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A Bacterial Analogue of the Mammalian Benzodiazepine Receptor in *Rhodobacter sphaeroides*

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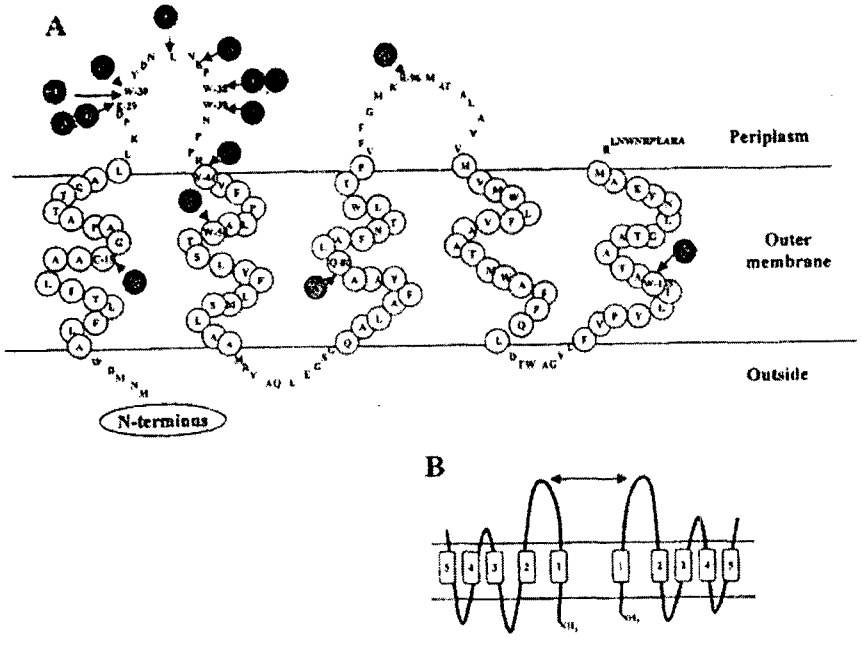
The Tryptophan Rich Sensory Protein (TspO) is encoded by the *tspO* gene, formerly designated *crtK* because of its location within the carotenoid (*crt*) biosynthetic gene cluster of *Rhodobacter sphaeroides*. This protein is approximately 17Kdal in size and contains approximately 9 Mole % L-Tryptophan and in addition it has a high content of Phenylalanine and Tyrosine. This protein is extremely hydrophobic and is predicted to be membrane-localized. It was subsequently demonstrated that the TspO protein is localized to the outer membrane of *R. sphaeroides* and through the analysis of a number of mutant organisms we have shown that it is not involved in the biosynthesis of carotenoids. Mutants lacking TspO were shown to accumulate carotenoids and bacteriochlorophyll during photosynthesis (PS) gene induction under conditions of low O_2 at a faster rate than the wildtype as the result of enhanced photosynthesis gene expression. Other than this observation there was no obvious phenotype of a TspO mutant. Thus, the question arose, how does an outer membrane protein affect the transcriptional regulation of PS genes? It was also revealed that the TspO protein is a member of a highly conserved family of proteins found in a numerous mammalian systems, which are referred to as the peripheral benzodiazepine receptors localized to the outer membrane of the mitochondrion, but nuclear encoded. This ancient family of proteins has now grown to include Drosophila, Arabadopsis, Synechocystis, etc.

This conservation of structure, e.g. rat protein and TspO (36% identity, 56% similarity) is further revealed to also include functional conservation. When the rat *c*DNA (pK18) is expressed in an *R. sphaeroides* mutant lacking TspO, the rat protein was found localized in the outer membrane of *R. sphaeroides* and it could function in the regulation of the same spectrum of downstream genes as the homologous bacterial protein. That is, the rat protein caused increased repression of the same genes as affected by TspO. In a collaboration with Dr. Karl Krueger of Georgetown University, it was observed that the rat protein in the outer membrane of *R. sphaeroides* could bind a series of benzodiazpines with the same K_d values as when present in rat mitochondria. Thus, the rat protein assumed the proper confirmation in the bacterial outer membrane as in the mitochondrion. To further understand the mechanism of action of TspO, and to gain further insight into the role of the mammalian protein, we began a study of porphyrin metabolism in *R. sphaeroides* since it has been suggested that the mammalian protein might be involved in porphyrin transport across the mitochondrial outer membrane. Thus, we examined the accumulation of various porphyrin intermediates in TspO plus/minus cells, and observed that TspO is

involved in the efflux of specific intermediates in tetrapyrrole biosynthesis. We further observed that the rat protein could also function in this capacity when present in *R. sphaeroides.*

When comparing members of the TspO family of proteins across a diversity of genera we find that there are a number of tryptophan residues (and other amino acids) which are highly conserved. We therefore embarked upon a series of studies involving the site-directed mutagenesis of these conserved amino acid residues. In this very extensive study we found that the ability of TspO to regulate downstream (PS) gene activity could be altered to varying degrees in the different mutant strains. See Fig. 1 for the proposed structure of TspO. We further observed an excellent correlation between residual TspO activity and the levels of tetrapyrrole efflux in these mutant strains. By following the efflux of different tetrapyrroles it appeared that the critical intermediate, as in the mitochondrion is coproporphyrinogen III. Thus, we concluded that excess accumulation of this tetrapyrrole in a TspO mutant somehow resulted in increased PS gene transcription. Therefore, we reasoned that if we were to overexpress the *hemN* gene encoding coproporphyrinogen III oxidase we would make the wild type organism phenotypically resemble the TspO mutant. This turned out to be the case.

These investigations have enabled us to describe a model whereby TspO, by regulating the efflux of specific tetrapyrroles, namely coproporphyrinogen, results in maintaining the cellular level of an intermediate in tetrapyrrole synthesis, most likely protoporphyrin, which is employed as a corepressor involved in the regulation of gene expression of various genes involved in bacteriochlorophyll and carotenoid biosynthesis in *R. sphaeroides*. We are now attempting to determine the mechanism of action of TspO in the outer membrane.



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