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# NOTES

## Correction of the DNA Sequence of the *regB* Gene of *Rhodobacter capsulatus* with Implications for the Membrane Topology of the Sensor Kinase RegB

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**We corrected the previously published sequence for the *regB* gene, which encodes a histidine sensor kinase in *Rhodobacter capsulatus*. The deduced RegB amino acid sequence has an additional putative transmembrane domain at the N terminus. Analysis of RegB-PhoA and RegB-LacZ fusion proteins supports a topology model for RegB with six membrane-spanning domains.**

Facultatively phototrophic bacteria, such as *Rhodobacter capsulatus*, perform aerobic respiration as long as oxygen is available. A decrease in oxygen tension below a threshold value results in the formation of a photosynthetic apparatus which is used to generate energy through anoxygenic photosynthesis (6). This demands the coordinated expression of many genes encoding pigment-binding proteins, enzymes for pigment synthesis, and regulatory proteins. A two-component system comprising the sensor kinase RegB and the response regulator RegA is involved in oxygen-dependent regulation of photosynthesis genes in *R. capsulatus* (reviewed in references 2 and 3). Dependent on the external oxygen signal, RegB undergoes autophosphorylation at a histidine residue and in turn phosphorylates an aspartate residue of RegA (13, 22). RegA binds to DNA sequences upstream of the *puf* and *puc* promoters and activates transcription (7, 11, 16). The *puf* and *puc* operons encode pigment-binding proteins of the reaction center and of the two light-harvesting complexes and are part of a cluster of photosynthesis genes (20). It is not known which signal (molecular oxygen or the redox state of other cellular components) is sensed by RegB and what the mechanism of sensing is. In order to learn more about the mechanism of sensing it is important to determine the membrane topology of RegB. Mosley et al. (22) suggested a model with five membrane-spanning regions for this sensor kinase based on the DNA sequence they published. In this model the N terminus of RegB is placed in the periplasm, which is an uncommon topology among bacterial sensor kinases.

**Correction of the sequence of the *regB* gene.** In an attempt to test the model of Mosley et al. (22), we constructed a number of *regB-lacZ* and *regB-phoA* fusions. During sequencing of these constructs we noticed an additional C in the 5′ region of *regB*, which was missed in the sequence published previously. If translation of the *regB* gene started at the ATG proposed by Mosley et al. (22), the addition of the C would result in a reading frame shift and the deduced amino acid sequence would no longer be similar to bacterial sensor kinases. Indeed, the paper of Mosley et al. (22) did not present any experimental evidence for the translational start of RegB, and the ATG selected by these authors is not preceded by a strong ribosome binding site. In the corrected RegB sequence, four ATG codons could function as translational start site, all of them upstream of the ATG proposed by Mosley et al. In order to determine the start site of RegB translation, we fused DNA sequences which extended to different positions of the putative RegB-coding sequence to the *lacZ* gene and quantified the β-galactosidase activity of the fusion proteins in *R. capsulatus*. The positions of the fusions and the β-galactosidase activities are shown in Fig. 1. Our data strongly suggest the start of translation of RegB to be at the two adjacent ATGs (Fig. 1). The putative ribosome binding site for the two ATGs was compared to the ribosome binding sequence which was deduced from the *R. capsulatus* 16S rRNA sequence. When we compared the deduced amino acid sequence of the corrected *regB* gene to that of the *R. sphaeroides* homologue *prrB* (9), it became obvious that the correction extends the similarity between the two proteins to the N terminus (Fig. 1).

Furthermore, when we cloned a DNA fragment harboring the *regB* sequence with the additional C into the *Kpn*I-*Ecl*136 sites of plasmid pRK415 (15) and transferred the resulting construct, pRK4RegB1, by conjugation (17) into the *regB* mutant strain CSM01 (22), spectral analysis of extracts from equal amounts of cells revealed that plasmid pRK4RegB1 was able to restore wild-type levels of photosynthetic complexes the strain (data not shown). This demonstrates that the additional C nucleotide was not an artifact of PCR amplification.

**Implication of the corrected RegB sequence for its orientation in the membrane.** By the addition of the C nucleotide the predicted sequence of the RegB protein was extended by 19 amino acids (Fig. 1). When the complete corrected RegB sequence was analyzed, some computer programs (HMMTOP [26], TopPred 2 [27], ProtScale [18], and Split [14]) predicted six membrane-spanning segments with the N terminus in the cytoplasm (Fig. 2), although some others (PHDhtm [10] and Sosui [25]) predicted five membrane-spanning segments with the N terminus in the periplasmic space. All computer programs give similar results for the prediction of four transmembrane domains extending from positions 28 ± 2 to 47 ± 2, 56 ± 2 to 75 ± 1, 131 ± 1 to 150 ± 3, and 163 ± 2 to 186 ± 1

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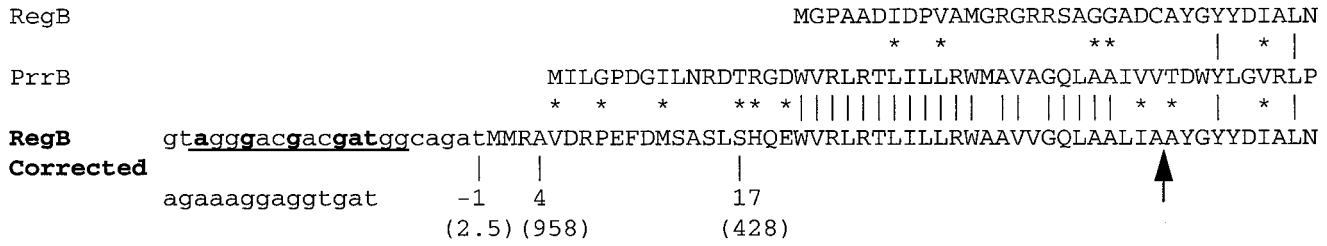


FIG. 1. Alignment of the amino acid sequences of the RegB protein from *R. capsulatus* as previously published (22), of the RegB protein as deduced from our corrected DNA sequence, and RegB homologue PrrB (9) of *R. sphaeroides*. The positions of fusion to the *lacZ* gene and the β-galactosidase activities of the fusion proteins (where synthesized) in *R. capsulatus* are indicated. The strategy for construction of the gene fusions is identical to the strategy used for the other *regB-lacZ* fusions as described in the text and shown in Fig. 2. Vertical lines identify identical amino acids, and asterisks indicate similar amino acids. The putative ribosome binding site is underlined.

(positions refer to amino acid residues of the corrected RegB sequence as shown in Fig. 2).

**Analysis of the membrane topology of RegB.** In order to test this model, we used the approach of studying RegB-PhoA and RegB-LacZ fusion proteins (8, 19). For creation of the RegB-PhoA fusions we cloned the 1.4-kb *Bam*HI fragment from plasmid pSWFII (8) comprising the *phoA* gene into the *Bam*HI site of plasmid pRK415 (15). We amplified DNA fragments comprising 36 nucleotides upstream of the new *regB* start codon and sequences of different lengths from the 5′ part of *regB* by PCR using plasmid pRK4RegB1 as a template. The PCR primers introduced new restriction sites which were used to insert the *regB* fragments into the *Hin*dIII and *Xba*I sites of the pRK415 derivative containing the *phoA* gene. Our cloning strategy allowed the in-frame fusion of *regB* to *phoA* and transcription by the *lac* promoter of plasmid pRK415. The positions of the fusions between RegB and PhoA are indicated in Fig. 2.

For the construction of the RegB-LacZ fusions we amplified DNA fragments spanning the same sequences as used for the PhoA fusions. By using different primers for the PCR, we created different restriction sites which allowed cloning of the PCR products into the *Kpn*I and *Hin*dIII sites of plasmid pPHU236 (12). This cloning strategy created in-frame fusions to the *lacZ* gene. These constructs gave no β-galactosidase activity in *Escherichia coli* or *R. capsulatus*, indicating that no promoter for the *regB* gene is present on the amplified DNA fragment. In order to allow analysis of our fusion constructs as well in *R. capsulatus* as in *E. coli*, we cloned a PCR product containing the *aph* promoter (amplified from plasmid pUC4-KIXX [1] with primers 5′GAAAGCAGGTACCTTGCA and 5′CAGATCTGGTACCCCTGC) into the *Kpn*I sites of the pPHU236 derivatives harboring the *regB-lacZ* fusion genes.

We analyzed all *regB-phoA* fusions in *E. coli* strain CC118 (19), which does not harbor an endogenous alkaline phosphatase. RegB-PhoA fusion plasmids were also transferred into *R. capsulatus* strain 37b4, but only very low activities of alkaline phosphatase were detected (data not shown). This is surprising, since *prrB-phoA* fusions yielded even higher levels of alkaline phosphatase in *R. sphaeroides* than in *E. coli* and the *lac* promoter is known to be expressed in *R. capsulatus* (24) (expression of RegB from plasmid pRK4RegB1 is described in this paper). However, it was previously shown that the alkaline phosphatase activities in *E. coli* reflect the values obtained in

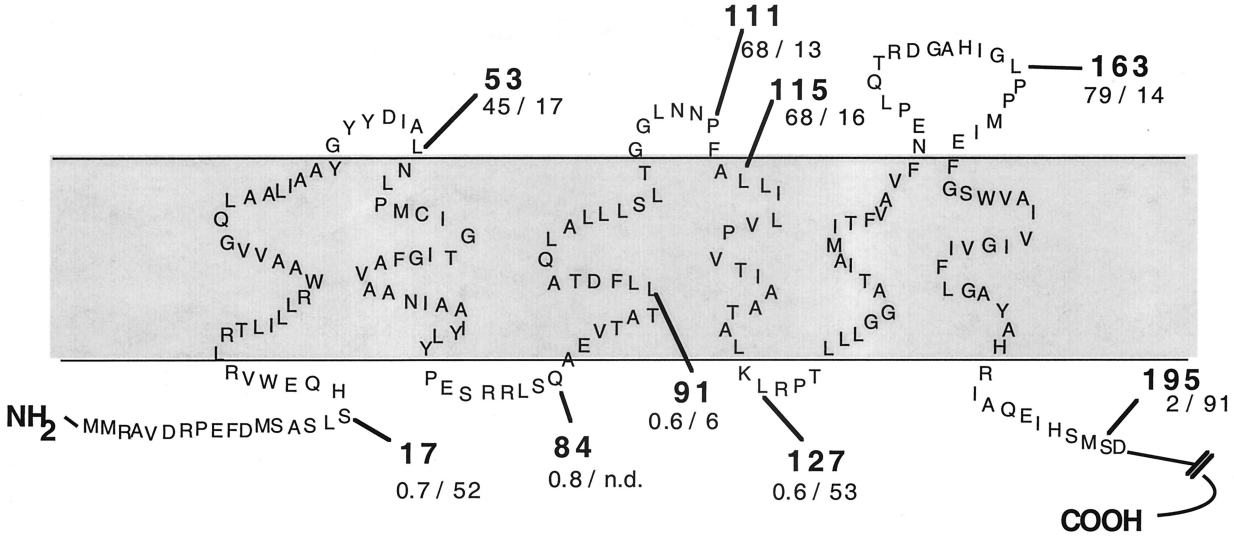


FIG. 2. Schematic representation of the membrane topology of the sensor kinase RegB. The bold numbers give the position of the last amino acid of RegB, which was fused to LacZ (first amino acid Ala) or to PhoA (first amino acid Ser). Two additional amino acids have been introduced by the cloning procedure. The units of activity for β-galactosidase and alkaline phosphatase in *E. coli* are given below the number for the fusion position. The activities measured in parental strains were subtracted, and average values from at least three measurements were calculated. *E. coli* cultures were grown semiaerobically in Luria-Bertani medium to late exponential phase. n.d., not determined.

*Rhodobacter* and that proteins fold in a very similar manner in both bacterial species (23). The *regB-lacZ* fusions were expressed in *E. coli* strain MC1061 [5] as well as in *R. capsulatus*. The activities determined for the different fusion proteins (4, 21) expressed in *E. coli* and *R. capsulatus* are given in Fig. 2. For fusion proteins with the β-galactosidase extending into the periplasm, the activities determined for *R. capsulatus* were in the same range as determined for *E. coli*. Fusion proteins with the β-galactosidase extending into the cytoplasm gave three- to ninefold-higher activity in *R. capsulatus* than in *E. coli*. Our results clearly support the model, which is in agreement with the topology analysis performed for the *R. sphaeroides* PrrB protein (23).

Our results are not in accordance with any alternative model, including the five-transmembrane-helix model proposed earlier (22), and strongly suggest that the membrane topologies of the *R. capsulatus* RegB protein and the *R. sphaeroides* PrrB protein show high similarity. These models now provide a basis for future experiments designed to determine the RegB domains involved in sensing of an oxygen-dependent signal.

**Nucleotide sequence accession number.** The corrected *regB* sequence has been assigned GenBank accession number AF189160.

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