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Evolution

Eubacteria, halobacteria, and the origin of photosynthesis: The photocytes

(evolution/ribosome structure/parsimony/eocytes/eukaryotes)

JAMES A. LAKE*, MICHAEL W. CLARK*, ERIC HENDERSON*, SHAWN P. FAY*, MELANIE OAKES*, ANDREW SCHEINMAN*, J. P. THORNBER*, AND R. A. MAH†

*Molecular Biology Institute and Department of Biology, and †School of Public Health, University of California, Los Angeles, CA 90024

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ABSTRACT **The halobacteria and the photosynthetic members of the eubacteria have previously been classified in two separate urkingdoms—the archaebacteria and the eubacteria, respectively. They were thought to be no more closely related to each other than they each were to the eukaryotes. In accord with this earlier classification, photosynthesis was thought to have originated twice by independent events—once within the eubacteria and once within the archaebacteria. In this paper, however, using three-dimensional ribosome structure as a probe of evolutionary divergences, we show that the eubacteria and the halobacteria are more closely related to each other than they are to any other known organisms. The simplest interpretation of our data is that all extant photosynthetic cells are descended from a single common ancestor that possessed a primeval photosynthetic mechanism. Numerous data on the occurrence of related biochemical processes in halobacteria and eubacteria support this theory. Essential components of the photosynthetic apparatus, such as carotenoids, are present in both halobacteria and in eubacteria, including the nonphotosynthetic eubacteria, suggesting that photosynthesis could be a primitive property of both groups. Our data indicate that together the eubacteria and the halobacteria form a monophyletic group for which we propose the name "photocytes." If other techniques of phylogenetic analysis confirm this evolutionary tree, we propose that the photocytes be given urkingdom status.**

Photosynthetic bacteria have previously been classified in two urkingdoms. The halobacteria are classified as archaebacteria (1), whereas all other groups of photosynthetic bacteria are classified as eubacteria (2). As a result, it has been thought that photosynthesis was invented twice, once by archaebacteria and once by eubacteria. In this paper, we present evidence that photosynthesis, as exemplified by extant photosynthetic bacteria, could have been invented once.

Recently, techniques have been developed to use three-dimensional ribosome structure to probe evolutionary divergences and most parsimoniously to determine uprooted evolutionary trees (3–5). In particular, it has been shown that a rift, even deeper than that between the eubacteria and the archaebacteria, separates the eocytes (a group of sulfur metabolizing bacteria) from the other bacteria (5). This prompted us to investigate the details of the relationship between the methanogenic and halophilic branches of the archaebacteria and to analyze their specific relationships with eubacteria.

In this paper, we present data showing that the eubacteria and the halobacteria are more closely related to each other than they are to any other known organisms. We interpret this to imply that both could be derived from a common photosynthetic ancestor corresponding to a single invention of photosynthesis. This interpretation is supported by data on the common denominators of photosynthesis, such as the occurrence of identical types of carotenoids in both groups and their syntheses by essentially identical mechanisms (for reviews, see refs. 6 and 7). If this interpretation is correct, then all halobacteria and all eubacteria, including nonphotosynthetic eubacteria, could be descendants of the same photosynthetic ancestor.

Evolutionarily, the eubacteria and halobacteria compose a monophyletic group, for which we propose the name "photocytes." As a group, the photocytes are more closely related to each other than they are to members of any other urkingdom including the remaining archaebacteria (methanogens), the eukaryotes, or the eocytes. Hence, we propose that halobacteria should be removed from the archaebacterial urkingdom and that urkingdom status be given to the combined photosynthetic group, the "photocyta," provided that other studies on their molecular properties confirm the proposed evolutionary tree.

MATERIALS AND METHODS

Ribosomes and ribosomal subunits from eubacteria, archaebacteria, eocytes, and eukaryotes were prepared as described (3, 5). Substitution of the buffer used to isolate halobacterial ribosomes for that used to isolate eubacterial 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, eocytic, and eubacterial subunits were determined by electron microscopy of pair-wise mixtures of subunits from the groups.

RESULTS

Electron micrographs of ribosomal subunits from representative photosynthetic and nonphotosynthetic eubacteria and from halobacteria are shown in Fig. 1. Small subunits are shown in the first two columns and large subunits are shown in the last two columns. These are interpreted in diagrams below the columns from each group. The small subunits are shown in the "asymmetric projection" (8) and large subunits are shown in the "quasi-symmetric projection" (8). Both projections are particularly useful for comparative purposes and have been used previously to compare the three-dimensional structures of archaebacterial, eubacterial, eocytic, and eukaryotic subunits (5, 9).

The representative photosynthetic eubacteria include a green nonsulfur bacterium (row A), a purple sulfur bacterium (row B), a purple nonsulfur bacterium (row C), and a cyanobacterium (row D). For comparison, micrographs of a nonphotosynthetic Gram-positive bacterium are also included (row E). Ribosomes from *Halobacterium cutirubrum* and from *Halococcus morrhuae* are shown in rows F and G, respectively. The structures of the halobacterial ribosomal

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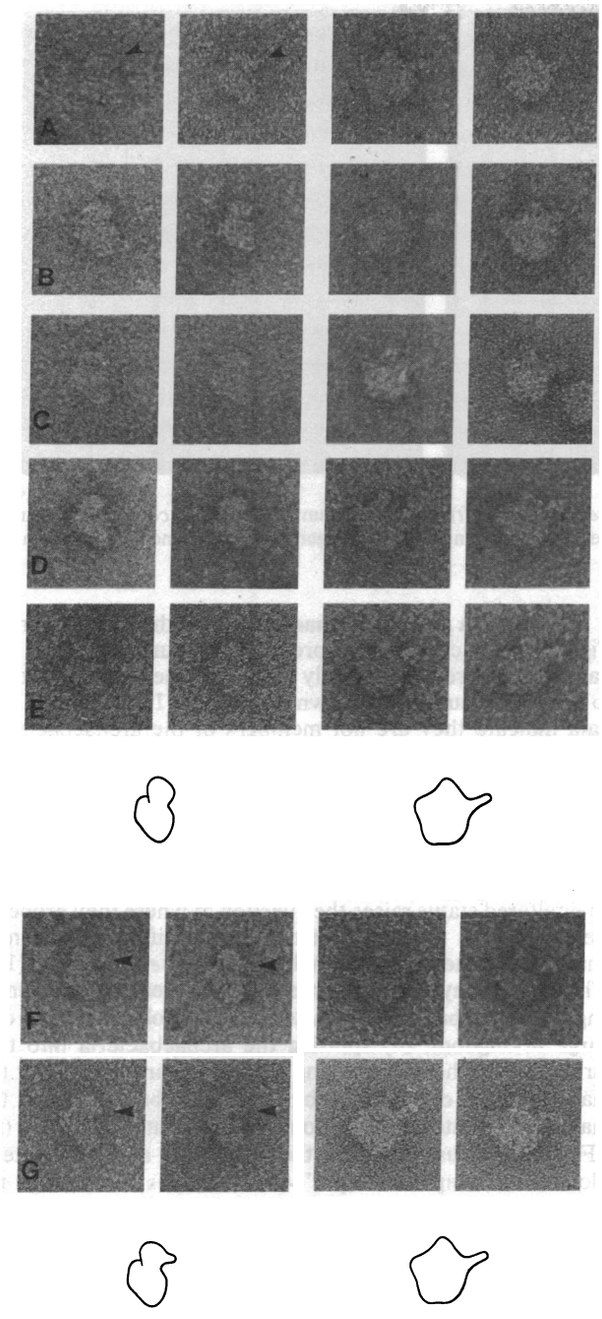

FIG. 1. Electron micrographs of eubacterial (rows A–E) and halobacterial (rows F and G) ribosomal subunits. Small subunits in the asymmetric projection are shown in the first two columns, and large subunits in the quasi-symmetric projection (the L7/L12 stalk is to the right of the subunit) are shown in the third and fourth columns. The archaebacterial bills are indicated by arrows in rows F and G. The organisms represented are *Chloroflexus aurantiacus* (a green nonsulfur bacterium, row A), *Thiocapsa pfennigii* (a purple sulfur bacterium, row B), *Rhodopseudomonas viridis* (a purple nonsulfur bacterium, row C), *Synechocystis 6701* (a cyanobacterium, row D), and *Bacillus stearothermophilus* (a nonphotosynthetic Gram-positive thermophilic bacterium, row E). The halobacteria are *Halobacterium cutirubrum* (a halobacterium, row F) and *Halococcus morrhuae* (a halococcus, row G). (×225,000.)

subunits are nearly indistinguishable from those of the eubacteria (9), except that the small subunit of halobacterial ribosomes contains a significant bill, whereas eubacterial small subunits contain only a vestigial bill. The structure of the eubacterial–halobacterial ribosome is shown in Fig. 4 (the bill is diagonally shaded).

In contrast, ribosomes of methanogens, eocytes, and eukaryotes (Fig. 2) are distinctly different from the eubacterial–halobacterial type (see Table 1). Their separate types (9) are illustrated beneath the micrographs in Fig. 2. Features present in the three types of small subunits but lacking, or present in a significantly modified form, in eubacterial and halobacterial subunits include lobes at the base of the subunit (nearly absent in methanogens, of intermediate size in eocytes, and large in eukaryotes), a bifurcation of the platform, and a gap at the base of the platform. Fields of small subunits from a methanogen, a eubacterium, and a halobacterium are shown from left to right, respectively, in Fig. 3. In large ribosomal subunits two features, lacking in eubacteria and halobacteria, are present in subunits from eocytes and eukaryotes. These are a lobe at the base of the subunits and a prominent bulge above the lobe and separated from it by a gap in eocytes. The gap is filled in eukaryotes. The profiles of the eocytic subunits, where they

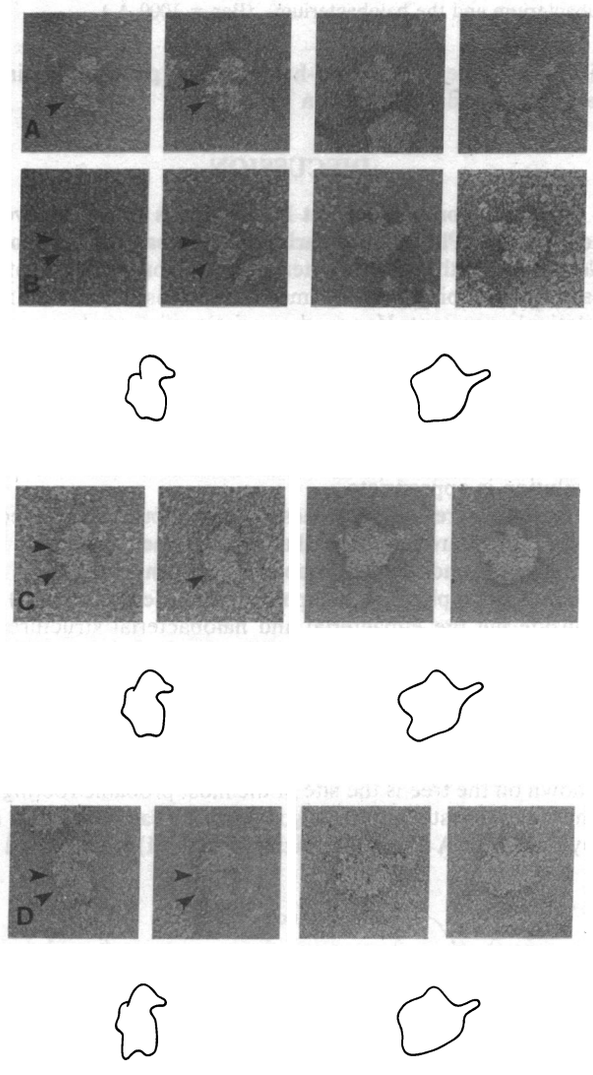

FIG. 2. Ribosomal subunits from the archaebacterial (rows A and B), eocytic (row C), and eukaryotic (row D) kingdoms. As in Fig. 1, small subunits are in the left two panels and large subunits are in the right two panels. The organisms are *Methanosarcina barkeri* (a heterotrophic methanogen, row A). *Methanobacterium thermoautotrophicum* (a thermophilic autotrophic methanogen, row B), *Thermoproteus tenax* (a thermophilic autotrophic sulfur-reducing bacterium, row C), and *Saccharomyces cerevisiae* (a yeast, row D). Subunit profiles are diagrammed beneath each group. (×225,000.)

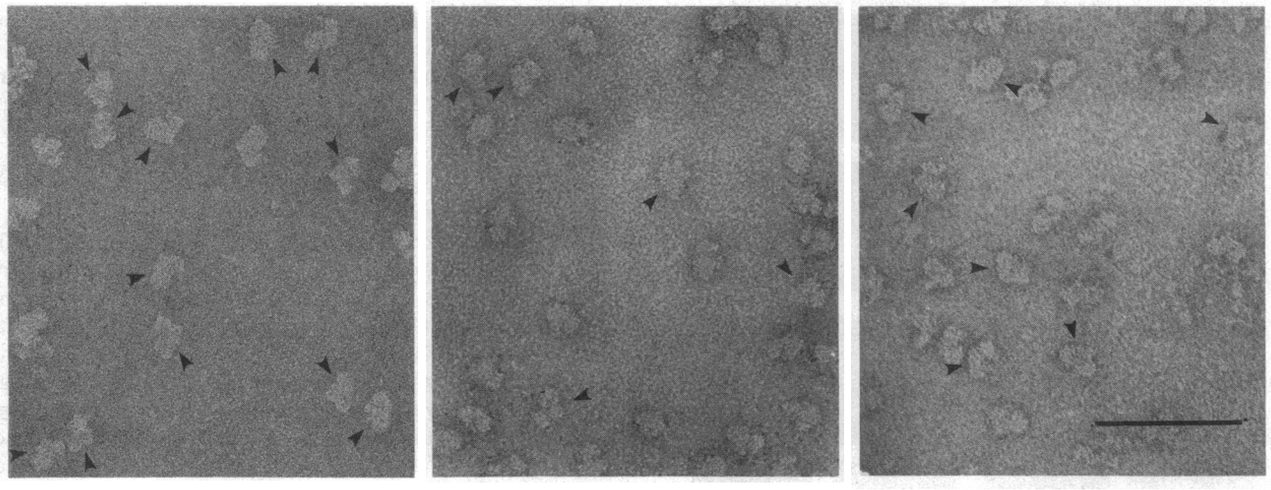

FIG. 3. Fields of small subunits from a methanogen (*Methanobrevibacter smithii*; *Left*), a eubacterium (*Escherichia coli; Center*), and a halobacterium (*Halobacterium cutirubrum; Right*). Arrows indicate the site of the gap (in the methanogen) or the absence of a gap (in the eubacterium and the halobacterium). (Bar = 1000 Å.)

differ from the eubacterial–halobacterial pattern, are indicated by thin dashed lines in Fig. 4.

DISCUSSION

Determination of the Most Parsimonious Unrooted Evolutionary Tree Places Eubacteria and Halobacteria as Closest Neighbors. Within the eubacterial and halobacterial and other bacterial groupings, three-dimensional ribosomal structure is relatively constant. Hence, the variations in structure among groups provide a phylogenetic basis for relating their evolution (Table 1). If, as the constancy of ribosome structure within lines and at the resolution limit of our images suggests (3), the individual structural features of each ribosomal type arose only once, then a parsimony analysis of ribosomal evolution is appropriate.

The structures of ribosomes have previously been used to derive the unrooted tree that related the eocytes to the eukaryotes and the archaebacteria (then defined as the methanogens plus the halophiles) to the eubacteria (5). In considering the eubacterial and halobacterial structures in more detail, two new 30S structural features are added in the analysis. These are a gap at the base of the platform and a split, or bifurcated, platform. The unrooted tree that most parsimoniously fits these data is shown in Fig. 5. It is not the only interpretation of our data but it is the simplest. Also shown on the tree is the site of the most probable rootings of the tree suggested by the oligonucleotide catalogues (10) and by DNA·rRNA cross hybridization data (11) as discussed (5).

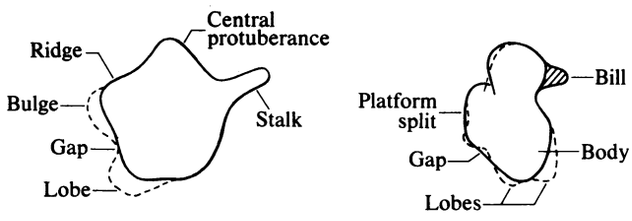

FIG. 4. Summary of the ribosomal features of the eubacteria and halobacteria. Structures common to both are shown by solid lines. The bill, found on the halobacterial and as a vistigial element on the eubacterial small subunit, is diagonally shaded. Additional structures found on the eocytic ribosomal subunits, but not on photocytic subunits, are shown as lightly dashed lines. A large subunit is on the left and a small subunit is on the right.

Halobacteria Are Not Archaebacteria. If the dendrogram in Fig. 5 is the correct interpretation of our data, then the halobacteria are more closely related to the eubacteria than to any other currently known organisms. In particular, the data indicate they are not members of the archaebacterial urkingdom.

URKINGDOM PHOTOCYTA

Eubacteria and Halobacteria Form a Natural Group, the Photocytes. If the halobacteria are not archaebacteria, then their altered status raises the question of where they properly belong. There are three alternative classification schemes that would generate monophyletic groups *sensu* Hennig (13). These monophyletic groupings could be created by (*i*) lumping the archaebacteria, eubacteria, and halobacteria into one large urkingdom; (*ii*) splitting the archaebacteria into two urkingdoms, thereby creating a separate urkingdom for the halobacteria; or (*iii*) combining the eubacteria and the halobacteria into an urkingdom. We advocate alternative (*iii*) (Fig. 6), because grouping the eubacteria and halobacteria does not "lump" or "split"—i.e., it does not change the number of highest level categories, and it generates a group that is at the same branching level as the archaebacteria (minus the halophiles). For this urkingdom we propose the name photocytes (light + cell) to emphasize the photosynthetic abilities of both subgroups, the eubacteria and the halobacteria. In addition, a classification that naturally accommodates photosynthesis (whether it occurred singly or multiply) makes good biological sense (14), because this clearly represents a landmark in biochemical evolution.

The Photocytes May Be Descended from a Photosynthetic Ancestor. If eubacteria and halobacteria are evolutionarily

Table 1. Structural features of ribosomes

	Small subunit				Large subunit		
	Bill	Lobes	Gap	Platform split	Lobe	Gap full	Bulge
Eubacteria	–	–	–	–	–	–	–
Halobacteria	+	–	–	–	–	–	–
Archaebacteria	+	±	+	+	±	–	±
Eocytes	+	+	+	+	+	–	++
Eukaryotes	+	++	+	+	+	+	+

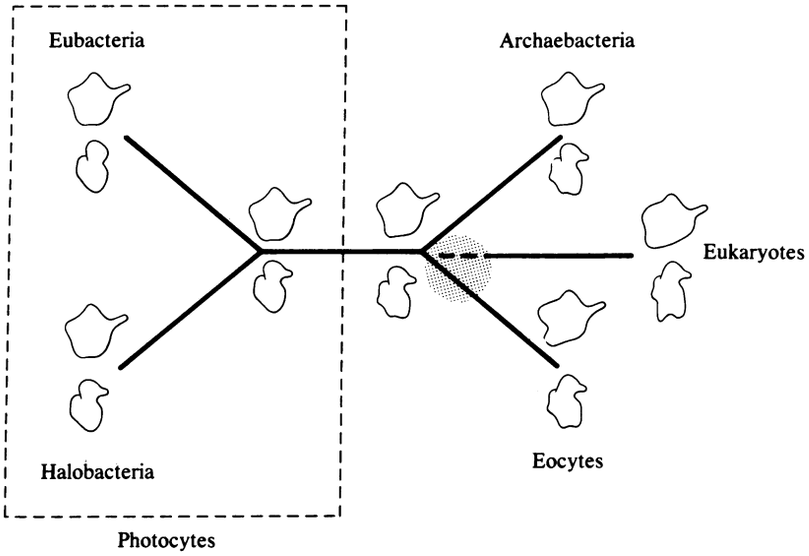

FIG. 5. The unrooted tree relating the steps in evolution indicates eubacteria and halobacteria are topologically closest neighbors. The group corresponding to the photocytes is enclosed by a dashed line. This tree most parsimoniously (26) fits the ribosomal structural data in Table 1. The assignment of the eukaryotic branch is tentative. Characters listed as ++ in Table 1 are assumed to represent ordered transitions (10) from ++ to + ± to −. The most likely rootings of this tree are shown in the shaded area.

nearest neighbors, then other data on the molecular properties of these two groups should exist that support this tree. Indeed, a considerable body of data supports this relationship. Surprisingly, however, many of the properties unique to eubacteria and halobacteria are related to the molecular details of photosynthesis. The simplest interpretation of their distribution is that the common ancestor of both the halobacteria and eubacteria was photosynthetic.

The general outline of photosynthesis is similar in the two branches of the photocytes. In photosynthetic eubacteria, the central event is a chlorophyll-dependent charge separation in the reaction centers of photosynthetic membranes, which produces an electrochemical proton gradient across the membrane. This gradient powers the phosphorylation of ADP (15) as well as the exchange of ions and nutrients. In halobacteria, the retinal protein bacteriorhodopsin functions as a light-driven proton pump (16, 17) to establish an electrochemical proton gradient. As in the eubacteria, this gradient powers phosphorylation of ADP. This argues that at the time of the separation of the two sublines of the photocytes, the general scheme of photosynthesis as a light-driven proton pump could have been established.

The halobacteria share many metabolic similarities with the purple nonsulfur eubacteria, and Osterhelt and Krippahl (15) have summarized them quite well. "A comparison of the . . . (two groups) reveals the following similarities: both groups exhibit photoorganotrophic growth, both are able to live in habitats of variable oxygen tension and both respond by a variable extent of expression of respiratory and photosynthetic activity. Most species of the *Rhodospirillaceae* grow as anaerobic phototrophs but can also grow under microaerophilic conditions or as aerobic chemoorganotrophs. Most species of *Halobacteriaceae* grow as chemoorganotrophs and develop the photosynthetic bacteriorhodopsin system under reduced oxygen tension. Under anaerobic conditions, they have been shown to increase in cell count by a factor of about 10, indicating their capacity for anaerobic phototrophic growth."

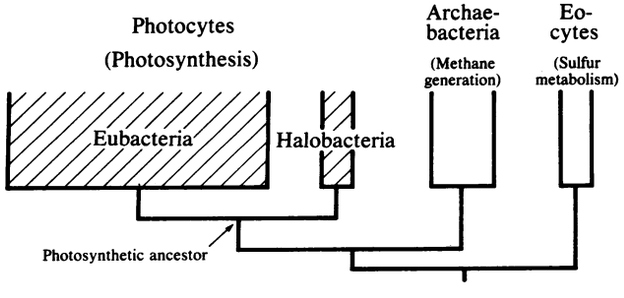

FIG. 6. Rooted evolutionary tree illustrating eubacteria and halobacteria as sublines of the monophyletic group, the photocytes. This tree shows the phylogenetic relationships among the photocytes, archaebacteria, and eocytes. The eubacterial and halobacterial branches of the photocytes are marked by diagonal lines. The photosynthetic common ancestor is indicated. This tree was rooted as described in ref. 5.

The electron transport chains of eubacteria and halobacteria contain some proteins that have not yet been found in the other bacterial groups. The 2Fe–2S ferredoxins, for example, are iron–sulfur containing soluble electron transport proteins found in both eubacteria and halobacteria. The primary sequence of halobacterial 2Fe–2S ferredoxin is closely related to that of eubacterial 2Fe–2S ferredoxin, except that the halobacterial molecule contains an additional 22 amino- and 6 carboxyl-terminal residues, indicating that both molecules are derived from a common ancestor (18). Functionally, the two are also similar. In photosynthetic eubacteria (including chloroplasts), ferredoxin is a part of photosystem 1 (the more primitive photosystem) where it carries the electrons that are used to reduce $NADP^+$. Halobacterial ferredoxin can function in place of its eubacterial counterpart in a hybrid system using chloroplast membranes and halobacterial ferredoxin. The hybrid system is inefficient, however, and halobacterial ferredoxin does not form a complex with chloroplast ferredoxin–$NADP^+$ reductase (19).

Similarities of photosynthesis in eubacteria and halobacteria extend to other individual molecular components and to their biosynthetic pathways. In halobacterial photosynthesis, a carotenoid derivative plays a central role in the generation of the protonmotive gradient, whereas in eubacterial photosynthesis their role is secondary to that performed by the chlorophylls. Carotenoid pigments do not occur in archaebacteria and eocytes but are broadly distributed in eubacteria (in both photosynthetic and nonphotosyn-

thetic members) and in halobacteria. The main pigment in halobacteria—bacterioruberin, a 50-carbon carotenoid having four hydroxyl groups—is also found (20) in the nonphotosynthetic eubacterium *Corynebacterium poinsettiae*, an actinomycete. Other 50-carbon carotenoids occur in *Halobacterium* and in the nonphotosynthetic eubacteria *Flavobacterium dehydrogenans* and *Corynebacterium poinsettiae* (21). Membranes of halophiles also contain many of the 40-carbon carotenes that are broadly distributed across the eubacteria. These range from phytoene to β-carotene and include retinal (22), a derivative of β-carotene and a part of the halobacterial light-driven proton pump. Furthermore, the biochemical pathway of carotenoid synthesis in halobacteria generally follows that in eubacteria (22, 23).

These details are most simply interpreted as showing that eubacteria and halobacteria diverged from the ancestor of the photocytes after 2Fe–2S ferredoxins were present, after the biochemical pathways of the 40- and 50-carbon carotenoids had been established, and after carotenoids (but before chlorophylls) had been incorporated into a primitive photosynthetic mechanism. Alternatively, photosynthesis could have evolved more than once within the photocytes.

Photosynthesis Very Likely Evolved After an Electrophosphorylation Chain Was Established. It is thought that the methanogens use a proton-driven mechanism for ATP generation (24). Hence, the last common ancestor of the photocytes and archaebacteria (see the phylogenetic tree in Fig. 6) probably generated ATP by using a protonmotive force. The system at this time may have already contained cytochromes, because *b*-type cytochromes are present in *M. barkeri* and in all methanogens capable of methyl group oxidation (25). Thus, with a preexisting electrophosphorylation system, the first photosynthetic apparatus needed to be little more than a primitive photoproton pump capable of supplying only a small fraction of the cell's requirement for ATP.

All Known Molecular Properties of Halobacteria and Eubacteria Are Consistent with Their Being Evolutionarily Closest Neighbors. A number of molecular properties were previously thought to support the placement of the halobacteria with the methanogens (2, 11). These include a large number of "noneubacterial" characters. Properties that are shared by halobacteria, methanogens, and eocytes, but not by the eubacteria, include ether-linked lipids, methionine carried by initiator tRNAs, elongation factors that react with diphtheria toxin, a ribosomal "bill," significant genealogical closeness as measured by oligonucleotide catalog derived S_{AB}s, similarly designed ribosomal A proteins, and a lack of peptidylglycan cell walls. These characters are phylogenetically uninformative, because they are shared by three groups (halobacteria, methanogens, and eocytes) and differ in only the eubacteria. In cladistic terms (13), they represent plesiomorphic characters. The data supporting our proposed photocyte evolutionary tree, in contrast, are shared derived (or synapomorphic) characters that occur in both halobacteria and eubacteria. Thus, we know of no data that conflict with our evolutionary proposal.

Photocytes as a Kingdom Make Good Biological Sense. One measure of the value of a new classification is whether it can redirect our thinking. Gould (25) explains "Taxonomy is often regarded as the dullest of subjects, fit only for mindless ordering and sometimes denigrated within science as mere 'stamp collecting'. . . . If systems of classification were neutral hat racks for hanging the facts of the world, this disdain might be justified. But classifications both reflect and direct our thinking. The way we order represents the way we think."

Phylogenetically, it is clear that the halobacteria do not belong in the archaebacteria. Grouping the halobacteria together with the eubacteria, however, makes good sense cladistically (13) and biologically (14), and it has the potential to "reflect and direct" our thinking about photosynthesis, surely one of the most important biochemical developments in evolution. In our proposed view of bacterial evolution (Fig. 6), each of the three urkingdoms correspond to significant biochemical innovation—i.e., photosynthesis (photocytes), methanogenesis (archaebacteria), and sulfur metabolism (eocytes). We believe that a case can be made at the highest classification level (i.e., urkingdoms) for using biochemical innovation as an important benchmark. Significantly, these three groups all represent biochemical innovations that are generally thought to have occurred before the last common ancestor of the eukaryotes appeared. We hope that our proposal will not be viewed as a final answer, but simply be seen as one of many steps taken toward developing a natural phylogenetically based classification of organisms.

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