seems appropriate to propose that an early branch of the primate tree may have developed the power of flight long before the hominid branch even dreamed of it.

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15. In two animals supplementation with Nembutal (pentobarbitone sodium) was used.
16. In *Pteropus* and *Macroderma* the vertical meridian was horizontally displaced from the blind spot approximately 18° and 20°, respectively. These values can be obtained from Fig. 1, given that in *Pteropus* 1 mm = 4.3° and in *Macroderma* 1 mm = 8° on the retina.
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Structure of Ribosomal Subunits of *M. vannielii*: Ribosomal Morphology as a Phylogenetic Marker

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On the basis of ribosomal morphology, it has been proposed that the sulfur-metabolizing archaebacteria constitute a group (the eocytes) with a phylogenetic importance equal to that of the eubacteria, archaebacteria, and eukaryotes. It has been further proposed that eocytes should be given kingdom status. Ribosomal subunits from the methanogenic archaebacterium *Methanococcus vannielii* were examined by electron microscopy, and their structures were compared to those of other archaebacterial, eubacterial, and eukaryotic ribosomes. 30*S* subunits from *M. vannielii* showed the elongated contour and the one-third to two-thirds partition characteristic of such subunits. In addition, the angled asymmetric projections of those subunits showed a squarish base and a beak on the head. 50*S* subunits from *M. vannielii* were seen in both crown and kidney views. In crown views, the L1 protuberance was frequently pronounced and split; an incision below this protuberance and a protrusion at the base of the particle were also observed. Although previous studies suggested that certain of these structural features were found exclusively in ribosomes from sulfur-metabolizing archaebacteria, these new results indicate that such features also occur in ribosomes from a typical methanogenic archaebacterium and thus may not be reliable phylogenetic markers.

IT HAS BEEN PROPOSED THAT ANALYSIS of the structure of ribosomal subunits by electron microscopy is a rapid and accurate method for the classification of organisms (*1*). Five different ribosome structures have been described and have been assigned to four independent evolutionary lineages (*2–4*). We present here electron micrographs of ribosomal subunits from *Methanococcus vannielii* that are inconsistent with the assignment of one of these lineages (*2*).

The ribosomes used for this study were prepared as described (*5, 6*), and their activities in poly(U)-dependent polyphenylalanine synthesis were assessed (*6*). The sub-

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units could be reassociated to form active 70*S* particles.

Electron micrographs of 50*S* subunits from *M. vannielii* are shown in Fig. 1. Of the several hundred particles evaluated, 10 percent were seen in the kidney view (Fig. 1c), and 90 percent in the crown view (Fig. 1, b and d through g). Many crown views of the 50*S* subunits show, however, structural details that differ from those seen in 50*S* subunits from *Escherichia coli*. In 30 to 50 percent of these views, the L1 protuberance is larger than that found in 50*S* subunits from *E. coli*, revealing a clear incision between this protuberance and the body of the subunit (Fig. 1d). More than 25 percent of the 50*S* particles from *M. vannielii* have a small protrusion at the base of the particle on the same side as the L1 protuberance (Fig. 1f); occasionally, a splitting of the L1 protuberance can be seen (Fig. 1e). Some of the particles show several of these structural features simultaneously (Fig. 1g).

Lake and co-workers (*2, 3*) observed similar structural details in 50*S* subunits from *Sulfolobus acidocaldarius*, *Thermoproteus tenax*, *Desulfurococcus mucosus*, *Thermococcus celer*, and *Thermophilum pendens*. They referred to the enlargement of the L1 protuberance as the "eocytic bulge," to the incision below this protuberance as the "eocytic gap," and to the protrusion at the base of the 50*S* particle as the "eocytic lobe" (*3*). These features of the ultrastructural morphology of 50*S* ribosomal subunits were the principal phylogenetic markers used by Lake *et al.* (*2*) to distinguish the sulfur-metabolizing archaebacteria (the "eocytes") from other archaebacteria, eubacteria, and eukaryotes. It was proposed on this basis that the eocytes constitute a new phylogenetic kingdom (*2*). The observation of the same structural features in 50*S* ribosomal subunits from *M. vannielii*, a typical methanogenic archaebacterium, contradicts this proposal.

Small ribosomal subunits from *M. vannielii*, *Sulfolobus solfataricus*, and *Halobacterium marismortui* have also been studied (*7*). The images observed in electron micrographs of small subunits from *M. vannielii* (Fig. 2) correspond to the three characteristic projections that have been described for 30*S* preparations from *E. coli* (*8*). Of the 400 particles that were evaluated, more than 70

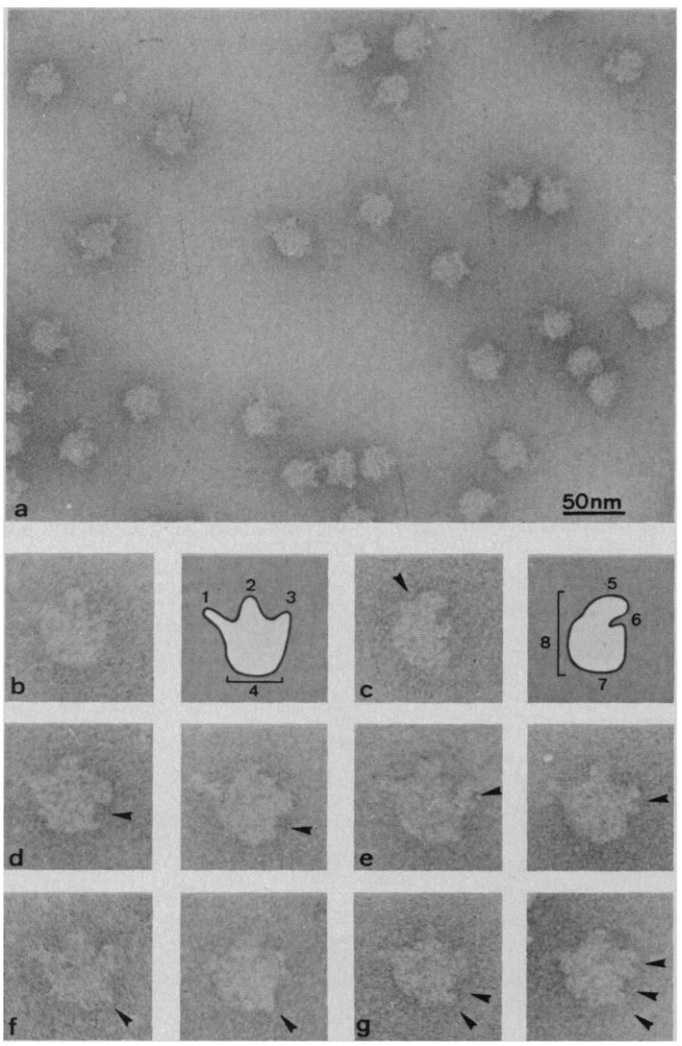

Fig. 1 (left). Electron micrographs of 50*S* subunits of *M. vannielii*, negatively contrasted with uranyl formate by the double layer carbon technique (*17*). (a) General view. (b through g) Selected images showing the particle in (b and d through g) the crown view and (c) the kidney view. Arrows indicate characteristic structural features. The schematic drawings of the two characteristic views identify typical structural features: 1, rodlike appendage or stalk; 2, central protuberance; 3, L1-protuberance; 4, base; 5, pointed end; 6, notch; 7, blunted end; 8, back.

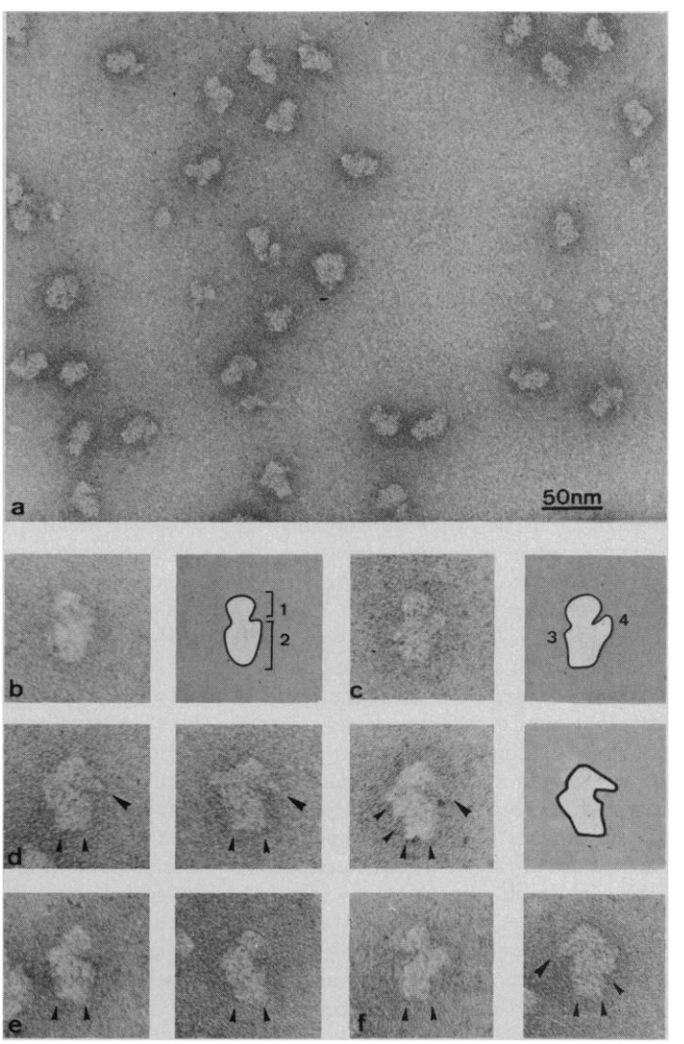

Fig. 2 (right). Electron micrographs of 30*S* subunits from *M. vannielii*. (a) General field. (b through f) Selected images showing the 30*S* particle in (b) the quasi-symmetric projection, (c) the cloven asymmetric projection, and (d through f) the angled asymmetric projection. The schematic drawings identify typical structural features: 1, head; 2, body; 3, small lobe; 4, large lobe.

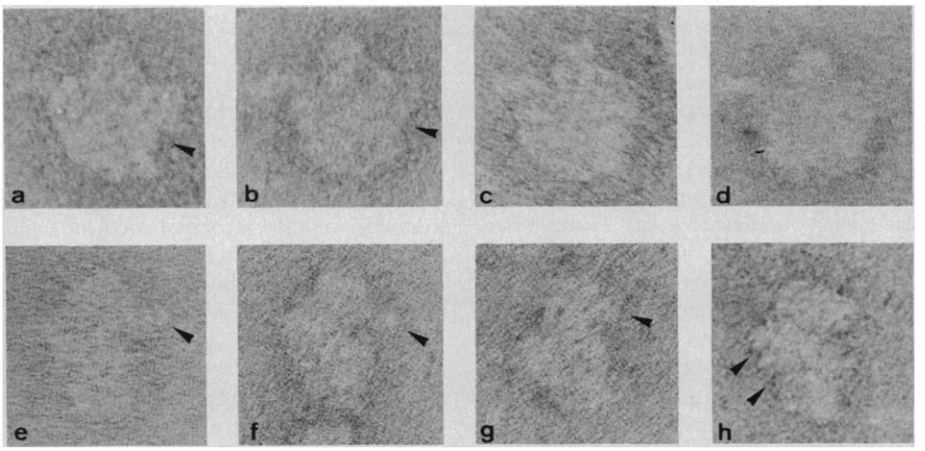

Fig. 3. Electron micrographs of crown views of 50*S* subunits from (a and c) *M. vannielii* and (b and d) *E. coli*, and electron micrographs of the angled asymmetric projection of the 30*S* subunit from (e) *M. vannielii*, (f and h) *B. stearothermophilus*, and (g) *E. coli*.

percent were seen in the angled asymmetric or lateral view (Fig. 2, d through f), with the large lobe oriented predominantly to the left (Fig. 2, d and e). Approximately 10 percent were seen in the quasi-symmetric view (Fig. 2b), and 10 percent in the cloven asymmetric view (Fig. 2c). The remaining images of 30*S* subunits did not conform to these three projections.

The quasi-symmetric and the cloven asymmetric projections of small subunits from *M. vannielii* are similar to those found in small subunits from *E. coli*; however, images of small subunits from *M. vannielii* show considerable variation in their gross features (for example, the absolute length of the particle and the relative proportions of head and body). The angled asymmetric projections of *M. vannielii* subunits are more uniform in their appearance, and they clearly differ from corresponding projections of subunits from *E. coli* (*8*). The characteristic features seen in these projections are the beaklike structure on the head and the squarish base, which make these projections look similar to lateral views of small ribosomal subunits from eukaryotes (*9*). A squarish base in the angled asymmetric projection of the small subunit (called the "intermediate lobes") was another specific eocytic feature of ribosome structure cited by Lake *et al.* [table 1 in (*2*)]. However, in a subsequent paper [figure 2 in (*4*)] Lake and co-workers observed that "intermediate lobes" also occur in small ribosomal subunits from methanogenic archaebacteria; in the same paper they proposed that halophilic archaebacteria and eubacteria constitute another new kingdom, the "photocytes."

Other features of the ultrastructural morphology of the small ribosomal subunit proposed by Lake *et al.* (*4*) to be characteristic for both sulfur-metabolizing and methanogenic archaebacteria were a bifurcation of the large lobe and an incision below it. We find that approximately only half of the 30*S* subunits from *M. vannielii* exhibit these features (*10*).

Thus, the structural details of 30*S* and 50*S* subunits from *M. vannielii* show considerable variation. In addition to the characteristics of the 50*S* subunit described by Lake *et al.* [the "eocytic" gap, bulge, and lobe (*3*)], we also observed a small population (approximately 15 percent) of 50*S* subunits that were similar to 50*S* subunits from *E. coli*. Further, not all the angled asymmetric projections of the 30*S* subunit showed a beaklike structure on the head (Fig. 2e). To evaluate electron microscopic images of ribosomal subunits, one needs information about the frequency of occurrence of observed structural details. Such information can be obtained by computer-assisted image analysis (*11, 12*).

Clearly, ribosomal subunits from *M. vannielii* and sulfur-metabolizing archaebacteria have structural features in common (*7*), but most of these structural details have also been observed in ribosomes from the eubacteria *E. coli* and *Bacillus stearothermophilus*, at least in a rudimentary form. For example, 50*S* subunits from *E. coli* show a vestigial bulge and a gap (Fig. 3b) (*13*). Also, in 30*S* subunits from *E. coli* and *B. stearothermophilus*, a beaklike structure has been observed. In the latter organism, the beak is occasionally pronounced (Fig. 3f); whereas, in *E. coli*, it is considerably shorter than that seen in archaebacteria and eukaryotes (Fig. 3g). A bifurcation of the large lobe (platform) and a gap at the base of this structure is clearly seen in small ribosomal subunits from *B. stearothermophilus* (Fig. 3h). The presence of these structures in eubacteria has been confirmed by computer-assisted image analysis (*11*). Thus, they may be general features of all small ribosomal subunits.

Structural details that are more pronounced in ribosomal subunits from archaebacteria than in those from eubacteria (the "archaebacterial" bill and the "eocytic" gap, bulge, and lobe) might result from the higher number of proteins present in archaebacterial ribosomes or from specific features of archaebacterial ribosomal RNA's (*5, 14, 15*). It is also possible, however, that the presence of these features in images of ribosomal subunits from archaebacteria is due to differences in adsorption behavior or in the stability of the ribosomal subunits prepared from different organisms. Such structural details could even be artifacts; for example, they could be due to variations of the *p*H or ionic strength of the buffers used during ribosome isolation and specimen preparation.

The archaebacteria have been grouped into a separate kingdom on the basis of a number of different characteristics, all of which are defined in molecular terms (*16*). Our results suggest that structural details of ribosomal subunits that are seen at the limit of resolution of electron microscopy and that are not understood at the molecular level should not be used as a basis for proposing new kingdoms or phyla.

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