The Phylogeny of *Methanopyrus kandleri*

MARIA C. RIVERA* AND JAMES A. LAKE

Molecular Biology Institute and Department of Biology, University of California, Los Angeles, Los Angeles, California 90024

The phylogenetic position of *Methanopyrus kandleri* has been difficult to determine because reconstructions of phylogenetic trees based on rRNA sequences have been ambiguous. The most probable trees determined by most algorithms place the genus *Methanopyrus* at the base of a group that includes the halobacteria and the methanogens and their relatives, although occasionally some algorithms place this genus near the eocytes (the hyperthermophilic, sulfur-metabolizing prokaryotes), suggesting that it may belong to this lineage. In order to resolve the phylogeny of the genus *Methanopyrus*, we determined the sequence of an informative region of elongation factor 1-alpha that contains an 11-amino-acid insertion in eocytes and eukaryotes which is replaced by a 4-amino-acid insertion in methanogens, halobacteria, and eubacteria. On the basis of the results of our elongation factor 1-alpha gene analysis, we concluded that the genus *Methanopyrus* diverged from the eocyte branch before the eukaryotic and eocyte lineages separated and therefore is not an eocyte.

*Methanopyrus kandleri* is a rod-shaped, gram-positive, methanogenic bacterium that was isolated from a deep thermal vent in the Gulf of California (16). This organism is the first known hyperthermophilic methanogen that grows at temperatures up to 110°C (16). Although extreme thermophily has been found in three phylogenetically different groups of prokaryotes (the eubacteria, the methanogens and their relatives, and the eocytes [hyperthermophilic, principally sulfur-metabolizing prokaryotes]), members of the genus *Pyrodictium* are the only other prokaryotes that are known to grow at a temperature of 110°C or above (37, 38). On the basis of the results of a 16S rRNA and protein synthesis elongation factor 1-alpha (EF-1α) sequence analysis, the genus *Pyrodictium* was classified as an eocyte taxon (24, 32, 33, 37); therefore, we wanted to determine whether the genus *Methanopyrus* is also an eocyte taxon.

*Methanopyrus kandleri* appears to represent a unique lineage within the phylogenetically diverse methanogens (18, 30), and, consistent with this, the results of a Jukes-Cantor distance and parsimony analysis of the 16S rRNA sequence of this organism have shown that it is only distantly related to the other methanogens (8). A suggestion that *M. kandleri* may be related to the eukaryotes resulted from the discovery that a eukaryotic-like topoisomerase I molecule is present in *Methanopyrus* cells (35).

The most probable tree determined in our analysis of small-subunit rRNA sequences in which the Jukes-Cantor (19), Kimura (21) and paralinear distance (27) algorithms were used is shown in Fig. 1; the probabilities of this eocyte tree are 72.5, 60.5, and 63.5%, respectively. The most probable location of the genus *Metha-

*Corresponding author.*

Evidence for the phylogenetic position of the genus *Methanopyrus*

In a previous study, the phylectic distribution of the two variants of EF-Tu and EF-1α was used to infer an immediate relationship between the eocytes and the eukaryotes (32). Previously, this relationship had been difficult to analyze by direct sequence comparisons because of the extremely long divergence times between the prokaryotes and the eukaryotes. In that study, we showed that the members of the genus *Pyrodictium* and the other eocytes, as well as all of the eukaryotes, contain an 11-amino-acid segment in EF-1α that is not present in other prokaryotes. In the methanogens, the halobacteria, and the eubacteria this segment is replaced by a 4-amino-acid segment. In light of the questionable phylogenetic position of the genus *Methanopyrus*, determining the sequence of the EF-1α gene of a member of this taxon could reveal if it is also an immediate eukaryotic relative (containing the 11-amino-acid segment and corresponding to tree branch C) or if it is more closely related to the methanogens and halobacteria (containing the 4-amino-acid segment and corresponding to either tree branch A or tree branch B).

As previously noted (22, 28, 31), EF-1α appears to be one of the best molecules available for studying deep divergences. This molecule is a slowly evolving protein found in all cells, where it binds, transports, and participates in the selection of the correct aminoacyl-tRNA. In addition, EF-1α interacts with cellular components encoded by genes dispersed throughout the genome, including aminoacyl-tRNAs, ribosomal proteins, 16S and 18S rRNAs, and elongation factor EF-Ts; therefore, it is unlikely that EF-1α is transferred laterally between organisms.

To determine whether the genus *Methanopyrus* is an eocyte genus, we isolated by PCR, cloned, sequenced, and analyzed a fragment in the GDP-binding domain of the gene coding for EF-1α from several taxa whose sequences had not been determined previously. Total genomic DNAs were isolated from frozen cell pastes of *Methanopyrus kandleri, Archaeoglobus fulgidus* (36), and *Thermococcus maritimus* by using the sodium dodecyl sulfate-proteinase K lysis method (14). Total nucleic acids were extracted from *Dactylococcopsis salina* (40) and purified by CsCl centrifugation (1). Degenerate oligonucleotide primers were designed on the basis of conserved amino acid motifs in all known EF-Tu and EF-1α sequences (Fig. 2) and used for PCR amplification (34). The primers spanned the
FIG. 1. Rooted tree showing the three possible positions of the Methanopyrus lineage. This tree is the most probable tree derived from an analysis of 16S and 18S rRNA sequences in which the Kimura (21), Jukes-Cantor (19), and parsimonious linear distance (25, 27) algorithms were used. The tree is rooted in the branch leading to the eubacteria, as proposed by other workers (12, 17). The three possible locations of the genus Methanopyrus that have finite probabilities are designated branches A, B, and C. The eubacterium used was Thermotoga maritima. Abbreviations: H. volc., Halobacterium volcanii; Ms.hun., Methanospirillum hungatei; Mc. vann., Methanococcus vannielii; Archae., Archaeoglobus fulgidus.

FIG. 2. Comparison of the Methanopyrus kandleri EF-1a sequence with sequences of methanogens, halobacteria, eubacteria, eocytes, and eukaryotes. The 4- and 11-amino-acid segments are underlined. The lowercase letters represent sequences used as the PCR primers. The methanogens and their relatives which we used are shown in Fig. 3. The deduced amino acid sequence of the Methanopyrus kandleri EF-1a fragment was aligned with the sequences of the homologous fragments of eocytes, methanogens, and their relatives, halobacteria, eubacteria, and eukaryotes (Fig. 2). The Methanopyrus kandleri sequence contained the 4-amino-acid segment (GVMP) characteristic of eubacteria, halobacteria, and methanogens, and it lacked the 11-amino-acid segment found in eocyte and eukaryotic sequences (Fig. 2).

The absence of the 11-amino-acid segment in the genus Methanopyrus is most parsimoniously consistent with branches A and B on Fig. 1. On either branch A or branch B, only a single change from the 4-amino-acid segment to the 11-amino-acid segment (Fig. 1, solid rectangle) is needed to explain the observed distribution of insertions. In comparison, one additional change from the 11-amino-acid segment to the 4-amino-acid segment on branch C would be required to explain the branch C alternative. Therefore, these results strongly indicate that the genus Methanopyrus is not an eocyte genus and is less closely related to the eukaryotes. In addition, it should be noted that all of the new sequences described in this paper are consistent with and provide additional support for the eocyte tree, on which the eocytes are the closest relatives of the eukaryotes.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Organism</th>
<th>11 amino acid segment</th>
<th>4 amino acid segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanogens &amp; Relatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. kand.</td>
<td>KNMITGAGAAILLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>T. celer</td>
<td>KNMITGAGAAAVLVAATD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>P. woei</td>
<td>KNMITGAGAAAVLVAATD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>A. fulg.</td>
<td>KNMITGAGAAALLVIVVE</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>M. vanillii</td>
<td>KNMITGAGAAALLVIVVE</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>T. acid.</td>
<td>KNMITGAGAAALLVIVVE</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Halobacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. maris.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>QTEK</td>
</tr>
<tr>
<td>Eubacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th. mar.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>QTEK</td>
</tr>
<tr>
<td>D. sal.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>QTEK</td>
</tr>
<tr>
<td>E. coli</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>QTEK</td>
</tr>
<tr>
<td>Eocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. d. mar.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>P. occult.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>D. muc.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>A. inf.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>S. acid.</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Eukaryotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giardia</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Tetrahymena</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Yeast</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Tomato</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Drosophila</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Rat</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
<tr>
<td>Human</td>
<td>KNMITGAGAAALLVVAAD</td>
<td>---GVMP</td>
<td>qtreh</td>
</tr>
</tbody>
</table>

The nucleotide sequences of the methanogen Methanopyrus kandleri, Archaeoglobus fulgidus, the halophilic cyanobacterium Dactyloccocus salina, and the eocyte Thermotoga maritima are shown in Fig. 3. The deduced amino acid sequence of the Methanopyrus kandleri EF-1a fragment was aligned with the sequences of the homologous fragments of eocytes, methanogens, and their relatives, halobacteria, eubacteria, and eukaryotes (Fig. 2). The Methanopyrus kandleri sequence contained the 4-amino-acid segment (GVMP) characteristic of eubacteria, halobacteria, and methanogens, and it lacked the 11-amino-acid segment found in eocyte and eukaryotic sequences (Fig. 2).
Dactylococopsis salina, Pyrococcus kandleri, cur in eukaryotes and eocytes and 4-amino-acid segments or include the sequences of the extremely halophilic eubacterium

A) the fact that this pattern continues to be found in new se-

quences from taxa that occupy other critical phylogenetic po-

tions, such as Giardia lamblia (13), provides additional evi-

dence that the EF-1α segment is useful as a phylogenetic

marker. We concluded that the genus Methanopyrus is either a

member of the true archaea (corresponding to branch A) or a member of the nonarchaeabacterial, parapathic clade of organisms (including organisms like Thermococcus celer and Pyrococcus woesei) which are located before the node which gives rise to the eocytes and eukaryotes (branch B).

We thank Karl Stetter for providing Methanopyrus, Archaeoglobus, and Thermococcus cells and thank Anthony Walsby for providing Dactylococopsis cells.

This work was supported by a grant from the National Science Foundation to J.A.L.

REFERENCES


11. Creli, R., F. Catterlina, O. Toboni, A. M. Sanangelantoni, P. Palm, and P. Cammarano. 1991. Nucleotide sequence of a DNA region comprising the gene for elongation factor 1 from the ultrathermophilic archaeaet Pyrococ-

12. Gogarten, J. P., H. Kibak, P. Dittrich, L. Taix, B. J. Bowman, M. F. Manol-


ophila melanagaster with continuous and stage specific expression. Nucleic Acids Res. 16:3175–3194.


24. Lake, J. A. 1988. Origin of the eukaryotic nucleus determined by rate-


33. Runnegar, B. 1993. Proterozoic eukaryotes: evidence from biology and ge-


