Eocytes: A new ribosome structure indicates a kingdom with a close relationship to eukaryotes

(eocyta/eocyte gap/parsimony analysis/unrooted dendrogram/electron microscopy)

JAMES A. LAKE, ERIC HENDERSON, MELANIE OAKES, AND MICHAEL W. CLARK

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

Communicated by Everett C. Olson, March 12, 1984

ABSTRACT Ribosomal large and small subunits are organized in four general structural patterns. The four types are found in ribosomes from eubacteria, archaebacteria, eukaryotes, and a group of sulfur-dependent bacteria (eocytes), respectively. All four ribosomal types share a common structural core, but each type also has additional independent structural features. The independent features include the eucytic lobes and the archaebacterial bill on the smaller subunit. On the larger subunit, they include the eocyte lobe, eocyte gap, and eocyte bulge and a modified central protuberance. These data are most parsimoniously fit by a single unrooted evolutionary tree. In this tree eocytes are closely related to eukaryotes, while archaebacteria and eubacteria are closest neighbors. The tree is consistent with currently known molecular biological properties and indicates that eocytes have a phylogenetic importance equal to that of the three known kingdoms. When other properties and molecular mechanisms of these organisms are better defined, we suggest that an appropriate kingdom name for this group would be the Eocyte.

In recent years, understanding the evolution of organisms has increasingly been based on phylogenies determined by using fundamental molecular properties. Notably, it has been proposed on the basis of oligonucleotide cataloging of ribosomal RNA sequences that archaebacteria, eubacteria, and eukaryotes represent three very primitive lines of cellular descent (1). Numerous data on fundamental cellular properties support this proposal (for a collection of papers, see ref. 2). In particular, ribosomes from organisms within the eubacterial, archaebacterial, and eukaryotic lineages each have different three-dimensional structures (3). In this paper we report a fourth type of ribosomal structure found in the sulfur-dependent bacteria that thrive in thermal springs at temperatures above 90°C (4–6).

In the following sections, we describe these major patterns of ribosome structure in order to use the differences among them as indicators of deep evolutionary divergences. The differences between these four types of ribosomes are appropriately sensitive to delineate even the evolution of lineages (3) and are used to derive an unrooted evolutionary tree relating taxa from each group. The four ribosome structures are consistent in a most parsimonious way with only a single unrooted tree. Eukaryotes and the sulfur-dependent organisms (eocytes) are adjacent in the unrooted tree, and the archaebacteria and eubacteria are adjacent. This tree seems to be well supported by known information on other fundamental molecular properties. Based on their unique phylogenetic positions, it appears to us that the sulfur-dependent bacteria (4) consisting of the subdivisions Sulfolobales and Thermoproteales have a phylogenetic importance equal to that of the other three kingdoms. If this interpretation is confirmed by subsequent additional data for these organisms, we propose that an appropriate kingdom name for these organisms would be Eocyte (dawn + cell).

MATERIALS AND METHODS

Ribosomes and ribosomal subunits of eubacteria, archaebacteria, and eukaryotes were prepared as described (3). In addition, eocyta ribosomal subunits were resuspended in the following buffer: 200 mM NH₄Cl/5 mM Tris-HCL, pH 7.6/10 mM MgCl₂. Substitution of the buffer used for eubacterial ribosomes for use with archaebacterial ribosomes, eukaryotic ribosomes, or eocyte ribosomes produced no differences in ribosomal profiles. Subunits in these buffers were negatively stained by the double-layer carbon method. Relative sizes of eukaryotic, archaebacterial, eocyte, and eubacterial subunits were determined by electron microscopy of pairwise mixtures of subunits from the four groups.

RESULTS

Representative electron micrographs of ribosomal subunits from eubacteria, archaebacteria, eocytes, and eukaryotes are shown in the first through fourth columns, respectively,
of Fig. 1. Small subunits are illustrated in row A and large subunits are in row C. These are interpreted in diagrams below each figure (rows B and D). Large subunits are shown in a projection that is useful for comparative purposes, the "quasisymmetric" projection (7). Identification of this projection of the eocytic and eukaryotic large subunits was determined by comparison with the eubacterial structure. The small subunit profile that is shown is the "asymmetric projection" of the small subunit. This is the same projection that has been used previously to compare the three-dimensional structures of archaeabacterial, eukaryotic, and eubacterial small subunits (3).

Two fields of large subunits are shown in Fig. 2. In both fields the predominant subunit view is the quasisymmetric projection. Ribosomal large subunits from the eocyte Thermoproteus tenax are shown in Fig. 2B. Subunits in the quasisymmetric projection in this field can be recognized by the presence of an indentation, the "eocytic gap," that gives these ribosomes their characteristic shape. This gap separates a region of density at the bottom of the subunit, the "eocytic lobe," from a second region on the side of the subunit, the "eocytic bulge." For comparison, a field of large subunits from the archaeabacterium Halobacterium cutirubrum is shown in Fig. 2A. In both archaeabacteria and eubacteria, the lobe and bulge are absent, or nearly so.

The eocytic gap is indicated by arrows in micrographs of the large subunits from five eocytic ribosomes in Fig. 3B Left. In micrographs of representative eukaryotic large subunits shown in Fig. 3B Right, both the lobe and the bulge are present, and the gap between them is filled. The eocytic lobe and bulge are present in the large ribosomal subunits of both eocytes and eukaryotes but the gap is unique to eocytic taxa.

In the small subunit, the lobes at the base of the eocytic subunit are modified so that their structure, although pronounced, is intermediate between that of the archaeabacterial and the eukaryotic small subunits. Both this modification in Sulfolobus acidocaldarius small subunits and the lack of eukaryotic lobes in small subunits of eubacterial and archaeabacterial subunits have been noted previously (3). For comparison, the lobes are indicated by arrows in micrographs of five eocytic small subunits in Fig. 3A Left and in micrographs of representative eukaryotic small subunits in Fig. 3A Right. These features are summarized in Fig. 4.

DISCUSSION

Four Designs of Ribosomes Are Most Parsimoniously Fit by Only One Unrooted Dendrogram. Within each of these four types, three-dimensional ribosomal structure is relatively constant (for a discussion, see ref. 3). Hence, the variations in structure between these four groups (outlined in Table 1)

![Diagram](image-url)

Fig. 2. (A) An electron micrograph of a field of large ribosomal subunits from the archaeabacterium Halobacterium cutirubrum. (B) An electron micrograph of a field of large ribosomal subunits from the eocyte Thermoproteus tenax. The eocytic gap is indicated by arrowheads. Both micrographs are at the same magnification, indicated by the 500-Å bar.
provide a phylogenetic basis for relating their evolution. If, as the constancy of ribosome structure within lines and at the resolution limit of our images suggests (3, 9), the individual structural features of each ribosomal type arose only once, then a parsimony analysis is appropriate. Of the three possible unrooted dendrograms for connecting four taxa, only one dendrogram most parsimoniously fits the data in Table 1 (10). This tree, shown in Fig. 5, places the eukaryotes and eocytes on one side and the eubacteria and archaebacteria on the other. It is not the only possible interpretation of our data, but it is the simplest.

Data of Others Suggest Three Possible Rootings for the Tree. Structural data alone cannot root the tree; however, in combination with the oligonucleotide catalog-derived $S_{AB}$ data (12) and DNA-rRNA hybridization data (13) summarized in Fig. 6, the tree can be rooted. According to this analysis, the three right-most branches of Fig. 5 are the most likely roots. In both of the trees shown in Fig. 6 A and B, the distance between the transition organism Thermoplasma and the archaebacteria is greater than that between any two different archaebacterial taxa and less than that between Sulfolobus (an eocyte) and archaebacteria. Hence, we conclude that the branching of eubacteria is that shown in Fig. 6C. The rooted trees consistent with these constraints are the three trees in Fig. 6 D–F.

If these arguments are correct, then certain ribosomal features, such as the bill, must be primitive. The three rooted trees also imply that eocytes and eukaryotes are “older” than (Fig. 6 D and E), or at least as old as (Fig. 6F), the archaebacteria and eubacteria. It is interesting to note that all three rooted trees are consistent with the proposals (15, 16) that the ability to splice RNA is a primitive property.

Considered as one of four kingdoms, eocytes are a monophyletic group. If we attempt to include eocytes in with either the archaebacteria or the eubacteria, then the larger group defined in this way becomes paraphyletic. Consider, for example, the tree shown in Fig. 6F. If eocytes are to be lumped with archaebacteria, then both the eukaryotic and eubacterial kingdoms would be derived from the archaebacterial kingdom so that these two derived groups would have a phylogenetic status inferior to that of archaebacteria. (Similar arguments apply to the four other rooted trees.) If kingdoms are to represent monophyletic groups, it is necessary to give eocytes kingdom status. Ultimately, it is the special relationship of eocytes to eukaryotes, shown in the unrooted tree, that forms the basis for classifying eocytes in a separate kingdom.

The Phylogeny and Molecular Properties of Eocytes Indicate a New Kingdom. The close, phylogenetic relationship of eocytes to eukaryotes, when compared with archaebacterial and eubacterial features, is supported by the similarity of many molecular processes in eocytes and eukaryotes. Both the fundamental nature of these common properties and the variety of the processes involved strongly support the proposed phylogeny and classification.

Many molecular properties of eocytes are more like those of eukaryotes than they are like those of archaebacteria and

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<th>Large subunit</th>
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<tr>
<td>Lobe</td>
<td>Filled gap</td>
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<tr>
<td>Eubacteria</td>
<td>-</td>
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<tr>
<td>Archaebacteria</td>
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<tr>
<td>Eukaryotes</td>
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<tr>
<td>Eocytes</td>
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<td>Thermofilum</td>
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<td>Sulfolobus</td>
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<td>Thermoproteus</td>
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<td>Thermoplasma</td>
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Within eocytes, the size of the 50S lobe varies from large (+ + in Thermoproteus tenax) to intermediate (+ in Thermoplasma acidophilus). For this reason we regard Thermoplasma as representing a transitional organism. In archaebacteria, and to a lesser extent in eubacteria (see ref. 8), a small 50S lobe is present. Details of the structural organization of eocytes will be discussed elsewhere.
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Fig. 5. The unrooted dendrogram relating the steps in the evolution of taxa from the four lineages. This is the most parsimonious tree (11) relating the four groups. Characters listed as "++" in Table 1 are assumed to represent ordered transitions (11) from ++ to + to --. The ribosomal subunits shown at the nodes correspond to the ribosomes present in the most parsimonious interpretation.

Eubacteria. The DNA-dependent RNA polymerases of eocytes are composed of protein subunits with molecular weights and immunological properties that resemble those of eukaryotic polymerase A(I) more closely than the patterns found in archaeabacteria and eubacteria (14). Introns similar to those found in eukaryotic tRNA genes have been found in tRNA genes in the eocyte Sulfolobus (17), whereas none has yet been found in archaeabacteria or eubacteria. The secondary structures of 5S ribosomal RNA from Sulfolobus, and from Thermoplasma to a lesser degree, while related to the eukaryotic 5S pattern, differ more from the archaeabacterial and the eubacterial patterns than does even eukaryotic 5S tRNA (18). The initiation tRNAs of archaeabacteria show a closeness to eu- bacterial (for example, the sequence of the initiator tRNA of Halo- cococcus resembles those of eubacteria more than those of eucaryotes), while the sequence of Sulfolobus initiator tRNA is closer to those of eucaryotes, and that of Thermoplasma is intermediate (19). Significant amounts of long polyadenylated sequences are found in Sulfolobus RNA that are similar to those in eukaryotic mRNAs, whereas only much lower amounts (1/30th) are found in eubacteria (20).

The modes of cell division in eocytes also mimic those found in eucaryotes. Eocytes do not divide equally by septum formation as the archaeabacteria and eubacteria do, rather they divide unequally by budding, constriction, branching, and other mechanisms (4). While archaeabacteria and eucaryotes both utilize glycerolipids with ether linkages, significant differences are found between the kingdoms. The thermo- trophic membrane lipids of eocytes (Sulfolobus and Thermoplasma to a lesser extent) contain cyclopentanol C40-biphytan chains. In addition, their neutral lipids also contain branched alkyl benzenes (21). The membrane lipids of archaeabacteria and eubacteria contain neither. The lipids of eucaryotes contain a high percentage of tetrathers, in contrast with archaeabacteria, which contain relatively less or none (21). Thus, although the molecular mechanisms of the eocyte are only starting to be explored, it appears that eocytes are a group with unique cellular properties appropriate for the phylogeny and kingdom classification proposed in this paper.

We think that the reasons for considering eocytes to be a separate kingdom are as compelling as those for the choice of the other three. The evolutionary tree is supported by data on a broad range of basic molecular properties. In this tree eocytes occupy a special position with respect to the eucaryotes and, hence, as a group are likely to be extremely useful in future investigations into the origin of the eukaryotic cell. Although only a small number of eocytes have been isolated, both anaerobic and aerobic taxa are known, indicating phylogenetic diversity. Furthermore, many eocytes are capable of a novel anaerobic, purely chemolithoautotrophic metabolism utilizing H2, CO2, and elemental sulfur as a terminal electron acceptor (6). Hence, they prominently occupy niches appropriate for modern day descendants of primitive organisms. We think their crucial evolutionary position and unique molecular biological properties, when more fully explored, will support the designation of independent kingdom status.

Note Added in Proof. The unrooted evolutionary tree described in this paper differs from the alternative advanced by Woese and Fox (ref. 1; also see ref. 12). In their proposal, the earliest divisions are into the three lines consisting of the archaeabacteria, the eubacteria, and the eucaryotes. In their scheme, Sulfolobus (and the eocytes) subsequently branch from the archaeabacterial lineage. We have referred to the unrooted tree corresponding to this sequence of events (shown below) as the "archaeabacterial tree." (For the rooted version of this tree see ref. 12.) Also shown is the tree proposed in this paper, the "eocytes tree." When more extensive data become available for taxa from all four groups, we anticipate that they will permit further testing of these two alternative theories. Because evolutionary distances between taxa normally are interpreted by assuming constant rates of evolution (an assumption not likely to apply when groups as diverse as eubacteria, archaeabacteria, and eucaryotes are considered), we emphasize that it is the topology of the tree that must be tested and not the distances.
We thank J. Washizaki for expert electron microscopy and photograph assistance and R. Dickerson, D. Eisenberg, W. Fitch, W. Zillig, A. Matheson, E. Smith, and A. Klug for discussions. We thank J. William Schopf for suggesting the name eocytes. We thank W. Zillig for providing cells from eocytes, A. Matheson for providing cells and ribosomal subunits from archaebacteria and eocytes, and C. Brunk for providing cells from Tetrahymena. This work was supported by research grants from the National Science Foundation (PCM 76-14710 to J.A.L.) and the National Institute of General Medical Science (GM 24034 to J.A.L.).
