

# Search for a *Methanopyrus*-proximal last universal common ancestor based on comparative-genomic analysis

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**Abstract** The origin of life is a long-standing mystery puzzling many people. This mystery possesses not only philosophical but also important biological significance. To unveil this mystery, the searches for the root of life, or the last universal common ancestor (LUCA), based on comparative-genomic analysis have been intensively performed on rRNAs, tRNAs and proteins sequences. The current search pointed to a *Methanopyrus*-proximal LUCA, which opens up the reconstruction of Lucan biology and helps to delineate the evolutionary pathways.

**Keywords** LUCA · *Methanopyrus* · Genetic evolution · Comparative-genomic analysis

## Introduction

Since the genetic code constitutes the foundation of the protein world, the near universality of the standard genetic code among known living organisms suggests that a single tree of life encompasses all extant organisms which are

descendants from a last universal common ancestor, or LUCA. Biologists have subscribed to the powerful, unifying idea that all life on Earth arose from a common ancestor (Wong 2005, 2009). Initially, nothing concrete could be said about the nature of this ancestor. Only when biology could be defined on the level of molecular sequences would it become possible to seriously enquire into the nature of this ancestor. Rooting the universal phylogenetic tree and thereby hunting for LUCA has been of central importance for biology in the past decade. In the forward direction of time, knowledge of LUCA is a prerequisite to the analysis of early evolutionary events and will help to uncover the evolutionary process that gave rise to Archaea, Bacteria and Eukarya, the three fundamental living domains on Earth. In the backward direction, LUCA marks the finish line of the origin of life process (Xue et al. 2003; Woese 1998; Doolittle 1999). Accordingly, there has been an intensive search of LUCA in recent years. Comparative analysis of molecular sequences has become a powerful approach to construct phylogenetic tree, determine the universal evolutionary relationship and search for LUCA. However, it has been estimated that >99% of microorganisms observable in nature are typically not cultivatable by standard techniques (Somerville et al. 1989). Therefore, such a search for LUCA relying on comparative-genome analysis should not be restricted to the cultivatable microorganisms. Instead, it has to be conducted on the genomes of cultivatable microorganisms, as well as uncultivable ones present on the planet. With technological advances, especially in PCR and sequencing techniques, biologists have extensively applied molecular techniques to the direct retrieval of genome sequences in natural systems, obviating the need for cell culture. To date, tons of organism genes and genomes have been sequenced and deposited, rendering the fundamental capability and

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feasibility for biologists to search for LUCA on the basis of comparative-genome analysis. Brown and Doolittle (1995) used comparative analysis of paralogous protein sequences to favor a rooting of the tree of life in the Bacterial domain. Lake et al. (2008), in contrast, through the comparative analysis of directed indels, insertions and deletions within paralogous genes, located the root to an internal branch between a clade consisting of the Firmicutes plus the Archaea and a clade consisting of the Actinobacteria plus the double-membrane prokaryotes. Moreover, Di Giulio (2007) proposed to root the universal phylogenetic tree to Nanoarchaeota based on the comparative analysis of the uniqueness and ancestrality of a trait that is the existence of split tRNA genes codifying for the 5' and 3' halves only within *Nanoarchaeum equitans*, supported by the finding of the absence of operons. The nature of LUCA and the root of the tree of life are so far still open questions. In this communication, genes or genomes commonly chosen for comparative analysis in LUCA search were critically reviewed, and the search pointed to a *Methanopyrus*-proximal LUCA (Xue et al. 2003, 2005).

### Building of universal tree based on rRNA

In the mid-1970s, Woese and his collaborators began to assemble the massive database of sequence information on small subunit ribosomal RNA (SSU rRNA) on which the current universal tree tests and discovered a third domain of living organisms, the Archaea (Fox et al. 1977a, b; Woese and Fox 1977). Later on, they proposed a phylogenetic classification system for prokaryotic species based on the nucleotide sequences comparison of SSU rRNA molecules. They reported that the SSU rRNA gene sequence could be used for comparative analysis between different species to provide a tree of relatedness based on common ancestry or genealogy. Significantly, as both prokaryotic and eukaryotic cells contain SSU rRNA genes, phylogenetic analysis could also be used to compare prokaryotic as well as eukaryotic species, giving rise to Archaea, Bacteria and Eukarya, the three fundamental living domains on Earth (Woese 1987; Woese et al. 1990). In the following decades, rRNA genes were the most widely used to build a universal tree based on sequence comparison and provided insight into the distribution and phylogenies of organisms in environmental samples (DeLong 1992; Barns et al. 1996; Hohn et al. 2002). To locate the origin on a sequence tree, outgroup sequence is needed. A paralog generally serves as the outgroup (Schwartz and Dayhoff 1978). rRNAs are devoid of paralogs, and therefore cannot be utilized to find the root of the universal phylogenetic tree. The rRNAs also show the dilemma of discrepant phylogenies from SSU and large subunit (LSU) rRNAs. For red algae and cryptomonads, for

example, SSU and LSU rRNAs present dissimilar tree topologies, and a combination of SSU and LSU rRNAs favors one or the other of these topologies on the basis of the tree algorithm (Van der Auwera et al. 1998). SSU and LSU rRNAs also give rise to different tree topologies for euryarchaeotes (Nesbo et al. 2001), and evidence demonstrates that SSU rRNA trees could be far less robust than LSU rRNA ones (De Rijk et al. 1995). All these findings indicate that the rooting of the universal phylogenetic trees must be sought from an analysis of transfer RNA (tRNA) or protein genes.

### Rooting based on tRNAs

In contrast to the lack of rRNA paralogs, tRNAs have paralogs and can be used to root the sequence tree. Analysis of tRNA genes suggests that tRNA evolution began from a closely clustered group of sequences that became increasingly dispersed with time (Xue et al. 2003; Wong 1975, 1976, 2005; Di Giulio 1995, 2000). Accordingly, a minimum genetic distance or 'alloacceptor distance' between tRNAs accepting different amino acids, or alloacceptor tRNAs, within an organism would be a mark of extreme primitiveness of the tRNA genotypes. It is commonly held that single tRNA sequences, owing to their short lengths, are not useful for phylogenetic tree construction and LUCA search. This situation has been fundamentally overcome by our laboratory through comparison of total tRNA ensembles rather than any single tRNA. The tRNA tree built from ensemble comparisons displayed well-separated clades for the three domains just as delineated by SSU rRNA phylogeny (Xue et al. 2003). In that study, analysis was performed on 2,878 tRNA sequences from 60 different genomes, 34 Bacteria, 18 Archaea and 8 Eukarya. Overall, the genetic distances between alloacceptor tRNAs yielded estimates of how closely clustered in sequence space are the tRNAs in a genome. Among the 60 genomes analyzed, *Methanopyrus kandleri* AV19 (Mka) yielded the lowest alloacceptor distances, or the most primitiveness, indicating an Mka-proximal LUCA.

### Rooting based on protein paralogs

Protein sequences are also widely used for phylogenetic studies because they have paralogs and can provide a great deal of sequence information. Graur and Li (2000) reported that amino acids change less frequently during evolution than nucleotides. Since there are 20 amino acids and only four nucleotides, the chance of two sites on a sequence being identical is lower with amino acids. Several poten-

tially paralogous protein pairs, including ATPase subunits, elongation factors, signal recognition particle and its receptor, aminoacyl-tRNA synthetases, aspartate and ornithine transcarbamoylases, were chosen to construct paralogously rooted phylogenetic trees (Gogarten et al. 1989; Gogarten and Olendzenski 1999; Iwabe et al. 1989; Kollman and Doolittle 2000; Brown and Doolittle 1995). Based on reciprocal rootings of the phylogenetic trees for protein paralogs that likely stemmed from a primordial gene duplication, the proposal was made that the root of life was located in the Bacteria domain (Iwabe et al. 1989; Woese et al. 1990; Brown and Doolittle 1995). However, the number of archaeal protein sequences employed in arriving at a bacterial siting of LUCA in these studies was meager, and protein trees are also known to be highly susceptible to horizontal gene transfers that could invalidate such paralogous protein tree rootings (Philippe and Forterre 1999). In fact, when a large number of ValRS and IleRS sequences were employed for reciprocal rootings, the IleRS tree was found to be burdened with interdomain mixings of sequences that would render any rooting of the IleRS tree hazardous. The ValRS tree in contrast included only minor interdomain mixings, and use of IleRS sequences as outgroup rooted the ValRS tree within the Archaea domain (Wong et al. 2007). The protein paralog method of rooting the tree has been questioned due to the misconstruction of life histories on the tree resulting from the fact that bacterial sequences have been changing much faster than archaeal and eukaryotic sequences (Brinkmann and Philippe 1999; Philippe and Forterre 1999). It was concluded that protein paralogous rootings were susceptible to artifacts arising from long branch attraction, mutational saturation, and horizontal gene transfers (HGT), and have given unreliable results (Gribaldo and Philippe 2002; Wolf et al. 2002).

### Interparalog distances between aminoacyl-tRNA synthetases (aaRS)

Considering the difficulties of paralogous protein trees, our laboratory has proposed an alternative interparalog-distance method for rooting the universal phylogenetic tree and

searching for LUCA based on a comparison of the genetic distances between ancient protein paralogs within different genomes. The expectation is that these interparalog distances would increase with the evolved distance of a species away from LUCA: the lower the evolved distance, the more primitive would be the divergence as a genetic trait (Wong and Xue 2002; Xue et al. 2005). Since interparalog distances depend only on intraspecies, and not at all on interspecies, comparisons, they are intrinsic to each species and uninfluenced by other species. The interparalog genetic distance represents a criterion of primitivity for the search of LUCA. Aminoacyl-tRNA synthetases (aaRS) are particularly attractive candidates for interparalog-distance analysis because all free-living organisms possess a complete set of aaRS for protein amino acids besides Gln, Asn and Cys (Wong and Xue 2002; Sauerwald et al. 2005). To screen for potential paralogs between aaRS all-to-all BLASTP (Altschul et al. 1997) was employed to yield the bit-scores as indicators of homology for all possible aaRS pairs in 60 species. Analysis yielded ten potentially paralogous aaRS pairs. For each species, its bit-scores for the ten aaRS pairs were compared with those of other species to generate a ranking of the species in terms of each aaRS pairs. Among those analyzed species, Mka yielded the highest overall bit-score, displaying the least genetic distance evolved away from LUCA, which is an exact agreement with the evidence from tRNA genes locating the root of life closest to this organism (Xue et al. 2005).

### Deeper search using ValRS-IleRS interparalog distance

*Methanopyrus kandleri* AV19 (Mka) is rod-shaped, Gram-positive and grows at temperature up to 110°C (Huber et al. 1989). Because of its location in the 16S rRNA gene (rDNA) tree near the root of the Archaea, Mka is considered to be primitive (Burggraf et al. 1991) among the Archaea, which is supported by its unique lipids (Hafenbradl et al. 1993). These properties of Mka are consistent with our tRNA and aaRS paralogous gene comparison studies that congruously pointed to an Mka-proximal LUCA (Xue et al. 2003, 2005). Further searches

**Table 1** Several *Methanopyrus* isolates collected over the years

Organism	Year	Origin	Ocean
Mka	1988	Guaymas Basin, Mexico	Pacific Ocean
GC34	1991	Guaymas Basin, Mexico	
GC37	1991	Guaymas Basin, Mexico	
KOL6	1988	Kolbeinsey Ridge, Iceland	Atlantic Ocean
TAG1	1993	Mid Atlantic Ridge, 'TAG' site	
TAG11	1993	Mid Atlantic Ridge, 'TAG' site	
SNP6	1993	Mid Atlantic Ridge, 'SNP' site	

for organisms even closer to LUCA than Mka based on comparative-genomic analysis may also begin with members of *Methanopyrus* genus, or organisms that are close relatives of the *Methanopyrus*. In view of the finding that among all aaRS gene pairs in 60 organisms analyzed the bit-score of 506.5 displayed by valyl-tRNA synthetase (ValRS)-isoleucyl-tRNA synthetase (IleRS) interparalog distance of Mka was remarkably high for a paralogous pair that diverged prior to the emergence of LUCA some 3 billion years ago (Xue et al. 2005), this interparalog genetic distance could be employed as a measure of primitivity to search for organisms even closer to LUCA than Mka. During the last 14 years, several isolates of *Methanopyrus* sp. (Table 1) and environmental *Methanopyrus* gene fragments (Takai et al. 2004a, b) have been collected from different locations. Comparison of ValRS-IleRS interparalog distances between those different *Methanopyrus* members showed that different *Methanopyrus* members differ significantly with respect to ValRS-IleRS divergence, and one *Methanopyrus* member from the Indian Ocean mixed environmental sample represents the most primitive *Methanopyrus*, phylogenetically the oldest living lineage closest to LUCA, or this *Methanopyrus* member is closer to LUCA than Mka (Yu et al. 2009; Yu 2010).

## Conclusions

The second half of the twentieth century witnessed the birth of molecular biology and the subsequent development of powerful methods to analyze the primary sequences and structures of nucleic acids and proteins, allowing biologists to answer biological questions through comparative-genomic analysis. For evolutionists, one of the most momentous consequences of this unprecedented progress in life sciences was Woese's discovery of a third domain of living organisms, the Archaea, on the basis of comparative-genomic analysis of rRNA gene which has been proven to be the most useful marker for determining the distribution and phylogenies of organisms (Fox et al. 1977a, b; Woese and Fox 1977). However, on account of the lack of paralogs, rRNAs cannot be employed for finding out the root of the organisms or searching for LUCA. Proteins and tRNAs in contrast have paralogs and therefore can be used for rooting and searching for LUCA. However, it was found that protein paralogous rootings were susceptible to artifacts generated by long branch attraction, mutational saturation, and horizontal gene transfers (HGT), thereby giving rise to unreliable results (Gribaldo and Philippe 2002; Wolf et al. 2002). On the other side, tRNAs turn out to evolve more slowly than proteins, and be less subject to variations caused by lifestyle adaptations. Comparative-genomic analysis based on total tRNA ensembles for the search for

LUCA yielded an Mka-proximal LUCA (Xue et al. 2003), which is an exact agreement with the result from our aaRS interparalog distance rooting also locating LUCA closest to Mka (Xue et al. 2005). Further search based on ValRS-IleRS interparalog genetic distance showed that one *Methanopyrus* member from the Indian Ocean is closer to LUCA than Mka from the Pacific Ocean (Yu et al. 2009; Yu 2010). The exact congruence between tRNA and aaRS genes in identifying *Methanopyrus* as being endowed with the most primitive genotypes among genome-sequenced organisms, supported by different lines of polyphasic evidence from anticodon usages, gene contents, metabolism and geochemistry (Wong et al. 2007), provides exceptional evidence for a close genotypic relationship between *Methanopyrus* and LUCA. This identification not only puts LUCA near *Methanopyrus* on the universal phylogenetic tree but also, insofar as substantial genotypic changes are in all likelihood essential to the adaptation of any organism to a different ecological niche, suggests that LUCA itself might be a hyperthermophilic archaeal methanogen. It opens the door to an examination of the biology and evolution of LUCA, and helps to define the evolutionary process leading up to and radiating from LUCA.

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