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Plant Gene Register

A *ompR* Gene in the Plastid Genome of *Rhodella violacea*[1]

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The prokaryotic "two-component" regulatory systems consist of His-kinase sensors and their associated response-regulator proteins. Such systems are implied in the regulation of various processes such as chemotaxis, osmoregulation, sporulation, virulence, and responses to nitrogen, phosphorus, or oxygen depletion. They act through a common mechanism involving the detection of environmental changes by a sensor protein and the transfer of this information to the regulator protein via protein phosphorylation processes. The response-regulator family is defined by a conserved N-terminal domain of about 100 to 120 amino acids. Sequence alignments between the N-terminal domains of any two response regulators show 20 to 30% identity. The response regulators can be further classified into subfamilies based on sequence similarities of their C-terminal domains (for review, see Stock et al., 1989b). The *ompR* subfamily includes, for example, *ompR*, *phoP*, and *phoB*, implicated in osmoregulation and phosphate regulation. The gene product of each member of the *ompR* subfamily is a DNA binding protein, which mediates the interaction between DNA and the RNA polymerase. Their function is to regulate the expression of specific target genes at the transcriptional level.

We report the nucleotide sequence of an open reading frame (ORF_{246}) obtained from the plastid genome of the red alga *Rhodella violacea* (Table I). ORF_{246} was characterized from a 1.7-kb *Hin*dIII fragment, located 154 bp downstream of the *rpeBA* operon encoding the β and α subunits of phycoerythrin (Bernard et al., 1992) but transcribed on the opposite strand. ORF_{246} might encode a polypeptide of 246 amino acids. An AUG codon preceded by a putative GAAG ribosome binding site is found at nucleotide 163. However, based on the amino acid sequence homologies, this Met codon is located within the coding region of the ORF_{246} gene. Thus, we tentatively chose as a possible initiation codon the UUG located at nucleotide 100 and preceded by a putative GAGG ribosome binding site. UUG initiation codons were first identified by Thach et al. (1966) as being functional in *Escherichia coli* but with a much lower efficiency than AUG. Subsequently, they have been described in other species (Kozak, 1983). In the ORF_{246} a surprisingly long stretch of Asp residues follows the putative UUG initiation codon. Thus, we cannot exclude that the first 120 to 130 nucleotides in fact correspond to the 3′ extremity of an intron as found in the sequence of the *rpeB* gene of *R. violacea* (Bernard et al., 1992).

Table I. *Characteristics of the ORF_{246} gene from R. violacea*

Organism:
Rhodella violacea, rhodophyta.
Location in Genome:
Plastidial genome, downstream of the *rpeBA* operon encoding the β and α subunits of phycoerythrin.
Gene Function:
Encodes a putative DNA-binding protein of a two-component response-regulator system.
Techniques:
Partial DNA libraries constructed by ligation of *Hin*dIII plastid DNA fragments (1.5–2.5 kb) into the *Hin*dIII site of pTZ18R. The isolated clone was sequenced on both strands by the dideoxy chain-termination method.
Sequence Identification:
DNA and protein sequence comparisons with bacterial CheY, PhoP, OmpR, and rhodophytan OmpR.
Features of the Sequence:
UUG initiation codon.
Features of Predicted Amino Acid Sequence:
Open reading frame of 246 amino acids. Sequence and secondary structure similarities with response-regulator proteins.

The predicted amino acid sequence of the *R. violacea* ORF_{246} shares 45.1% identity with the *phoP* gene product from *Bacillus subtilis* (Seki et al., 1987), 37.1% with the *ompR* gene product from *E. coli* (Wurtzel et al., 1982), and respectively 75.9 and 68.4% with the OmpR protein from two atypical red algae, *Porphyridium aerugineum* and *Cyanidium caldarium* (Kessler et al., 1992). The presence of a *trsB* gene (*ompR*-like) has been mentioned on the chloroplast genome of *Porphyra purpurea* (Reith and Munholland, 1993). The precise function of the red algal *ompR* genes is still unknown. The *"ompR"* terminology, based on amino acid sequence homologies, does not preclude any osmotic regulatory function. It is interesting to note that such regulatory-response genes have not yet been identified in chloroplast genomes of higher plants (Sugiura, 1992).

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Active sites of proteins generally correspond to the most highly conserved domains in the amino acid sequences. Thus, we compared the amino acid sequences of a number of response-regulator proteins with the deduced sequence from the ORF_{246} gene. Stock et al. (1989a) have solved the three-dimensional structure of the CheY protein from *Salmonella typhimurium*, a response-regulator protein implicated in cell motility. This protein is composed of a central core of five parallel β-strands surrounded by five α-helices (Stock et al., 1989b). The active sites of this protein (Asp^{12}, Asp^{13}, and Asp^{57}), located at the C-terminal ends of $\beta1$ and $\beta3$ (Stock et al., 1989a), are also found in ORF_{246} and might correspond to the active sites of this protein.

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