitive entities from which the cenancestor evolved may be derived from the analysis of paralogous gene families. The number of sequences that have undergone such duplications prior to the divergence of the three lineages includes genes encoding for a variety of enzymes that participate in widely different processes such as translation, DNA replication, CO_2 fixation, nitrogen metabolism, and biosynthetic pathways. Whole-genome analysis has revealed the impressive expansion of sequences involved in membrane transport phenomena, such as ABC transporters, P-type ATPases, and ion-coupled permeases (Clayton et al. 1997). Structural studies of proteins provide evidence of another group of paralogous duplications. A number of enzymes—including protein disulfide oxidoreductase (PDO) (Ren et al. 1998), the large subunit of carbomoyl phosphate synthetase (Alcántara et al. 2000), and HisA, a histidine biosynthetic isomerase (Alifano et al. 1996)—are formed by two tandem homologous modules. This indicates that the size and structure of a number of proteins are the evolutionary outcome of paralogous duplications followed by gene fusion events that took place prior to the divergence of the three primary kingdoms.

A third group of paralogous genes can be recognized. All cells are endowed with different sets formed by a relatively small number of paralogous sequences. The list includes, among others, the pair of homologous genes encoding the EF-Tu and EF-G elongation factors (Iwabe et al. 1989). Another is formed by the duplicated sequences encoding the F-type ATPase hydrophilic alpha and beta subunits (Gogarten et al. 1989). No cell is known that is endowed with only one EF factor or only one type of F-type ATPase hydrophilic subunit. However, the extraordinary conservation of these duplicates implies that the LCA was preceded by a simpler cell with a smaller genome in which only one copy of each of these genes existed, that is, by cells in which protein synthesis involved only one elongation factor and with ATPases with limited regulatory abilities.

Paralogous families of metabolic genes support the proposal that anabolic pathways were assembled by the recruitment of primitive enzymes that could react with a wide range of chemically related substrates, that is, the so-called patchwork assembly of biosynthetic routes (Jensen 1976). Such relatively slow, unspecific enzymes may have represented a mechanism by which primitive cells with small genomes could have overcome their limited coding abilities.

GENOMICS AND THE TRACES OF THE RNA/PROTEIN WORLD

Studies of deep phylogenies (Brown et al. 2001, Daubin et al. 2001, Doolittle 2000, Moreira & López-García 2006) and comparative genomics (**Table 1**) can provide important insights into the gene complement of the LCA. However, if the term universal distribution is restricted to its most obvious sense, that is, that of traits found in all completely sequenced genomes, then quite surprisingly the resulting repertoire

Table 1 Estimates of the LCA gene complement based on quantitative genomic analysis

Cenancestral traits	Methodology	Number of sequences and functional categories
LCA		**80 universally conserved COGs (50 of them exhibiting three domain phylogenies)**
Relatively efficient transcription and ribosome structure; functions linked to membranes; ability to synthesize long DNA molecules (Harris et al. 2003)	Identification of universally conserved COGs showing three domain phylogenies	Translation and transcription: (63/80) DNA replication and repair: (5/80) Membrane-associated proteins: (1/80) Nucleotide and sugar metabolism: (4/80) Amino acid metabolism: (1/80) Protein management: (2/80) Others: (2/80)
LUCA		**~600 genes assigned to LUCA (COGs)**
Nearly sufficient genes to sustain a functioning organism (Mirkin et al. 2003)	Construction of parsimonious scenarios for individual sets of COGs based on species trees	Translation and transcription: (112/600) DNA replication and repair: (30/600) Membrane-associated proteins and metabolism: (287/600) Protein management: (25/600) Others: (94/600)
LUCA		**~63 ubiquitous genes (proteins)**
Simple, with a small number of genes; lack of a modern-type DNA genome and replication system (Koonin 2003)	Sequence comparison of ~100 genomes	Translation and transcription: (56/63) DNA replication and repair: (3/63) Membrane-associated proteins: (3/63) Protein management: (1/63)
LCA		**49 universally distributed protein folds (SCOP superfamilies)**
With a very sophisticated genetic inventory of structural equipment (Yang et al. 2005)	Distribution of SCOP protein superfamilies in 174 complete genomes	Translation and transcription: (32/49) DNA replication and repair: (5/49) Metabolism: (5/49) Protein management: (1/49) Others: (5/49)
LCA		**~115 protein domains (Pfam domains)**
Similar to extant cells in genetic complexity (Delaye et al. 2005)	BLAST sequence comparison of 20 genomes and orthologous identification based on the Pfam database	Translation and transcription: (56/115) DNA replication and repair: (6/115) Membrane-associated proteins: (7/115) Nucleotide and sugar metabolism: (33/115) Amino acid metabolism: (12/115) Protein management: (1/115)

Table 1 (*Continued*)

Cenancestral traits	Methodology	Number of sequences and functional categories
LUCA		**20 described motifs (octapeptides in proteins with known 3D structure)**
Total amount of ancestral octamers may be in the order of several hundreds (Sobolevsky & Trifonov 2006)	Identification of omnipresent (or nearly so) octamer protein motifs	Translation and transcription; DNA replication and repair; protein management; membrane-associated proteins
LUCA		**~1000 genes, with a minimum of 561 to 669 sequence/function categories (proteins)**
Fairly complex genome similar to those of free-living prokaryotes (Ouzounis et al. 2006)	Identification of homologous sequences among 184 genomes, using a method that corrects for gene losses	Translation and transcription: (34/659) DNA replication and repair: (35/659) Membrane-associated proteins: (120/659) Metabolism: (309/659) Others: (161/659)
LUCA		**140 ancestral protein domains (CATH superfamiles)**
Genetically complex entity, with practically all essential traits present in extant organisms (Ranea et al. 2006)	Distribution of CATH protein superfamilies in 114 complete genomes	Translation and transcription: (52/140) DNA replication and repair: (12/140) Membrane associated proteins: (2/140) Metabolism (46/140) Others: (28/140)

Acronyms: BLAST, basic local alignment search tool; CATH, protein structure classification; COGs, clusters of orthologous genes; LCA, last common ancestor; LUCA, last universal common ancestor; SCOP, structural classification of proteins.
For an extended version of this table, follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**.

is formed by relatively few features and by incompletely represented biochemical processes (**Table 1**). Reconstructions of gene complements of distant ancestors are mere statistical approximations of the biological past because their accuracy depends upon manifold factors, including the possible biases in the construction of genome databases, the levels of HGT, the significant variations in substitution rates of different proteins, and the degree of secondary losses, as well as methodological caveats (Becerra et al. 1997, Mirkin et al. 2003).

As shown in **Table 1**, the results of different attempts to characterize the LCA include gene sequences from incompletely represented basic biological processes, such as transcription, translation, energy metabolism, biosynthesis of nucleotides and amino acids, and folding of proteins, as well as some sequences related to replication, repair, and cellular transport. Despite the different methodological approaches, these inventories provide significant insights into (*a*) the biological complexity of the LCA, (*b*) evidence pertaining to the chemical nature of the cenancestral genome, and (*c*) the existence of an ancient RNA/protein world.

RNA/protein world: an early stage in cell evolution during which ribosome-mediated protein synthesis had already evolved but DNA genomes had not emerged

Although estimates may vary, many of the conserved domains correspond to proteins that interact directly with RNA (such as ribosomal proteins, DEAD-type helicases, aminoacyl tRNA synthetases, and elongation factors, among others) or take

part in RNA and nucleotide biosyntheses, including the DNA-dependent RNA polymerase β and β′ subunits, dimethyladenosine transferase, adenyl-succinate lyases, dihydroorotate oxidase, and ribose-phosphate pyrophosphokinase, among many others (Anantharam et al. 2002, Delaye & Lazcano 2000, Delaye et al. 2005). Together with the high conservation of ATP-dependent RNA helicases (see below), the presence of these sequences is consistent with the hypothesis that the cenancestor was an evolutionary outcome of the so-called RNA/protein world.

ATP-dependent RNA helicases are highly conserved proteins that participate in a variety of cellular functions involving the unwinding and rearrangement of RNA molecules, including translation initiation, RNA splicing, ribosome assembly, and degradosome-mediated mRNA decay (Schmid & Linder 1992). The degradosome is a multienzymatic complex involved in mRNA processing and breakdown that is formed by polynucleotide phosphorylase, polyphosphate kinase, ATP-dependent DEAD/H-type RNA helicase, and enolase, a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate and water (Blum et al. 1997).

Although RNA hydrolysis is an exergonic process, degradosome-mediated mRNA turnover plays a key role as a regulatory mechanism for gene expression in both prokaryotes and eukaryotes (Blum et al. 1997). A possible explanation for the conservation of DEAD-type RNA helicases may lie in their role in protein biosynthesis and in mRNA degradation. If this interpretation is correct, then it could be argued that degradosome-mediated mRNA turnover is an ancient control mechanism at RNA level that was established prior to the divergence of the three primary kingdoms. Together with other lines of evidence, including the observation that the most highly conserved gene clusters in several (eu)bacterial genomes are regulated at RNA level (Siefert et al. 1997), the results reported here are fully consistent with the hypothesis that during early stages of cell evolution, RNA molecules played a more conspicuous role in cellular processes, that is, the so-called RNA/protein world (**Figure 1**).

THE NATURE OF THE CENANCESTRAL GENOME: DNA OR RNA?

Because all extant cells are endowed with DNA genomes, the most parsimonious conclusion is that this genetic polymer was already present in the cenancestral population. Although it is possible to recognize the evolutionary relatedness of various orthologous proteins involved with DNA replication and repair (ATP-dependent clamp loader proteins, topoisomerases, gyrases, and 5′-3′ exonucleases) across the entire phylogenetic spectrum (Edgell & Doolittle 1997, Harris et al. 2003, Leipe et al. 1999, Olsen & Woese 1997, Penny & Poole 1999), comparative proteome analysis has shown that (eu)bacterial replicative polymerases and primases lack homologs in the two other primary kingdoms.

The peculiar distribution of the DNA replication machinery has led to suggestions not only of an LCA endowed with an RNA genome, but also of the polyphyletic origins of DNA and of many enzymes associated with its replication (Koonin & Martin 2005, Leipe et al. 1999) in which viruses may have played a central role (Forterre 2006). Koonin & Martin (2005) have argued that the LCA was an acellular

entity endowed with high numbers of RNA viral-like molecules that had originated abiotically within the cavities of a hydrothermal mound. This idea, which has little, if any, empirical support, does not take into account the problems involved with the abiotic synthesis and accumulation of ribonucleotides and polyribonucleotides, nor does it explain the emergence of functional RNA molecules.

Forterre (2006) also argued that the ultimate origins of cellular DNA genomes lie in viral systems, which gave rise to polyphyletic deoxyribonucleotide biosyntheses. According to a rather complex hypothetical scheme, gene transfers mediated by viral takeovers took place three times, giving origin to the DNA genomes of the three primary kingdoms. The invasion of the ancestor of the bacterial domain by a DNA virus eventually led to a replacement of its cellular RNA genes by DNA sequences, whereas the archaeal and eucaryal DNA replication enzymes resulted from an invasion by closely related DNA viruses (Forterre 2006).

We find it difficult to accept these different schemes. There are indeed manifold indications that RNA genomes existed during early stages of cellular evolution (Lazcano et al. 1988b), but, as argued below, it is likely that double-stranded DNA genomes had become firmly established prior to the divergence of the three primary domains. The major arguments supporting this possibility follow:

1. In sharp contrast to other energetically favorable biochemical reactions (such as phosphodiester backbone hydrolysis or the transfer of amino groups), the direct removal of the oxygen from the 2′-C ribonucleotide pentose ring to form the corresponding deoxy-equivalents is a thermodynamically much-less-favored reaction that considerably reduces the likelihood of multiple, independent origins of biological ribonucleotide reduction.
2. Demonstration of the monophyletic origin of ribonucleotide reductases is greatly complicated by their highly divergent primary sequences and the different mechanisms by which they generate the substrate 3′-radical species required for the removal of the 2′-OH group. However, sequence analysis and biochemical characterization of archaeal ribonucleotide reductases have shown their similarities with their bacterial and eucaryal counterparts, confirming their ultimate monophyletic origin (Stubbe et al. 2001).
3. Sequence similarities shared by many ancient, large proteins found in all three domains suggest that considerable fidelity existed in the operative genetic system of their common ancestor. Despite claims to the contrary (Poole & Logan 2005), such fidelity is unlikely to be found in RNA-based genetic systems (Lazcano et al. 1992, Reanney 1987) that do not replicate using the multiunit cellular DNA-dependent RNA polymerase.

THE DNA POLYMERASE I PALM DOMAIN: A MOLECULAR PENTIMENTO

Enzyme evolution often involves the acquisition of new catalytic or binding properties by an existing protein scaffold. The structural homology of functional domains of DNA and RNA helicases (Caruthers et al. 2000, Theis et al. 1999) suggests that DNA helicases evolved from a nonspecific helicase inherited from the RNA/protein world.

However, this model of enzyme evolution cannot account for the diversity of extant polymerases. Although it has been argued that all polymerases have a monophyletic origin (Lazcano et al. 1988a), the available evidence suggests that this is not the case, as shown by the identification of several nonhomologous classes of polymerases: primases, DNA polymerases, DNA-dependent RNA polymerases, replicases, and poly(A) polymerase, among others (Steitz 1999).

Detailed analysis of the three-dimensional structures of the DNA pol I, DNA pol II, pol Y, reverse transcriptases, and several viral replicases has shown that they all share homologous palm subdomains, which catalyze the formation of the phosphodiester bond (Steitz 1999). Homologous palm subdomains have also been identified in the viral T7 DNA and RNA polymerases (Jeruzalmi & Steitz 1998), indicating that they can catalyze the template-dependent polymerization of either ribo- or deoxyribonucleotides.

These observations are consistent with the hypothesis that the conserved palm subdomain, which is formed by approximately 150 amino acids, is one of the oldest recognizable components of an ancestral cellular polymerase that may have been both a replicase and a transcriptase during the RNA/protein world stage (Delaye et al. 2001). This hypothesis, which Koonin (2006) incorporated into his proposal on the evolution of viral and cellular polymerases, is supported by the presence of homologs of this domain in adenylate cyclase, the eukaryotic RNA recognition protein, pseudouridine synthetase, and several ribosomal proteins (Aravind et al. 2002).

If our hypothesis is correct, the lack of absolute template and substrate specificity of polymerases (Chaput et al. 2003, Lazcano et al. 1988a) suggests that relatively few mutations would have been required for the evolution of this RNA replicase into a DNA polymerase prior to the divergence of the three domains. If the palm domain was part of the ancestral replicase during the RNA/protein world stage, then the presence of the *Escherichia coli* replicative DNA pol III (DNA pol C) and its homologs can be explained by a nonorthologous displacement (Delaye et al. 2001). By analogy with the yeast and animal mitochondrial RNA polymerases, which play a dual role in transcription and in the initial priming required for DNA replication (Schinkel & Tabak 1989), we propose that the original RNA polymerase endowed with the palm domain described above catalyzed the formation of the RNA primer required for DNA replication. This ancestral polymerase may have also acted as a transcriptase during the RNA/protein stage, but the distribution of the highly conserved sequences of the oligomeric DNA-dependent RNA polymerase (**Table 1**) indicates that prior to the evolutionary divergence of the three primary kingdoms, a modern type of transcription had evolved. How this complex oligomeric transcriptase came into being can only be surmised for the time being.

WAS THE LAST COMMON ANCESTOR AN EXTREMOPHILE?

The discovery of a number of archaeal and bacterial species that thrive under extreme environmental conditions including high temperature and low pH values has led to suggestions that extremophiles may be considered models of primordial organisms (Di Giulio 2001, 2003). Additionally, the discovery has led to speculation that their

lifestyles may provide insights into extraterrestrial habitats where life could develop (Cleaves & Chalmers 2004). With the exception of heat-loving prokaryotes, however, the phylogenetic distribution of other extremophiles in molecular cladograms does not provide clues of possible antiquity.

Within the Bacteria, the earliest branching organisms are represented by Aquificales and Thermotogales, whereas among the Archaea, the deepest and shortest branches, that is, the slowest-evolving clades, correspond to the Nanoarchaeota, Pyrodictiacea, and Methanopyraceae (Stetter 2006). The position and length of the branches of these thermophiles and hyperthermophiles in rRNA trees support the idea that the LCA was a thermophile (Di Giulio 2001, Stetter 2006).

However, the recognition that the deepest branches in rooted rRNA universal phylogenies are occupied by hyperthermophiles does not provide by itself conclusive proof of a heat-loving LCA. The hypothesis for a thermophilic LCA is further weakened by evidence that calls into question the early branching of *Thermotoga* and *Aquifex*, two bacterial thermophilic species (Daubin et al. 2001, Forterre et al. 1993, Gogarten & Townsend 2005, Klenk et al. 1994). A nonhyperthermophilic LCA is also supported by the analysis of optimal growth temperatures in prokaryotes correlated with the G+C nucleotide content of 40 rRNA sequences. This has led Galtier et al. (1999) to conclude that the universal ancestor was a mesophile, although alternative opinions exist (Di Giulio 2001).

The possibility that the LCA was not a heat-loving entity is further supported by recent phylogenetic studies of the above-mentioned PDO sequences. These enzymes are involved in dithiol-disulfide exchange reactions (Pedone et al. 2004, Ren et al. 1998), and computational analysis of genomic data suggests that they play a major role in the formation and/or stabilization of intracellular protein disulfide bonds in disulfide-rich thermophiles (Ladenstein & Ren 2006). The available evidence suggests that the paralogous duplication that led to PDOs, which are formed by two homologous domains, first took place in the crenarcheaota and spread from them into the Bacteria by HGT via the euryarchaeota (Becerra et al. 2007), confirming previous suggestions that the bacterial PDOs have an archaeal origin (Pedone et al. 2004). This is consistent with the hypothesis that significant gene exchanges took place between archaea and thermophilic bacteria (Forterre et al. 2000, Makarova & Koonin 2003).

CENANCESTRAL METABOLISM AND MEMBRANES: TWO OPEN ISSUES

The number of highly conserved metabolic genes is surprisingly small (**Table 1**). The inventory includes many sugar-metabolism-related sequences, such as enolase, thioredoxin (*trxB*), phosphoribosyl-pyrophosphate synthase (*prs*), and UDP-galactose 4-epimerase (*galE*). Very likely, the evolutionary conservation of the *trxB* and *prsA* genes is best understood in terms of the key roles they play in nucleotide biosynthesis. The role of UDP-galactose 4-epimerase in complex carbohydrate synthesis via the interconversion of the galactosyl and glucosyl groups is well known. Although the uniqueness of the enzyme mechanism has been acknowledged, it is possible that

the conservation of UDP-galactose 4-epimerase is due to an unknown participation in other basic processes, as is the case for enolase.

The conclusion that the LCA was a prokaryote-like organism similar to extant bacteria does not say much about its mode of energy acquisition and carbon sources. The patchy distribution of metabolic genes hinders our understanding of the sources of carbon, nitrogen, and energy for the LCA and its immediate predecessors (Moreira & López-García 2006), and for now it is difficult to assess the cenancestral metabolism. However, if multiple copies of every major gene family are assumed to have been present in the LCA genome (Glansdorff 2000), then the observed complex distribution patterns of bioenergetic and biosynthetic genes can be explained as the outcome of polyphyletic gene losses as the cenancestor descendants adapted to a wide variety of environments under different selection pressures (Castresana 2001).

It has been argued that the LCA was an acellular entity (Koga et al. 1998, Koonin & Martin 2005). However, the high conservation and wide phylogenetic distribution of membrane-bound proteins and multiunit enzymes such as the ATPase hydrophilic subunits (Gogarten et al. 1989), signal recognition particles (Gribaldo & Cammarano 1998), and ABC transporters (Delaye et al. 2005) are consistent with the idea that the cenancestor was a membrane-bounded cell, which may have been endowed with heterochiral lipids composed of a mixture of glycerol-1-phosphate and glycerol-3-phosphate (Peretó et al. 2004, Wächstershäuser 2003).

The conservation of membrane-bound proton pump ATPase subunits (**Table 1**) suggests that the cenancestor produced a chemically driven protein gradient across its cell membrane using a variety of oxidized inorganic molecules as molecular acceptors (Castresana & Moreira 1999). The conservation of ABC transporters, P-type ATPases, and ion-coupled permeases is an indication of the high conservation of membrane transport phenomena throughout evolution (Clayton et al. 1997). The conservation of ABC transporters (**Table 1**) involved in the import of metabolic substrates is also consistent with the possibility of a heterotrophic LCA that depended upon external sources of organic compounds.

CONCLUSIONS AND OUTLOOK

Regardless of the qualitative and quantitative differences in the methodological approaches used to identify the gene complement of the cenancestor, the inventories shown in **Table 1** indicate an overlap that reflects an impressive level of conservation for a significant number of sequences involved in basic biological processes. Current descriptions of the LCA are limited by the scant information available: It is hard to understand in full the evolutionary forces that acted upon our distant ancestors, whose environments and detailed biological characteristics are forever beyond our ken. By definition, the node located at the bottom of a cladogram is the root of a phylogenetic tree and corresponds to the common ancestor of the group under study. But names may be misleading. What we have been calling the root of the universal tree is in fact the tip of its trunk: Inventories of LCA genes include sequences that originated in different precenancestral epochs. For instance, a number of highly conserved ribosomal proteins may have originated during the RNA/protein world

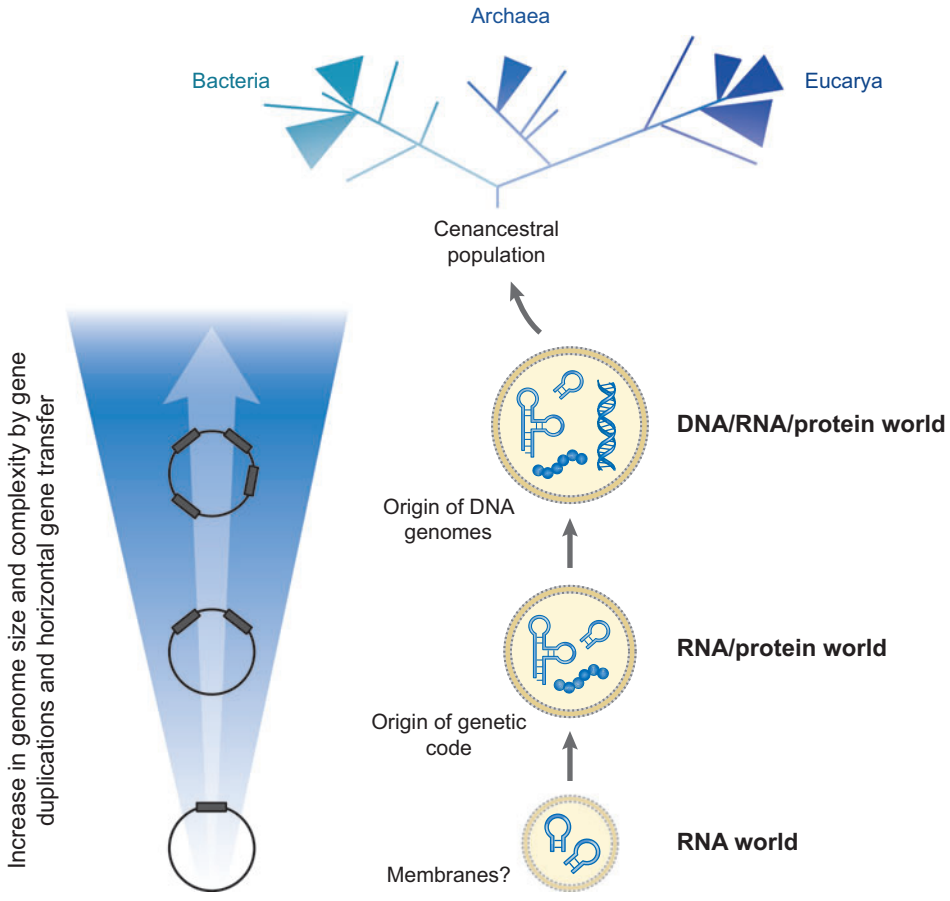

Figure 2

Evolutionary events that may have preceded the last common ancestor and the divergence of the three major cell lineages.

stage, whereas thymidine kinase and thioredoxin reductase, which are involved in deoxyribonucleotide biosynthesis, evolved at a later stage.

Although we favor a bacterial-like cenancestor, for now it is difficult to assess the sources of energy and carbon used by the LCA or the environmental conditions in which it thrived. Biological evolution prior to the divergence of the three domains was not a continuous, unbroken chain of progressive transformation steadily proceeding toward the LCA (**Figure 2**). RNA-binding domains (Delaye & Lazcano 2000) and invariant sequences that exhibit a surprising degree of conservation, such as GHVDHGKT, DTPGHVDF, and GAGKSTL (Goto et al. 2002), are among the oldest recognizable motifs found in extant databases and may provide insights onto the nature of the evolutionary processes that shaped ribosome-mediated protein biosynthesis. Older stages are not yet amenable to phylogenetic analysis, and it is

difficult to see how the applicability of molecular cladistics and comparative genomics can be extended beyond a threshold that corresponds to a period of cellular evolution in which protein biosynthesis was already in operation. Many details of the processes that led to the beginnings of life are shrouded in mystery and may remain so, but current developments in comparative genomics suggest that important insights on very early stages of biological evolution can be achieved, even if the possible intermediates that may have once existed have long since vanished.

SUMMARY POINTS

1. Theoretical estimates of the gene content of the LCA's genome suggest that it was not a progenote or a protocell, but an entity similar to extant prokaryotes.
2. The presence of a core of highly conserved RNA-related sequences supports the hypothesis that the LCA was preceded by earlier entities in which RNA molecules played a more conspicuous role in cellular processes and in which ribosome-mediated protein synthesis had already evolved.
3. Whole-genome analysis has revealed high levels of sequence redundancy, which demonstrates the significance of paralogous duplications in shaping the size and complexity of cell genomes. This redundancy suggests that during early stages of biological evolution, anabolic pathways and other biological processes were catalyzed by less-specific enzymes that could react with a wide range of chemically related substrates.
4. The chemistry of ribonucleotide reduction, combined with sequence analysis, supports the hypothesis of a monophyletic origin of DNA that took place prior to the evolutionary divergence of the three main cell domains. The available evidence suggests that the bacterial DNA polymerase I palm domain, and its homologs, is a descendant of a component of an ancestral RNA-dependent RNA polymerase that may have played dual roles as a replicase and a transcriptase during the RNA/protein stage.
5. The availability of genomic data has revealed major discrepancies with the topology of rRNA trees. This has led researchers to question the early branching of *Thermotoga* and *Aquifex*, two bacterial thermophilic species. These conclusions suggest not only that heat-loving bacteria may have been recipients of archaeal hyperthermophilic traits, but also that the LCA was not an extremophile.

FUTURE ISSUES

1. The identification and proper annotation of highly conserved open reading frames found in all cell genomes remain important open issues.

2. Characterization of the cenancestor requires proper assessments of the frequency of ancestral events, including lateral gene transfer, gene losses, and paralogous duplications.
3. Understanding of the evolution of central metabolic pathways is hampered by the unexplained absence of one or more biosynthetic genes in the genomes of manifold free-living prokaryotes that have been sequenced.
4. The development of models of the carbon sources and energy acquisition mechanisms of the LCA and its immediate predecessors should be addressed.
5. It is important to assess the oldest paleontological evidence for the domain Archaea.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are indebted to Dr. Janet Siefert for her careful editing of the manuscript and many useful suggestions. Support from CONACYT-México (Project 50520-Q) to A.L. is gratefully acknowledged.

LITERATURE CITED

Alcántara C, Cervera J, Rubio V. 2000. Carbamate kinase can replace in vivo carbamoyl phosphate synthetase. Implications for the evolution of carbamoyl phosphate biosynthesis. *FEBS Lett.* 484:261–64

Alifano P, Fani R, Liò P, Lazcano A, Bazzicalupo M, et al. 1996. Histidine biosynthetic pathway and genes: structure, regulation, and evolution. *Microbiol. Rev.* 60:44–69

Altermann W, Kazmierczak J. 2003. Archean microfossils: a reappraisal of early life on Earth. *Res. Microbiol.* 154:611–17

Anantharaman V, Koonin EV, Aravind L. 2002. Comparative genomics and evolution of proteins involved in RNA metabolism. *Nucleic Acids Res.* 30:1427–64

Aravind L, Mazumderer R, Vasudevan S, Koonin EV. 2002. Trends in protein evolution, inferred from sequence and structure analysis. *Curr. Opin. Struct. Biol.* 12:392–99

Becerra A, Delaye L, Lazcano A, Orgel L. 2007. Protein disulfide oxidoreductases and the evolution of thermophily: Was the last common ancestor a heat-loving microbe? *J. Mol. Evol.* In press

Becerra A, Islas S, Leguina JI, Silva E, Lazcano A. 1997. Polyphyletic gene losses can bias backtrack characterizations of the cenancestor. *J. Mol. Evol.* 45:115–18

Becerra A, Lazcano A. 1998. The role of gene duplication in the evolution of purine nucleotide salvage pathways. *Orig. Life Evol. Biosph.* 28:539–53

Blum E, Py B, Carpousis AJ, Higgins CF. 1997. Polyphosphate kinase is a component of the *Escherichia coli* RNA degradosome. *Mol. Microbiol.* 26:387–98

Brown JR, Douady CJ, Italia MJ, Marshall WE, Stanhope MJ. 2001. Universal trees based on large combined protein sequence datasets. *Nat. Genet.* 28:281–85

Canfield DE. 2006. Biochemistry: gas with an ancient history. *Nature* 440:426–27

Caruthers JM, Johnson ER, McKay DB. 2000. Crystal structure of yeast initiation factor 4A, a DEAD-box RNA helicase. *Proc. Natl. Acad. Sci. USA* 97:13080–85

Castresana J. 2001. Comparative genomics and bioenergetics. *Biochem. Biophys. Acta* 1506:147–62

Castresana J, Moreira D. 1999. Respiratory chains in the last common ancestor of living organisms. *J. Mol. Evol.* 49:453–60

Chaput JC, Ichida JK, Szostak JW. 2003. DNA polymerase-mediated DNA synthesis on TNA template. *J. Am. Chem. Soc.* 125:856–57

Clayton RA, White O, Ketchum KA, Venter CJ. 1997. The genome from the third domain of life. *Nature* 387:459–62

Cleaves HJ, Chalmers JH. 2004. Extremophiles may be irrelevant to the origin of life. *Astrobiology* 4:1–9

Daubin V, Gouy M, Perriere G. 2001. A phylogenomic approach to bacterial phylogeny: evidence for a core of genes sharing a common history. *Genome Res.* 12:1080–90

Delaye L, Becerra A, Lazcano A. 2005. The last common ancestor: What's in a name? *Orig. Life Evol. Biosph.* 35:537–54

Delaye L, Lazcano A. 2000. RNA-binding peptides as molecular fossils. In *Origins from the Big-Bang to Biology: Proceedings of the First Ibero-American School of Astrobiology*, ed. J Chela-Flores, G Lemerchand, J Oró, pp. 285–88. Dordrecht: Klüwer Acad.

Delaye L, Vázquez H, Lazcano A. 2001. The cenancestor and its contemporary biological relics: the case of nucleic acid polymerases. In *First Steps in the Origin of Life in the Universe*, ed. J Chela-Flores, T Owen, F Raulin, pp. 223–30. Dordrecht: Kluwer Acad.

Di Giulio M. 2001. The universal ancestor was a thermophile or a hyperthermophile. *Gene* 281:11–17

Di Giulio M. 2003. The universal ancestor and the ancestor of bacteria were hyperthermophiles. *J. Mol. Evol.* 57:721–30

Doolittle WF. 2000. The nature of the universal ancestor and the evolution of the proteome. *Curr. Opin. Struct. Biol.* 10:355–58

Edgell RD, Doolittle WF. 1997. Archaea and the origins of DNA replication proteins. *Cell* 89:995–98

Fitch WM, Upper K. 1987. The phylogeny of tRNA sequences provides evidence of ambiguity reduction in the origin of the genetic code. *Cold Spring Harbor Symp. Quant. Biol.* 52:759–67

Forterre P. 2006. Three RNA cells for ribosomal lineages and three DNA viruses to replicate their genomes: a hypothesis for the origin of cellular domain. *Proc. Natl. Acad. Sci. USA* 103:3669–74

Forterre P, Benachenhou-Lahfa N, Confalonieri F, Duguet M, Elie C, Labedan B. 1993. The nature of the last universal ancestor and the root of the tree of life, still open questions. *BioSystems* 28:15–32

Forterre P, Bouthier de la Tour C, Philippe H, Duguet M. 2000. Reverse gyrase from hyperthermophiles: probable transfer of a thermoadaptation trait from Archaea to bacteria. *Trends Genet.* 16:152–54

Galtier N, Tourasse N, Gouy M. 1999. A nonhyperthermophilic common ancestor to extant life forms. *Science* 283:220–21

Glansdorff N. 2000. About the last common ancestor, the universal life-tree and lateral gene transfer: a reappraisal. *Mol. Microbiol.* 38:177–85

Gogarten JP, Kibak H, Dittrich P, Taiz L, Bowman EJ, et al. 1989. Evolution of the vacuolar H^+-ATPase, implications for the origin of eukaryotes. *Proc. Natl. Acad. Sci. USA* 86:6661–65

Gogarten JP, Townsend JP. 2005. Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* 3:679–87

Goto N, Kurokawa K, Yasunaga T. 2002. Finding conserved amino acid sequences among prokaryotic proteomes. *Genome Inform.* 13:443–44

Gribaldo S, Cammarano P. 1998. The root of the universal tree of life inferred from anciently duplicated genes encoding components of the protein-targeting machinery. *J. Mol. Evol.* 47:508–16

Harris JK, Kelley ST, Spiegelman GB, Pace NR. 2003. The genetic core of the universal ancestor. *Genome Res.* 13:407–12

Iwabe N, Kuma K, Hasegawa M, Osawa S, Miyata T. 1989. Evolutionary relationship of archaebacteria, eubacteria, and eukaryotes inferred from phylogenetic trees of duplicated genes. *Proc. Natl. Acad. Sci. USA* 86:9355–59

Jensen RA. 1976. Enzyme recruitment in evolution of new function. *Annu. Rev. Microbiol.* 30:409–25

Jeruzalmi D, Steitz TA. 1998. Structure of T7 RNA polymerase complexed to the transcriptional inhibitor T7 lysozyme. *EMBO J.* 17:4101–13

Joyce GF. 2002. The antiquity of RNA-based evolution. *Nature* 418:214–21

Kandler O. 1994. The early diversification of life. In *Early Life on Earth*, *Nobel Symp. 84*, ed. S Bengtson, pp. 152–60. New York: Columbia Univ. Press

Klenk HP, Palm P, Zillig W. 1994. DNA-dependent RNA polymerases as phylogenetic marker molecules. *Syst. Appl. Microbiol.* 16:638–47

Koga Y, Kyuragi T, Nishihara M, Sone N. 1998. Did archaeal and bacterial cells arise independently from noncellular precursors? A hypothesis stating that the advent of membrane phospholipids with enantiomeric glycerophosphate backbones caused the separation of the two lines of descent. *J. Mol. Evol.* 46:54–63

Koonin EV. 2003. Comparative genomics, minimal gene-sets and the last universal common ancestor. *Nat. Rev.* 1:127–36

Koonin EV. 2006. Temporal order of evolution of DNA replication systems inferred by comparison of cellular and viral DNA polymerases. *Biol. Direct.* 1:39

Koonin EV, Martin W. 2005. On the origin of genomes and cells within inorganic compartments. *Trends Genet.* 21:647–54

Kyrpides N, Overbeek R, Ouzounis C. 1999. Universal protein families and the functional content of the last universal common ancestor. *J. Mol. Evol.* 49:413–23

Ladenstein R, Ren B. 2006. Protein disulfides and protein disulfide oxidoreductases in hyperthermophiles. *FEBS J.* 273:4170–85

Lazcano A, Fastag J, Gariglio P, Ramírez C, Oró J. 1988a. On the early evolution of RNA polymerase. *J. Mol. Evol.* 27:365–76

Lazcano A, Fox GE, Oró J. 1992. Life before DNA: the origin and early evolution of early Archean cells. In *The Evolution of Metabolic Function*, ed. RP Mortlock, pp. 237–95. Boca Raton, FL: CRC

Lazcano A, Guerrero R, Margulis L, Oró J. 1988b. The evolutionary transition from RNA to DNA in early cells. *J. Mol. Evol.* 27:283–90

Leipe DD, Aravind L, Koonin EV. 1999. Did DNA replication evolve twice independently? *Nucleic Acids Res.* 27:3389–401

Line MA. 2002. The enigma of the origin of life and its timing. *Microbiology* 148:21–27

Lollar BS, McCollom TM. 2006. Biosignatures and abiotic constrainst on early life. *Nature* 444:E18

Makarova KS, Koonin EV. 2003. Comparative genomics of archaea: How much have we learned in six years, and what's next? *Genome Biol.* 4:115–45

Mirkin BG, Fenner TI, Galperin MY, Koonin EV. 2003. Algorithms for computing parsimonious evolutionary scenarios for genome evolution, the last common ancestor, and dominance of horizontal gene transfer in the evolution of prokaryotes. *BMC Evol. Biol.* 3:2

Moreira D, López-García P. 2006. The last common ancestor. *Earth Moon Planets* 98:187–93

Olsen G, Woese CR. 1997. Archaeal genomics: an overview. *Cell* 89:991–94

Ouzounis AC, Kunin V, Darzentas N, Goldovsky L. 2006. A minimal estimate for gene content of the last universal common ancestor—exobiology from a terrestrial perspective. *Res. Microbiol.* 157:57–68

Pedone E, Ren B, Ladenstein R, Rossi M, Bartolucci S. 2004. Functional properties of the protein disulfide oxidoreductase from the archaeon *Pyrococcus furiosus*. *FEBS Eur. J. Biochem.* 271:3437–48

Penny D, Poole A. 1999. The nature of the last common ancestor. *Curr. Opin. Gen. Dev.* 9:672–77

Peretó J, López-García P, Moreira D. 2004. Ancestral lipid biosynthesis and early membrane evolution. *Trends Biochem. Sci.* 29:469–77

Philippe H, Forterre P. 1999. The rooting of the universal tree of life is not reliable. *J. Mol. Evol.* 49:509–23

Poole A, Logan DT. 2005. Modern mRNA proofreading and repair: clues that the last universal common ancestor possessed an RNA genome? *Mol. Biol. Evol.* 22:1444–55

Ranea AG, Sillero A, Thorton MJ, Orengo AC. 2006. Protein superfamily evolution and the last universal common ancestor (LUCA). *J. Mol. Evol.* 63:513–25

Reanney DC. 1987. Genetic error and genome design. *Cold Spring Harbor Symp. Quant. Biol.* 52:751–57

Ren B, Tibbelin G, de Pascale D, Rossi M, Bartolucci S, Ladenstein R. 1998. A protein disulfide oxidoreductase from the archaeo *Pyrococcus furiosus* contains two thioredoxin fold units. *Nat. Struct. Biol.* 7:602–11

Schinkel AH, Tabak HF. 1989. Mitochondrial RNA polymerase: dual role in transcription and replication. *Trends Genet.* 5:149–54

Schmid SR, Linder P. 1992. D-E-A-D protein family of putative RNA helicases. *Mol. Microbiol.* 6:283–92

Schopf JW. 1993. Microfossils of the early Archaean Apex chert: new evidence for the antiquity of life. *Science* 260:640–46

Shen Y, Buick R, Canfield DE. 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature* 410:77–81

Siefert JL, Martin KA, Abdi F, Wagner WR, Fox GE. 1997. Conserved gene clusters in bacterial genomes provide further support for the primacy of RNA. *J. Mol. Evol.* 45:467–72

Sobolevsky Y, Trifonov EN. 2006. Protein modules conserved since LUCA. *J. Mol. Evol.* 63:622–34

Steitz TA. 1999. DNA polymerases: structural diversity and common mechanisms. *J. Biol. Chem.* 274:17395–98

Stetter KO. 2006. Hyperthermophiles in the history of life. *Philos. Trans. R. Soc. London Ser. B* 361:1837–43

Stubbe J, Ge J, Yee CS. 2001. The evolution of ribonucleotide reduction revisited. *Trends Biochem. Sci.* 26:93–99

Theis K, Chen PJ, Skorvaga M, Van Houten B, Kisker C. 1999. Crystal structure of UvrB, a DNA helicase adapted for nucleotide excision repair. *EMBO J.* 18:6899–907

Tice MM, Lowe DR. 2004. Photosynthetic microbial mats in the 3416-Myr-old ocean. *Nature* 431:549–52

Ueno Y, Yamada K, Yoshida N, Maruyama S, Isozaki Y. 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature* 440:516–19

Wächstershäuser G. 2003. From precells to Eukarya—a tale of two lipids. *Mol. Microbiol.* 47:13–22

Woese CR. 1998. The universal ancestor. *Proc. Natl. Acad. Sci. USA* 95:6854–59

Woese CR, Fox GE. 1977. The concept of cellular evolution. *J. Mol. Evol.* 10:1–6

Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms, proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. USA* 87:4576–79

Yang S, Doolittle RF, Bourne PE. 2005. Phylogeny determined by protein domain content. *Proc. Natl. Acad. Sci. USA* 102:373–78

Zhaxybayeva O, Gogarten PJ. 2004. Cladogenesis, coalescence and the evolution of the three domains of life. *Trends Genet.* 20:182–87

Annual Review of Ecology, Evolution, and Systematics

Volume 38, 2007

Contents

Evolution of Animal Photoperiodism
William E. Bradshaw and Christina M. Holzapfel 1

Virus Evolution: Insights from an Experimental Approach
Santiago F. Elena and Rafael Sanjuán 27

The Social Lives of Microbes
Stuart A. West, Stephen P. Diggle, Angus Buckling, Andy Gardner, and Ashleigh S. Griffin 53

Sexual Selection and Speciation
Michael G. Ritchie 79

Kin Selection and the Evolutionary Theory of Aging
Andrew F.G. Bourke 103

Climate Change and Invasibility of the Antarctic Benthos
Richard B. Aronson, Sven Thatje, Andrew Clarke, Lloyd S. Peck, Daniel B. Blake, Cheryl D. Wilga, and Brad A. Seibel 129

Spatiotemporal Dimensions of Visual Signals in Animal Communication
Gil G. Rosenthal 155

Gliding and the Functional Origins of Flight: Biomechanical Novelty or Necessity?
Robert Dudley, Greg Byrnes, Stephen P. Yanoviak, Brendan Borrell, Rafe M. Brown, and Jimmy A. McGuire 179

How Mutational Networks Shape Evolution: Lessons from RNA Models
Matthew C. Cowperthwaite and Lauren Ancel Meyers 203

How Does It Feel to Be Like a Rolling Stone? Ten Questions about Dispersal Evolution
Ophélie Ronce 231

Exploring Cyanobacterial Mutualisms
Kayley M. Usher, Birgitta Bergman, and John A. Raven 255

Human Impacts in Pine Forests: Past, Present, and Future
David M. Richardson, Philip W. Rundel, Stephen T. Jackson, Robert O. Teskey, James Aronson, Andrzej Bytnerowicz, Michael J. Wingfield, and Şerban Procheş 275

Chemical Complexity and the Genetics of Aging
Scott D. Pletcher, Hadise Kabil, and Linda Partridge 299

A Global Review of the Distribution, Taxonomy, and Impacts of Introduced Seaweeds
Susan L. Williams and Jennifer E. Smith 327

The Very Early Stages of Biological Evolution and the Nature of the Last Common Ancestor of the Three Major Cell Domains
Arturo Becerra, Luis Delaye, Sara Islas, and Antonio Lazcano 361

Functional Versus Morphological Diversity in Macroevolution
Peter C. Wainwright 381

Evolutionary Game Theory and Adaptive Dynamics of Continuous Traits
Brian J. McGill and Joel S. Brown 403

The Maintenance of Outcrossing in Predominantly Selfing Species: Ideas and Evidence from Cleistogamous Species
Christopher G. Oakley, Ken S. Moriuchi, and Alice A. Winn 437

Sympatric Speciation: Models and Empirical Evidence
Daniel I. Bolnick and Benjamin M. Fitzpatrick 459

The Evolution of Color Polymorphism: Crypticity, Searching Images, and Apostatic Selection
Alan B. Bond 489

Point, Counterpoint: The Evolution of Pathogenic Viruses and their Human Hosts
Michael Worobey, Adam Bjork, and Joel O. Wertheim 515

The Evolution of Resistance and Tolerance to Herbivores
Juan Núñez-Farfán, Juan Fornoni, and Pedro Luis Valverde 541

Plant-Animal Mutualistic Networks: The Architecture of Biodiversity
Jordi Bascompte and Pedro Jordano 567

Gene Flow and Local Adaptation in Trees
Outi Savolainen, Tanja Pyhäjärvi, and Timo Knürr 595

The Evolution of Multicellularity: A Minor Major Transition?
Richard K. Grosberg and Richard R. Strathmann 621

Developmental Genetics of Adaptation in Fishes: The Case for Novelty
J.T. Streelman, C.L. Peichel, and D.M. Parichy 655

Terrestrial Carbon–Cycle Feedback to Climate Warming
Yiqi Luo 683

Shortcuts for Biodiversity Conservation Planning: The Effectiveness of Surrogates
Ana S.L. Rodrigues and Thomas M. Brooks 713

Understanding the Effects of Marine Biodiversity on Communities and Ecosystems
John J. Stachowicz, John F. Bruno, and J. Emmett Duffy 739

Stochastic Dynamics of Plant-Water Interactions
Gabriel Katul, Amilcare Porporato, and Ram Oren 767

Evolutionary Endocrinology: The Developing Synthesis between Endocrinology and Evolutionary Genetics
Anthony J. Zera, Lawrence G. Harshman, and Tony D. Williams 793

The Role of Behavior in the Evolution of Spiders, Silks, and Webs
Fritz Vollrath and Paul Selden 819

Applications of Flow Cytometry to Evolutionary and Population Biology
Paul Kron, Jan Suda, and Brian C. Husband 847

Indexes

Cumulative Index of Contributing Authors, Volumes 34–38 877

Cumulative Index of Chapter Titles, Volumes 34–38 881

Errata

An online log of corrections to *Annual Review of Ecology, Evolution, and Systematics* articles may be found at http://ecolsys.annualreviews.org/errata.shtml