Determination of the *Paracoccus denitrificans* SOS box

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By gel retardation experiments with crude cell extracts of *Paracoccus denitrificans* it was demonstrated that a protein specifically binds to the promoter of the *P. denitrificans* *recA* gene. PCR mutagenesis of the *recA* promoter showed that the GAACN₃GAAC motif is required for the formation of the DNA–protein complex. This protein also binds to the GTTCN₃GTTC motif, which is present in the promoter of the *P. denitrificans* *uvrA* gene. Mutational analysis of the promoter regions of both *P. denitrificans* *recA* and *uvrA* genes indicated that the GAACN₃GAAC and GTTCN₃GTTC sequences are required for DNA-damage-mediated induction of these two genes *in vivo*. Furthermore, the *P. denitrificans* *recA* gene was DNA-damage-inducible when introduced into cells of the phylogenetically related phototrophic bacterium *Rhodobacter sphaeroides*, although this inducibility was lost in mutants in the GAACN₃GAAC motif. These results indicate that *P. denitrificans* possesses the same SOS box as *R. sphaeroides*, which, in agreement with previous work, is proposed as being the GTTCN₃GTTC motif.

Keywords: *recA* gene, *uvrA* gene, SOS box, *Paracoccus denitrificans*, DNA damage

INTRODUCTION

DNA damage induces in *Escherichia coli* cells the expression of a set of genes whose encoded proteins are directly involved in DNA repair or in enabling the cell to tolerate the lesion until repair occurs (Little & Mount, 1982; Walker, 1984). This set of genes, which constitutes the SOS system, is under the control of both RecA and LexA proteins. The LexA protein is the common repressor of the SOS network, which also includes both *recA* and *lexA* genes. RecA promotes proteolytic inactivation of LexA in DNA-damaged cells (Little, 1991). Several studies indicate that the RecA protein binds to single-stranded DNA regions, originated by DNA-damage-mediated replication inhibition or enzymic processing of broken DNA ends, and is converted into an active conformation (Sassanfar & Roberts, 1990). Once activated, RecA facilitates autocatalytic cleavage of LexA, giving rise to the expression of the SOS genes. When DNA has been repaired, the RecA protein is no longer activated and the level of the LexA repressor increases, again blocking expression of the SOS genes.

Similar DNA repair systems have been reported in other

bacteria (Miller & Kokjohn, 1990). In this way, the regulatory genes of the SOS system, *lexA* and *recA*, of different Gram-positive and Gram-negative bacteria have been characterized (Raymond-Denise & Guillen, 1991; Garriga *et al.*, 1992; Roca & Cox, 1997). *E. coli* and *Bacillus subtilis* LexA proteins bind to a specific site located at the 5' end of their respective SOS genes which is known as the SOS box. So far, three different SOS boxes have been identified. The first one, which was initially described in *E. coli*, displays the dyad symmetrical sequence 5' CTGT-(AT)₄-ACAG 3' (Wertman & Mount, 1985; Lewis *et al.*, 1994). This *E. coli*-like SOS box is present in a great number of Gram-negative bacterial species belonging to several taxonomic families (*Enterobacteriaceae*, *Pseudomonadaceae* and *Pasteurellaceae*, among others). The second SOS box known, whose consensus sequence is 5' CGAACRNR-YGTTYC 3' (Winterling *et al.*, 1998), was first reported in *B. subtilis* (Cheo *et al.*, 1991), although it is also present in many other Gram-positive bacteria (Movahedzadeh *et al.*, 1997; Durbach *et al.*, 1997; Johnston *et al.*, 1997). Finally, a third SOS box belonging to the phototrophic bacterium *Rhodobacter sphaeroides* has recently been identified (Fernández de Henestrosa *et al.*, 1998). This displays the GTTCN₃GTTC motif, and is the first SOS box known whose sequence is a direct repeat.

The GenBank/EMBL/DBJ accession number for the sequence determined in this work is AJ007707.

Paracoccus denitrificans is an aerobic soil bacterium which, taxonomically, has been grouped in the α -subclass of the *Proteobacteria* together with *R. sphaeroides* (Woese *et al.*, 1984). The *recA* gene of *P. denitrificans* has been isolated and sequenced, and it has been shown to be DNA damage-inducible despite its promoter lacking an *E. coli*-like SOS box (Fernández de Henestrosa *et al.*, 1997). In this work, to determine whether the *R. sphaeroides* SOS box is uniquely present in this organism or is also found in other members of the *Proteobacteria*, the promoters of the *P. denitrificans* *recA* and *uvrA* genes were mutagenized and their behaviour *in vitro* and *in vivo* was analysed.

METHODS

Bacterial strains, plasmids and growth conditions. The bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* was grown at 37 °C in Luria-Bertani (LB)

medium (Miller, 1991), and *P. denitrificans* and *R. sphaeroides* strains were normally grown at 30 °C in Brain-Heart Infusion (BHI) (Miller, 1991) or 290A (Calero *et al.*, 1994) media, respectively. Antibiotics were added to the culture media at the appropriate concentrations for each bacterial species (Miller, 1991).

General genetic techniques. Plasmid DNA was transformed into competent *E. coli* cells as described by Silhavy *et al.* (1984). Bacterial matings were carried out as reported previously (Fernández de Henestrosa & Barbé, 1994). Mitomycin C treatment was performed as described previously (Barbé *et al.*, 1985).

Biochemical, and RNA and DNA methods. β -Galactosidase activity assays, RNA extraction, DNA techniques and gel mobility experiments were as reported previously (Fernández de Henestrosa *et al.*, 1998). In all shift mobility experiments, the protein concentration of the crude cellular extract, when used, was 2.5 $\mu\text{g ml}^{-1}$. In the competition experiments with a specific DNA, a 100-fold molar excess of unlabelled DNA was always added to the reaction mixture.

To determine the transcriptional starting point of the *P. denitrificans* *recA* gene, the oligonucleotide 5' GAACTGCC-

Table 1. Bacterial strains and plasmids used in this work

Strain/plasmid	Description	Source/reference
Strains		
<i>E. coli</i> K-12		
DH5 α F'	<i>recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1</i> Δ (<i>lacZYA-argF</i>)U169 <i>deoR</i> ϕ 80d <i>lacZ</i> M15 F'	Clontech
MC1061	Δ <i>lacX74 hsdR mcrB araD139</i> Δ (<i>araABC-leu</i>)7679 <i>galU galK rpsL thi</i>	M. Casadaban, University of Chicago, USA
<i>P. denitrificans</i>		
PD1222	Rif ^R derivative of the wild-type strain	J. D. Wall, University of Missouri, USA
UAP10	PD1222 but <i>recA::</i> Ω Km	Fernández de Henestrosa <i>et al.</i> (1997)
<i>R. sphaeroides</i>		
UA8000	Rif ^R derivative of the wild-type strain	Calero <i>et al.</i> (1994)
Plasmids		
pBSK(+)	Amp ^R	Stratagene
pGEM-T	Amp ^R	Promega
pUJ8	Promoterless vector for making <i>lacZ</i> fusions, Amp ^R	de Lorenzo <i>et al.</i> (1990)
pLV106	Broad-host-range plasmid, Tc ^R Mob ⁺	Lee & Kaplan (1992)
pRK2013	Tra ⁺ ColE1 replicon Km ^R	H. D. Helinski, University of California, USA
pUA 617	pBSK(+) carrying <i>P. denitrificans</i> <i>recA</i> gene in a 3 kb <i>EcoRI</i> fragment	Fernández de Henestrosa <i>et al.</i> (1997)
pUA 699	As pLV106 but carrying the <i>P. denitrificans</i> wild-type <i>recA::lacZ</i> fusion	This work
pUA 700	As pLV106 but carrying the <i>P. denitrificans</i> OL <i>recA::lacZ</i> fusion	This work
pUA 701	As pLV106 but carrying the <i>P. denitrificans</i> OR <i>recA::lacZ</i> fusion	This work
pUA 694	As pLV106 but carrying the <i>P. denitrificans</i> wild-type <i>uvrA::lacZ</i> fusion	This work
pUA 702	As pLV106 but carrying the <i>P. denitrificans</i> OL <i>uvrA::lacZ</i> fusion	This work
pUA 703	As pLV106 but carrying the <i>P. denitrificans</i> OR <i>uvrA::lacZ</i> fusion	This work

Table 2. Oligonucleotide primers used in this work

Primer	Sequence*	Position†	Use
L1	5' TCGAAAAGTGTGCCCC 3'	+20	Lower primer, 5' digoxigenin end-labelled; used in the construction of probes for mobility shift assays (Fig. 2b)
LF	5' GGATCCTCGAAAAGTGTGCCCC 3'	+20	Lower primer used in <i>recA</i> fusion constructions
LPE	5' GAACTGCCGTTTCGATCTGGGCC 3'	+96	Lower primer used for primer extension experiments
UR1	5' GAGGCGCAAATAGGAACA 3'	-90	Upper primer used to obtain P1 probe (Fig. 2b)
UR2	5' CGTGAACAAAGTGGCGG 3'	-69	Upper primer used to obtain P2 probe (Fig. 2b)
UF1	5' GAATTCAGGACTTGCATTTTCGGCG 3'	-112	Upper primer used to obtain fusion constructions
UF2	5' GAATTCAGGACTTGCATTTTCGGCGCAATG AGGCGCAAATAGGCCCAACCCGTG 3'	-112	Upper primer used to obtain the mutagenized <i>recA</i> OL probe; also used in mutagenized fusion constructions
UF3	5' GAATTCAGGACTTGCATTTTCGGCGCAATG AGGCGCAAATAGGAACAACCCGTGCCCA AAGTGG 3'	-112	Upper primer used to obtain the mutagenized <i>recA</i> OR probe; also used in mutagenized fusion constructions
LUF1	5' AGGACTTGCCCGAGC 3'	+122	Lower primer used to obtain wild-type <i>uvrA</i> fusion
LUF2	5' GGATCCGCCCGAGCCCGACAGGCCCG 3'	+111	Lower primer used to obtain <i>uvrA</i> probes; also used in mutagenized fusion
UV1	5' GCCGCATTGGCAGCCGCC 3'	-189	Upper primer used to obtain wild-type <i>uvrA</i> fusion
UV2	5' CCTGCATGGTTGGGGCAGGG 3'	-126	Upper primer used to obtain <i>uvrA</i> probe (Fig. 2b)
UV3	5' GAATTCCTGCATGGTTGGGGCAGGGCTG CCAGGGGCCGCAAGCCGGAATGCCCTG TGATG 3'	-126	Upper primer used to obtain the mutagenized <i>uvrA</i> OL probe; also used in mutagenized fusion constructions (Fig. 2b)
UV4	5' GAATTCCTGCATGGTTGGGGCAGGGCT GCCAGGGGCCGCAAGCCGGAATGTTCTT GTGATGCCCTGTTT 3'	-126	Upper primer used to obtain the mutagenized <i>uvrA</i> OR probe; also used in mutagenized fusion constructions (Fig. 2b)

* Nucleotide changes are in bold and underlined. Added restriction sites are in italics.

† Distance from the 5' end of the oligonucleotide to either the transcriptional or the translational starting point of *P. denitrificans recA* and *uvrA* genes, respectively.

GTTCGATCTGGGCC 3', which hybridizes to nucleotides +75 to +96 relative to the translational starting site, was used. This primer was synthesized and 5'-end-labelled with digoxigenin-*N*-hydroxysuccinimide ester (MWG-Biotech). Primer extension was carried out as previously reported (Tapias *et al.*, 1997).

The nucleotide sequence of the *P. denitrificans uvrA* gene was determined from both DNA strands by the dideoxy method (Sanger *et al.*, 1977) on an ALF Sequencer (Pharmacia Biotech).

Primers used in the construction of the different *P. denitrificans recA* and *uvrA* probes, supplied by Boehringer Mannheim, are presented in Table 2. To facilitate subcloning of PCR-DNA fragments and construction of the *lacZ* fusions, specific restriction sites (shown in Table 2) were incorporated into the oligonucleotide primers. The DNA sequence of all PCR-mutagenized fragments was obtained in all cases from both DNA strands.

Construction of *lacZ* fusions. To construct the several *recA-lacZ* fusions used in this work a 250 bp PCR product containing the *recA* promoter and 138 nt of its coding region of either the wild-type or the desired mutant was cloned in the pGEM-T vector (Promega). In the same way, the *uvrA-lacZ* fusions were constructed with a 240 bp PCR-amplified fragment of either the wild-type promoter or the desired mutants

(Table 2) containing 114 bp of the *uvrA* coding region. For each fusion, desired restriction fragments were recovered from the appropriate pGEM-T derivative and subcloned into the pUJ8 plasmid upstream of the promoterless *trp'-lacZ* region. Afterwards, the *NotI* fragment harbouring the created fusion was recovered from the agarose gel, filled with T4 DNA polymerase to obtain blunt ends and inserted into the single *SmaI* cloning site of the pLV106 plasmid. The presence of the desired mutations was again tested by DNA sequencing of each fusion using the 5' fluorescein CGAACGGCCAGTGA-ATCCG 3' primer, which extended from nucleotides +32 to +14, with respect to the translational starting point of the *E. coli lacZ* gene. Finally, plasmids containing the constructed fusions were introduced by mating into *P. denitrificans* cells.

RESULTS

Characterization of the *Paracoccus denitrificans recA* transcriptional starting point

It has been reported that some *recA* genes are expressed from a polycistronic transcript (Martin *et al.*, 1995). To determine whether this was the case in the *P. denitrificans recA* gene, and also to locate its putative regulatory sequences, the transcriptional starting point

Fig. 1. (a) Determination of the 5' end of the *P. denitrificans* *recA* transcript by primer extension. Lanes T, C, G and A contain products of sequencing reactions of plasmid pUA617 carried out with the same primer as used for primer extension. The transcriptional starting point is indicated (+1). (b) Nucleotide sequence of the *P. denitrificans* *recA* upstream region. The +1 position, the predicted -10, -35 and ribosome-binding site (RBS) sequences, and the translational starting point are indicated. The GAACN₇GAAC direct repeat present in the upstream region of the *recA* gene is underlined.

of this gene was identified by primer extension experiments using total RNA isolated from *P. denitrificans* PD1222 cells in the presence of mitomycin C at 10 µg ml⁻¹. The main signal obtained was the A nucleotide located 72 bp upstream of the site previously predicted as the putative translational starting codon (Fig. 1a). In agreement with these results, the putative -35 and -10 sites could be TTGCAT and CAAATA, respectively (Fig. 1b).

Isolation of the *P. denitrificans* *uvrA* gene

To determine whether a given sequence is the regulatory element of a gene network, this sequence must be present in all genes of the network. For this reason, it was decided to isolate the *P. denitrificans* *uvrA* gene. In this way, the promoter sequences of both genes could be

compared and the relationship existing between them analysed *in vivo* and *in vitro*. To isolate the *P. denitrificans* *uvrA*, the same strategy used to clone its *recA* gene was employed, taking advantage of the phylogenetic relationship existing between *P. denitrificans* and *R. sphaeroides* (Fernández de Henestrosa *et al.*, 1997). Thus, a gene bank of *P. denitrificans* constructed in the λGEM12 vector was probed with a 0.3 kb *Sac*II internal fragment of the *R. sphaeroides* *uvrA* gene (Mackenzie *et al.*, 1995). Among 1200 plaques screened, four reacted with the probe. One of these positive phage was analysed by restriction enzyme digestion and Southern blot hybridization. A 5 kb *Sma*I fragment of this phage, which hybridizes with the *R. sphaeroides* probe, was isolated and ligated into the single *Sma*I site of the pBluescript SK(+) plasmid. Further subcloning and sequencing experiments have enabled us to obtain the nucleotide sequence of the promoter and the 5' end coding region of the *P. denitrificans* *uvrA* gene.

It is known that not all *uvrA* genes are DNA-damage-inducible. Thus, it has been demonstrated that expression of the *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* *uvrA* genes is not triggered following DNA damage (Rivera *et al.*, 1997; Black *et al.*, 1998). For this reason, it was decided to analyse the behaviour of the *P. denitrificans* *uvrA* promoter towards DNA injury. To do this, the pUA694 plasmid containing a *uvrA-lacZ* fusion (constructed as reported in Methods) was introduced into a Rif^R mutant of *P. denitrificans*. Addition of mitomycin C to *P. denitrificans* (pUA694) cells induced the expression of the *uvrA-lacZ* fusion in RecA⁺ but not in RecA⁻ cells, indicating that this inducibility is *recA*-dependent (data not shown).

Electrophoretic mobility of the *P. denitrificans* *recA* and *uvrA* promoters

The above-established +1 position of the *P. denitrificans* *recA* gene overlaps with the GAACN₇GAAC direct repeat (Fig. 1b). This motif, GAACN₇GAAC, is also present upstream of the *R. sphaeroides* *recA* gene and controls its DNA-damage-mediated expression (Fernández de Henestrosa *et al.*, 1998). Gel retardation experiments were therefore carried out with different derivatives of the *P. denitrificans* *recA* promoter to determine whether a protein binds to its GAACN₇GAAC motif. Fig. 2(b) shows that the mobility of a DNA fragment containing the GAACN₇GAAC sequence shifts in the presence of *P. denitrificans* cell extract. On the other hand, no changes were detected in the mobility of a fragment lacking the GAACN₇GAAC motif (Fig. 2b, lane 4). This DNA-protein complex is formed by the binding of a sequence-specific protein to the probe since the addition of unlabelled pBSK-DNA did not affect the formation of the DNA-protein complex (Fig. 2b, lane 6), whereas the presence of unlabelled wild-type *recA* promoter inhibited it (Fig. 2b, lane 7). Furthermore, the addition of competitor fragments carrying a mutation in any of the two GAAC

Fig. 2. (a) Sequence of the operator regions of the *P. denitrificans* *recA* and *uvrA* genes. P1 and P2 indicate the starting point of the upper primers used to obtain fragments containing the *recA* gene employed in gel mobility experiments. The changes introduced by PCR mutagenesis in the GAACN₇GAAC and GTTCN₇GTTC direct repeats present in the operators of the *recA* and *uvrA* genes, respectively, are also indicated. (b) Gel mobility of the *recA* promoter of *P. denitrificans* under several experimental conditions. Lanes 1–4 show the behaviour of P1 and P2 fragments in the absence (–) or in the presence (+) of *P. denitrificans* crude cell extract. Lanes 5–9 show the mobility of the P1 fragment (lane 5) in the presence of *P. denitrificans* crude extract as well as the effect on this mobility of unlabelled 100-fold molar excess of pBSK-DNA (lane 6), P1 fragment (lane 7), or mutants RL and RR of the P1 fragment (lanes 8 and 9, respectively). The effect of an excess of unlabelled-fragment containing either the wild-type or mutant derivatives of the *P. denitrificans* *uvrA* promoter on the mobility of the P1 fragment in the presence of crude extract is also shown (lanes 12–14). All binding assays were done simultaneously, although competitive experiments with *uvrA* fragments (lanes 12–14) were run in a different gel. For this reason, and as an experimental control, the mobility of the P1 fragment is also included in lanes 10–11.

submotifs of the GAACN₇GAAC sequence does not affect the band shift (Fig. 2b, lanes 8 and 9). All these data indicate that the binding of the protein requires the presence of the GAACN₇GAAC sequence. It is worth noting that the same protein binds to the *P. denitrificans* *uvrA* promoter since its presence prevents *P.*

denitrificans *recA*–protein complex formation (Fig. 2b, lane 12).

The gel retardation experiments indicate that a protein binds the promoters of both *recA* and *uvrA* of *P. denitrificans*. Between the –66 and –91 positions, with

Table 3. Effects of mutations on the expression of the *P. denitrificans* *recA* and *uvrA* genes measured as the β -galactosidase specific activity of *recA-lacZ* and *uvrA-lacZ* fusions

Plasmid	β -Galactosidase specific activity (units)*		
	Basal level	Induced level	Induction factor†
pUA699 (<i>recA</i> wild-type)	1200	2600	2.2
pUA700 (<i>recA</i> OL ⁻)	6500	6600	1
pUA701 (<i>recA</i> OR ⁻)	6400	6500	1
pUA694 (<i>uvrA</i> wild-type)	1600	6800	4.2
pUA702 (<i>uvrA</i> OL ⁻)	4800	5000	1
pUA703 (<i>uvrA</i> OR ⁻)	6600	6700	1

* β -Galactosidase specific activities were measured in the absence of mitomycin C (basal level) or 180 min after the addition of this compound at $10 \mu\text{g ml}^{-1}$ (induced level). All determinations are the means of at least three experiments (each in triplicate), and a single standard error of any value was never greater than 10%.

† The induction factor is the ratio between the specific activity of β -galactosidase of mitomycin C-treated cells and the specific activity in non-treated cells for each strain.

respect to the *uvrA* translational starting point, the *uvrA* promoter contains the GTTCN₇GTTC sequence, which corresponds to the reverse and complementary sequence of the GAACN₇GAAC motif present in the *P. denitrificans* *recA* gene (Fig. 2a). The *R. sphaeroides* *uvrA* promoter also contains a GTTCN₇GTTC motif which controls its DNA-damage-mediated induction (Fernández de Henestrosa *et al.*, 1998). For this reason, the electrophoretic mobility of mutant derivatives of the *P. denitrificans* *uvrA* promoter was studied. A *P. denitrificans* *uvrA* promoter mutagenized in either the first or the second direct repeat of the GTTCN₇GTTC sequence did not inhibit the band shift detected when the *P. denitrificans* *recA* promoter was incubated in the presence of crude cell extract (Fig. 2b, lanes 13 and 14).

Effect of operator mutations upon *P. denitrificans* *recA* and *uvrA* gene expression

The results shown above demonstrate that the same protein binds to the GAACN₇GAAC and GTTCN₇GTTC sequences present upstream of the *P. denitrificans* *recA* and *uvrA* genes, respectively. To determine whether these sequences are the regulatory elements of both genes, the effect of several mutations in these sequences upon the *in vivo* expression of these two genes was analysed. To do this, different *lacZ* fusions were constructed with derivatives of both *recA* and *uvrA* promoter fragments carrying the same mutations as those used in the gel mobility experiments. The fusion constructs were introduced into *P. denitrificans* using the pLV106 plasmid. Table 3 shows that cells carrying any of the mutations tested demonstrated a constitutive and high level of β -galactosidase synthesis, indicating that the control of *P. denitrificans* *recA* and *uvrA* genes requires the GAACN₇GAAC and GTTCN₇GTTC sequences, respectively. The reason why the basal level of the deregulated *recA* mutants is higher than the induced level achieved by the wild-type fusion following mitomycin C treatment is unknown. However, one

possibility could be that the concentration of mitomycin C used was not the optimal amount to obtain the maximum DNA-damage-mediated induction of the *recA* gene. In fact, similar behaviour has been described in *E. coli* cells (Krueger *et al.*, 1983; Wertman & Mount 1985). Finally, the expression of the wild-type and OR⁻ *P. denitrificans* *recA* promoters is either DNA-damage-inducible or constitutive when introduced into *R. sphaeroides* cells, respectively (data not shown).

DISCUSSION

We have previously shown that GTTCN₇GATC and GAACN₇GAAC motifs control the DNA-damage-mediated induction of the *R. sphaeroides* *recA* gene. Likewise, we have also demonstrated that the GTTCN₇GTTC motif regulates the induction of the *R. sphaeroides* *uvrA* gene. Since the GAACN₇GAAC motif is the reverse and complementary sequence of GTTCN₇GTTC, together with the fact that the GTTCN₇GATC and GTTCN₇GTTC motifs are also present in both DNA-damage-inducible genes, we have proposed the GTTCN₇GTTC motif as the SOS box of *R. sphaeroides* (Fernández de Henestrosa *et al.*, 1998). In the present study, it has been established that the GAACN₇GAAC and GTTCN₇GTTC motifs control the expression of the *P. denitrificans* *recA* and *uvrA* genes, respectively. Thus it is clear that the *R. sphaeroides* and *P. denitrificans* *uvrA* genes have the same regulatory sequence (GTTCN₇GTTC), whereas the *recA* genes of these two organisms differ in the presence of a second regulatory sequence in one of them: in both *recA* genes the GAACN₇GAAC sequence is found, but the *R. sphaeroides* *recA* gene also has the GTTCN₇GTAC motif. Furthermore, expression of the *P. denitrificans* *recA* gene is triggered by DNA damage in *R. sphaeroides* cells unless the GAACN₇GAAC motif is mutagenized. These data are in agreement with the fact that *P. denitrificans* and *R. sphaeroides* have both

been phylogenetically classified within the α -subclass of the *Proteobacteria*.

In this context, and in agreement with our previous work (Fernández de Henestrosa *et al.*, 1998), we propose the GTTCN₇GTTC motif as the *P. denitrificans* SOS box. From a biological point of view, and despite the sequences being different with respect to the transcriptional sense, the mechanism by which the LexA repressor of *P. denitrificans* binds to either *recA* (GAACN₇GAAC) or *uvrA* (GTTCN₇GTTC) operators must be the same since, in fact, the sequences of both motifs (which are a direct repeat) are complementary and reverse. In this respect, it is also worth noting that the sequence of the *R. sphaeroides* and *P. denitrificans* SOS box shows several specific and different properties from either of the other SOS boxes or protein-binding sequences described. The first is that this sequence is a direct repeat instead of a palindrome. The second is that, to our knowledge, this is the only case shown where the controlling sequences of two genes of the same regulatory network are found in the opposite orientation in each of them. Because of these characteristics, the model LexA repressor-SOS box of *P. denitrificans* may be very useful for progressing knowledge of DNA-protein interactions in bacteria.

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