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G.E. Fox: INSIGHTS INTO THE PHYLOGENETIC POSITIONS OF  
PHOTOSYNTHETIC BACTERIA OBTAINED FROM 5S rRNA AND 16S rRNA SEQUENCE  
DATA

Comparisons of complete 16S ribosomal ribonucleic acid (rRNA) sequences have established that the secondary structure of these molecules is highly conserved. Earlier work with 5S rRNA secondary structure revealed that when structural conservation exists the alignment of sequences is straightforward. Furthermore the constancy of structure implies minimal functional change. Under these conditions a reasonably uniform evolutionary rate can be expected so that conditions are favorable for phylogenetic tree construction.

The sequences of all the 16S ribosomal RNA oligonucleotides produced by complete digestion with ribonuclease T1 allows the construction of rRNA "catalogs" from a large variety of organisms. Binary comparisons of these catalogs are used to generate a matrix of association coefficients ( $S_{AB}$  values) from which dendrograms, thought to be partial phylogenies, can be constructed. Complete 5S rRNA sequences exist for many of the same organisms such that separate dendrograms can be made from 5S rRNA data.

The major finding of these studies is that three well-separated evolutionary lines of descent can be detected which are at present represented by eukaryotes, archaebacteria, and eubacteria (Fig. I-7). Presumably, all three lines of descent have diverged from the same ancestral bacterial populations. This branching may have occurred, however, before the evolution of the genetic apparatus was complete. These three lineages differ in morphology of the small ribosomal subunit as well (Fig. I-8).

Within the two prokaryotic lines of descent at least twelve major groups are detected; two archaebacterial and ten eubacterial. Five of the eubacterial groups contain photosynthetic organisms so that it is apparent that the various types of photosynthesis are polyphyletic (Fig. I-9). Only seven species of cyanobacteria are represented but even so it is clear that this cyanobacterial lineage includes both *Prochloron* and the rhodoplast of *Porphyridium*. The plant and *Euglena* chloroplasts share a common ancestry with those seven cyanobacteria as they are currently represented on these trees. Together then the cyanobacteria and chloroplasts define a single major group of photosynthetic eubacteria. The photosynthetic flexibacterium *Chloroflexus aurantiacus* and the Chlorobiaceae apparently represent unrelated groups. The recently discovered *Heliothrix* belongs to the Gram positive bacteria and for the first time brings photosynthesis to that order. The nonsulfur

purple photosynthetic bacteria cluster exclusively into the fifth group with many non-photosynthetic respiring bacteria (Fig. I-10).

The three best-characterized branches of the purple bacteria are referred to as the alpha, beta, and gamma subgroups. The majority of the Rhodospirillaceae contain lamellar or vesicular intercytoplasmic membranes and are in the alpha subgroup where they can be further subdivided according to criteria such as helical form, asymmetric cell division, and the presence of a split 23S rRNA. Each of the subgroups contains non-photosynthetic genera, suggesting that photosynthesis has been gained and/or lost several times during bacterial evolution. The Rhodospirillaceae with tubular intracytoplasmic membranes are similarly interspersed among non-photosynthetic organisms in the "beta" subgroup (Fig. I-10). The Chromatiaceae, including members of the genera *Chromatium*, *Thiocapsa*, and *Ectothiorhodospira* are localized in the "gamma" subgroup.

The purple group is of special interest because several of its members have been suggested as being involved in endosymbiotic relationships which ultimately gave rise to eukaryotic mitochondria. 5S rRNA sequence has already given strong support for the origin of plant mitochondria from the alpha subgroup. Mitochondria are, however, probably of polyphyletic origin, as they can be readily divided between those with tubular cristae and those with flattened (lamellar or vesicular) cristae. Since the alpha and beta subgroups of the purple bacteria can be distinguished by the same criteria, it seems likely that each subgroup may have separately given rise to some eukaryotic mitochondria. In the future it may be possible to localize mitochondrial origins in these two subgroups with even greater precision.

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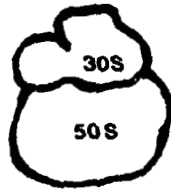
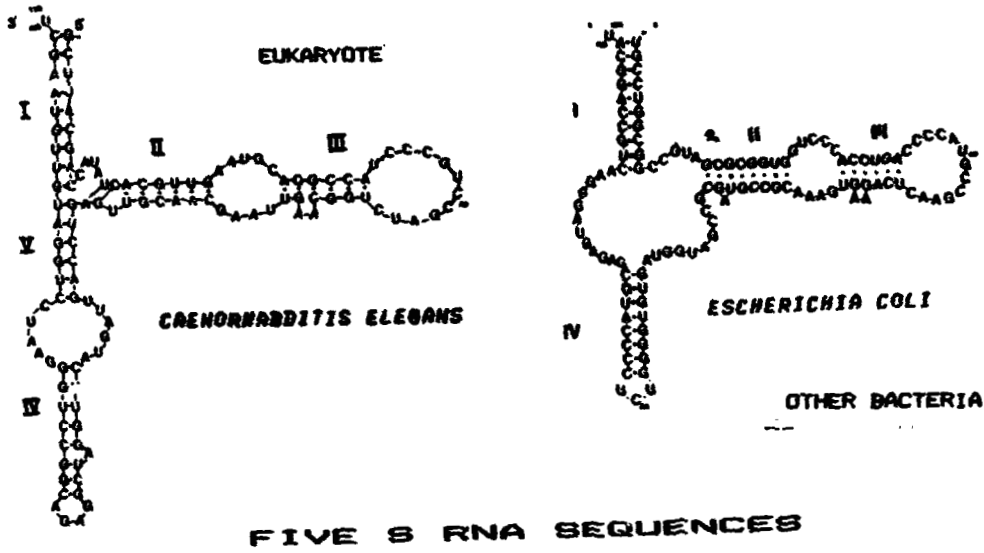
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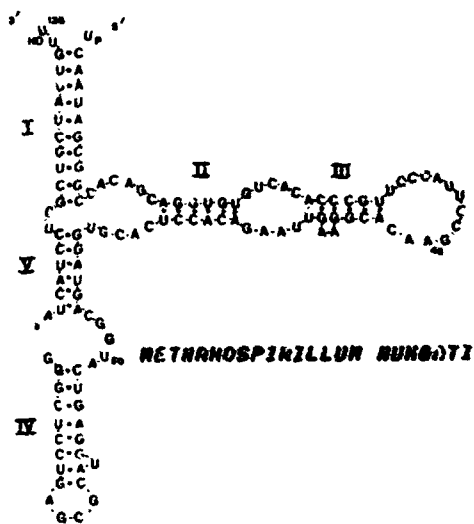
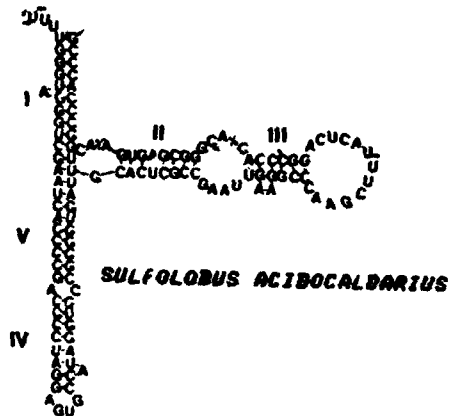
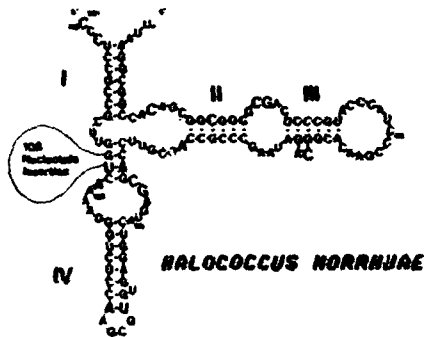
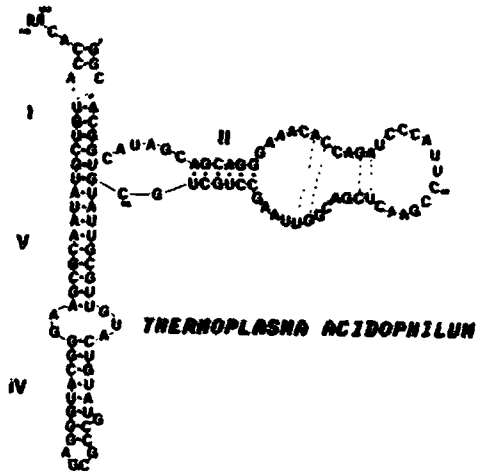
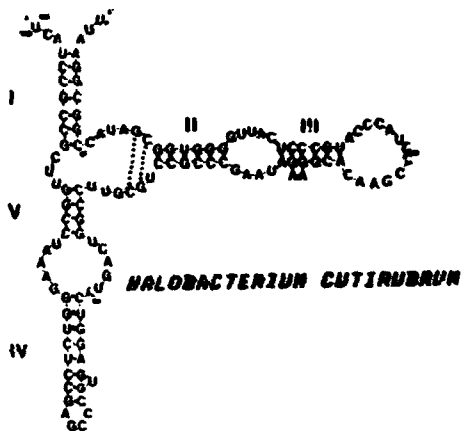


**COMPOSITION OF BACTERIAL RIBOSOMES**

Subunits	Type of molecules	Number of molecules	Size of subunits
50S	23S rRNA	1	2900 nucleotides 120 nucleotides different proteins (L1-L22)
	5S rRNA	1	
	proteins	34	
30S	16S rRNA	1	1540 nucleotides different proteins (S1-S22)
	proteins	22	

**Figure 1-7. Comparison of ribosomal macromolecules.** Five S ribosomal RNA sequences showing structures of (left) a nematode (*C. elegans*) and *E. coli*, a eubacterium. A summary of the structure and macromolecular composition of bacterial ribosomes is shown at the bottom. (Right) Five S RNA from five "archaeobacteria." Note the 108 nucleotide insertion in *Halococcus*, nearly the size of the rest of the 5S rRNA molecule.

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**ARCHAEBACTERIA**

**FIVE S RNA SEQUENCES**

**Table I-4.**  
**ARCHAEBACTERIA**

**Defining Criteria of Archaeobacterial Prokaryotes**

1. Major lipids are ether linked with phytanyl side chains (C<sub>20</sub>). In other bacteria and eukaryotes major lipids are ester linked.
2. Based on 16S ribosomal RNA oligonucleotide catalogues these organisms are more closely related to each other than they are to eukaryotic or other bacterial 16S RNAs. (16S rRNA from small ribosomal subunits is about 1540 nucleotides long).
3. Based on the sequence of nucleotides in 5S ribosomal RNA these organisms are more closely related to each other than they are to eukaryotic or other bacterial 5S RNAs. (5S rRNA from large ribosomal subunit is about 120 nucleotides long, Fig. I-7).
4. Archaeobacteria lack peptidoglycan in their cell walls.
5. A single DNA dependent-RNA polymerase with complex subunit structure (more than 6 subunits) is present in archaeobacteria.
6. Archaeobacteria ribosomes have a distinctive shape. (Fig. I-8). (Lake, 1983.)
7. Archaeobacteria include:

All methanogenic bacteria

Halophilic and thermoacidophilic bacteria:

*Sulfolobus*

*Halococcus*

*Thermoproteus*

*Halobacterium*

*Desulfurococcus*

*Thermoplasma acidophilum*

**Table I-4.**  
continued

Ranges of  $S_{ab}$  values: 16S ribosomal RNA

The lower limit of the range of  $S_{ab}$  values from oligonucleotide catalogues amongst the archaeobacteria are 0.18 to 0.24, amongst the eubacteria 0.17 - 0.22. The data for the chloroplasts (0.28 - 0.32 with cyanobacteria) and rhodoplasts (0.40 - 0.43 with cyanobacteria) are included. The  $S_{ab}$  values between the archaeobacteria and eubacteria are between 0.06 to 0.10, between the eukaryotes and archaeobacteria 0.07-0.10, and between the eukaryotes and eubacteria 0.06 - 0.10. Catalogues are available for about 400 genera.

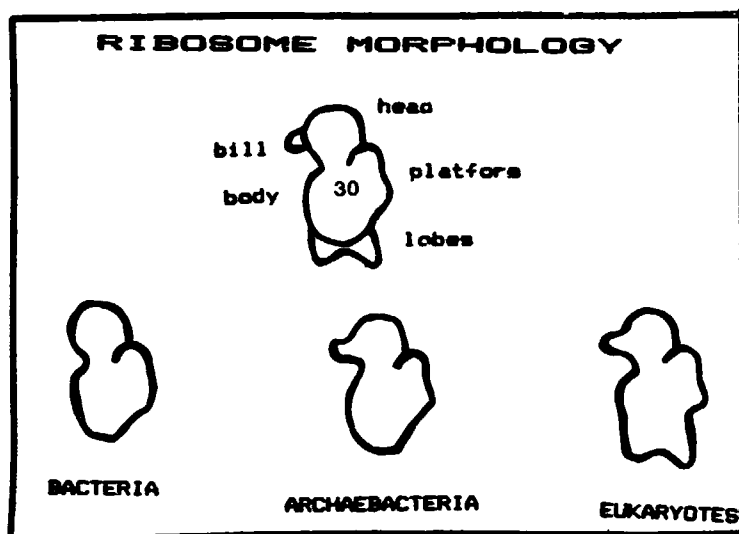


Figure I-8.

**SMALL  
RIBOSOMAL  
SUBUNITS**

Comparative  
morphology of  
the 30S ribosomal  
subunit.

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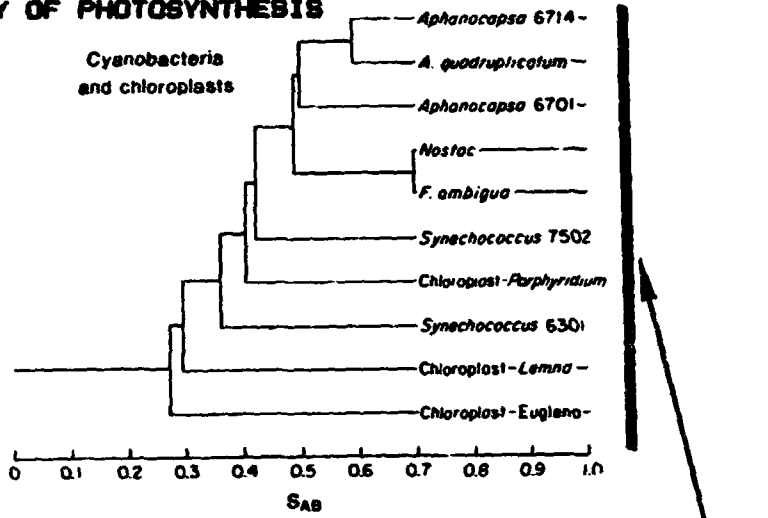
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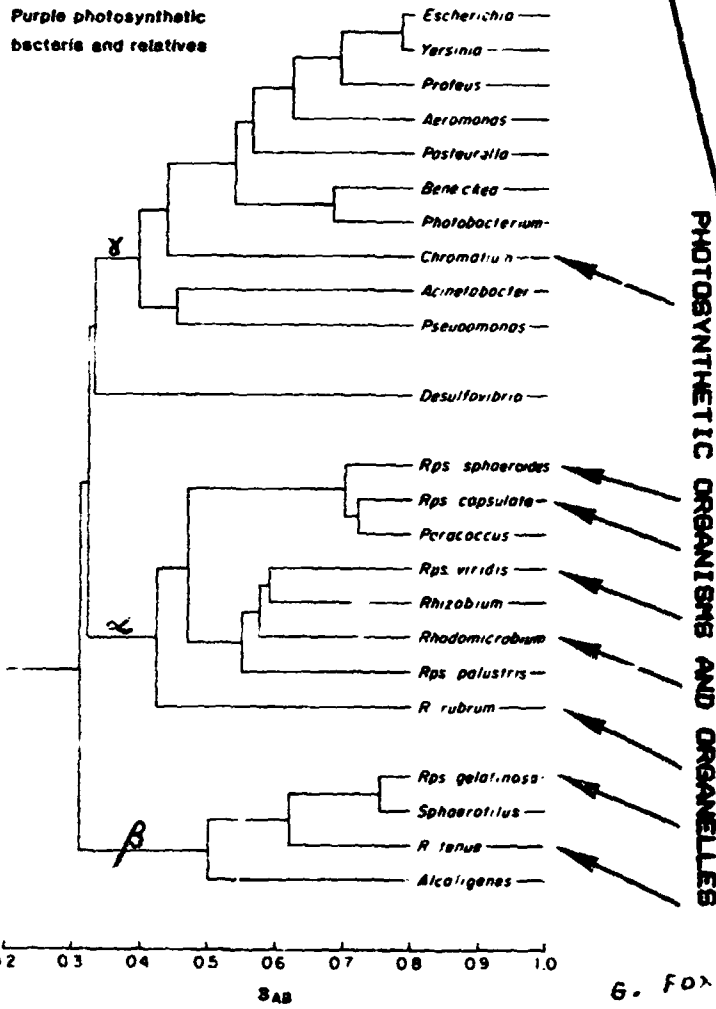
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**POLYPHYLY OF PHOTOSYNTHESIS**



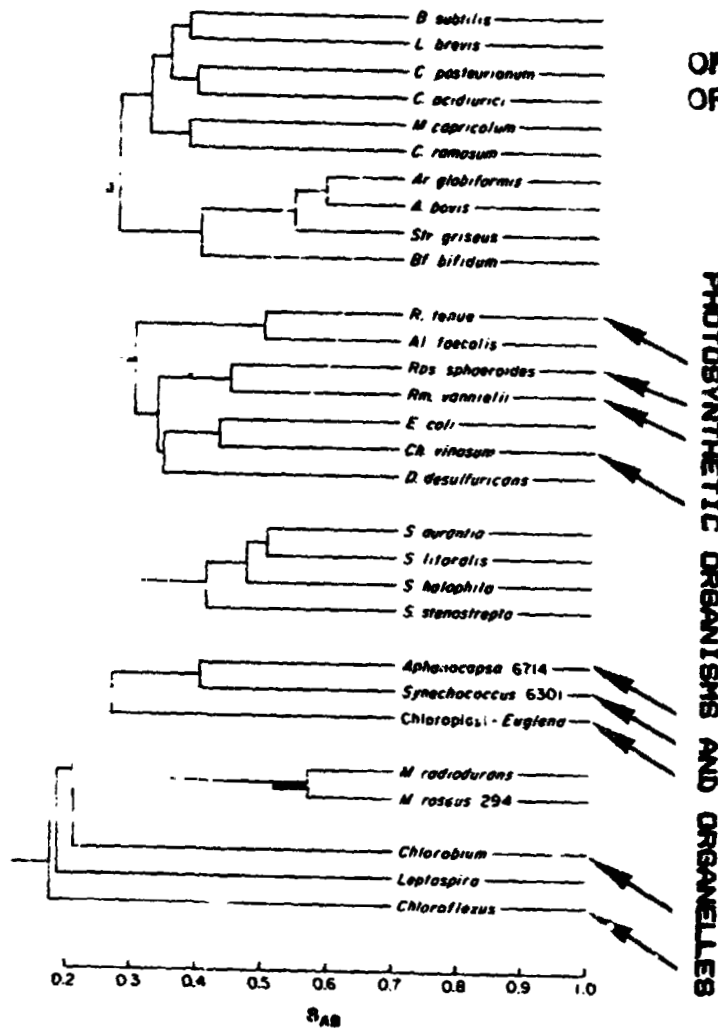
**Purple photosynthetic bacteria and relatives**



Similarity coefficients based on 16S ribosomal RNA oligonucleotide catalogues



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Similarity coefficients based on 16S ribosomal RNA  
oligonucleotide catalogues

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POLYPHYLY OF PHOTOSYNTHESIS

Figure I-9. Partial phylogenies based on 16S RNA comparative data suggest that photosynthesis evolved separately in different bacterial lineages. It probably evolved at least three times (green photosynthetic bacteria, e.g., *Chlorobium* (left), purple photosynthetic bacteria (left and right) and cyanobacteria/plastids (left and right). The relationship between certain respiring and their presumed photosynthetic relatives is shown in more detail at right.

