pathogenicity. Both visna virus and equine infectious anemia virus (EIAV) are believed to avoid elimination by host immune defense mechanisms by undergoing progressive changes in their envelope proteins during the course of infection (*13*). It is not yet known whether a similar type of immunologic escape occurs with HTLV-III, but the existence of neutralizing antibodies in infected individuals (*14*) and the relatedness of HTLV-III to visna virus and EIAV make this a serious consideration.

Another question is why some individuals infected with HTLV-III develop AIDS, others ARC, and still others no disease at all (*15*). Viral genotype, host immune response, and other factors are likely to be determinants of clinical outcome. From our analysis of the 18 HTLV-III isolates, we were unable to identify any disease-specific restriction pattern. In fact, each of the 18 viral isolates was different from the next and none were identical to HTLV-III$_B$, LAV, or ARV. Further nucleotide sequence analyses and deletion mutant studies (*16*) will be needed to identify regions of the HTLV-III genome responsible for the virus's biologic effects and for correlating viral genotype with clinical outcome.

Finally, it is of interest that in most patients only one predominant form of the AIDS virus was identified. If this is not the result of selective pressures introduced by cultivation in vitro, it suggests that some sort of interference process may occur, since these patients, especially the hemophiliacs, homosexuals, and intravenous drug addicts, are subject to repeated exposures to genotypically diverse viruses. The data would also then suggest that if genotypic variation is being generated in vivo as occurs with visna virus and EIAV (*13*), then either it is a rather slow process or the preexisting viral strains are largely eliminated during the disease course. Analyses of viral isolates from the same patient at different time points and from donor-recipient pairs of individuals would help to address these questions.

References and Notes

1. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; M. G. Sarngadharan *et al.*, *ibid.*, p. 506; F. Wong-Staal and R. C. Gallo, *Blood* **65**, 253 (1985).
2. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983); F. Brun-Vezinet, *et al.*, *Lancet* **1984-I**, 1253 (1984); L. Montagnier *et al.*, in *Human T-Cell Leukemia/Lymphoma Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1984), pp. 363–370; J. Levy *et al.*, *Science* **225**, 840 (1984).
3. B. H. Hahn *et al.*, *Nature (London)* **312**, 166 (1984); G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
4. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985); S. Wain-Hobson *et al.*, *Cell* **40**, 9 (1985); R. Sanchez-Pescador *et al.*, *Science* **227**, 484 (1985).
5. L. Ratner, R. C. Gallo, F. Wong-Staal, *Nature (London)* **313**, 636 (1985).
6. B. H. Hahn *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
7. S. Z. Salahuddin *et al.*, *ibid.*, in press.
8. See M. Popovic *et al.* in (*1*), p. 498.
9. L. Ratner *et al.*, in preparation.
10. B. Starcich *et al.*, in preparation.
11. G. M. Shaw *et al.*, *Science* **227**, 177 (1985).
12. M. A. Gonda *et al.*, *ibid.*, p. 173.
13. J. E. Clements, F. S. Pederson, O. Narayan, W. A. Haseltine, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4454 (1980); R. C. Montelaro, B. Parekh, A. Orrego, C. J. Issel, *J. Biol. Chem.* **259**, 10539 (1984).
14. M. Robert-Guroff, M. Brown, R. C. Gallo, *Nature (London)*, in press; D. Ho *et al.*, *ibid.*, in press; R. A. Weiss *et al.*, *ibid.*, in press.
15. H. W. Jaffe *et al.*, *Ann. Intern. Med.* **102**, 627 (1985).
16. A. G. Fisher *et al.*, *Nature (London)*, in press.

17. We thank J. Oleske, J. Hoxie, S. Broder, J. Groopman, L. Epstein and R. Price for supplying clinical specimens, W. Parks for providing viral isolate MJ, and A. Mazzuca for editorial assistance.

3 April 1985; accepted 3 July 1985

Gram-Positive Bacteria: Possible Photosynthetic Ancestry

Abstract. *A 16S ribosomal RNA gene has been sequenced from* Heliobacterium chlorum, *the recently discovered photosynthetic bacterium that contains a novel form of chlorophyll. Comparisons with other 16S ribosomal RNA sequences show that the organism belongs to the Gram-positive bacteria (one of ten eubacterial "phyla")—more precisely to the so-called low G + C (G, guanine; C, cytosine) subdivision thereof. This brings to five the number of such phyla that contain photosynthetic species, the other four being the purple bacteria and relatives, the green sulfur bacteria, the green nonsulfur bacteria, and the cyanobacteria. The finding suggests that Gram-positive bacteria may be of photosynthetic ancestry, and it strengthens the case for a common photosynthetic ancestry for all eubacteria.*

C. R. Woese
B. A. Debrunner-Vossbrinck
H. Oyaizu
Department of Genetics and Development, University of Illinois, Urbana 61801
E. Stackebrandt
Christian-Albrechts-Universitat, Olshausenstr. 40/60, D-23 Kiel, Federal German Republic
W. Ludwig
Lehrstuhl fur Mikrobiologie, Technische Universitat Munchen, Arcisstr. 16, D-8000 Munchen 2, Federal German Republic

The role of photosynthetic bacteria in evolution, although poorly understood, is clearly central. Cyanobacteria and their endosymbiotic descendants, the chloroplasts, are responsible for the oxygen atmosphere surrounding this planet, for the evolution of plant life, and so indirectly, for animal life as well. In some earlier era devoid of oxygen, other photosynthetic bacteria may have had a comparable impact on the evolutionary course and the physical state of the planet. The role of photosynthetic bacteria in the origin of cellular life is not known. Although we are only now beginning to understand the natural relationships among bacteria, the biologist in the past has proposed theories regarding them, particularly theories regarding the relationship of photosynthetic bacteria to bacterial evolution. Oparin long ago argued (*1*) that the primitive oceans were a "soup" of energy-rich biochemicals, and consequently, the first organisms arising therein were nonphotosynthetic, fermentative heterotrophs—there being no need initially for biological systems to generate their own energy-rich compounds.

Fig. 1 (Opposite page). Sequence of *Heliobacterium chlorum* 16*S* rRNA (hc) aligned with three other eubacterial 16*S* rRNA's—(bs) *Bacillus subtilis* (*14*), (an) *Anacystis nidulans* (*15*), and (ec) *Escherichia coli* (*16*). A gene for rRNA was selected by plaque hybridization procedures from a library of *H. chlorum* DNA in lambda phage produced by shotgun cloning of a Sau 3A partial restriction digest (*17*). The gene was subcloned into the phage M13 system as two Eco RI restriction fragments (*18*). [An Eco RI site is located in the sequence in the vicinity of position 680.] The sequence was determined by the dideoxy method, using both the normal primer for the M13 system and a variety of primers produced (for the most part by the University of Illinois DNA Synthesis Facility) specifically for use in sequencing eubacterial 16*S* ribosomal RNA's (*19*). The oligonucleotide catalog for the 16*S* rRNA itself was determined as described (*20*). The starting material for these studies was a frozen cell pellet of *H. chlorum* cells harvested anaerobically in early log phase (*13*). Sequences were aligned by the method indicated in (*21*). Numbering accords with that for the *E. coli* sequence (*16*), each line being 100 positions long by this measure. Our *H. chlorum* sequence begins at *E. coli* position 15 because the original clone (Sau 3A site) began at this point. The 16*S* rRNA oligonucleotides are shown by overlining the appropriate portions of the corresponding gene sequence. Discrepancies between the rRNA catalog and the sequence (discussed in text) are indicated by lowercase symbols for the bases inserted into the overlined segments (to indicate the catalog sequences). An oligonucleotide predicted by the sequence but not found in the catalog is indicated by xxx... in the overlining.

```
00 hc ............ ....GAUCCU GGCUCAGGAC GAACGCUGGC GGCAUGCCUA ACACAUGCAA GUCGAACGGA GAGCGGAAGU ..UUCG..AU GGAAGCUCUU
   bs .UUUAUCGGAGA GUUUGAUCCU GGCUCAGGAC GAACGCUGGC GGCAUGCCUA AUACAUGCAA GUCGAGCGGA CAGGUGGGAG ..CUUG...C UCCCGAUGUU
   an .CAAAAUGGAGA GUUUGAUCCU GGCUCAGGAU GAACGCUGGC GGCGUGCUUA ACACAUGCAA GUCGAACGGG CUC....... ..UUCG.... .....GAGCU
   ec ..AAAUUGAAGA GUUUGAUCAU GGCUCAGAUU GAACGCUGGC GGCAGGCCUA ACACAUGCAA GUCGAACGGU AACAGGAAGA AGCUUGCUUC UUUGCUGACG

      g                              u    g                                                    a        ga c u    ac
01 hc AGUGGCGGAC GGGUGAGUAA CGCGUGGACAA CCUACCGGAG AGUGGGGAU AACAGUCCGA AAGGGCUGCU AAUACCGCAUAACGUUCCUGAAGGACAUCCUUCAGG.AACCAAAGGA
   bs AGCGGCGGAC GGGUGAGUAA CACGUGGGUAA CCUGCCUGUA AGACUGGGAU AACUCCGGGA AACCGGGGCU AAUACCGGAUGGU.UGUUUGAACCGCAUGGUUCAAA.CAUAAAAGGU
   an AGUGGCGGAC GGGUGAGUAA CGCGUGAGAA. UCUGCCUACA GGACGGGGAC AACAGUUGGA AACGACUGCU AAUACCCGAUG.UGCC........GAGA.........GGUGAAACA.
   ec AGUGGCGGAC GGGUGAGUAA UGUCUGGGAA. ACUGCCUGAU GGAGGGGGAU AACUACUGGA AACGGUAGCU AAUACCGCAUAACGUC........GCAA.........GACCAAAGAG

               .....       xxxxxxx
02 hc .......GCA A.....UCCG  CUUUCCGAUG GGUCCACGUC  CGAUUAGCUA GUUGGUAGGG  UAAAGGCCUA CCAAGGCGAC  GAUCGGUAGC CGGCCUGAGA
   bs GGC....UUC G..GCUACCA  CUUACAGAUG GACCCGCGGC  GCAUUAGCUA GUUGGUGAGG  UAACGGCUCA CCAAGGCAAC  GAUGCGUAGC CGAUCUGAGA
   an .......UUU A......UGG  CCUGUAGAUG AGCUCGCGUC  UGAUUAGCUA GUUGGUGGGG  UAAGGGCCUA CCAAGGCGAC  GAUCAGUAGC UGGUCUGAGA
   ec GGGGACCUUC GGGCCUCUUG  CCAUCGGAUG UGCCCAGAUG  GGAUUAGCUA GUAGGUGGGG  UAACGGCUCA CCUAGGCGAC  GAUCCCUAGC UGGUCUGAGA

03 hc GGGUGAACGG CCACACUGGG  ACUGAGACAC GGCCCAGACU  CCUACGGGAG GCAGCAGUGG  GGAAUCUUCC GCAAUGGGCG  AAAGCCUGAC GGAGCAAUGC
   bs GGGUGAUCGG CCACACUGGG  ACUGAGACAC GGCCCAGACU  CCUACGGGAG GCAGCAGUAG  GGAAUCUUCC GCAAUGGACG  AAAGUCUGAC GGAGCAACGC
   an GGAUGAUCAG CCACACUGGA  ACUGAGACAC GGCCCAGACU  CCUACGGGAG GCAGCAGUGG  GGAAUUUUCC GCAAUGGGCG  CAAGCCUGAC GGAGCAACGC
   ec GGAUGACCAG CCACACUGGA  ACUGAGACAC GGUCCAGACU  CCUACGGGAG GCAGCAGUGG  GGAAUAUUGC ACAAUGGGCG  CAAGCCUGAU GCAGCCAUGC

04 hc CGCGUGGGGG AUGAAGGUCU  UCGGAUUGUA AACCCUUGUC  UUCGGGGAAG AAG........ .......... ......UUUU  GACGGUACCC GAGGAGGAAG
   bs CGCGUGAGUG AUGAAGGUUU  UCGGAUCGUA AAGCUCUGUU  GUUAGGGAAG AACAAGUACCG UUCGAAUAGG GCGGUACCUU  GACGGUACCU AACCAGAAAG
   an CGCGUGGGGG AGGAAGGUUU  UUGGACUGUA AACCCCUUUU  CUCAGGGAAG AAGA....... .......... ......AAGU  GACGGUACCU GAGGAAUAAG
   ec CGCGUGUAUG AAGAAGGCCU  UCGGGUUGUA AAGUACUUUC  AGCGGGGAGG AAGG.GAGUAA AGUUAAUACC UUUGCUCAUU  GACGUUACCC GCAGAAGAAG

05 hc CCCCGGCUAA CUACGUGCCA  GCAGCCGCGG UAAUACGUAG  GGGGCAAGCG UUGUCCGGAA  UGACUGGGCG UAAAGCGCGU  GCAGGCGGAC AUGUAAGUCU
   bs CCACGGCUAA CUACGUGCCA  GCAGCCGCGG UAAUACGUAG  GUGGCAAGCG UUGUCCGGAA  UUAUUGGGCG UAAAGCGCUC  GCAGGCGGUU UCUUAAGUCU
   an CCUCGGCUAA UUCCGUGCCA  GCAGCCGCGG UAAUACGGGA  GAGGCAAGCG UUAUCCGGAA  UUAUUGGGCG UAAAGCGCCU  GCAGGCGGUU AAUCAAGUCU
   ec CACCGGCUAA CUCCGUGCCA  GCAGCCGCGG UAAUACGGAG  GGUGCAAGCG UUAAUCGGAA  UUACUGGGCG UAAAGCGCAC  GCAGGCGGUU UGUUAAGUCA

06 hc GAGGUGAAAG CUUGGAGCUC  AACUCCGAAA CGGCCUUGGA  AACUGGAUGU CUUGAGAGAU  GGAGAGGAUA GCGGAAUUCC  CGGUGUAGCG GUGAAAUGCG
   bs GAUGUGAAAG CCCCCGGCUC  AACCGGGGAG GGUCAUUGGA  AACUGGGGAA CUUGAGUGCA  GAAGAGGAGA GUGGAAUUCC  ACGUGUAGCG GUGAAAUGCG
   an GUUGUUAAAG CGUGGGGCUC  AACCUCAUAC AGGCAAUGGA  AACUGAUUGA CUAGAGUAUG  GUAGGGGUAG CGGGAAUUCC  AGGUGUAGCG GUGAAAUGCG
   ec GAUGUGAAAU CCCCGGGCUC  AACCUGGGAA CUGCAUCUGA  UACUGGCAAG CUUGAGUCUC  GUAGAGGGGG GUAGAAUUCC  AGGUGUAGCG GUGAAAUGCG

07 hc UAGAUAUCGG GAGGAACACC  CGUGGCGAAG GCGGCUAUCU  GGACAUUAUC UGACGCUGAG  GCGCGAAAGC GUGGGGAGCA  AACAGGAUUA GAUACCCUGG
   bs UAGAGAUGUG GAGGAACACC  AGUGGCGAAG GCGGCUCUCU  GGUCUGUAAC UGACGCUGAG  GAGCGAAAGC GUGGGGAGCG  AACAGGAUUA GAUACCCUGG
   an UAGAUAUCUG GAGGAACACC  AGCGGCGAAA GCGCGCUACU  GGGCCAUAAC UGACGCUCAU  GGACGAAAGC UAGGGGAGCG  AAAGGGAUUA GAUACCCCUG
   ec UAGAGAUCUG GAGGAAUACC  GGUGGCGAAG GCGGCCCCCU  GGACGAAGAC UGACGCUCAG  GUGCGAAAGC GUGGGGAGCA  AACAGGAUUA GAUACCCUGG

08 hc UAGUCCACGC CGUAAACGAU  GAGUGCUAGG UGUUGGGGGU  AUCGACCCCCC CGGUGCCGCA GUUCACGCAA UAAGCACUCC  GCCUGGGGAG UACGGCCGCA
   bs UAGUCCACGC CGUAAACGAU  GAGUGCUAAG UGUUAGGGGG  UUUCCGCCCCU UAGUGCUGCA GCUAACGCAU UAAGCACUCC  GCCUGGGGAG UACGGUCGCA
   an UAGUCCUAGC CGUAAACGAU  GAACACUAGG UGUUGCGUGA  AUCGACCCGCG CAGUGCCGUA GCCAACGCGU UAAGUGUUCC  GCCUGGGGAG UACGCACGCA
   ec UAGUCCACGC CGUAAACGAU  GUCGACUUGG AGGUUGUGCC  .CUUGAGGCGU GGCUUCCGGA GCUAACGCGU UAAGUCGACC  GCCUGGGGAG UACGGCCGCA

09 hc AGGUUGAAAC UCAAAGGAAU  UGACGGGGGC CCGCACAAGC  GUUGGAGCAU GUGGUUUAAU  UCGACGCAAC GCGAAGAACC  UUACCAAGGC UUGACAUCCU
   bs AGACUGAAAC UCAAAGGAAU  UGACGGGGGC CCGCACAAGC  GGUGGAGCAU GUGGUUUAAU  UCGAAGCAAC GCGAAGAACC  UUACCAGGUC UUGACAUCCU
   an AGUUGGAAAC UCAAAGGAAU  UGACGGGGGC CCGCACAAGC  GGUGGAGUAU GUGGUUUAAU  UCGAUGCAAC GCGAAGAACC  UUACCAGGGU UUGACAUCCC
   ec AGGUUAAAAC UCAAAUGAAU  UGACGGGGGC CCGCACAAGC  GGUGGAGCAU GUGGUUUAAU  UCGAUGCAAC GCGAAGAACC  UUACCUGGUC UUGACAUCCA

                                     a
10 hc CUGAAUCCGA UAGAGAUAGC GGAGUGCCCUU CGGGGAGCAGA GAGACAGGUG GUGCAUGGUU  GUCGUCAGCU CGUGUCGUGA  GAUGUUGGGU UAAGUCCCGC
   bs CUGACAAUCC UAGAGAUAGG ACGU.CCCCUU CGGGG.GCAGA GUGACAGGUG GUGCAUGGUU  GUCGUCAGCU CGUGUCGUGA  GAUGUUGGGU UAAGUCCCGC
   an CCGAAUCUCU UGGAAACGAG AGAG.UGCCUU CGGGA.GCGGG GAGACAGGUG GUGCAUGGCU  GUCGUCAGCU CGUGUCGUGA  GAUGUUGGGU UAAGUCCCGC
   ec CGGAAGUUUU CAGAGAUGAG AAUG.UGCCUU CGGGA.ACCGU GAGACAGGUG CUGCAUGGCU  GUCGUCAGCU CGUGUUGUGA  AAUGUUGGGU UAAGUCCCGC

11 hc AACGAGCGCA ACCCUUAUCC  CUAGUUGCCA GCGAGAGAGU  CGGGGACUCU AGGGAGACUG  CCCGGGACGA CCGGGAGGAA  GGCGGGGAUG ACGUCAAAUC
   bs AACGAGCGCA ACCCUUGAUC  UUAGUUGCCA GCAU.UCAGU  UGGGCACUCU AAGGUGACUG  CCGGUGACAA ACCGGAGGAA  GGUGGGGAUG ACGUCAAAUC
   an AACGAGCGCA ACCCACGUUU  UUAGUUGCCA UCAU.UCAGU  UGGGCACUCU AGAGAAACUG  CCGGUGACAA ACCGGAGGAA  GGUGUGGACG ACGUCAAGUC
   ec AACGAGCGCA ACCCUUAUCC  UUUGUUGCCA GCGGUCCGGC  CGGGAACUCA AAGGAGACUG  CCAGUGAUAA ACUGGAGGAA  GGUGGGGAUG ACGUCAAGUC

12 hc AUCAUGCCCC UUAUGUCUUG  GGCUACACAC GUGCUACAAU  GGGCGGUACA AACCGAAGCG  AAGCCGAGAG GUGGAGCGAA  CCGGAGAAAG CCGUUCCCAG
   bs AUCAUGCCCC UUAUGACCUG  GGCUACACAC GUGCUACAAU  GGACAGAACA AAGGGCAGCG  AAACCGCGAG GUUAAGCCAA  UCCCACAAAU CUGUUCUCAG
   an AUCAUGCCCC UUACAUCCUG  GGCUACACAC GUACUACAAU  GCUCCGGACA GCGAGACGCG  AAGCCGCGAG GUGAAGCAAA  UCUCCCAAAC CGGGGCUCAG
   ec AUCAUGGCCC UUACGACCAG  GGCUACACAC GUGCUACAAU  GGCGCAUACA AAGAGAAGCG  ACCUCGCGAG AGCAAGCGGA  CCUCAUAAAG UGCGUCGUAG

13 hc UUCGGAUUGC UCUCUGCAAC  UCGAGAGCAU GAAGGCGGAA  UCGCUAGUAA UCGCGGGUCA  GCAUACCGCG GUGAAUACGU UCCCGGGCCU UGUACACACC
   bs UUCGGAUCGC AGUCUGCAAC  UCGACUGCGU GAAGCUGGAA  UCGCUAGUAA UCGCGGAUCA  GCAUGCCGCG GUGAAUACGU UCCCGGGCCU UGUACACACC
   an UUCAGAUUGC AGGCUGCAAC  UCGCCUGCAU GAAGGCGGAA  UCGCUAGUAA UCGCAGGUCA  GCAUACUGCG GUGAAUACGU UCCCGGGCCU UGUACACACC
   ec UCCGGAUUGG AGUCUGCAAC  UCGACUCCAU GAAGUCGGAA  UCGCUAGUAA UCGUGGAUCA  GAAUGCCACG GUGAAUACGU UCCCGGGCCU UGUACACACC

                                                         u   u
14 hc GCCCGUCACA CCACGAAAGU  CGGCAACACC CGAAGUCGGU GAGGUAACC.U UUCAGGAGCCA GCCGCCGAAG GUGGGGUCGA  UGAUUGGGGU GAAGUCGUAA
   bs GCCCGUCACA CCACGAGAGU  UUGUAACACC CGAAGUCGGU GAGGUAACC.U UUU.AGAGCCA GCCGCCGAAG GUGGGACAGA  UGAUUGGGGU GAAGUCGUAA
   an GCCCGUCACA CCAUGGAAGU  UGGCCAUGCC CGAAGUCGUU ACCCUAACCGU UCGCGGAGGGG GGCGCCGAAG GUAGGGCUGA  UGACUGGGGU GAAGUCUGAA
   ec GCCCGUCACA CCAUGGGAGU  GGGUUGCAAA AGAAGUAGGU AGCUUAACC.U UCG.GGAGGGC GCUUACCACU UUGUGAUUCA  UGACUGGGGU GAAGUCGUAA

15 hc CAAGGUAGCC GUAUCGGAAG  GUGCGGCUGG AUCACCUCCU  UUCU
   bs CAAGGUAGCC GUAUCGGAAG  GUGCGGCUGG AUCACCUCCU  UUCU
   an CAAGGUAGCC GUACCGGAAG  GUGUGGCUGG AUCACCUCCU  UU..
   ec CAAGGUAACC GUAGGGGAAC  CUGCGGUUGG AUCACCUCCU  UA..
```

Photosynthetic species arose only later, as the oceanic supply of energy-rich biochemicals became exhausted. Oparin's view was taken to mean that the first bacteria were heterotrophs and that photosynthetic bacteria arose only later, as one particular subline in the otherwise nonphotosynthetic bacterial world (*2*). Although we still have no idea whether such an idea is correct, it tends to be presented to each new generation of microbiologists as unassailable truth.

Ribosomal RNA (rRNA) sequence comparisons have begun to reveal the overall pattern of bacterial evolution (*3*). It is already evident that photosynthetic bacteria are neither confined to one of the eubacteria sublines, nor phylogenetically isolated from their nonphotosynthetic counterparts. Of the ten known major eubacterial groups (the bacterial equivalent of metazoan phyla or divisions), four contain photosynthetic species—the purple bacteria and relatives, the green sulfur bacteria, the green nonsulfur bacteria, and the cyanobacteria (*3–6*). Such a broad phylogenetic distribution of photosynthetic species suggests either that most if not all eubacteria have arisen from a common photosynthetic ancestor, or that photosynthesis has arisen a number of times among the eubacteria.

Although the classical photosynthetic phenotypes have been recognized for more than a century, two new ones have been discovered within the last decade, *Chloroflexus aurantiacus* (*7*) and *Heliobacterium chlorum* (*8*). The first of these resembles the classical green photosynthetic bacteria in its chlorosomes (*7*), but is unlike them in the structure of its photoreaction center (*9*); and together with its nonphotosynthetic relatives the organism holds a distinctive phylogenetic position (*6*). The second, *Heliobacterium*, a strict anaerobe, contains a new type of bacterial chlorophyll (*8*), but its phylogenetic position is unknown. We now show that *H. chlorum* is not specifically related to any other photosynthetic bacteria, but is, surprisingly, a member of the Gram-positive bacteria as defined phylogenetically.

The *H. chlorum* 16*S* rRNA gene sequence is aligned with those of three other eubacteria in Fig. 1. Corresponding T1 oligonucleotide sequences, determined from the rRNA itself, are indicated by overlining their presumed counterparts in the gene sequence. Some discrepancy exists between the oligonucleotides predicted from the DNA sequence and those found in the rRNA catalog. Most, if not all, of this disagreement would seem to reflect sequence heterogeneity among the various copies of the 16*S* rRNA gene in the *H. chlorum* genome. The existence of multiple cistrons of slightly different sequence can be deduced from the rRNA catalog. For example, UAACCUUUUCUG (U, uracil; A, adenine; C, cytosine; G, guanine) (vicinity of position 1450) occurs in the catalog along with lesser amounts of UAACCUUUUUAG and UAACCUUUUAUG, obvious sequence variants; the gene sequence shows yet a fourth version, UAACCUUUCAG. In the vicinity of position 130, the gene sequence is ACAACCUACCG, but its counterpart in the catalog is AUAACCUG (an oligonucleotide that is actually more typical of the general sequence pattern in this area than that found in the DNA sequence). CCCUUCG is the gene sequence in the vicinity of position 1030, but CCCUUAG is found in the catalog. It would appear that the 16*S* rRNA gene we have sequenced is a minor variant of the predominant rRNA gene in this organism.

The *H. chlorum* sequence is most closely related to that of *B. subtilis* and vice versa; they are 85 percent homologous. Homology for the remaining pairs of sequences in Fig. 1 ranges from 76 to 81 percent. (Only those 1470 positions in the alignment that are represented in all four sequences are scored in this calculation.)

Too few eubacterial 16*S* rRNA sequences have been determined for us to analyze the phylogeny of *H. chlorum* in any detail. However, this phylogeny is spelled out by the 400 or so oligonucleotide catalogs that now exist for a variety of prokaryotic species. Table 1 is an oligonucleotide signature (*4–6*) that places *H. chlorum* within the Gram-positive eubacterial "phylum." Oligonucleotides from *H. chlorum* 16*S* rRNA are found elsewhere most frequently among the catalogs of Gram-positive bacteria. In some of the examples in Table 1 a more generalized form of the *H. chlorum* sequence is also included to further em-

Table 1. Oligonucleotide signature showing the specific relationship of *H. chlorum* to the Gram-positive eubacterial "phylum." The table contains all sequences from the *H. chlorum* 16*S* rRNA oligonucleotide catalog heptamer and larger that meet the following criteria: (i) they occur in at least one other eubacterial catalog and (ii) they have been found in less than five of the ten eubacterial "phyla" (*4*). Their percentage occurrence in each of the eubacterial "phyla"—the Gram-positive "phylum" being split into its two subdivisions (*3, 4*)—appears in the various columns. In some cases a more generalized form of a given *H. chlorum* sequence, shown in brackets, is also included, to further demonstrate the specificity of the relationship. The number of catalogs in each group (*4*) is shown under each column heading (where appropriate) in parentheses. Abbreviations are LG+, low G+C subdivision of the Gram-positive eubacteria (*3*); HG+, high G+C subdivision of the Gram-positive eubacteria (3); SPR, spirochetes and their relatives (*12*); PUR, purple bacterial "phylum" (*5*); DSM, sulfate-reducing bacteria and relatives (*22*); CFB, bacteroides, cytophagas, and their relatives (*23*); CYN, cyanobacterial "phylum" (*3, 4*); RAD, radioresistant micrococci, (*3, 24*); OTH, the remaining eubacterial phyla (*3, 4*); Y, pyrimidine; and R, purine. A dot signifies no occurrence of the sequence in a group.

Sequence	Percent occurrence in								
	LG+ (94)	HG+ (61)	SPR (22)	PUR (100)	DSM (18)	CFB (22)	CYN (9)	RAD (3)	OTH
CACUCCG	53	·	·	5	6	·	·	·	·
CAUACCG	·	·	·	·	·	·	·	100	·
CAAUCCG	2	·	·	1	·	·	·	·	·
CAUAACG	·	·	·	3	·	·	·	·	·
AUAACAG	13	·	9	1	11	·	·	·	·
CAAUAAG	18	·	5	·	·	·	·	·	·
UUCCCAG	2	·	·	2	·	·	·	·	·
ACUCUAG	2	·	·	·	·	23	22	·	·
CUCUCUG	28	·	·	2	5	·	·	·	·
CUCUUAG	1	·	·	7	·	·	·	·	·
AUAACCUG*	6	·	5	·	11	5	·	·	·
UAACCUUU· ·	26	·	·	·	·	·	·	·	·
[UAACCYYY· ·]	(40)	·	(5)	·	(6)	(5)	·	·	·
CAACACCCG	15	16	·	·	·	·	·	·	·
[YAAYACCCR]	(67)	(85)	·	·	·	·	·	·	·
CCCCUUAUG	89	93	18	·	·	·	·	·	·
AAUCUUCCG	24	·	45	·	·	·	·	·	·
[AAUCUUC· · ·]	(55)	·	(55)	(1)	·	·	·	(100)	·
CUCAACUCCG	3	5	·	1	·	·	11	·	·
ACAUCCUCUG	12	·	·	·	·	·	·	·	·
[ACAUYYYCU· ·]	(21)	(2)	·	·	·	·	·	·	·
UCACACCACG	62	·	·	·	70	·	·	·	·
AACCUUACCAAG	6	62	·	·	·	18	67	·	·
UCAAAUCAUCAUG	84	51	14	·	·	·	·	·	·

* In catalog only; sequence counterpart ACAACCUACCG.

phasize the relation to the Gram-positive bacteria. For example, of the eight possible specific sequences of the general form UAACCYYY . . . , five are seen among the catalogs of the "clostridial" subdivision of the Gram-positive bacteria, and these represent at least six phylogenetically independent occurrences. Similarly, six of the eight possible versions of YAAYACCCR are found in this same group, representing at least eight phylogenetically independent occurrences (*10*).

Heliobacterium chlorum appears closer to the "clostridial" (low G + C) than to the "actinomycete" (high G + C) subdivision (*3*) of the Gram-positive bacteria by this measure. Although the organism should probably be considered a member of this particular subdivision, a definitive statement regarding this must await full sequence analysis of a representative "actinomycete," because the relative lack of closeness in this case may merely reflect rapid evolution in the "actinomycete" subline (*11*). In Table 1, *H. chlorum* also shows some relationship to the spirochete "phylum" (*12*). A possible distant but specific relationship between the spirochetes and Gram-positive bacteria has previously been noted (*4*).

To date nothing we know about the *H. chlorum* phenotype suggests a relationship to the Gram-positive bacteria (or to any other eubacteria for that matter). On preliminary examination, the organism's cell wall is not of the Gram-positive type (*13*).

The association of *H. chlorum* with the Gram-positive bacteria is surprising only in terms of our prejudices regarding relationships between photosynthetic and nonphotosynthetic bacteria. Precedent for the observed relationship definitely exists in the phylogeny of eubacteria determined by rRNA cataloging (*3–4*). The purple photosynthetic bacteria are part of a unit, a phylum, that includes many nonphotosynthetic phenotypes (*5*). The known members of the green nonsulfur "phylum" are predominantly nonphotosynthetic (*6*). The interesting question now is whether there exist other, as yet undiscovered, photosynthetic eubacteria belonging to "phyla" not now known to contain photosynthetic species.

Given that five of the ten recognized eubacterial "phyla" (*4*) have now been shown to contain (each a different type of) photosynthetic species, it would seem likely that the ancestor common to all eubacteria was itself photosynthetic. The point, although strengthened by our results, is, however, intrinsically unprovable. Therefore, although our conclusion is not a compelling one, it does demand that the archaic and now suspect notion that all bacteria have arisen from a common nonphotosynthetic ancestor no longer be accepted and perpetuated as dogma.

References and Notes

1. A. I. Oparin, *The Origin of Life* (Macmillan, New York, 1938).
2. C. B. van Niel, *Cold Spring Harbor Symp. Quant. Biol.* **1**, 285 (1946); R. Y. Stanier and C. B. van Niel, *J. Bacteriol.* **42**, 437 (1941); R. Y. Stanier, *Ann. Inst. Pasteur* **101**, 297 (1961).
3. G. E. Fox *et al.*, *Science* **209**, 457 (1980); E. Stackebrandt and C. R. Woese, in *Molecular and Cellular Aspects of Microbial Evolution*, M. J. Carlile *et al.*, Eds. (Cambridge Univ. Press, London, 1981), pp. 1–31.
4. C. R. Woese, E. Stackebrandt, T. J. Macke, G. E. Fox, *Syst. Appl. Microbiol.*, in press; C. R. Woese, in *The Evolution of Prokaryotes*, K. H. Schleifer and E. Stackebrandt, Eds. (Academic Press, London, 1985); E. Stackebrandt, in *ibid.*
5. J. Gibson *et al.*, *Curr. Microbiol.* **3**, 59 (1979); C. R. Woese *et al.*, *Syst. Appl. Microbiol.* **5**, 315 (1984); C. R. Woese *et al.*, *ibid.*, p. 327; C. R. Woese *et al.*, *ibid.* **6**, 25 (1985).
6. J. Gibson *et al.*, *Syst. Appl. Microbiol.*, in press.
7. B. K. Pierson and R. W. Castenholz, *Arch. Microbiol.* **100**, 5 (1974).
8. H. Gest and J. L. Favinger, *ibid.* **136**, 11 (1983).
9. R. G. Feick and R. C. Fuller, *Biochemistry* **23**, 3693 (1984).
10. C. R. Woese *et al.*, unpublished data.
11. C. R. Woese, J. Maniloff, L. B. Zablen. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 494 (1980); C. R. Woese, E. Stackebrandt, W. Ludwig, *J. Mol. Evol.* **21**, 305 (1985).
12. B. J. Paster, E. Stackebrandt, R. B. Hespell, C. M. Hahn, C. R. Woese. *Syst. Appl. Microbiol.* **5**, 337 (1984).
13. H. Gest, personal communication. We thank Professor Gest for a frozen pellet of *H. chlorum* cells.
14. C. J. Green, G. C. Stewart, M. A. Hollis, B. S. Vold, K. R. Bott, *Gene*, in press.
15. N. Tomioka and M. Sugiura, *Mol. Gen. Genet.* **191**, 46 (1983).
16. J. Brosius, J. L. Palmer, J. P. Kennedy, H. F. Noller, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4801 (1978).
17. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982); W. A. M. Loenen and W. J. Brammar. *Gene* **20**, 249 (1980).
18. J. Messing, *Methods Enzymol.* **101**, 20 (1983).
19. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); D. Yang *et al.*, *ibid.* **82**, 4443 (1985).
20. E. Stackebrandt, E. Seewaldt, W. Ludwig, K.-H. Schleifer, B. A. Huser, *Zentral. Bakteriol. Hyg., Abt. I. Orig.* **C3**, 90 (1982); V. J. Fowler, W. Ludwig, E. Stackebrandt, in *The Use of Chemotaxonomic Methods for Bacteria*, M. Goodfellow and D. E. Minnikin, Eds. (Academic Press, New York, in press).
21. C. R. Woese, R. R. Gutell, R. Gupta, H. F. Noller, *Microbiol. Rev.* **47**, 621 (1983).
22. R. B. Hespell, B. J. Paster, T. J. Macke, C. R. Woese, *Syst. Appl. Microbiol.* **5**, 196 (1984); E. Stackebrandt and W. Ludwig, in preparation.
23. B. J. Paster *et al.*, *Syst. Appl. Microbiol.* **6**, 34 (1985).
24. B. W. Brooks, R. G. E. Murray, J. L. Johnson, E. Stackebrandt, C. R. Woese, G. E. Fox, *Int. J. Syst. Bacteriol.* **30**, 627 (1980).

25. Supported by grants from NASA and NSF (C.R.W.) and by the Gesellschaft fuer Biotechnologische Forschung to support the German Collection of Microorganisms (E.S.).

5 March 1985; accepted 30 April 1985

Antibody-Directed Urokinase: A Specific Fibrinolytic Agent

Abstract. *A specific fibrinolytic agent was synthesized by covalently coupling urokinase to a monoclonal antibody that was fibrin-specific and did not cross-react with fibrinogen. The antibody was raised against a synthetic peptide representing the seven amino-terminal residues of the beta chain of human fibrin. The urokinase-antifibrin conjugate retained the original binding specificity of the antibody and showed 100-fold increased fibrinolysis in vitro when compared to unmodified urokinase. The presence of human fibrinogen at plasma concentration did not influence these properties.*

CHRISTOPH BODE
GARY R. MATSUEDA
KWAN Y. HUI
EDGAR HABER
Cardiac Unit, Massachusetts General Hospital, Boston 02114

Coronary arteriographic studies indicate that 87 percent of transmural myocardial infarctions are caused by coronary thrombosis (*1*). Although thrombolytic agents currently available can lyse coronary artery thrombi in the early hours of coronary thrombosis and thereby diminish myocardial injury, their clinical application has been attended by significant problems. Both urokinase and streptokinase activate the conversion of plasminogen to the fibrinolytic enzyme plasmin. Plasmin, in turn, not only affects lysis of the fibrin in the thrombus but also promotes generalized fibrinogenolysis, at times resulting in severe bleeding (*2*). Human tissue plasminogen activator may be more fibrin-specific than urokinase (*3*). In order to target urokinase to a fibrin-containing clot, we coupled this plasminogen activator to a monoclonal antibody that was raised against a synthetic peptide representing the seven amino-terminal residues of the beta chain of human fibrin. This antibody is specific for fibrin and does not cross-react with fibrinogen (*4*). Fibrinolysis was promoted to a much greater extent with the conjugate than with unmodified urokinase.

Reduced urokinase was coupled to fibrin-specific monoclonal antibody 64C5 by means of its intrinsic sulfhydryl groups, with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as a cross-linking agent (*5*). The cross-linking agent (20 m*M* in 0.05 ml of absolute ethanol) was added to the antibody [6.3 mg in 3.0 ml of phosphate-buffered saline (PBS)