(data not shown). Therefore, whereas SQDG-deficiency arrested the growth of SD1 cells, the *de novo* biosynthesis of DNA and chlorophyll were not completely inhibited. These results suggested some novel function of SQDG in the cell separation of *Synechocystis* PCC6803. The interaction of SQDG with the cell separation mechanism remains to be clarified at the molecular level.

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CHOROFLEXUS AURANTIACUS AND THE ORIGIN OF OXYGENIC, TWO-LIGHT REACTION PHOTOSYNTHESIS IN FAILURE TO SWITCH BETWEEN TYPE I AND TYPE II REACTION CENTRES

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Keywords: Photosynthetic bacteria, cyanobacteria, redox regulation, gene expression, oxygen evolution, cell evolution, photosystem I, photosystem II

Two types of light reaction cooperate to give oxygen evolution in plant and algal chloroplasts and cyanobacteria: photosystem I produces soluble reductant, and photosystem II passes electrons on to photosystem I by removing electrons and protons from water, thus liberating oxygen (Hill & Bendall 1960). In anaerobic, photosynthetic bacteria, only one light reaction drives electron transport, and water is not oxidised to oxygen (Van Niel 1954). Primary electron transfer in photosynthesis occurs in reaction centres of one of two types, termed I and II (Pierson & Olson 1987). Type I reaction centres are found in photosystem I and in "green" bacteria such as *Chlorobium* species and heliobacteria (Baymann et al 2001, Hauska et al 2001, Nitschke & Rutherford 1991). Type I centres have iron-sulphur electron acceptors, and contain a large membrane-extrinsic domain to carry electrons out into the soluble phase, to ferredoxin and $NADP^+$. Type II reaction centres, in contrast, are found in water-oxidising photosystem II, and in the "purple" photosynthetic proteobacteria (Heathcote et al 2002, Nitschke & Rutherford 1991, Rutherford & Faller 2003). Type II centres have quinone electron acceptors, and, in bacteria, participate in cyclic electron transport. by passing electrons to a proton-motive cytochrome b-c_1 complex that is shared with respiratory electron transport. Electrons from type II centres either move back the donor side, giving the bacterial, cyclic pathway, or move on to the donor side of the type I reaction centre of photosystem I.

From structural studies it is now clear that type I and type II photosynthetic reaction centres had a common ancestor (Blankenship 2002, Olson & Blankenship 2004). We propose that the prototype reaction centre shared the properties of both, being able to supply electrons as reduced quinone species into a proton-pumping complex (type II behaviour) shared with a respiratory chain as well to reduce soluble electron acceptors, via ferredoxin, using electrons supplied from a donor such as H_2S (type I behaviour). These two functions are required under different environmental conditions. Photoautotrophy requires net reduction of CO_2, and is typical of photosynthesis using type I centres, becoming possible when an environmental electron donor such as H_2S is readily available, although electrons from ferredoxin may also be fed back into a respiratory-type protonmotive quinone-cycle. Thus light energy is used predominantly for net oxidation-reduction in organisms with type I centres only, and ATP synthesis is coupled indirectly. In contrast, type II centres serve to pump protons, drive ATP synthesis, and, where they drive autotrophic metabolism, they do so by use of ATP and the proton motive force to reduce CO_2 at the expense of organic electron donors such as succinate, using reverse respiratory electron transport.

Clearly there is competition between the functions of type I and type II reaction centres, and, if the prototype reaction centre had both functions, there would have been selective advantage in specialisation for one or other function, according the availability of H_2S. The green filamentous bacterium *Chloroflexus aurantiacus* (Pierson & Castenholz 1974) is commonly supposed to possess only type II centres, but lives in environments with varying H_2S supply (Pierson & Castenholz 1974, 1995). We find that the draft genome of *Chloroflexus aurantiacus* contains contigs with the sequence of the *pscB* gene for the protein containing the Fe_4S_4 clusters A and B that function as secondary electron acceptors in type I-containing bacteria such *Chlorobium*, and in photosystem I (Fig. 1A). Although

A

```
>caur_25may01_Contig1085_revised_gene1417 Length=212
 Score=32.0 bits (71), Expect=0.005
 Identities=15/30 (50%), Positives=17/30 (56%), Gaps=1/30 (3%)

Query: 7  LCVGCGLCLDKCPPKVNAIGYKFYGDVQEG 36
          LC+ CG CL+ C P   AIG   YG V  G
Sbjct: 45 LCIRCGACLNAC-PVYQAIGGHAYGGVYSG 73
           FeS binding motif

 Score=21.6 bits (44), Expect=6.3
 Identities=8/20 (40%), Positives=10/20 (50%), Gaps=2/20 (10%)

Query: 41 YIDQAACISCSACFSGDECP 60
          Y +   CI C AC +   CP
Sbjct: 40 YAESLLCIRCGACLNA--CP 57
                FeS binding motif

>caur_25may01_Contig1075_revised_gene1189 Length=466
 Score=30.8 bits (68), Expect=0.010
 Identities=24/78 (30%), Positives=32/78 (41%), Gaps=27/78 (34%)

Query: 4  IENLCVGCGLCLDKCPPKVNAIGYKFYG-DVQEGGFRCYIDQAA---------------- 46
          I N CV CGLCL  CP       Y+  G ++     R Y+ +A
Sbjct: 30 IINTCVHCGLCLSSCPT------YRETGLEMASPRGRIYLMKAVDEGRISLASEVFQEQM 83
             FeS binding motif

Query: 47 --CISCSACFSGDECPSG 62
            C++C AC +   CPSG
Sbjct: 84 SLCLNCRACEA--VCPSG 99
           FeS binding motif
```

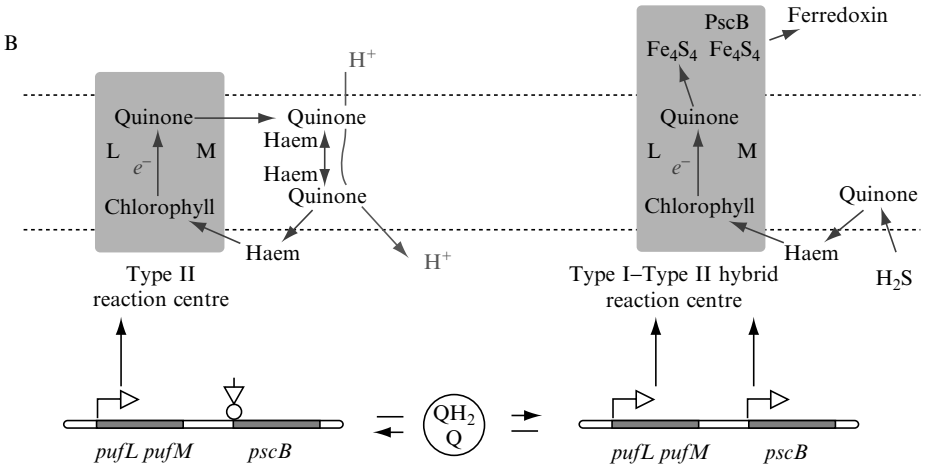

Figure 1: (A) Sequence alignment of the Fe_4S_4-binding motif of the type I electron acceptor protein PscB (Hauska et al 2001) of *Chlorobium tepidum* (Eisen et al 2002) with amino acid sequences predicted from named contigs in the draft genome of the type II-containing filamentous bacterium *Chloroflexus aurantiacus*. (B) Scheme for inducible PscB in *Chloroflexus aurantiacus*, adding PscB function and linear electron transport to a type II reaction centre and thus to sustain transient photoautolithtrophic growth in the presence of hydrogen sulphide. With H_2S, and upon reduction of the quinone pool, the *pscB* gene is upregulated, allowing photooxidation of H_2S to elemental sulphur and generation of soluble reductant as reduced ferredoxin. The type I core reaction proteins are absent, and the type II core reaction centre proteins L and M are constitutively synthesised in the light.

Chloroflexus aurantiacus grows best under photoheterotrophic conditions in the laboratory (Pierson & Castenholz 1995), it seems likely from its novel, predicted PscB protein, with two Fe_4S_4-binding motifs CXXCXXCXXXCP, that the *Chloroflexus* reaction centre is capable of both type I and type II behaviour. This would explain why *Chloroflexus* in natural environments is a facultative photoautolithotroph. We sugget that H_2S availabiliy is the main environmental factor initiating a quinone-level redox signal that determines which reaction centre and mode of metabolism prevails (Fig. 1B).

While such metabolic flexibility may be advantageous in environments with fluctuating supplies of H_2S, such as hot springs and in the vicinity of marine hydrothermal vents, most species of single-photosystem, anaerobic phototrophs seem to have opted for specialisation to type I (lithoautotrophic) or type II (chemoheterotrophic) behaviour. However, evolutionary divergence of type I and type II reaction centres need not have required loss of the complementary reaction centre and its genes (Pierson & Olson 1987). We propose that diverge also occurred within a single lineage of cells, giving division of labour an adaptation to different environemntal condition (Fig. 2).

In such an organism, a mechanism for water oxidation may have arisen as a sluggish reaction serving to maintain redox poise of type II cyclic electron transport. However, any mutation producing constitutive expression of both type I and type II genes would then

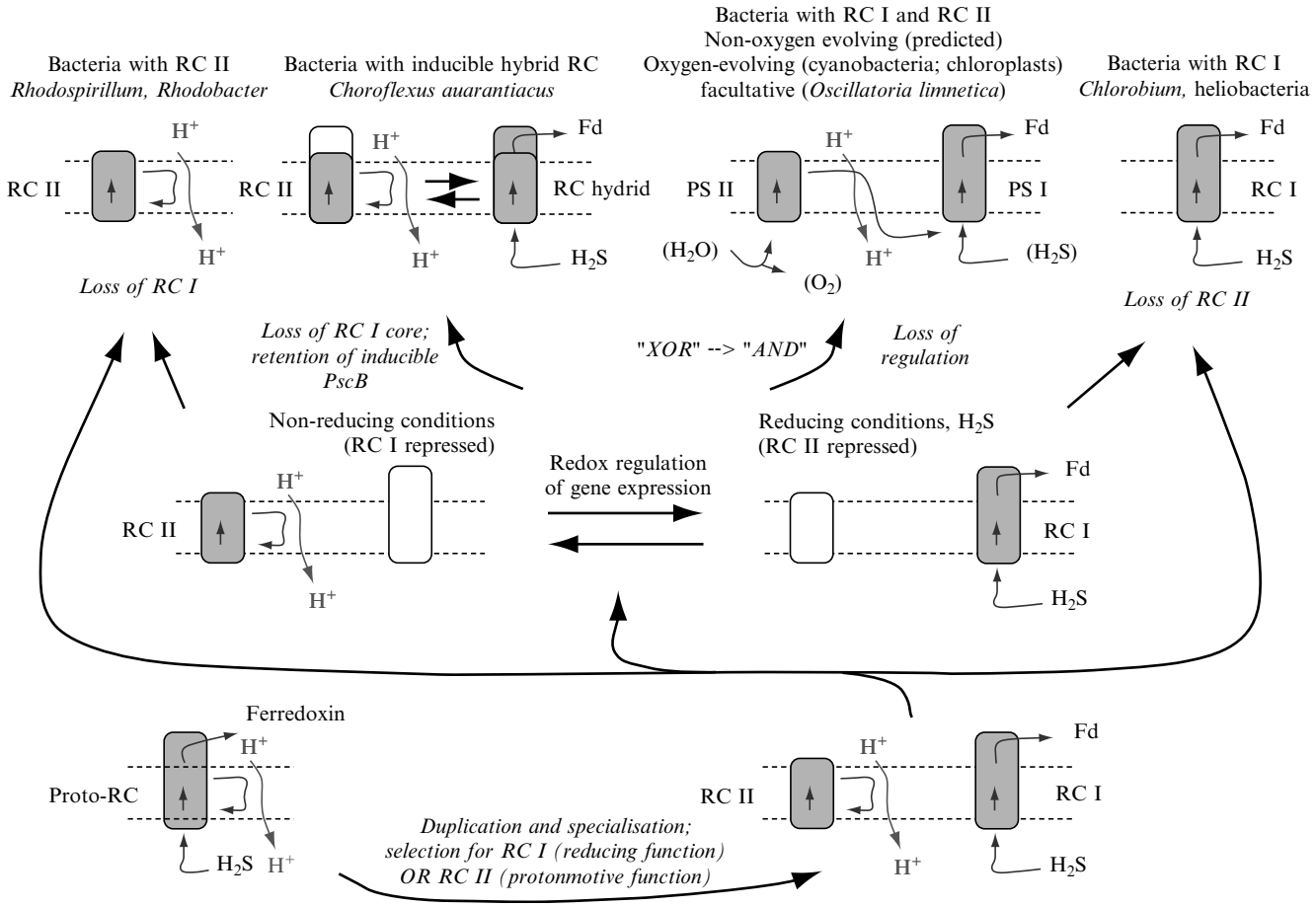

Figure 2: Separation of type I and type II reaction centres, allowing specialisation with eventual loss of each redundant reaction centre in photoautolithotrophic (type I-containing) lineages (e.g., *Chlorobium*, *Heliobacillus* spp.) and in photoheteroorganotrophic (type II-containing) lineages (e.g., *Rhodobacter*, *Rhodospirillum* spp.). *Chloroflexus* retains genes for a type I Fe_4S_4 acceptor side in the absence of a type I core, and is proposed (Fig. 1) to retain the capability of making a hybrid reaction centre in response to a redox signal initiated by H_2S. Retention of genes for both type I and type II reaction centres is proposed in another versatile, facultatively chemoautotrophic, photosynthetic bacterium, where expression of type I centre genes in the presense of H_2S is accompanied by silent type II genes, themselves induced under the non-reducing conditions where type I genes become repressed. Loss of regulatory control by a simple mutation is allows co-existence of type I and type II reaction centres, with complementary functions: in place of H_2S, the type II centre, as photosystem II, oxidises water, liberating oxygen, and donating electrons to the type I centre, as photosystem I. The cyanobacterium *Oscillatoria limnetica* switches between ''type I'' and ''type I plus type II'' photosynthesis, since it exhibits sulphide-dependent repression of photosystem II (Oren & Padan 1978). Developmental repression of photosystem II is also widely distributed, as seen in cyanobacterial heterocysts, and the bundle sheath chloroplasts of C_4 plants.

provide new functions for the two reaction centres, because of their coupling to a single quinone pool. The acceptors for the type II centre, oxidising water, became the donors for the type I centre, reducing ferredoxin. This coupling provided the these bacteria with the advantages of both modes of photosynthesis (ATP synthesis and reduction of soluble electron carriers), and released them from dependency on H_2S for autolithotrophic growth. We propose (Fig. 2) that the origin of the ''Z-scheme'' of two light reactions, coupled in series, occurred by these means.

The appearance of oxygen-evolving photosynthesis at 2.3 to 2.6 Gya was probably the most profound and far-reaching event in the history of life in Earth (Lane 2002). Redox control of gene expression has been conserved in cell evolution and may explain the retention of cytoplasmic genomes in eukaryotes (Allen 2003).

Our hypothesis of an origin for the Z-scheme in a failure to switch between type I and type II reaction centres has many testable predictions, chief of which is the existence of a two-light reaction, phototrophic, anaerobe that retains the capacity to switch between sulphide-oxidising, lithoautotrophic, type I photosynthesis, and sulphide-independent, chemoheterotrophic, type II photosynthesis. *Chloroflexus aurantiacus* appears to have genes only for type II reaction centre core proteins (PufL and PufM) and not for type I cores (PscA), but its otherwise surprising retention of the type I electron acceptor PscB (homologous with PsaC of cyanobacterial and chloroplast photosytem I) strengthens the case for facultative type I and type II photosynthesis in an anaerobic bacterium, either yet undiscovered, or not fully characterised in terms of its photosynthetic adaptability.

METHODS

The *Chloroflexus aurantiacus* genome database, housed by the Oak Ridge National Laboratory (ORNL) Computational Biology Group was searched using the BLAST service from the same computational group: http://maple.lsd.ornl.gov/microbial/caur/

The search used the BLAST program protein-protein Blast: (BLASTP).

The Query sequence used was the C-terminal stretch of the *Chlorobium tepidum pscB* gene product, which contains the conserved Fe_4S_4 binding motifs. The *Chorobium tepidum pscB* gene sequence was taken from the complete genome (Eisen et al 2002).

Sequences denoted in Fig. 1A as subjects are hits recovered from the *Chloroflexus aurantiacus* database: http://genome.ornl.gov/cgi-bin/Blast/blastform.cgi?blastorgs=caur

The first two alignment are from the same contig, but from different positions and are aligned with first and second Fe_4S_4 binding motifs of *Chlorobium* PscB respectively. The third alignment contains both Fe_4S_4 motifs in a continuous sequence and is from a different contig.

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DISTRIBUTION OF GREEN SULFUR BACTERIA TYPE FNR IN VARIOUS BACTERIA AND ARCHAEA

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Keywords: ferredoxin, thioredoxin, ferredoxin-$NADP^+$ reductase, green sulfur bacteria, *Bacillus subtilis*

INTRODUCTION

Ferredoxin-NAD(P)H oxidoreductase (FNR) catalyzes the electron transfer between ferredoxin (flavodoxin) and NAD(P)H. FNR is present not only in phtosynthetic organisms but in non-photosynthetic ones participating in nitrogen assimilation, reduction of cyt P450 etc. Plant-type FNR contained in cyanobacteria and bacteria-type FNR contained in *Escherichia coli* and *Azotobacter vinelandii* have been related in their 3-D structure and amino acid sequence. These two types of FNRs can be, in a broader sense, classified as the plant-type FNR (Ceccarelli et al 2004). Adrenodoxin reductase and FNR from the bacterium *Mycobacterium tuberculosis* (Fischer et al 2002) have a similarity in the certain amino acid sequence with the plant- and bacteria-type FNRs. However, 3-D structure of adrenodoxin reductase resembles to those of glutathione reductases to a certain extent (Ziegler et al 1999).

In the library of the whole genome sequences of many bacterial species, genes similar to that of FNRs has not been found. Recently, we reported the occurrence of a novel type of FNRs in the green sulfur bacterium *Chlorobium tepidum* (Seo & Sakurai 2002) and gram positive bactrium *Bacillus subtilis* (Seo et al 2004). These enzyme share high amino acid sequence similarity with NAD(P)H-thioredoxin reductases, although they lack the di-cysteine motif essential to thioredoxin reduction. According to BLAST search genes homologous to these FNRs are not present in gram positive bacteria and archaea. We propose that *C. tepidum* FNR and *B. subtilis* FNR should be classified as a new category of FNR in addition to plant-type, bacteria-type, and mitochondria-type FNRs.

MATERIALS AND METHODS

Isolation of ferredoxin NADPH reductases. *C. tepidum* TLS culture was kindly provided by Dr. M. T. Madigan of Southern Illinois University and *B. subtilis* 168 by Drs. K. Kobayashi and N. Ogasawara of NAIST, Japan. Purification procedures were described before (Seo & Sakurai 2002, Seo et al 2004).

Enzyme activity. Affinity to NAD(P)H were determined by diaphorase activities with 2,6-dichlorophenol-indophenol (DCPIP) as electron acceptor. NAD(P)H oxidase activity was detected by the oxidation of NAD(P)H in an air-saturated buffer. Molecular mass was determined by SDS-PAGE and gel-permeation chromatography with a Superdex 200 HR 10/30 column.