karyotic cell from the blue-green algae. To see this problem resolved through sequence analysis one will have to wait for perfectly comparable data coming from the three eukaryotic cell compartments (for example, partial sequences of the large rRNA's). If they show clearly incompatible cladistic patterns, the symbiotic theory will then really be favored; if not, the autogeneous theory.

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9. I have used Aitken's alignment [see (*8*)] which takes into account all the blue-green algal sequences known, and I have counted every deletion as one mutation and each of the last two residues of *Euglena* as an addition. A slightly inferior total number of mutations might be obtained if large deletions were counted as a single mutation. It would, however, in no way change the relative order of parsimony of the three trees considered.
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Demoulin raises a number of interesting questions that we address in order below. The major conclusions we drew from the sequence data (*1*), that the eukaryote mitochondria and chloroplasts were acquired by protoeukaryote host organisms through symbioses and that oxygen-releasing photosynthesis evolved in one line late in the proliferation of the major bacterial groups, remain very probably correct and are bolstered by new sequence data.

Demoulin's major objection to the conclusions we draw from the ferredoxin and c-type cytochrome trees is that each is based on a set of proteins "that are not entirely orthologous." The only published sequence data we could find for Demoulin's examples are the 45 amino-terminal residues of the 8Fe-8S ferredoxin from *Azotobacter vinelandii* (*2*). This sequence is consistent with our tree. Some general comments about gene duplications need to be made. In deducing the position of each branch on a tree, it is sufficient that the sequence used be directly descended from the ancestral gene in the earliest ancestor on the branch. If there have been gene duplications in the descendants on the branch, any one of them will be useful, regardless of their multiplicity. Thus, if we were reconstructing a tree using bacterial globins (if such existed) and wanted to place the eukaryote line, a sequence of either myoglobin or hemoglobin would be useful, because the myoglobin-hemoglobin divergence occurred on the animal branch, much later than the first eukaryote. Recent sequence studies have revealed several duplications in the plant-type ferredoxins (*3*). Most of them are quite local; none affects the configuration of the main branches of our tree. In order for us to have been misled in placing the branch to the oxygen-releasing photosynthetic forms on the ferredoxin tree, a duplication would have to have occurred prior to their divergence from the *Bacillus-Desulfovibrio* branch, and we would have to have selected the wrong plant and blue-green algal ferredoxins. If so, the real divergence would have been more recent than that shown, not earlier. The blue-green algae would still share considerable evolution with the bacteria. A newly determined sequence, the 8Fe-8S ferredoxin from *Pseudomonas putida*, and experimental corrections to the sequence from *Desulfovibrio gigas* are now available (*4*). New calculations place the divergence of the *Pseudomonas* branch very close to the divergence of *Desulfovibrio* and *Bacillus*. The branch leading to the blue-green algae and eukaryote chloroplasts still cannot be accurately placed on this tree due to the large amount of difference between the sequences of these 2Fe-2S ferredoxins and the others with 4Fe-4S clusters.

None of the available sequence data contradict our supposition that the cytochrome c_2 and c_6 genes are descended from the same gene present at the time of the divergence of the chloroplast-blue-green algal line from the pseudomonad-Rhodospirillaceae-mitochondrial line.

Demoulin is correct in pointing out that the reliability with which the configuration of an evolutionary tree is inferred decreases sharply if branch lengths are very long compared with internodal distances. There is not sufficient information to be certain of the exact topology of the cytochrome c_6 subtree. However, if there was only one symbiosis to form a single ancestor from which all photosynthetic eukaryotes were descended, then *Plectonema* and *Spirulina* must be adjacent on one branch of the cytochrome c_6 subtree. In our calculations, the first configuration in which this occurs is tenth (of 105 possible) in order of tree size and includes a branch −4 PAM's (accepted point mutations per 100 residues) long. We therefore suggested that there may have been more than one symbiotic event. We certainly agree with Demoulin that many additional carefully chosen sequences are needed to sort out the early divergences of the blue-green algae and the eukaryote chloroplasts. In order to expeditiously investigate crucial sequences it is helpful to have a hypothesis regarding the evolutionary tree. This was one of our goals in writing the article.

A much more precise picture of chloroplast-blue-green algal evolution is emerging from the ferredoxin sequences than is possible from the cytochromes because there appears to have been a duplication early in the proliferation of blue-green algal types (*5*). This also allows us to place the earliest divergence in this subtree. The location of a *Porphyra umbilicalis* branch, although not compelling, is consistent with the symbiosis leading to the chloroplasts of the red algae being separate from that of the green algae and higher plants (*6*).

In constructing a tree based on 5*S* ribosomal RNA (rRNA), Hori and Osawa (*7*) use a sequential methodology, adding a branch at a time; that is entirely different from the matrix method we used. Nevertheless, the only major difference in the configurations of the two trees is that Hori and Osawa place the *Escherichia-Photobacterium* branch on the *Pseudomonas* branch. In addition, we place the point of earliest time on the

branch to *Clostridium* in conformance with our ferredoxin tree.

With respect to Demoulin's criticism of our composite tree [figure 5 in (*1*)], it is clear in our references that we had available to us sequences of 5*S* rRNA from *Bacillus stearothermophilus* and cytochrome c_{551} from *Pseudomonas fluorescens*. This brings the number of identical species appearing on two trees to five. We regret any confusion caused by our picturing only representative sequences on those two trees. For the "plant chloroplast" branch, sequences were not available from the same species. However, comparable data in the context of major phylogenetic branches mean that the sequences and the species in which they are found are sure to be directly descended from the same gene in the first ancestor on the branch. We assumed that the green algae and vascular plants share a common chloroplast ancestor and therefore used the c_6 sequence from *Euglena* and the ferredoxin from *Scenedesmus* to locate this branch in the composite tree. In combining the ferredoxin and 5*S* rRNA trees, we assumed that the two coccoid blue-green algae, *Aphanothece* and *Anacystis*, shared an ancestor more recently than the time of divergence of both from *Pseudomonas*.

Our suggestion that the development of aerobic respiration preceded that of oxygen-releasing photosynthesis is not proved by sequence data. However, our reasoning does not involve arguments about whether the ancestor of *Pseudomonas* and *Anacystis* is more like one or the other organism. Most of the Rhodospirillaceae, the pseudomonads, *Escherichia*, and some species of blue-green algae such as *Nostoc* sp. strain MAC can live heterotrophically in aerobic conditions. It therefore seems reasonable to suggest that their most recent common ancestor possessed a rudimentary form of aerobic respiration. On our composite tree, we place the development of some important components of a respiratory chain slightly earlier, near the divergence of the *Bacillus* and *Desulfovibrio* branch from the trunk of the tree.

Both of these organisms possess respiratory metabolisms. However, *Desulfovibrio* respires anaerobically using sulfate as the terminal electron acceptor, whereas *Bacillus* respires aerobically. It is certainly possible that aerobic respiration evolved separately in all of these lines. However, that is not the simplest explanation of our composite tree. Contrary to Demoulin's commonsense argument, Schopf (*8*) has pointed out that it is difficult to imagine the development of oxygen-releasing photosynthesis prior to the development in that line of a rudimentary mechanism for coping with oxygen, as oxygen is produced intracellularly in photosynthesis. This is not to say that aerobic respiration developed in anything like the present atmosphere. The high level of free oxygen in our present atmosphere is almost certainly due to oxygen-releasing photosynthesis.

Our composite tree clearly supports a symbiotic origin for the eukaryotes. It pictures the branches that contribute to the eukaryote host and organelles as distinctly separate, with each being closely related to contemporary free-living prokaryotes. Demoulin states that a resolution of the question of how eukaryotes originated "will have to wait for perfectly comparable data coming from the three eukaryotic cell compartments (for example, partial sequences of the large rRNA's)." We would welcome the elucidation of additional sequence data for its value in reconstructing a highly probable evolutionary schema. However, no information is perfect. A tree based on partial rRNA sequences might well suffer all the criticisms Demoulin has made here. If there is any suggestion of gene doubling in the sequences, the possibility that nonorthologous segments are being compared could be raised. One could raise questions about the accuracy of the tree in a small region and suggest that the accuracy of the overall tree is suspect. Alternative methods, whether or not sound, could be used to demonstrate the unreliability of any tree based on the segments.

In the nearly 3 years since we first presented our composite tree (*9*), we have found it an excellent working hypothesis with which to organize new sequence data and our ideas. We hope that it is as useful to others in the many disciplines for which it has implications.

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Long-Term Choline Treatment of Memory-Impaired Elderly Patients

Davis *et al.* (*1*) and Sitaram *et al.* (*2*) have reported an improvement in human memory following the administration of single doses of certain cholinomimetic agents (1 mg of physostigmine, 4 mg of arecholine, and 10 g of choline chloride). In both studies, the enhancement of memory was demonstrated in normal volunteers by means of a verbal serial learning task. These results, coupled with evidence that reduced cholinergic function may be related to the memory decline of the elderly and senile, led the authors of both reports to suggest that treatment with cholinergic agents might benefit elderly patients with memory impairment.

Since there are no clearly efficacious treatments currently available for age-related memory impairment (*3*), we find the data in (*1*) and (*2*) to be encouraging and agree that the potential use of cholinergic agents in senility should be investigated. However, the likelihood of suc-

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Protein and Nucleic Acid Sequence Data and Phylogeny

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