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Mapping evolution with ribosome structure: intralineage constancy and interlineage variation

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Correction. In the article "Mapping evolution with ribosome structure: Intralineage constancy and interlineage variation" by James A. Lake, Eric Henderson, Michael W. Clark, and A. T. Matheson, which appeared in number 19, October 1982, of *Proc. Natl. Acad. Sci. USA* (**79**, 5948–5952), the reproduction of the electron micrographs in Figs. 1–3 was inadequate. The figures and their legends are printed here.

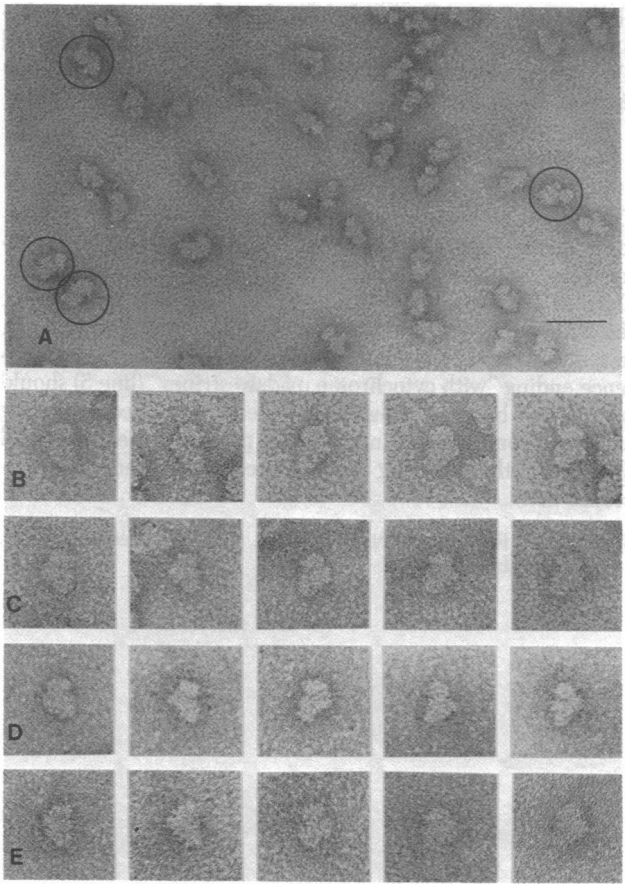

FIG. 1. Electron micrographs of small ribosomal subunits from the three principal lineages of eubacteria. (*A*) A field of *E. coli* subunits; also contains four circled (one is not circled) eukaryotic subunits for use as a control. The scale bar represents 500 Å. Rows *B–E* contain ribosomal small subunits in the asymmetric projection from the following sources: (*B*) *Thermus aquaticus*, a Gram-negative thermophilic bacterium; (*C*) *Bacillus stearothermophilus*, a Gram-positive thermophilic bacterium; (*D*) *Synechocystis 6701*, a cyanobacterium; and (*E*) *Spinacia oleracea* chloroplast, a spinach.

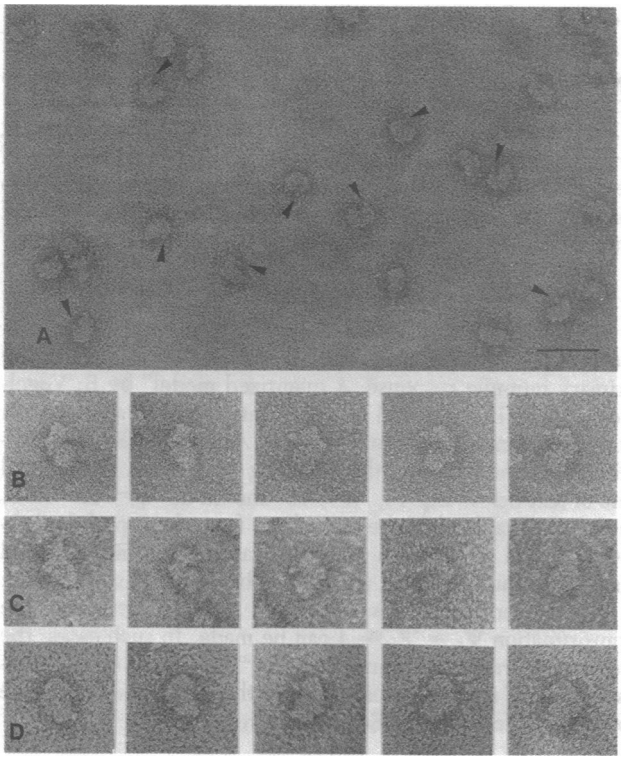

FIG. 2. Electron micrographs of small ribosomal subunits from the three lineages of archaebacteria. In the field in *A*, small subunits from *Sulfolobus* are marked with arrows to indicate the archaebacterial bill. The scale bar is 500 Å. Rows *B–D* illustrate the asymmetric projection of small subunits from the following sources: (*B*) *Methanobacterium thermoautothrophicum*, a methanogenic bacterium; (*C*) *H. cutirubrum*, an extreme halophile; and (*D*) *Sulfolobus acidocaldarius*, a thermoacidophile.

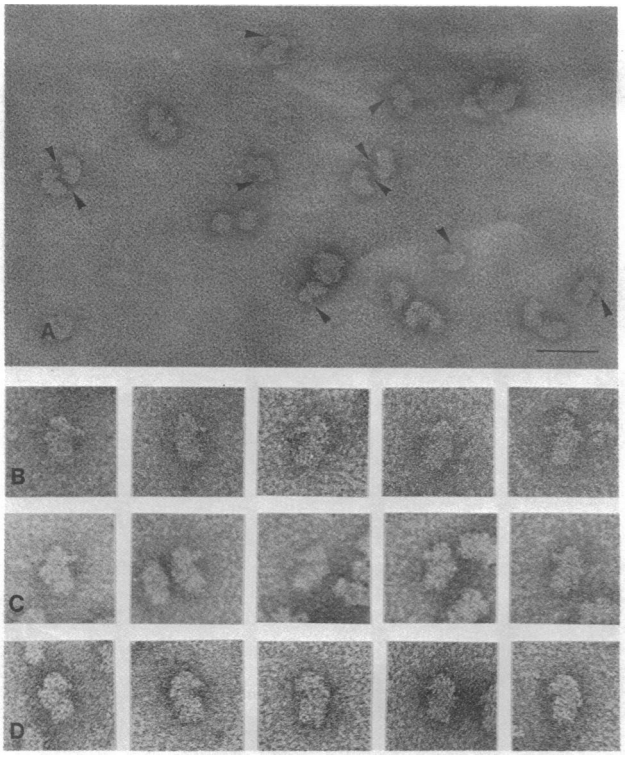

FIG. 3. Electron micrographs of small subunits of cytoplasmic ribosomes from three main divisions of eukaryotes. In the field in *A*, small subunits from *Triticum aestivum* (wheat germ) are marked with arrows to indicate the archaebacterial bill. The scale bar is 500 Å. Rows *B–D* illustrate the asymmetric projection of the small subunits from the following sources: (*B*) *Saccharomyces cerevisiae*, a yeast; (*C*) *Triticum aestivum*, a wheat; and (*D*) *Rattus rattus*, a rat.

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Mapping evolution with ribosome structure: Intralineage constancy and interlineage variation

(archaebacterial bill/eukaryotic lobes/unrooted dendrogram/endokaryotic hypothesis)

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ABSTRACT **Ribosomal small subunits are organized in three general structural patterns that correspond to the eubacterial, archaebacterial, and eukaryotic lineages. Within each of these lineages, ribosomal structure is highly conserved. Small subunits from all three lineages share a common overall structure except for the following differences: (*i*) small subunits from archaebacteria and from the cytoplasmic component of eukaryotes both contain a feature on the head of the subunit, the archaebacterial bill, that is absent in eubacteria, and (*ii*) eukaryotic small subunits contain additional regions of density at the base of the subunit, the eukaryotic lobes, that are absent in archaebacteria and in eubacteria. We interpret the intralineage conservation of ribosomal three-dimensional structure as forming a phylogenetic basis for regarding archaebacteria, eubacteria, and eukaryotes as primitive lines. Although our data are separate and independent from those of Woese and Fox, they lend further support to their proposal [Woese, C. R. & Fox, G. E. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5088–5090]. These data also provide a simple, rapid, and accurate method for classifying organisms and for identifying new lineages. Finally, interlineage variation of ribosomal structure is used to establish a rigorous framework for considering the evolution of these three lines.**

It has been proposed that archaebacteria, eubacteria, and eukaryotes represent three aboriginal lines of cellular descent (1). The phylogenetic relationships of these lines have been unclear, however. In certain aspects archaebacteria resemble eukaryotes, whereas in others they resemble the eubacteria (2–7). Phylogenetic trees based on accumulated nucleotide differences in rRNA sequences show that all three lines diverged at approximately the same time (1), but due to extensive divergence of the sequences the technique cannot determine their phylogenetic relationships. Hence a firm conclusion regarding the evolution of these three lineages is currently lacking.

Three-dimensional molecular structure has been successfully used to measure bacterial evolution within lineages (8). We propose that major reorganizations of ribosome structure, in particular, are indicators of evolutionary divergences that are sufficiently sensitive to delineate even the evolution of lineages. In the following section we show that, within each of the three lineages, the structure of the small ribosomal subunit is remarkably constant. Two independent structural features are present in only certain lineages. A region at the bottom of the small subunit, consisting of the "eukaryotic lobes", is present in only the eukaryotic lineage and a second structure located on the head of the subunit resembling a duck bill, the "archaebacterial bill," is present in only the eukaryotic and archaebacterial lineages. Aside from the presence or absence of these features, the balance of ribosome structure is conserved across lineages.

Conservation of ribosomal three-dimensional structure within each of the three lineages implies that ribosome structure has great evolutionary stability. We interpret this to be evidence that each lineage is descended from an ancestor having ribosomes representative of that line. Hence our morphological data form a phylogenetic basis [separate and independent from that of Woese and Fox (1)] for regarding archaebacteria, eubacteria, and eukaryotes as three separate lineages and lend further support to that proposal (1). Furthermore, they provide a simple, rapid method for classifying new organisms and potentially for defining new lineages. Finally, we have used the archaebacterial bill and eukaryotic lobes to assign characters to the unrooted dendrogram representing the evolution of these three lineages and have developed a framework for investigating the evolution of lineages.

MATERIALS AND METHODS

Ribosomes and ribosomal subunits of the following were prepared as described: eubacteria (9); archaebacteria (3); yeast (10); wheat germ (11); rat liver (12); and chloroplasts (13). Ribosomal subunits were resuspended in the following buffers: eubacteria and archaebacteria, except *Halobacterium cutirubrum*, 200 mM NH_4Cl/5 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$; *H. cutirubrum*, 3.0 M KCl/500 mM NH_4Cl/20 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$; and eukaryotes, 50 mM KCl/20 mM Tris·HCl, pH 7.6/5 mM $MgCl_2$. Substitution of the bacterial buffer for use with eukaryotic ribosomes (see Fig. 1*A*) or vice versa (data not shown) produced no difference in ribosomal profiles. Subunits in these buffers were negatively stained by the double-layer carbon method (14). The relative sizes of eukaryotic, archaebacterial, and eubacterial subunits were determined by electron microscopy of pair-wise mixtures of subunits (see Fig. 1*A*) from the three lineages and also from a triple mixture (data not shown).

RESULTS

Electron micrographs of small ribosomal subunits from eubacteria, from archaebacteria, and from eukaryotes (cytoplasmic ribosomes) are shown in the fields and galleries in Figs. 1, 2, and 3, respectively. Because ribosomal subunits may be randomly oriented on the carbon support film used for electron microscopy, the images vary depending upon the orientation of each subunit. The three-dimensional structure of the eubacterial subunit and, hence, the angular relationships between its characteristic projections, or images, have been determined by using antibody labels (for a review, see ref. 15). The eukaryotic structure and images corresponding to its characteristic projections have also been identified by analogy with the prominent eubacterial projections and with the eubacterial structure (12, 16–19).

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No micrographs of archaebacterial ribosomes have been previously published. However, all observed archaebacterial projections (unpublished results) correspond to the characteristic eubacterial projections. Differences between the eukaryotic, archaebacterial, and eubacterial structures are most apparent in the projection corresponding to the asymmetric (90°) projection of the eubacterial subunit (20). That projection, and its enantiomorph, is illustrated in the galleries that follow.

A field of eubacterial (*Escherichia coli*) small subunits is shown in Fig. 1*A*. Also present are several eukaryotic small subunits that have been included as size markers. They are quite apparent because of their larger size and different shape. (Four of them have been circled and a fifth is left unmarked for the reader to identify). A striking feature of small subunits from eubacteria is the constancy of the characteristic projections. For comparison, the asymmetric profiles of a Gram-negative bacterium (Fig. 1*B*), a Gram-positive bacterium (Fig. 1*C*), a cyanobacterium (Fig. 1*D*), and a chloroplast (Fig. 1*E*) are shown. These profiles, representing the three major divisions of the eubacteria (1), are nearly indistinguishable. A generalized eubacterial profile is shown schematically in Fig. 4*A*.

A field of archaebacterial small ribosomal subunits is shown in Fig. 2*A*. These subunits contain a structure that resembles a duck bill—the archaebacterial bill. The bill is not present in eubacterial ribosomes. It extends from the head of the subunit

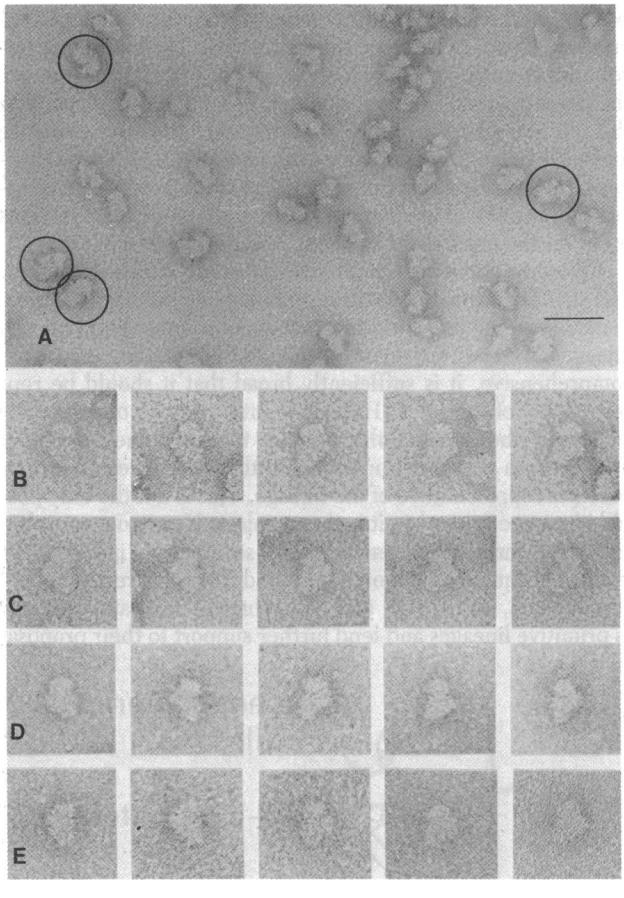

FIG. 1. Electron micrographs of small ribosomal subunits from the three principal lineages of eubacteria. (*A*) A field of *E. coli* subunits; also contains four circled (one is not circled) eukaryotic subunits for use as a control. The scale bar represents 500 Å. Rows *B–E* contain ribosomal small subunits in the asymmetric projection from the following sources: (*B*) *Thermus aquaticus*, a Gram-negative thermophilic bacterium; (*C*) *Bacillus stearothermophilus*, a Gram-positive thermophilic bacterium; (*D*) *Synechocystis 6701*, a cyanobacterium; and (*E*) *Spinachia oleracea* chloroplast, a spinach.

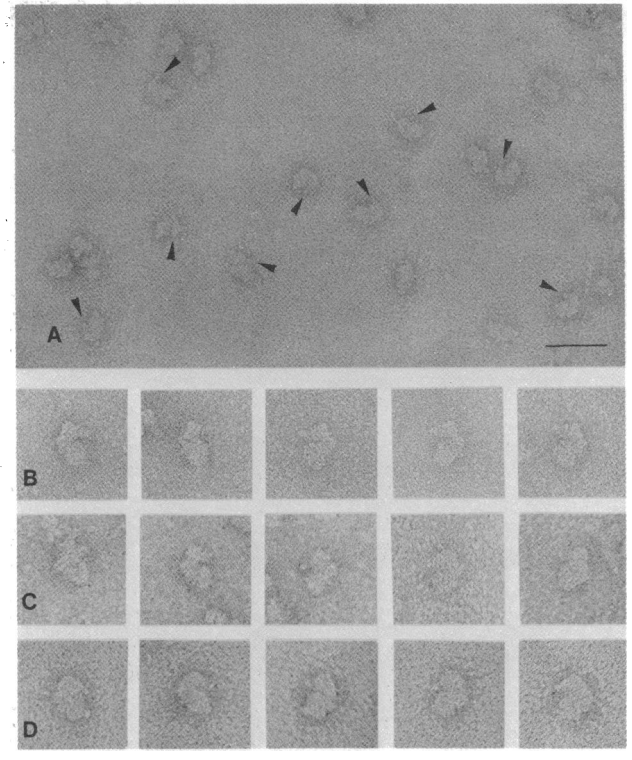

FIG. 2. Electron micrographs of small ribosomal subunits from the three lineages of archaebacteria. In the field in *A*, small subunits from *Sulfolobus* are marked with arrows to indicate the archaebacterial bill. The scale bar is 500 Å. Rows *B–D* illustrate the asymmetric projection of small subunits from the following sources: (*B*) *Methanobacterium thermoautothrophicum*, a methanogenic bacterium; (*C*) *H. cutirubrum*, an extreme halophile; and (*D*) *Sulfolobus acidocaldarius*, a thermoacidophile.

and is estimated from a comparison of its size (in several projections) with that of the L7/L12 stalk (21) to have a molecular weight (±SEM) of 44,000 ± 7,000. The maximum dimensions of archaebacterial and eubacterial subunits, as determined by micrographs of mixtures of both subunits (data not shown), are, within measurement, the same. The asymmetric profile of small subunits from a methanobacterium (Fig. 2*B*), from a halobacterium (Fig. 2*C*), and a thermo-acidophilic bacterium (Fig. 2*D*) represent the three archaebacterial lines and are quite similar.‡ Indeed, the structure of the archaebacterial small subunit (shown schematically in Fig. 4*B*) is that of the eubacteria with the addition of the bill.

A field of eukaryotic small subunits, from the cytoplasmic ribosomes of a plant, is shown in Fig. 3*A*. In the gallery that follows, subunits from three branches of the eukaryotic lineage are shown. These subunits derived from a yeast (Fig. 3*B*), a plant (Fig. 3*C*), and a mammal (Fig. 3*D*) are highly similar in organization. In addition to containing all the features of the eubacterial subunits, they also contain the archaebacterial bill and possess additional structures at the bottom of the subunit called the eukaryotic lobes (see Fig. 4*C*). Measurements of the lobes from several projections suggest they are large enough to contain (±SEM) 305 ± 20 nucleotides if they contained only RNA. These lobes are absent in both eubacteria and archaebacteria.

Thus it appears the three-dimensional structure of ribosomal subunits is nearly invariant *within* lineages. In all three lineages

‡ Ribosomal subunits from *Sulfolobus* differ slightly from the other two in having a small "bulb" present at the base of the subunit.

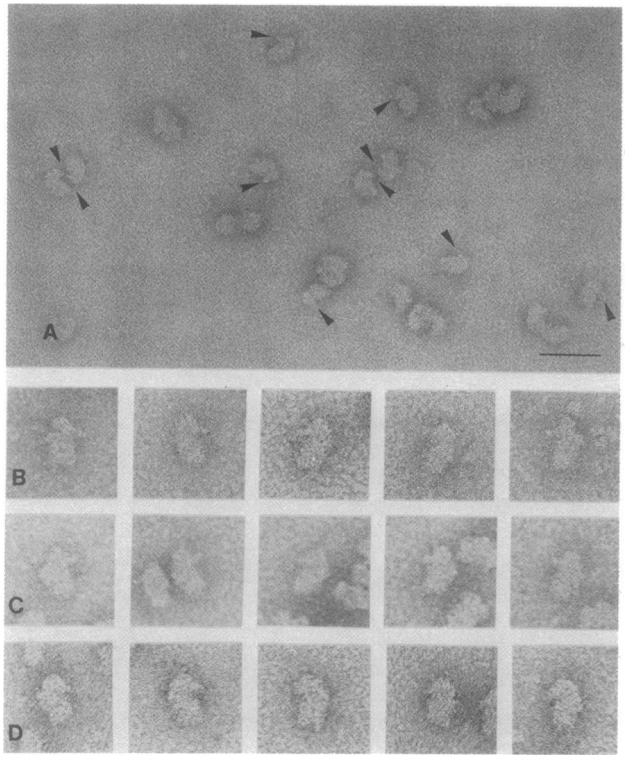

FIG. 3. Electron micrographs of small subunits of cytoplasmic ribosomes from three main divisions of eukaryotes. In the field in *A*, small subunits from *Triticum aestivum* (wheat germ) are marked with arrows to indicate the archaebacterial bill. The scale bar is 500 Å. Rows *B–D* illustrate the asymmetric projection of the small subunits from the following sources: (*B*) *Saccharomyces cerevisiae*, a yeast; (*C*) *Triticum aestivum*, a wheat; and (*D*) *Ratus ratus*, a rat.

the variations related to differences in sample preparation seen in micrographs are comparable with those that are due to species variation.

DISCUSSION

Properties of the Archaebacterial Bill and the Eukaryotic Lobes. Biochemical and structural evidence, although indirect, suggests the bill may function in the factor-related steps of protein synthesis. In small ribosomal subunits from the eubacteria *E. coli*, the site where the bill would be attached is a protein-rich region (for a review, see ref. 15). Proteins S10, S14, and S3 implicated in tRNA recognition and binding are found here (22), suggesting that the bill, too, may function in these aspects of protein synthesis. In addition, the region of the small subunit corresponding to the bill is adjacent to large subunit proteins L7/L12 (the "stalk") in monomeric ribosomes (20) and is also adjacent to the stalk in archaebacterial ribosomes (unpublished results) (see Fig. 5). This region—i.e., the region containing the bill and the L7/L12 proteins—very likely interacts with elongation factor G (23) and also with elongation factor Tu (15). In eukaryotes, the bill is also primarily composed of proteins, because several proteins have been mapped on it by immune electron microscopy (17). Hence the bill appears to be associated with ribosomal proteins involved in the binding and regulation of factors and also in the L7/L12-mediated coordination of protein synthesis.

The eukaryotic lobes are thought to be composed primarily of RNA and to contain the equivalent of ≈300 nucleotides. Three-dimensional reconstruction of the RNA and protein distributions in eukaryotic ribosomes (24) has indicated that the region of the small subunit containing the lobes is predominantly RNA. Consistent with their being composed of RNA, in *E. coli* no small subunit proteins have yet been mapped on the bottom of the small subunit either by immune electron microscopy (22) or by neutron diffraction (25). The lobes may correspond to eukaryotic "inserts" (blocks of 18S rRNA sequence that are not found in 16S sequences and do not correspond to the 16S secondary structure pattern) although direct evidence for this is lacking. The function of the lobes is not known. However, they probably do not have a significant role in the translational aspects of protein synthesis because these functions occur on the head at the opposite end of the subunit (26).

Ribosome Structure Is Nearly Constant Within Lineages. The stability of small subunit morphology within lineages is remarkable, when one considers that major subgroupings of each lineage have been surveyed. Although the sampling is far from comprehensive, it is sufficiently broad that it should be representative of the lines. Hence we interpret the observed intralineage ribosomal stability to imply that each line descended from its own common ancestor and that this ancestor had ribosomes representative of the lineage. This is not the only possible interpretation, but it is the simplest. Hence our morphological data form a phylogenetic basis—separate and independent from the sequence data of Woese and Fox—for regarding archaebacteria, eubacteria, and eukaryotes as separate lines and as primitive lineages and lend further support to their proposal

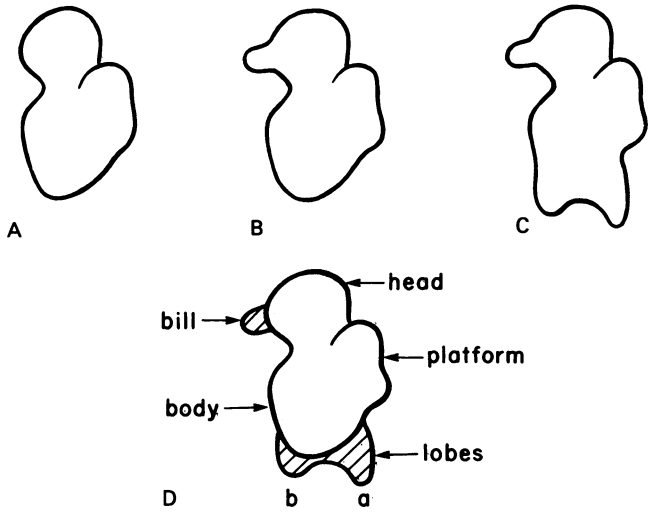

FIG. 4. Generalized profiles of small ribosomal subunits, in the asymmetric profile. The eubacterial, archaebacterial, and eukaryotic profiles are shown in *A*, *B*, and *C*, respectively. The common ribosomal regions are named in *D*, and the archaebacterial bill and eukaryotic lobes (a and b) are shown in diagonal stripes.

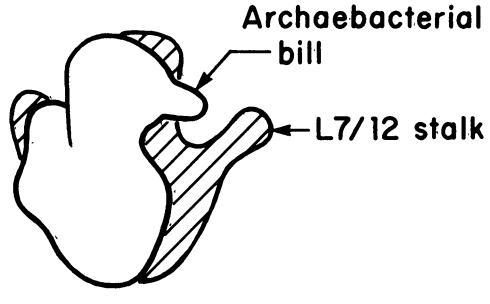

FIG. 5. Diagrammatic representation of the 70S archaebacterial ribosome showing the location of the archaebacterial bill, as inferred from the eubacterial 70S model and from preliminary results on *H. cutirubrum* 50S subunits and 70S ribosomes (unpublished results). The physical proximity of the bill and of the L7/L12 stalk is indicated.

(1). Ribosome structure, conserved as it is within lineages, provides a simple, rapid, and accurate method for classifying new organisms. In addition, new lineages, if they existed, could be detected by identifying ribosomes that do not fit the three currently recognized groups.

The "Endokaryotic Hypothesis"—The Nucleus May Represent an Engulfed Urkaryote. It may be possible, by using ribosome structure, to discover present-day, anucleate members of the eukaryotic lineage. Woese and Fox (1) proposed that a hypothetical group of organisms representing an *engulfing* species, the urkaryote, was the ancestor that contributed its ribosomes to the eukaryotic cell. We propose the opposite alternative—that the hypothetical ancestor that contributed its ribosomal subunits to the eukaryotic cell was an *engulfed* species. This proposal, which could be termed the *endokaryotic hypothesis* (27), posits that the nucleus, like the other eukaryotic organelles enclosed in double membranes (chloroplast and mitochondrion), has been derived through capture by an engulfing species. However, in the case of the nucleus the guest has taken over the host. The virtue of this proposal is that it is simple. It explains the origin of all double-membrane organelles through a single mechanism rather than requiring two separate and different mechanisms. We suggest the name urkaryote should also be applied to this organism. In both instances, the ribosomes of urkaryote-like organisms, if such exist today, are expected to be of the eukaryotic type.

Interlineage Ribosomal Alterations Suggest Steps in the Evolution of Lineages. The unusual stability of the archaebacterial bill and eukaryotic lobes over long time periods make them ideal markers to probe the creation of lineages. These two structures do not appear to have been significantly altered since lineages originated, possibly as long ago as the oldest (3.5 billion years) microfossils of bacteria (28). The topology of the formation of these lineages can be represented by a single dendrogram, or tree. This tree, with character assignments, is shown in Fig. 6. Because it is unrooted, no flow of time can be inferred. If we assume that the bill and the lobes represent unique evolutionary events—i.e., that each one was formed (or lost) only once—then there is only one character assignment for the central organism, or "paleocyte," that fulfills this requirement. That choice is shown in Fig. 6. In general, the paleocyte represents a now extinct organism that gave rise to two of the lineages. We cannot predict which two lineages, unless we root the dendrogram (for rooted dendrograms, see ref. 27).

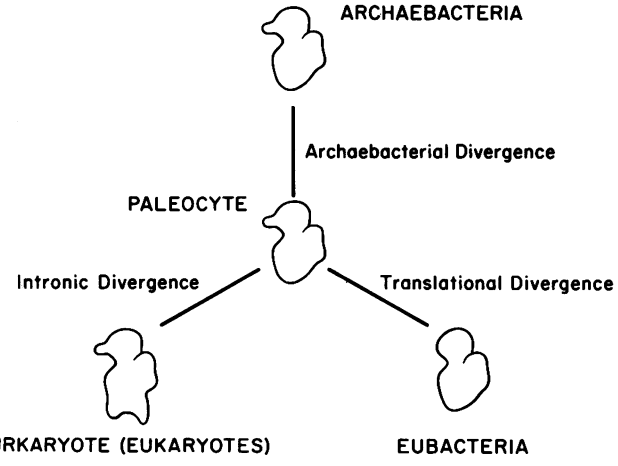

FIG. 6. Unrooted dendrogram representing the evolution of the three lineages. Ribosomal characters have been mapped on the tree. The central character, containing the bill and lacking the lobes, is the only solution that does not require multiple introduction of the bill or the lobes. Phylogenic data based on nucleotide differences (1) suggest that the divergences between any two of the three lineages are significantly deeper than they are within any single lineage. If this is true, then the characters labeled archaebacteria, eubacteria, and eukaryotes can be interpreted as corresponding to the single organisms that were ancestors to the three lineages.

Although currently available data are not adequate to distinguish between the three possible rooted trees, they nevertheless make specific predictions about the nature of the common ancestor. For example, the intronic dendrogram (corresponding to rooting the tree in the intronic arm) is the only tree that is consistent with the "genes in pieces" proposal (29, 30) because in that proposal the eukaryotic arrangement is primitive.

The unrooted tree clarifies some phylogenic relationships among the three lineages. Using the dendrogram as a guide, we interpret cellular properties present in eubacteria but absent in archaebacteria and eukaryotes (urkaryotes) as altered during the paleocyte-to-eubacteria transition. We refer to this branch of the dendrogram as the translational divergence, because during this period the archaebacterial bill was modified. Similarly, features shared by archaebacteria and eubacteria but not by urkaryotes and features shared by urkaryotes and eubacteria but not by archaebacteria evolved during the intronic and archaebacterial divergences, respectively.

The great majority of cellular rearrangements appears to have occurred during the translational and intronic divergences. The translational divergence produced major changes in translation (3, 31–33), in transcription (34), and in genome organization (6). Similarly, the intronic divergence produced major changes in rRNA organization (ref. 2; §; C. R. Woese, personal communication) and modifications in genome organization (35, 36). In contrast, few properties were altered during the archaebacterial divergence (37). Other properties, such as membranes (38), cannot be assigned until a better understanding of the urkaryote-to-eukaryote transition is obtained. Hence rather than view properties common to two of three lineages as evidence that eukaryotes evolved from archaebacteria (or some other conclusion), we see them as changes occuring during a particular divergence of the dendrogram.

In conclusion we note that our phylogenic data provide independent support for the concept of three (or possibly more) lineages. In addition, they constitute a rapid and reliable method for classifying organisms and define a criterion for identifying new lineages. Finally, we note that the archaebacterial bill and eukaryotic lobes have led to the development of a framework for studying the formation of lineages.

Noted Added in Proof. It turns out that the idea that the nucleus is derived from an engulfed organism is not a new proposal. Quite early on (1900–1910), Pfeffer, Boveri, and Mereschowsky all considered a possible symbiotic origin for the nucleus (for a discussion, see ref. 39). The most recent publication that the authors could find to reconsider this idea appeared in 1974 (40). Of these reports, none has noted the double membrane organization of the nuclear membrane and none has considered the possibility that this organization may reflect a common mechanism for the evolution of all double membrane-bounded organelles, including the nucleus.

Oligonucleotide cataloging of 16S rRNA suggests that the deepest known subgrouping within the eubacterial lineage is represented by *Chloraflexus aurantiacus* (7). Recent experiments done in collaboration with B. Pierson on the structure of *Chloroflexus* small ribosomal subunits show that they are nearly identical to the ribosomal subunits of all other eubacteria.

§ Zimmermann, R. A., Thynlow, D. L., Prince, T. L., Marsh, T. L. & Chen, J.-K. (1981) Seventh European Molecular Biology Organization Annual Symposium, Heidelberg, Federal Republic of Germany, September 28–October 2, p. 100 (abstr.).

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