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Organization of *trpG*, *trpD* and *trpC* in *Azospirillum brasilense* Sp7

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Abstract

The genes of *trpG*, *trpD* and *trpC*, involved in tryptophan (Trp) biosynthesis, were found to be clustered and located separately from other *trp* genes in the *Azospirillum brasilense* Sp7 genome (Zimmer et al., 1991). Organization of the genes in strain Sp7 is similar to the situation in *Pseudomonas aeruginosa*, *P. acidovorans* or *Acinetobacter calcoaceticus*. In order to study the transcriptional organization of the gene cluster, eight different cosmids, carrying transposon Tn*5* in or adjacent to the *trpGDC* region, were transferred into TrpC$^-$ or TrpD$^-$ mutants of strain Sp7. As Trp-prototrophy could neither be restored in TrpC$^-$ mutants by cosmids carrying Tn*5* in *trpD* nor in TrpD$^-$ mutants by cosmids carrying Tn*5* in *trpC*, the genes *trpD* and *trpC* are proven to be cotranscribed. The surprising observation that cosmids carrying Tn*5* in *trpG* could restore Trp-prototrophy in both the TrpC$^-$ and TrpD$^-$ mutant, strongly suggests an initiation side for a *trpDC* transcript inside *trpG*.

Keywords: *Azospirillum*, tryptophan, *trpGDC*-cluster, Trp-auxotrophic mutants

1. Introduction

In the preceding papers (Zimmer and Elmerich, 1991; Zimmer et al., 1991) the transfer of a cosmid gene library of *A. brasilense* Sp7 into the low IAA

producing strain *A. irakense* KA3 was described. This led to the identification of the cosmid pAB1005 as being responsible for enhancing the Trp-dependent IAA-production in the recipient strain. pAB1005 contained an 18.5 kb insert of the genome of strain Sp7. Via Tn*5* mutagenesis the locus responsible for the phenotype could be reduced to a region of about 1 kb. Plasmids carrying Tn*5* in and just beside this locus were recombined into the genome of strain Sp7 leading to the Trp-auxotrophic mutants 7853 and 7854. Six open reading frames (ORF) were identified by sequencing this region and the adjacent parts of pAB1005. Deduced amino acid sequences of ORF2, ORF3 and ORF4 showed homology to TrpG, TrpD and TrpC, enzymes of tryptophan biosynthesis of other organisms (Zimmer et al., 1991). The locus which enhanced Trp-dependent IAA-production in *A. irakense* KA3 was identified as *trpD*. As TrpD catalyses an anthranilate consuming reaction the phenotype of strain KA3 could be explained, assuming an inhibitory effect of anthranilate on IAA biosynthesis (Zimmer et al., 1991). Results reported here show that in addition to the probable complete transcription of *trpGDC, trpDC* can be transcribed independently starting from an initiation site inside *trpG*.

2. Materials and Methods

Azospirillum brasilense mutant strains 7853 and 7854 (Zimmer et al., 1991) were grown in liquid minimal K-medium (Franche and Elmerich, 1981) supplemented with kanamycin (25 μg/ml) and tryptophan (100 μg/ml) or in petridishes containing the same medium with 1.5% agar. The different Tn*5* derivatives of pAB1005 were transferred from *Escherichia coli* strain S17.1 (Simon et al., 1983) into strain 7853 and 7854 via bacterial conjugation. Transconjugants were selected on minimal K-medium containing additions in the following concentrations (g/ml): kanamycin, 25; tetracycline, 5; tryptophan, 100.

3. Results and Discussion

The previously published sequence data of the *A. brasilense* Sp7 *trpGDC* cluster (Zimmer et al., 1991) showed that the stop codon of *trpG* overlapped the start codon of *trpD* and the stop codon of *trpD* overlapped the start codon of *trpC*, strongly indicating that *trpG*, *trpD* and *trpC* are cotranscribed. The gene cluster is separated from ORF's upstream and downstream by apparently non coding sequences of respectively 172 and 37 basepaires. The ORF's adjacent to the locus did not show any homology to other genes of the Trp-biosynthesis (Zimmer et al., 1991).

Comparison of this situation to the organization in other organisms (Table 1) revealed that *trpGDC* is organized similarly to *Pseudomonas aeruginosa*, *P. acidovorans* and *Acinetobacter calcoaceticus*. Surprisingly the organization is different in *Rhizobium meliloti*, whereas the deduced amino acid sequences of *A. brasilense* Sp7 *trpD* and *trpC* showed highest homology with TrpD and TrpC of *Rhizobium meliloti* (Zimmer et al., 1991) among the organisms listed in Table 1.

With the aim of proving that *trpGDC* in *Azospirillum brasilense* is organized in one transcription unit, eight different cosmids, carrying Tn*5* in or beside the *trpGDC* region, were transferred into the TrpD$^-$ mutant 7853 and the TrpC$^-$ mutant 7854 of strain Sp7 (Fig. 1). Trp-prototrophy was not obtained when mutant 7853 was complemented with a cosmid carrying Tn*5* in *trpC*. Similarly, the transfer of cosmids carrying Tn*5* in *trpD* into mutant 7854 did not lead to a Trp$^+$ phenotype. This strongly suggests cotranscription of *trpD* and *trpC*. In contrast, all cosmids carrying Tn*5* in *trpG*, unless the orientation of the Km^r gene of the Tn*5*, were able to reconstitute Trp-prototrophy in both mutant 7853 and 7854. As the stop codon of *trpG* overlaps the start codon of *trpD* (Zimmer et al., 1991), a transcription initiation site for a *trpDC* transcript must be located inside *trpG*.

In conclusion, there are two different transcripts possible from the *trpGDC*-locus: (1) a complete *trpGDC* transcript concluded from the overlapping start and stop codons, and (2) a *trpDC* transcript according to the complementation

Table 1. Organization of *trp* genes in bacteria

Group	Trp gene organization
Purple eubacteria	
Escherichia coli and relatives	E-(G)D-C(F)-B-A
Serratia marcescens	E-G-D-C(F)-B-A
Pseudomonas aeruginosa	E G-D-C F B-A
Pseudomonas acidovorans	E G-D-C F-B-A
Acinetobacter calcoaceticus	E G-D-C F-B-A
Rhizobium meliloti	E(G) D-C F-B-A
Caulobacter crescentus	E(G) D-C F-B-A
Azospirillum brasilense	? G-D-C ?
Gram-positive eubacteria	
Bacillus subtilis	G E-D-C-F-B-A
Brevibacterium lactofermentum	E-G-D-C(F)-B-A

Data except for *Azospirillum* are from Crawford (1989). Genes connected by a hyphen are organized in one transcription unit. (G)D, C(F) and E(G) indicate that the genes encode a fusion protein.

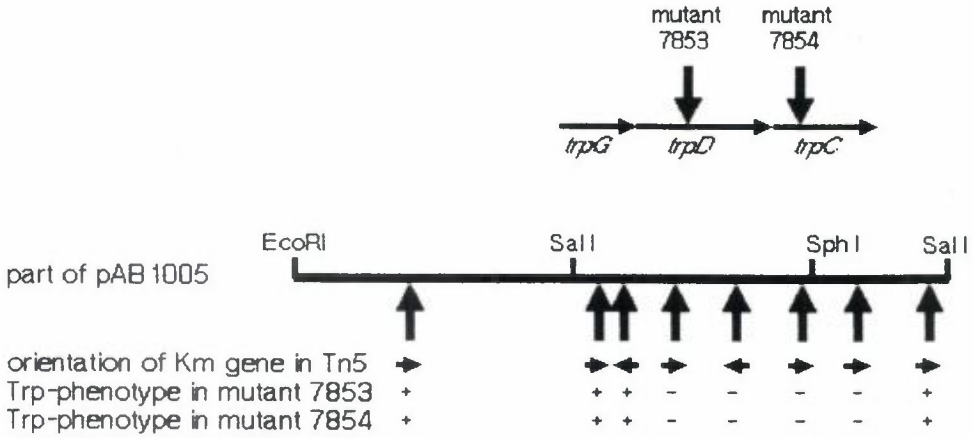

Figure 1. Complementation of *Azospirillum brasilense* mutants 7853 and 7854 with Tn*5* derivatives of pAB1005. Vertical arrows indicate the position of Tn*5* insertions in the genomic *trp* genes of mutant 7853 and 7854 and in the shown 5.2 kb part of pAB1005 carrying the copy of *trpGDC*.

experiments presented here. The ability to transcribe the *trpGDC* cluster with or without *trpG* is concomitant with the fact that TrpG is only essential for Trp-biosynthesis in cultures grown without ammonia (Crawford, 1989).

In order to study the regulation and transcription rate of the *trpGDC* cluster, mutagenesis with a Tn*5* derivative carrying a promotorless *lacZ* (Simon et al., 1989) was performed with the gene locus. After recombination into the Sp7 genome, expression studies of the β-galactosidase activity will help to elucidate which of the two possible transcripts, *trpGDC* or *trpDC*, is made, dependent on the chosen growth conditions.

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