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Horizontal Transfer of Genes Coding for the Photosynthetic Reaction Centers of Purple Bacteria

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Abstract. Phylogenetic trees were drawn and analyzed based on the nucleotide sequences of the 1.5-kb gene fragment coding for the L and M subunits of the photochemical reaction center of various purple photosynthetic bacteria. These trees are mostly consistent with phylogenetic trees based on 16S rRNA and soluble cytochrome *c*, but differ in some significant details. This inconsistency implies horizontal transfer of the genes that code for the photosynthetic apparatus in purple bacteria. Possibilities of similar transfers of photosynthesis genes during the evolution of photosynthesis are discussed especially for the establishment of oxygenic photosynthesis.

Key words: Reaction center — Purple bacteria — Photosynthesis — Gene transfer — Evolution — Phylogenetic tree — Nucleotide sequence — Polymerase chain reaction

Introduction

Photosynthetic apparatuses arose early in the evolution of bacteria (Woese 1987) and have adapted to environmental changes on the Earth. Much of the research on the nature and mechanisms of changes in photosynthesis genes have been aimed at understanding the course of the evolution of photosynthesis. Such studies promise to elucidate the relationship between the photosynthetic machinery and changes in the terrestrial environment.

Phylogenetic trees based on the nucleotide sequence of 16S rRNA have been generally accepted. In the late 1970s—still the age of classical bacterial taxonomy based on morphological and physiological characteristics—a number of amino acid sequences of *c*-type cytochromes were determined. This information allowed the prediction of phylogenetic relationships among members of the purple photosynthetic bacteria and their nonphotosynthetic relatives (Ambler et al. 1979a,b; Dickerson 1980). These and further studies demonstrated that a phylogenetic tree based on soluble cytochrome *c* is in good agreement with that based on 16S rRNA, leading to general acceptance of phylogenies deduced from 16S rRNA sequences as an indicator of the evolutionary relationships between organisms (Dickerson 1980; Woese et al. 1980). It is known that phylogenies based on the amino acid sequences of many kinds of proteins in bacteria support those based on 16S rRNA.

Phylogenetic trees based on 16S rRNA imply that the Cyanobacteria have a close relationship with purple bacteria (Woese 1987), despite the fact that the former carry out oxygenic photosynthesis similar to that in plants. Cyanobacteria have two distinct types of photosystems. One shows considerable similarity to that of purple bacteria; the other resembles that of green sulfur bacteria (Williams et al. 1984; Michel et al. 1986; Büttner et al. 1992; Liebl et al. 1993). The first lineage to branch from the tree of photosynthetic prokaryotes is that of the green filamentous bacteria, which have a reaction center re-

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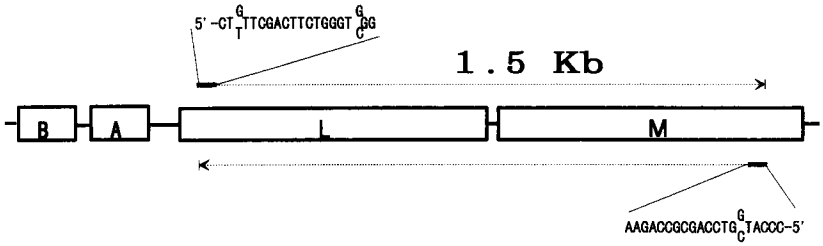

Fig. 1. Gene arrangement of *puf* operon. Boxes B, A, L, and M show the genes coding for the β and α subunits of the light-harvesting complex and the L and M subunits of the photochemical reaction center complex, respectively. The two primers for PCR were designed for the oligo nucleotide sequences of 5′ region of *pufL* and 3′ region of *pufM*. The amplified 1.5-kbp DNA fragment covered more than 90% of *pufL* and more than 85% of *pufM*.

sembling that of purple bacteria. The origin and the evolution of photosynthetic apparatus are not simply explained by the phylogeny based on the 16S rRNA sequence. Molecular evolutionary analysis of the photosynthetic proteins is one of the most useful ways to clarify the course of evolution of the photosynthetic apparatus.

The photochemical reaction center complex is a membrane protein that converts light energy into electrochemical energy through electron transfer and proton-pumping processes. The three-dimensional structures of the reaction center complexes have been clarified in two rather distantly related species of the purple bacteria, *Rhodopseudomonas viridis* (Deisenhofer et al. 1985, 1989) and *Rhodobacter sphaeroides* (Allen et al. 1987). The two complexes show considerable structural similarity, despite the fact that the identity between their amino acid sequences is only 50–58%; their subunit compositions also differ.

The reaction center complex of the purple bacteria is composed of at least three subunits: L, M, and H. In *R. viridis,* a cytochrome subunit is present in addition to these three subunits. Along with the genes for the light-harvesting complex, the genes encoding the L and M subunits form an operon called *puf,* as shown in Figure 1. In the present study, *pufL* and *M* of various purple photosynthetic bacteria were amplified via polymerase chain reaction (PCR), with primers based on consensus sequences from these genes. The amplified genes were then cloned and sequenced. The present study shows that phylogenetic trees based on nucleotide sequences of genes coding for subunits of the photochemical reaction center complex of purple photosynthetic bacteria provide important clues to the evolution of photosynthesis, suggesting the horizontal transfer of photosynthetic genes.

Materials and Methods

Cultivation of Bacteria

Rhodospirillum molischianum and *Rhodospirillum photometricum* were grown in a medium described previously (Nagashima et al. 1993a). *Rhodocyclus tenuis* was grown in SAY medium (0.3% succinate, 0.1% ammonium sulfate, 0.1% yeast extract (Difco, USA), 0.1% vitamin solution (1 g nicotinic acid, 1 g thiamine, 50 mg biotin, 0.5 g PABA, 10 mg vitamin B_{12}, 0.5 g calcium pantothenic acid, 0.5 g pyridoxine-HCl, 2.0 g EDTA, and 0.5 g folic acid per 1 l) and 1% basal salt solution (10 mM EDTA, 4 mM ferric sulfate, 100 mM magnesium sulfate, 5 mM calcium chloride, 500 mM sodium chloride, and 1% trace element solution, pH 7.0). The trace element solution contained 25 mM manganese sulfate, 5 mM zinc sulfate, 5 mM cobalt nitrate, 5 mM copper sulfate, 5 mM molybdic acid, 10 mM boric acid, and 50 mM EDTA. *Rhodomicrobium vannielii, Rhodobacter blasticus,* and *Chromatium vinosum* were grown in PYS medium [0.5% polypeptone (Wako, Japan), 0.1% yeast extract, 0.5% sodium succinate and 1% basal salt solution, pH 7.0]. *Rhodoferax fermentans* was grown in a medium containing 0.3% sodium malate, 0.1% ammonium sulfate, 15 mM phosphate, 0.1% vitamin solution, and 1% basal salt solution, pH 7.0. *Erythrobacter longus* was grown in a medium composed of 2% sodium chloride, 0.2% potassium phosphate, 0.4% magnesium sulfate ($7H_2O$), 0.03% calcium chloride ($2H_2O$), 0.001% ferric citrate, 0.2% yeast extract, 0.1% casamino acid (Difco, USA), 0.1% polypeptone, 0.1% glycerol, 0.03% trace element solution, and 0.1% vitamin solution (pH 7.7). All bacteria were grown photosynthetically at 30°C except *Erb. longus,* which was grown aerobically at 26°C in the dark.

Amplification of DNA Fragments Containing pufL *and* M *by PCR*

The genes coding for the L and M subunits of the reaction center were cloned using PCR as described previously (Hiraishi and Ueda 1994a; Hiraishi 1994). One of the primers has the sequence *5′-CT(G/T)TTCGACTTCTGGGT(G/C)GG-3′,* a motif that is well conserved in the 5′ region of *pufL* in all species so far examined. The other primer has the sequence *5′-CCCAT(G/C)GTCCAGCGCCAGAA-3′,* conserved at the 3′ region of *pufM*. The reaction was performed using the GeneAmp PCR Kit (Perkin-Elmer) in Zymoreacter (ATTO), following the protocol supplied by the manufacturers. Upon electrophoresis of the products of this reaction, we observed a band showing a mobility of 1.5 kbp, which corresponds to the region expected of *pufL* and *M* between the two primers. This band was recovered from the gel and cloned in the plasmids pT7Blue(R) or pUC18. Cloned and fractionated *puf* fragments were sequenced using a Taq Dye Primer Cycle Sequencing Kit and a 373A DNA Sequencer (Applied Biosystems), or an AutoCycle Sequencing kit and an A. L. F. DNA Sequencer (Pharmacia). The data obtained were processed using the DNASIS program (Hitachi).

Sequence Data of 16S rRNA and Soluble Cytochrome c

The sequence data for 16S rRNA for *Rhodobacter capsulatus, Rba. blasticus, Rba. sphaeroides, Rps. viridis, Rcy. tenuis, Rfx. fermentans,* and *Rubrivivax gelatinosus* were obtained from the previous works (Hiraishi and Ueda 1994a,b; Hiraishi 1994). The 16S rRNA sequence of *Rsp. photometricum* is from unpublished data generated by Hiraishi and Ueda, and that of *Thiocystis gelatinosa* was determined in the present study using the methods outlined in the previous works (Hiraishi 1992, 1994). All other data were obtained from the EMBL and GenBank

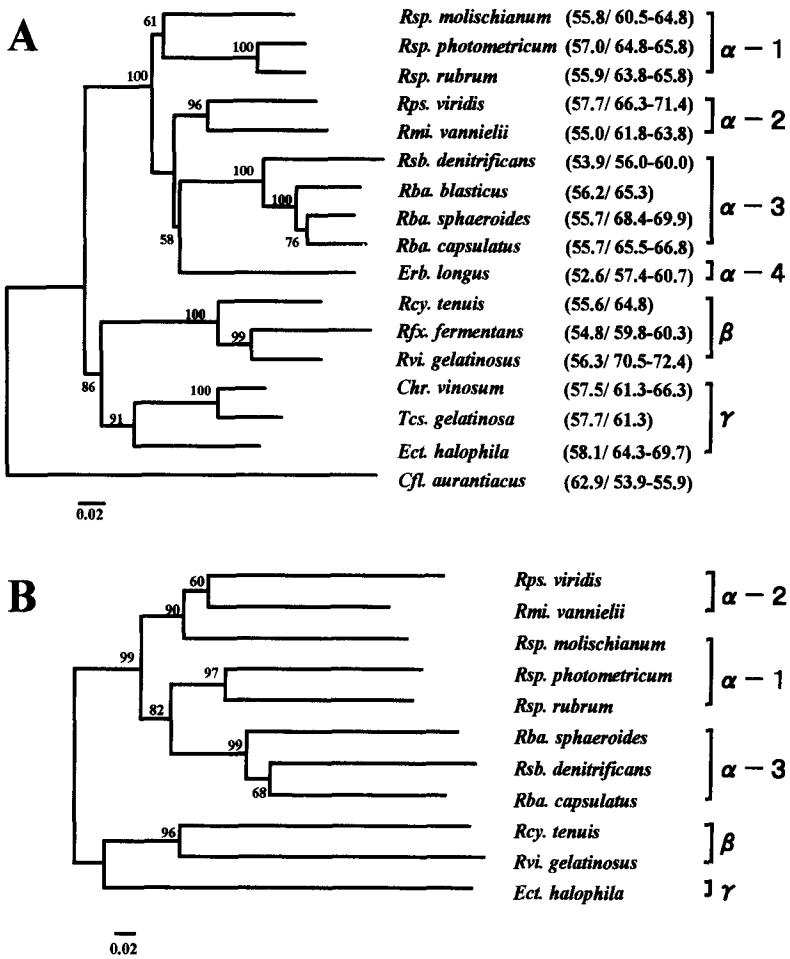

Fig. 2. Phylogenetic trees based on differences in nucleotide sequences of 16S rRNA (**A**) and amino acid sequences of soluble cytochrome *c* (**B**). The G+C content of the 16S rRNA gene of each species is shown in parenthesis; that of the total DNA follows the slash. The accession numbers of the sequences used for the construction of the 16S rRNA tree are follows: *Rsp. molischianum,* M59067; *Rsp. photometricum,* D30777; *Rsp. rubrum,* M32020; *Rps. viridis,* D25314; *Rmi. vannielii,* M34127; *Rsb. denitrificans,* M59063; *Rba. blasticus,* D16429; *Rba. sphaeroides,* D16425; *Rba. capsulatus,* D16428; *Erb. longus,* M59062; *Rcy. tenuis,* D16210; *Rfx. fermentans,* D16212; *Rvi. gelatinosus,* D16214; *Chr. vinosum,* M26629; *Ect. halophila,* M26630; *Cfl. aurantiacus,* M34116. The accession numbers of the amino acid sequences used for the construction of the phylogenetic tree of the soluble cytochrome *c* are follows: *Rps. viridis,* A00075; *Rmi. vannielii,* A00074; *Rsp. molischianum,* A00079; *Rsp. photometricum,* A00085; *Rsp. rubrum,* A00084; *Rba. sphaeroides,* A00087; *Rsb. denitrificans,* JT0008; *Rba. capsulatus,* A00086; *Rcy. tenuis,* A00090; *Rvi. gelatinosus,* A00089; *Ect. halophila,* A00115.

databases. The sequence data for soluble cytochrome *c* were all obtained from the EMBL, GenBank, SWISS-PROT and NBRF & PIR databases.

Tree Construction

The phylogenetic trees were drawn using the Clustal V (Higgins et al. 1992) and MEGA (Kumar et al. 1993) programs. The length of sequence alignment of 1.5-kb DNA fragments containing L and M subunit genes was 1604 nucleotides long. The number of gaps ranged from 32 to 77 in each sequence. All gaps in the sequence alignment were omitted pairwise in the calculation. Construction of the trees was performed by the neighbor-joining method, applying the Kimura 2-parameter distance for 16S rRNA, the *p*-distance for soluble cytochrome *c*, and the Tamura and Nei distance for photosynthetic gene fragment coding for L and M subunits of the reaction centers as distance estimators. In the tree of photosynthetic gene fragment, only transversional replacements were taken into account. A green filamentous bacterium, *Chloroflexus aurantiacus,* was chosen as an outgroup in the phylogenetic trees of 16S rRNA and photosynthetic genes.

Nucleotide Sequence Accession Numbers

The *pufL* and *M* DNA sequences determined in this study have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession numbers as follows: *Chr. vinosum,* D50647; *Erb. longus,* D50648; *Rba. blasticus,* D50649; *Rfx. fermentans,* D50650; *Rcy. tenuis,* D50651; *Rmi. vannielii,* D50652; *Tcs. gelatinosa,* D50653; *Rsp. photometricum,* D50681. The accession number for the nucleotide sequence of the *16S rRNA* gene of *Tcs. gelatinosa* is D50655.

Results

Figure 2A shows a phylogenetic tree based on the nucleotide sequences of 16S rRNAs of purple photosynthetic bacteria. These bacteria are divided into three major groups, called the α, β, and γ subclasses, respectively (Woese 1987). These subclasses are all grouped under the class Proteobacteria. The α subclass is further divided into four groups—α-1, α-2, α-3, and α-4 (Woese 1987)—as shown in Figure 2A. This classification is also supported by the phylogenetic tree based on the amino acid sequence of soluble cytochrome *c*, as shown in Figure 2B.

Table 1 summarizes identities among the deduced amino acid sequences of the L and M subunits. The table shows a clear tendency that species belonging to the α-1 and -2 groups show higher homology with those of the β and γ subclasses than with those of the other groups in the α subclass. This tendency is observed in both the L and M subunits. For example, the L subunit of *Rhodospirillum molischianum* exhibits 74–80% identity to those of five species in the β and γ subclasses, but 59–76%

Table 1. Identities in amino acid sequences of the L and M subunits

Organism	Sequence identity (%)															
	Rhodospirillum rubrum	*Rhodospirillum molischianum*	*Rhodospirillum photometricum*	*Rhodopseudomonas viridis*	*Rhodomicrobium vannielii*	*Rhodobacter capsulatus*	*Rhodobacter sphaeroides*	*Rhodobacter blasticus*	*Roseobacter denitrificans*	*Erythrobacter longus*	*Rubrivivax gelatinosus*	*Rhodoferax fermentans*	*Rhodocyclus tenuis*	*Chromatium vinosum*	*Thiocystis gelatinosa*	*Chloroflexus aurantiacus*
Rhodospirillum rubrum		69	67	56	67	67	67	67	66	57	70	69	70	69	70	47
Rhodospirillum molischianum	66		76	66	73	63	67	68	73	59	80	74	78	76	77	45
Rhodospirillum photometricum	65	59		63	71	60	62	65	68	56	73	70	73	72	73	47
Rhodopseudomonas viridis	60	59	54		62	56	56	59	60	52	64	63	65	63	62	46
Rhodomicrobium vannielii	68	64	63	62		66	64	64	72	61	73	72	71	68	70	48
Rhodobacter capsulatus	59	57	51	52	58		77	78	64	57	65	63	63	60	61	45
Rhodobacter sphaeroides	63	57	54	53	63	74		80	69	58	68	64	64	66	66	44
Rhodobacter blasticus	59	56	54	52	59	73	78		67	58	68	64	69	67	66	45
Roseobacter denitrificans	59	60	53	54	62	59	61	59		62	75	73	69	68	71	47
Erythrobacter longus	61	60	57	54	61	62	62	63	58		61	57	57	56	58	44
Rubrivivax gelatinosus	69	67	61	62	73	63	64	63	62	63		82	78	76	78	48
Rhodoferax fermentans	66	66	62	60	71	61	62	58	63	57	77		74	71	71	47
Rhodocyclus tenuis	70	66	62	59	75	57	61	57	60	63	73	70		77	77	45
Chromatium vinosum	71	68	62	61	72	54	58	57	61	60	73	67	71		89	46
Thiocystis gelatinosa	73	69	64	61	73	58	63	59	61	62	72	68	76	88		47
Chloroflexus aurantiacus	44	46	42	42	47	41	46	44	46	45	49	47	47	45	46	

The values on the upper right of the table body are for the L subunit and those on the lower left are for the M subunit

with those of the α subclass. Such a close relationship to the β and γ subclasses is not observed in species belonging to groups α-3 or -4. The L and M subunits of the green filamentous bacterium, *Chloroflexus aurantiacus,* exhibit low identity to those of the purple bacteria.

Figure 3 shows phylogenetic trees based on the nucleotide sequences of the 1.5-kb DNA fragments coding for the L and M subunits of the reaction centers of the species belonging to the α subclass only (Fig. 3A) and the α, β, and γ subclasses (Fig. 3B) of purple photosynthetic bacteria. In these phylogenetic trees, tree topology in each subclass is, in principle, consistent with that in the phylogenetic tree of 16S rRNA except that *Rsb. denitrificans* was positioned outside the cluster of the α-3 group in the tree of the α subclass (Fig. 3A). As shown in Fig. 3B, the species belonging to the β subclass (*Rubrivivax gelatinosus, Rhodoferax fermentans,* and *Rhodocyclus tenuis*) and to the γ subclass (*Chromatium vinosum* and *Thiocystis gelatinosa*) are positioned among members of the α-purple bacteria, in contrast to their positions in phylogenetic trees based on 16S rRNA (Fig. 2A) and soluble cytochrome *c* (Fig. 2B).

Discussion

The positions of species in the β and γ subclasses are clearly different in the phylogenetic trees based on the photosynthetic proteins from those of the 16S rRNA and cytochrome *c*. The major differences between phylogenetic trees derived from the different macromolecules found in the present study appear unlikely to be explained by methodological errors or convergent evolution, because the tree based on the photosynthetic proteins placed all the tested species in the β and γ subclasses among species in the α subclass as clusters conserving the tree topology. As suggested in our previous work for *Rvi. gelatinosus* (Nagashima et al. 1992, 1993b, 1994), these data are difficult to explain without invoking the idea of a horizontal transfer of photosynthetic genes between an ancestral species in the α subclass and in the β and γ subclasses.

As alternative explanations for these kinds of differences in phylogenetic trees, it has been suggested that substitutional biases in nucleotide sequences can cause the incorrect estimation of phylogenetic relationships, particularly between organisms that diverged long ago (Lockhart et al. 1992; Lockhart and Penny 1992). Differences in G+C content are thought to contribute significantly to such bias. Although the DNAs of the species used in this study have a high variation in G+C content (56–72.4 mol%), the values for the nucleotide sequences of the 16S rRNA are relatively low and constant (52.6–58.1%). The variation in G+C content is relatively high in the nucleotide sequences of genes coding for the L and M subunits (52.8–65.9%) compared to those of the 16S rRNAs. Such differences in G+C content could contribute to minor inconsistencies of branching topology in

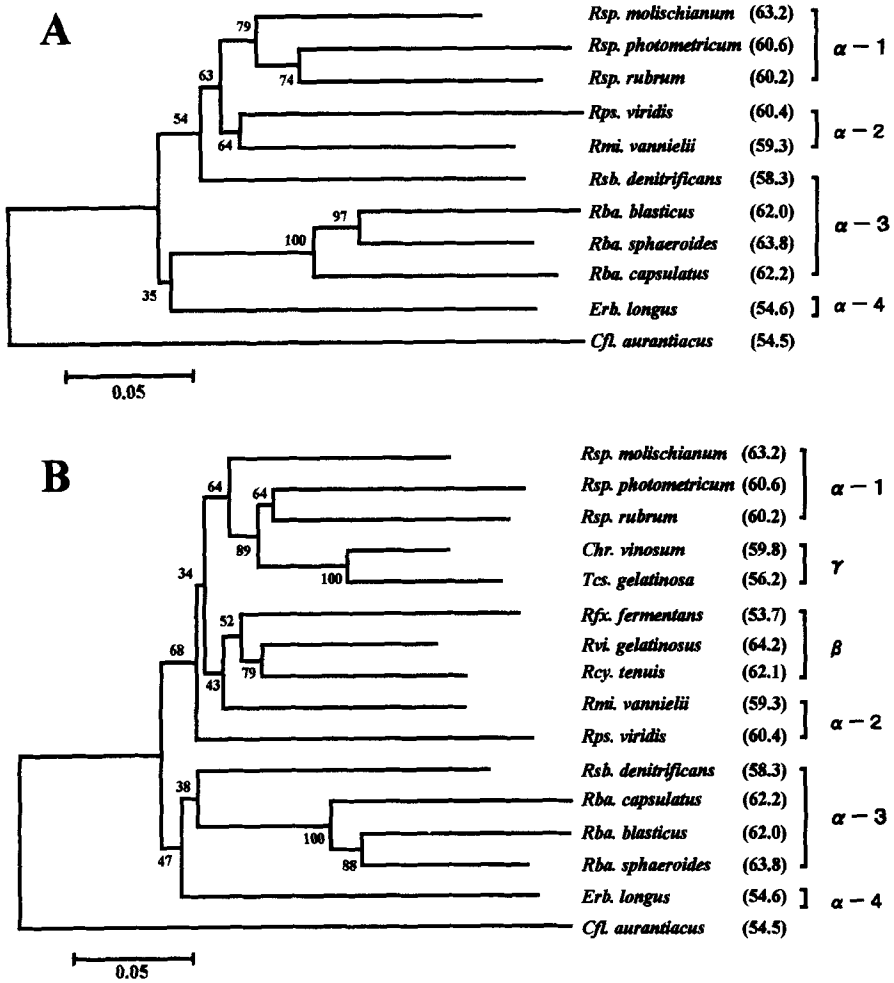

Fig. 3. Phylogenetic trees based on the nucleotide sequence of the 1.5-kb DNA fragment coding for the L and M subunits of the photochemical reaction center of purple bacteria. **(A)** Species belonging to the α subclass were chosen. **(B)** All species used in this study were included. The G+C content of the 1.5-kb DNA fragment of each species is shown in parenthesis. Bootstrap values are presented at the corresponding nodes. The accession numbers of the sequences used for the construction of the phylogenetic trees are as follows: *Rps. viridis*, X03915; *Rsp. molischianum*, D50654; *Rvi. gelatinosus*, D16822; *Rsb. denitrificans*, X57597; *Rba. sphaeroides*, M10206 and K00827; *Rba. capsulatus*, Z11165; *Rsp. rubrum*, J03731; *Cfl. aurantiacus*, X07847 and X14979. The accession numbers of the sequences determined in this study are shown in Materials and Methods.

some subgroups. However, there are no special similarities in G+C content of the L and M subunits among α-1, -2, β, and γ subclasses. Another possible factor that could affect tree topology is a bias resulting from functional evolutionary pressures in specific species. *Roseobacter denitrificans* and *Erythrobacter longus* produce photosynthetic apparatuses only under aerobic conditions, while the other species listed in this study are able to grow photosynthetically under anaerobic conditions. This means that photosynthetic apparatuses of these two species are not essential for any modes of their growth. *Rsb. denitrificans* is distantly positioned in the phylogenetic trees of photosynthetic genes, compared to its positions in the trees of 16S rRNA and soluble cytochrome *c*.

Thus, the functional evolutionary pressures and/or the differences in G+C content can hardly explain the major differences between the phylogenetic trees based on the photosynthetic genes and those on the 16S rRNA and soluble cytochrome *c*. The minor inconsistencies of branching topology can be explained with these factors and some other adaptive changes without introducing the idea of horizontal gene transfer. Thus, the present results imply that horizontal gene transfer probably once (or twice) occurred between an ancestral species of the α subclass, probably α-1 or -2, and an ancestor of the β and γ subclasses.

There are two possibilities for the direction of horizontal transfer of the photosynthetic genes. One is that the genes were transferred from an ancient species of the α subclass—possibly a relative of a *Rhodospirillum* or *Rhodomicrobium* species—to an ancestor of the β and γ subclasses. The other possibility is that the opposite transfer occurred. Since the branches of species belonging to the β and γ subclasses show lengths similar to those of the species of the α-subclass, the former appears likely to have occurred. Nevertheless, a definitive determination of the direction of horizontal gene transfer must await nucleotide sequence information on more species.

Horizontal gene transfer between bacterial species has been reported, for example, for the genes encoding endoglucanase (Guiseppi et al. 1991), nonspecific acid phosphatase (Groisman et al. 1992), and O antigen (Reeves 1993). These studies have been based on the discontinuity of G+C content and/or unexpected high homology between genes from distantly related species. In the present study, we present evidence for the horizontal transfer of photosynthetic genes in purple bacteria, based on clustered shifts of distantly related species in the phylogenetic trees of photosynthetic proteins in comparison to trees based on 16S rRNA and soluble cytochrome *c*. Blankenship (1992) have suggested that horizontal transfer of photosynthetic genes occurred between

purple photosynthetic bacteria and the green filamentous bacterium, *Cfl. aurantiacus*. Our data suggest, however, the *Cfl. aurantiacus* should be positioned outside the purple bacteria when the genes coding for the L and M subunits are used to construct the evolutionary tree. It is likely that no horizontal transfer of reaction center genes has been occurred between purple bacteria and *Chloroflexus*.

There are five classes that include photosynthetic species within Bacteria (formerly Eubacteria) (Woese 1987). Two of the five classes, green sulfur bacteria and Gram-positive bacteria, contain photosynthetic species having reaction center complexes that resemble that of the photosystem 1 of chloroplasts. In two of the remaining classes, purple bacteria and green filamentous bacteria, photosynthetic species have reaction centers that resemble the photosystem 2 reaction center except for an ability to generate oxygen. The two types of reaction centers show very low levels of identity in their primary structures, suggesting that they may have diverged very early in the evolutionary course or evolved independently. On the other hand, a phylogenetic tree derived from 16S rRNA sequences does not clearly divide these two groups. Cyanobacteria have both types of reaction center and evolved the ability to generate oxygen. The appearance of two types of reaction centers in one organism in the evolution of the photosynthesis can be explained by horizontal gene transfer. Horizontal gene transfer may be a rare phenomenon, but evidently must have occurred in this case.

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References

Allen JP, Feher G, Yeates TO, Komiya H, Rees DC (1987) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: the protein subunits. Proc Natl Acad Sci USA 84:6162–6166

Ambler RP, Daniel M, Hermoso J, Meyer TE, Bartsch RG, Kamen MD (1979) Cytochrome c_2 sequence variation among the recognized species of purple nonsulphur photosynthetic bacteria. Nature 278: 659–660

Ambler RP, Meyer TE, Kamen, MD (1979) Anomalies in amino acid sequences of small cytochrome *c* and cytochrome *c'* from two species of purple photosynthetic bacteria. Nature 278:661–662

Blankenship RE (1992) Origin and early evolution of photosynthesis. Photosynth Res 33:91–111

Büttner M, Xie D-L, Nelson H, Pinther W, Hauska G, Nelson N (1992) Photosynthetic reaction center genes in green sulfur bacteria and in photosystem1 are related. Proc Natl Acad Sci USA 89:8135–8139

Deisenhofer J, Epp O, Miki K, Huber R, Michael H (1985) Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3Å resolution. Nature 318:618–624

Deisenhofer J, Michel H (1989) The photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis*. EMBO J 8:2149–2170

Dickerson RE (1980) Evolution and gene transfer in purple photosynthetic bacteria. Nature 283:210–212

Groisman EA, Saier Jr MH, Ochman H (1992) Horizontal transfer of a phosphatase gene as evidence for mosaic structure of the *Salmonella* genome. EMBO J 11:1309–1316

Guiseppi A, Aymeric JL, Cami B, Barras F, Creuzet N (1991) Sequence analysis of the cellulase-encoding *celY* gene of *Erwinia chrysanthemi:* a possible case of interspecies gene transfer. Gene 106:109–114

Higgins DG, Beasby AJ, Fuchs R (1992) CLUSTAL V: improved software for multiple sequence alignment. Comp Appl Biosci 8:189–191

Hiraishi A, Ueda Y (1994a) Intragenetic structure of the genus *Rhodobacter:* transfer of *Rhodobacter sulfidophilus* and related marine species to the genus *Rhodovulum* gen. nov. Int J Syst Bacteriol 44:15–23

Hiraishi A, Ueda Y (1994b) *Rhodoplanes* gen. nov., a new genus of phototrophic bacteria including *Rhodopseudomonas rosea* as *Rhodoplanes roseus* comb. nov. and *Rhodoplanes elegans* sp. nov. Int J Syst Bacteriol 44:665–673

Hiraishi A (1994) Phylogenetic affiliations of *Rhodoferax fermentans* and related species of phototrophic bacteria as determined by automated 16S rDNA sequencing. Curr Microbiol 28:25–29

Hiraishi A (1992) Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett Appl Microbiol* 15:210–213

Kumar S, Tamura K, Nei M (1993) MEGA: Molecular Evolutionary Genetics Analysis, version 1.0, The Pennsylvania State University, University Park, PA 16802

Liebl U, Mockensturm-Wilson M, Trost JT, Brune DC, Blankenship RE, Vermaas W (1993) Single core polypeptide in the reaction center of the photosynthetic bacterium *Heliobacillus mobilis:* structural implications and relations to other photosystems. Proc Natl Acad Sci USA 90:7124–7128

Lockhart PJ, Penny D, Hendy MD, Howe CJ, Beanland TJ, Larkum AWD (1992) Controversy on chloroplast origins. FEBS Lett 301:127–131

Lockhart PJ, Penny D (1993) The problem of GC content, evolutionary trees and the origins of Chl-*a/b* photosynthetic organelles: are the procholorophytes a eubacterial model for higher plant photosynthesis? In: Murata N (ed) Research in Photosynthesis, Volume 3. Kluwer Academic Publishers, Dordrecht, pp 499–505

Michel H, Weyer KA, Gruenberg H, Dunger I, Oesterhelt D, Lottspeich F (1986) The 'light' and 'medium' subunits of the photosynthetic reaction centre from *Rhodopseudomonas viridis:* isolation of the genes, nucleotide and amino acid sequence. EMBO J 5:1149–1158

Nagashima KVP, Matsuura K, Shimada K (1992) Gene structure of the reaction center of *Rhodocyclus gelatinosus*. In: Murata N (ed) Research in photosynthesis, Volume 1. Kluwer Academic Publishers, Dordrecht, pp 365–368

Nagashima KVP, Itoh S, Shimada K, Matsuura K (1993a) Photooxidation of reaction center-bound cytochrome *c* and generation of membrane potential determined by carotenoid band shift in the purple photosynthetic bacterium, *Rhodospirillum molischianum*. Biochim Biophys Acta 1140:297–303

Nagashima KVP, Shimada K, Matsuura K (1993b) Phylogenetic analysis of photosynthetic genes of *Rhodocyclus gelatinosus:* Possibility of horizontal gene transfer in purple bacteria. Photosynth Res 36: 185–191

Nagashima KVP, Matsuura K, Ohyama S, Shimada K (1994) Primary structure and transcription of genes encoding B870 and photosynthetic reaction center apoproteins from *Rubrivivax gelatinosus*. J Biol Chem 269:2477–2484

Reeves P (1993) Evolution of *Salmonella* O antigen variation by interspecific gene transfer on a large scale. Trends Genet 9:17–22

Williams JC, Steiner LA, Feher G, Simon MI (1984) Primary structure of the L subunit of the reaction center from *Rhodopseudomonas sphaeroides*. Proc Natl Acad Sci USA 81:7303–7307

Woese CR, Gibson J, Fox GE (1980) Do genealogical patterns in purple photosynthetic bacteria reflect interspecific gene transfer? Nature 283:212–214

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271