

## Regulation of Expression of the 2-Deoxy-D-Ribose Utilization Regulon, *deoQKPX*, from *Salmonella enterica* Serovar Typhimurium

Mette Christensen,<sup>1</sup> Tudor Borza,<sup>2†</sup> Gert Dandanell,<sup>1</sup> Anne-Marie Gilles,<sup>2</sup> Octavian Barzu,<sup>2</sup> Rod A. Kelln,<sup>3</sup> and Jan Neuhard<sup>1\*</sup>

Department of Biological Chemistry, Institute of Molecular Biology, University of Copenhagen, DK-1307 Copenhagen K, Denmark<sup>1</sup>; Laboratoire de Chimie Structurale de Macromolécules, Institut Pasteur, 75724 Paris Cedex 15, France<sup>2</sup>; and Department of Chemistry and Biochemistry, University of Regina, Regina, Saskatchewan, Canada, S4S 0A2<sup>3</sup>

Received 20 March 2003/Accepted 16 July 2003

*Salmonella enterica*, in contrast to *Escherichia coli* K12, can use 2-deoxy-D-ribose as the sole carbon source. The genetic determinants for this capacity in *S. enterica* serovar Typhimurium include four genes, of which three, *deoK*, *deoP*, and *deoX*, constitute an operon. The fourth, *deoQ*, is transcribed in the opposite direction. The *deoK* gene encodes deoxyribokinase. In silico analyses indicated that *deoP* encodes a permease and *deoQ* encodes a regulatory protein of the *deoR* family. The *deoX* gene product showed no match to known proteins in the databases. Deletion analyses showed that both a functional *deoP* gene and a functional *deoX* gene were required for optimal utilization of deoxyribose. Using gene fusion technology, we observed that *deoQ* and the *deoKPX* operon were transcribed from divergent promoters located in the 324-bp intercistronic region between *deoQ* and *deoK*. The *deoKPX* promoter was 10-fold stronger than the *deoQ* promoter, and expression was negatively regulated by DeoQ as well as by DeoR, the repressor of the deoxynucleoside catabolism operon. Transcription of *deoKPX* but not of *deoQ* was regulated by catabolite repression. Primer extension analysis identified the transcriptional start points of both promoters and showed that induction by deoxyribose occurred at the level of transcription initiation. Gel retardation experiments with purified DeoQ illustrated that it binds independently to tandem operator sites within the *deoQ* and *deoK* promoter regions with  $K_d$  values of 54 and 2.4 nM, respectively.

Numerous bacteria can use the 2-deoxy-D-ribosyl moiety of 2'-deoxyribonucleosides as the sole carbon and energy source (15). Genetic and biochemical studies, using *Escherichia coli* and *Salmonella enterica* serovar Typhimurium as model organisms, have shown that the catabolic pathway involves four enzymes: thymidine phosphorylase (EC 2.4.2.4), purine nucleoside phosphorylase (EC 2.4.2.1), phosphopentomutase (EC 5.4.2.7), and deoxyriboaldolase (EC 4.1.2.4) (Fig. 1), encoded by the *deoA*, *deoD*, *deoB*, and *deoC* genes, respectively. In both organisms the four genes are organized as an operon, the *deoCABD* operon, located at approximately 99 centisomes on the chromosomes. The operon is transcribed from two promoters located 600 bp apart. Transcription initiation of the operon is highly regulated by a complex interplay of three regulatory proteins, of which two, DeoR and CytR, are repressors and the third is the cAMP-receptor protein (CAP). The genes encoding these proteins are unlinked to each other and to the *deoCABD* operon. The internal inducer for expression of the operon is 2-deoxy-D-ribose 5-phosphate (dRib5P) inactivating DeoR (8, 15) and cytidine (in *S. enterica* also uridine) inactivating CytR (27, 34)

In contrast, only a few bacteria have been shown to be able

to ferment 2-deoxy-D-ribose (dRib). They include *Lactobacillus plantarum* (11, 13), *Selenomonas ruminantium* (30), and serovar Typhimurium (17). Notably, *E. coli* K12 cannot use dRib as the sole carbon and energy source.

Deoxyribokinase, the first enzyme involved in dRib utilization, catalyzes the phosphorylation of deoxyribose to dRib5P. Its synthesis is induced in *S. enterica* following addition of dRib to the growth medium (17). Subsequently, dRib5P is cleaved by deoxyriboaldolase to glyceraldehyde 3-P and acetaldehyde (Fig. 1). The genes mediating fermentation of dRib were cloned from *S. enterica* serovar Typhi and shown to be located at 82.94 centisomes on the chromosome (38). The dRib system involved four genes, of which three, *deoK*, *deoP*, and *deoX*, were transcribed in one direction and the fourth, *deoQ*, was transcribed in the opposite direction. The *deoK* gene was shown to encode deoxyribokinase. In silico analyses determined that *deoP* encoded an inner-membrane protein, having 34.5% sequence identity with FucP of *E. coli*, and therefore most likely corresponded to the deoxyribose permease described by Hoffee (17). The *deoQ* gene was deduced to encode a putative regulatory protein with 37.4% amino acid sequence identity to the DeoR repressor of *E. coli*. No clear matches for the *deoX* gene product were found in the databases. The *deoQKPX* cluster is absent from the *E. coli* K12 genome.

In the present work we describe the transcriptional organization of the *deoQKPX* locus from serovar Typhimurium and show that *deoK*, *deoP*, and *deoX* constitute an operon and that transcription of both the *deoQ* gene and the *deoKPX* operon is

\* Corresponding author. Mailing address: Department of Biological Chemistry, Institute of Molecular Biology, University of Copenhagen, Sølvgade 83, DK-1307 Copenhagen K, Denmark. Phone: (45) 35 32 20 02. Fax: (45) 35 32 20 40. E-mail: neuhard@mermaid.molbio.ku.dk.

† Present address: Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011.

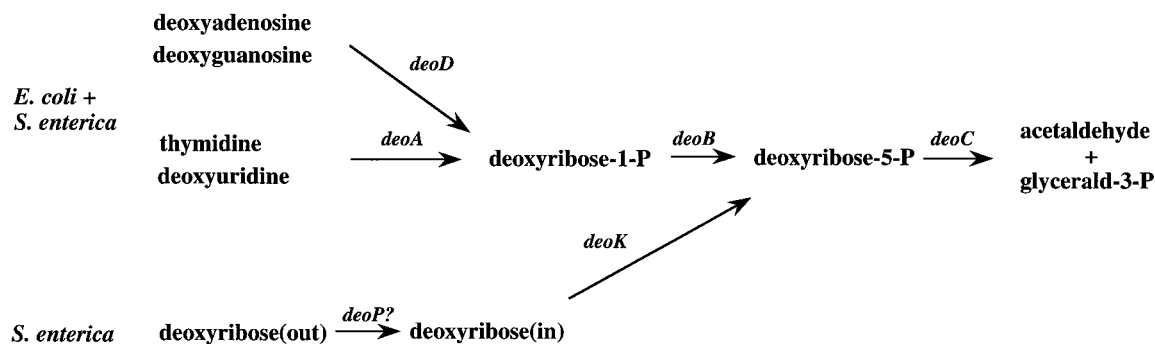


FIG. 1. 2-Deoxy-D-ribose and deoxynucleoside catabolism by *S. enterica* and *E. coli*. The individual enzymes are identified by the corresponding gene symbols: *deoA*, thymidine phosphorylase; *deoB*, phosphopentomutase; *deoC*, deoxyriboaldolase; *deoD*, purine nucleoside phosphorylase; *deoK*, deoxyribokinase; *deoP?*, inferred deoxyribose permease. Glyceraldehyde-3-P stands for glyceraldehyde 3-phosphate.

negatively controlled by the *deoQ* gene product through binding of DeoQ to the intercistronic region between *deoQ* and *deoK*.

#### MATERIALS AND METHODS

**Bacterial strains and plasmid vectors.** *E. coli* K12 strain MC1061 (*araD139*  $\Delta$ (*araABIOC leu*)7697  $\Delta$ *lacX74 galU galK rpsL hsdR*) (31) was used in all clonings, and CSH26 [ $F^-$  *ara*  $\Delta$ (*gtp-pro-cod-lac*)] (26) and its *deoR* derivative, MEC011, were used as hosts for expression of the *Salmonella deo* genes. For overproduction of recombinant proteins, strain BL21(DE3) was used (37). In most clonings, the low-copy-number vectors pWSK29 and pWSK129 (6 to 8 copies per cell), encoding resistance to ampicillin ( $Ap^r$ ) and kanamycin ( $Km^r$ ), respectively, were employed (39). For *lacZ* transcriptional fusions, the very-low-copy-number vector pFZY1 (9.6 kb) was used (21). It contains a promoterless *lacZ* gene and part of *lacY* from *E. coli*. Upstream of the *lacZ* gene is a multiple cloning region followed by a leader with stop codons in all three reading frames. The replication origin of pFZY1 is *oriF*, and the plasmid is present in one to two copies per genome and harbors a gene for ampicillin resistance. From pFZY1, pFLC (5.5 kb) was constructed by deleting the *lac* DNA (see below). Translational fusions were introduced using a single-copy system with *gal8* as a vector (12).

**Media and growth conditions.** Strains were grown at 37°C unless stated otherwise. Lysogenic strains harboring *gal8* derivatives were grown at 32°C. Media were NZY broth (modified LB medium [18]) or AB minimal medium (5) containing 0.2% of either glucose, glycerol, or 2-deoxy-D-ribose as the carbon source, thiamine (0.5  $\mu$ g/ml), and proline (50  $\mu$ g/ml). When used, 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside was added at a concentration of 40  $\mu$ g/ml; and isopropyl- $\beta$ -D-thiogalactopyranoside was added at 0.5 mM. Antibiotics were added at the following final concentrations: ampicillin, 100  $\mu$ g/ml, except with the very-low-copy-number plasmids, where the concentration was reduced to 30  $\mu$ g/ml; tetracycline, 10  $\mu$ g/ml; kanamycin, 30  $\mu$ g/ml; and chloramphenicol, 30  $\mu$ g/ml. Solid media were prepared by adding 1.5% agar to the respective liquid medium.

**DNA techniques.** *E. coli* was made competent by treatment with  $CaCl_2$  (31). Plasmids were isolated by the alkaline-sodium dodecyl sulfate lysis procedure (3). Endonuclease digestion, ligation of DNA, and 5'-end phosphorylation of DNA by polynucleotide kinase was done according to the suppliers' recommendations. All PCR-generated fragments were sequenced by the chain termination method (32) using the BigDye terminator cycle sequencing kit (PE Applied Biosystems, Warrington, Great Britain) and an ABI PRISM 310 genetic analyzer (PE Applied Biosystems).

**Cloning the *deoK* locus and plasmid constructions.** A plasmid library of serovar Typhimurium LT2 DNA containing from 8- to 15-kbp *Sau*3AI fragments in pBR328 was obtained from C. G. Miller (16) and amplified by one cycle of phage P22 propagation. The library was transformed into *E. coli* MC1061, and transformants capable of using dRib as the sole carbon source were selected. Restriction endonuclease mapping of plasmids isolated from dRib<sup>+</sup> candidates indicated that they harbored the entire *deoQKPX* locus with the exception of the last 200 bp of the 3' end of *deoX*. The missing DNA was obtained by PCR, making use of a unique *EcoRI* site located 230 bp from the 3' end of *deoX*, and the entire region, including *ilvN* and part of *ilvB*, was assembled as a 5.4-kbp fragment in pSU19 (2), yielding pSU*deoQKPX* (Fig. 2).

**pFLC derivatives.** The single-copy vector pFLC (5.5 kbp) was constructed by deleting all *lac* DNA (approximately 4.4 kbp) from pFZY1 by digesting pFZY1 with *Afl*III and *Hind*III, filling the 5' overhangs using the Klenow fragment, and ligating the blunt ends. A 4.7-kbp *Acl*I/*Bam*HI fragment, containing the entire *deoQKPX* region from pSU*deoQKPX*, was blunt ended with the Klenow fragment and ligated into filled-in *Bam*HI-digested pFLC, yielding pFLC*deoQKPX* (Fig. 2). The plasmid pFLC*deoQKPX'*, with 230 bp deleted from the 3' end of *deoX*, was constructed by ligating the 5.1-kbp *EcoRI* fragment of pSU*deoQKPX* into the unique *EcoRI* site of pFLC (Fig. 2). The plasmid pFLC*deoQK* (Fig. 2) was obtained by a three-point ligation of *Bam*HI/*EcoRI*-digested pFLC with the 2.2-kbp *EcoRI*/*Not*I fragment of pSU*deoQKPX*, containing *deoQ* and the proximal part of *deoK*, and a PCR fragment of the entire *deoK* gene with a built-in *Bam*HI site in the 3' primer, and digested with *Not*I/*Bam*HI. The plasmid pFLC*deoQ* was constructed from pFLC*deoQK* by deleting an internal 14-bp *Mlu*I/*Bss*HII fragment from the *deoK* gene (Fig. 2).

**pWSK derivatives.** Plasmids pWSK29*deoQKPX* and pWSK129*deoQKPX* were obtained by cloning the 4.7-kbp *Acl*I/*Bam*HI fragment from pSU*deoQKPX* into *Acl*I/*Bam*HI-digested pWSK29 and pWSK129, respectively. Digestion of pWSK129*deoQKPX* with *Sac*II (one *Sac*II site is located in the plasmid immediately downstream of the 3' end of *deoX*) resulted in deletion of almost the entire *deoX* gene, yielding pWSK*deoQK*. Plasmids pWSK*deoQK* and pWSK*deoQ* were obtained by transferring the *deo* fragments from pFLC*deoQK* and pFLC*deoQ* into pWSK129 as *EcoRI*/*Bam*HI fragments (Fig. 2).

**Translational fusions.** The intercistronic region between *deoQ* and *deoK*, including the first seven *deoQ* codons and the first two *deoK* codons, was amplified by PCR, and the 357-bp amplicon was separately cloned in each orientation into the *Sma*I site of the translational fusion vector pRAK80 (20). The resulting plasmids, pRAK80 $\Phi$ (*deoK-lacZ*)<sub>hybrid</sub> and pRAK80 $\Phi$ (*deoQ-lacZ*)<sub>hybrid</sub>, expressed the DeoK-LacZ and DeoQ-LacZ fusion proteins from the *deoK* and *deoQ* promoters located in the intercistronic region. The 3.7-kbp *EcoRI*/*Eco*NI fragments of pRAK80 $\Phi$ (*deoK-lacZ*)<sub>hybrid</sub> and pRAK80 $\Phi$ (*deoQ-lacZ*)<sub>hybrid</sub>, containing the intercistronic region and the entire *deo-lacZ* fusions, were blunt ended using the Klenow enzyme and ligated into the unique *Ecl*136II site of pGD519. pGD519 is a derivative of pGD56 (GenBank accession number X67018), with a unique *Ecl*136II site located between the B region and the *galK* gene of pGD519. This facilitated the transfer of the *deo-lacZ* gene fusions to *gal8* ( $\lambda$ *galETK*<sup>+</sup> c1857) by in vivo recombination (25). The resulting recombinant  $\lambda$  phages were used to construct single lysogens of CSH26 as described previously (7).

**Transcriptional fusions.** The 357-bp intercistronic region between *deoQ* and *deoK*, including the first few codons of the genes, was isolated from pRAK80 $\Phi$ (*deoK-lacZ*)<sub>hybrid</sub> and from pRAK80 $\Phi$ (*deoQ-lacZ*)<sub>hybrid</sub> as *EcoRI*/*Bam*HI fragments and ligated into the transcriptional fusion vector pFZY1 upstream of the promoterless *lacZ* gene, yielding pFZY $\Phi$ (*deoK-lacZ*<sup>+</sup>) and pFZY $\Phi$ (*deoQ-lacZ*<sup>+</sup>), respectively. The insertions were such that translation initiating from the *deoK* or *deoQ* start codon would terminate at the same stop codon within the *lacZ* upstream region. Plasmid pFZY $\Phi$ (*deoQKPX-lacZ*<sup>+</sup>), containing the *deoQ-deoK* intercistronic region, *deoK*, *deoP*, and part of *deoX* (Fig. 2), was obtained from pSU*deoQKPX* by digestion with *Psh*AI and *Rsr*II, filling of the 5' overhang of the latter site, and religating. From the resulting plasmid, the desired region was excised using *EcoRI* and cloned into the *EcoRI* site of pFZY1 upstream of *lacZ* (Fig. 2). Plasmid pFZY $\Phi$ (*deo'KPX-lacZ*<sup>+</sup>), lacking the *deoQ-*

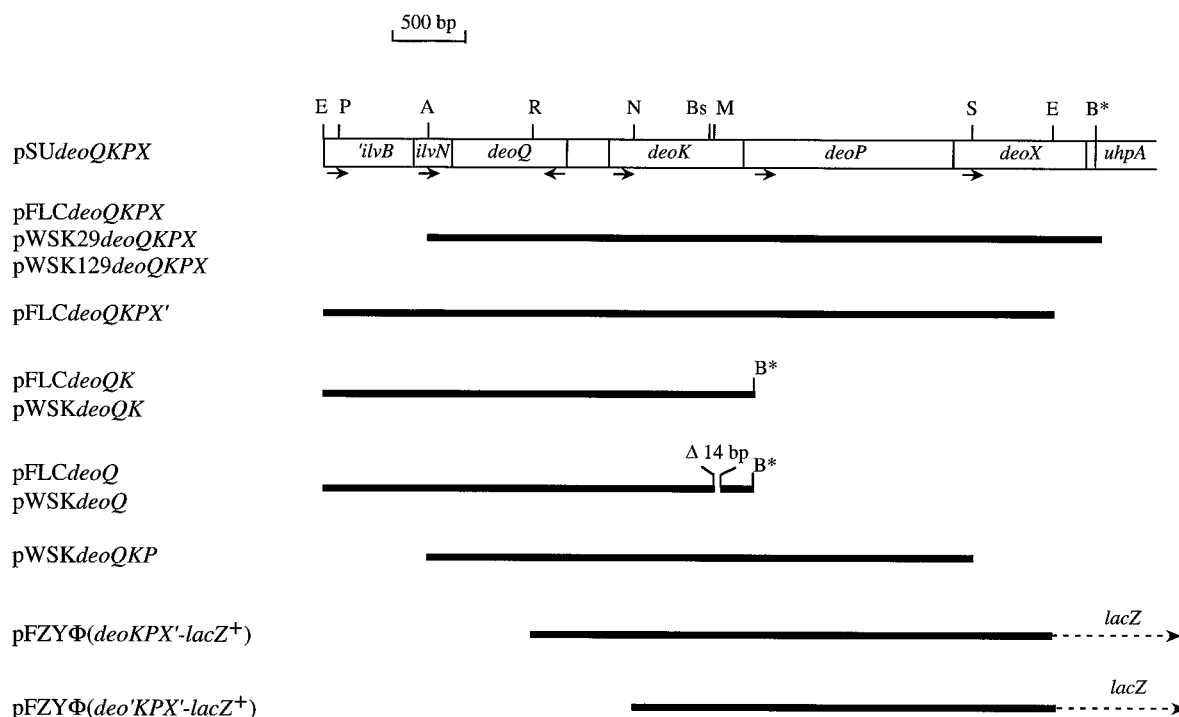


FIG. 2. Structure of specific plasmids. The open box represents the 5.4-kb serovar Typhimurium DNA cloned in pSU19. Above the box, relevant restriction enzyme sites are shown: A, *AclI*; B\*, *Bam*HI introduced by PCR; Bs, *Bss*HIII; E, *Eco*RI; M, *Mlu*I; N, *Not*I; P, *Psh*AI; R, *Rsr*II; S, *Sac*II. Arrows below the box indicate the direction of transcription. The thick solid lines pertain to cloned serovar Typhimurium DNA present in the individual plasmids. Dotted arrows indicate the plasmid-borne *lacZ* reporter gene.

*deoK* intergenic region and the first 46 codons of *deoK*, was constructed by deleting the *Psh*AI-*Not*I fragment from pSUdeoQKPX, filling the 5' overhangs, ligating, and moving the *Eco*RI fragment as described above into pFZY1 (Fig. 2).

**Preparation of cell extracts and enzyme assays.** Cell extracts were prepared by sonic disruption of cells suspended in 0.1 M potassium phosphate buffer (pH 7.5)–0.5 mM EDTA, followed by centrifugation to remove cellular debris.  $\beta$ -Galactosidase activity was determined at 28°C as described by Miller (26). Thymidine phosphorylase activity was measured at 37°C as described by Schwartz (33). One unit is defined as 1 nmol of product formed per min. Specific activities are given as units per optical density at 436 nm ( $OD_{436}$ ) of the bacterial suspension prior to sonication.

**Mapping of the transcriptional start sites.** Total cellular RNA was extracted from exponentially growing cells of MEC011/pWSK129deoQKPX using hot phenol, essentially as previously described (35). Primer extension analysis of the 5' ends of the *deoQ* and *deoK* transcripts was carried out using avian myeloblastosis virus reverse transcriptase (36). The primers used were complementary to codons 21 to 29 of *deoQ* (5'CGCGCGGCGTCTTTCAGATGGATTTCG) or to codons 16 to 25 of *deoK* (5'CCCCTTCTTTGGGCATCTGGTTGGTG) and were 5' labeled using [ $\gamma$ - $^{32}$ P]ATP and T4 polynucleotide kinase. The extension products were resolved on a 6% polyacrylamide–7 M urea gel alongside DNA sequence ladders produced with the same labeled primers.

**Expression and purification of the DeoQ protein.** The *deoQ* gene from serovar Typhi strain Ty2 was amplified by PCR using the following primers: forward, 5'GGCACGCATATGGAGACCAAGCAAAAAGAACG (*Nde*I restriction site underlined), and reverse, 5'AGGACTCGAGTTCACCTTCCGAATCAGGCGTG (*Xho*I restriction site underlined). (The amino acid sequence of the DeoQ protein from serovar Typhi is 100% identical with the DeoQ protein of serovar Typhimurium.) The amplicon was cleaved and inserted between the same two sites (*Nde*I-*Xho*I) in the expression vector pET22b (Novagen), creating an in-frame fusion between the 3' end of the *deoQ* gene and six histidine codons. The resulting plasmid, pAOT9b, was transformed into *E. coli* BL21(DE3). The recombinant His-tagged DeoQ protein was overproduced from cells growing exponentially at room temperature in NZY medium containing Ap. At an  $OD_{600}$  of 1.0, isopropyl- $\beta$ -D-thiogalactopyranoside (0.5 mM) was added, and incubation continued for 2 h, after which time the cells were harvested by centrifugation. Cells were suspended in 50 mM sodium phosphate (pH 8.0)–0.5 M NaCl and

sonically disrupted, and the cellular debris was removed by centrifugation. The supernatant was applied to a Ni-CAM (Sigma, St. Louis, Mo.) affinity column equilibrated in sonication buffer containing 25 mM imidazole, and the column was washed with 7 volumes of the same buffer. The DeoQ protein was eluted with 200 mM imidazole in sonication buffer and dialyzed against sonication buffer to remove the imidazole. Following addition of glycerol to 10%, the preparation was stored at –20°C. The protein concentration was calculated from the absorbance at 280 nm.

**Gel filtration.** The native molecular mass of purified DeoQ was estimated by gel filtration on a Superose 12HR 10/30 gel filtration column (Amersham Pharmacia Biotech) attached to a fast protein liquid chromatography apparatus (Bio-Rad). The column was equilibrated, and elution was carried out with 50 mM sodium phosphate (pH 6.5), 0.6 M NaCl, 2 mM EDTA, 1 mM dithiothreitol (DTT). Carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), and  $\beta$ -amylase (200 kDa) were used as molecular mass markers.

**Gel retardation assays.** The DNA probes used (a 386-bp fragment including the entire intergenic region between *deoQ* and *deoK*, a 189-bp fragment including the *deoK* promoter and 70 bp of its upstream region, and a 206-bp fragment with the *deoQ* promoter and 90 bp of its upstream region) were generated by PCR and radiolabeled at the 5' ends using [ $\gamma$ - $^{32}$ P]ATP and T4 polynucleotide kinase. The binding reaction contained, in a 10- $\mu$ l volume, the following: 10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 1 mM DTT (pH 8.2), 250  $\mu$ g of bovine serum albumin per ml, 5% glycerol, and 200 to 400 pM labeled probe. Different concentrations of DeoQ were used for each binding reaction. In the binding stoichiometry experiments, 0.2  $\mu$ M nonradioactive DNA of the same fragment was added in addition to the radiolabeled fragment. After incubation at 37°C for 15 min, the samples were electrophoresed at room temperature on a 5% polyacrylamide gel (10 cm) for 3 h at 8 V/cm. Dried gels were quantitated with a Packard cyclone storage phosphor system.  $K_d$  values were determined as the concentration of DeoQ required for 50% of the labeled probe to be retarded on the gel.

**DNA sequences.** The nucleotide sequence of the *deoQKPX* locus is located at coordinates 3991219 to 3995621 on the serovar Typhimurium LT2 genome (GenBank accession no. NC\_003197) and at coordinates 3853107 to 3857498 on the serovar Typhi genome (accession no. NC\_003198). The coordinates of the homologous regions of other bacteria are found under the following accession

TABLE 1. Effect of the *deoK*, *deoP*, and *deoX* genes on the growth rate of *E. coli* K12 with dRib as the carbon source at 37°C

Plasmid <sup>a</sup>	Doublings/h with:	
	0.08% dRib	0.2% dRib
pFLC	<0.10	<0.10
pFLC <i>deoQKPX</i>	0.68	0.63
pFLC <i>deoQKP</i>	0.32	0.57
pFLC <i>deoQK</i>	0.22	0.55

<sup>a</sup> The plasmids were contained in *E. coli* CSH26.

numbers: *Salmonella enterica* serovar Enteritidis, accession no. NC\_002962; *E. coli* CFT073, accession no. NC\_004431, coordinates 300643 to 305020; *L. plantarum*, accession no. NC\_004567; coordinates 442825 to 448118; *Pseudomonas syringa*, accession no. NC\_002949; *Agrobacterium tumefaciens*, accession no. NC\_003304, coordinates 2020602 to 2029321.

## RESULTS

**Cloning and phenotypic characterization of the *deoQKPX* region of serovar Typhimurium.** The capacity of serovar Typhi to ferment dRib was previously shown to be encoded on a 4,392-bp DNA fragment located between *ilvN* and *uhpA* (38). No homologous DNA is present in the *E. coli* K12 genome, and accordingly, *E. coli* K12 cannot grow with dRib as the sole carbon source. The nucleotide sequence of the serovar Typhimurium genome (24) indicated that this organism contained a homologous 4,403-bp DNA fragment, likewise located between *ilvN* and *uhpA*. The nucleotide sequence of this region is 98.7% identical with that of serovar Typhi, and the deduced amino acid sequences of the four open reading frames within the fragment are 100% identical for DeoQ, DeoK, and DeoP and 98.5% for DeoX.

From a partial *Sau3AI* library of serovar Typhimurium LT2 DNA in pBR328, most of the *deoQKPX* region was obtained by complementation in *E. coli*. The missing DNA was obtained by PCR, and the entire region was assembled as a 5.4-kb *EcoRI/BamHI* fragment in pSU19, yielding pSU*deoQKPX*. From this plasmid, all other plasmids used in the present work were derived as described in Materials and Methods and in Fig. 2.

It was previously shown that deoxyribokinase is required for growth of *S. enterica* or *E. coli* K12 with dRib as the sole carbon source (17, 38). To establish the importance of the *deoP* and *deoX* gene products in dRib utilization, various regions of the *deoQKPX* cluster were subcloned into the single-copy plasmid pFLC, and the ability of these plasmids to support growth of *E. coli* K12 with dRib as the carbon source was tested in liquid culture. As shown in Table 1, the presence of both *deoP* and *deoX* was required for the maximal growth rate with 0.08% dRib as the carbon source, whereas no significant difference was observed with 0.2% dRib.

***deoK*, *deoP*, and *deoX* constitute an operon.** Sequence analysis of the *deoQKPX* DNA suggested that the *deoK*, *deoP*, and *deoX* genes were transcribed as a single transcript from a promoter located in the *deoQ-deoK* intercistronic region. Accordingly, we observed that expression of  $\beta$ -galactosidase activity from the *deoX-lacZ* transcriptional fusion carried on pFZY $\Phi$ (*deoKPX'-lacZ*<sup>+</sup>) (Fig. 2) was 46.8 U/OD<sub>436</sub>, whereas it was only 0.8 U/OD<sub>436</sub> from pFZY $\Phi$ (*deo'KPX'-lacZ*<sup>+</sup>), which is lacking the *deoQ-deoK* intercistronic region and the first 46

codons of *deoK* (Fig. 2). A putative rho-independent transcriptional terminator, with a calculated stability of the stem-loop structure of -17.6 kcal per mol (23), was found 22 bp downstream of the *deoX* stop codon within the 74-bp intergenic region between *deoX* and *uhpA*.

**Regulation of *deoQ* and *deoKPX* expression.** To identify the divergent promoters responsible for expression of *deoQ* and *deoKPX*, the intergenic region between *deoQ* and *deoK* (including the first few codons of the genes) was fused in-frame to *lacZ* in each orientation, and the fusions were transferred to a lambda phage and integrated in single copy into the  $\lambda$  attachment sites of *E. coli* MEC011 and CSH26. The resulting four strains were grown in minimal medium with different carbon sources, and the levels of  $\beta$ -galactosidase activity, to monitor expression of *deoK/deoQ*, and thymidine phosphorylase (*deoA*) activity, to monitor expression of the DeoR controlled *deoCABD* operon, were determined. The results (Table 2) showed that expression of  $\beta$ -galactosidase from the two fusions was the same in the *deoR* genetic background (experiment 1) and that this expression was repressed by the presence of the *deoQ* gene in *trans*, the effect on the *deoK* fusion being the strongest (experiments 2 and 3). Addition of dRib to the growth medium resulted in about 15-fold induction of  $\beta$ -galactosidase synthesis from each fusion, whereas addition of thymidine had no effect (experiments 3, 4, and 5). The  $\beta$ -galactosidase activity levels observed when cells were grown with glucose as the carbon source (Table 2, experiment 6) indicated that *deoK* expression but not *deoQ* expression was regulated by catabolite repression.

The significant amino acid sequence identity between DeoQ and DeoR (36.3% for serovar Typhimurium) led us to look for possible cross-activity between the two repressors and their targets. Thus, the regulation of expression of the two fusions was also investigated in a *deoR*<sup>+</sup> genetic background (lower part of Table 2). The *E. coli* DeoR repressor was able to repress both *deoK* and *deoQ* expression to about the same extent as DeoQ (experiment 7 versus experiments 2 and 3). As expected, addition of thymidine, an effective inducer of the *deoCABD* operon, resulted in induction of DeoR-mediated repression (experiment 9). Since thymidine did not induce  $\beta$ -galactosidase synthesis in the presence of both DeoQ and DeoR (experiment 12), it appeared that DeoR was subordinate to DeoQ in repression of *deoK* and *deoQ* expression. In contrast, dRib, which is not an inducer of DeoR-mediated repression (experiment 8), was effective as an inducer of *deoK* and *deoQ* expression in a *deoQ*<sup>+</sup> *deoR*<sup>+</sup> genetic background (experiment 11 versus experiment 4). It is noteworthy that even though DeoR was capable of repressing expression of *deoK*, *deoQ*, and *deoA* (experiment 7), the reciprocal was not observed (experiment 3).

**Strength of the *deoK* and *deoQ* promoters.** Results obtained for translational fusions of different promoters to a reporter gene cannot be directly compared, since they reflect not only promoter strength but also different ribosomal binding sites and different  $\beta$ -galactosidase hybrid proteins. To gain more reliable information about the relative strengths of the *deoQ* and *deoK* promoters, transcriptional fusions were constructed. The intergenic region between *deoQ* and *deoK* including the first few codons of the genes was cloned in either orientation into pFZY1. The plasmids were transformed into MEC011,

TABLE 2. Effect of DeoQ and DeoR on the synthesis of  $\beta$ -galactosidase from the translational *deoK-lacZ* and *deoQ-lacZ* fusions and of thymidine phosphorylase from *deoA*<sup>a</sup>

Expt no.	Relevant host genotype	Relevant plasmid genotype <sup>b</sup>	Carbon source <sup>c</sup>	Activity (units/OD <sub>436</sub> )		
				$\beta$ -Galactosidase		Thymidine phosphorylase ( <i>deoA</i> ) <sup>d</sup>
				<i>deoK-lacZ</i>	<i>deoQ-lacZ</i>	
1	<i>deoR</i>	Parental	Gly	8.8	9.0	140
2	<i>deoR</i>	<i>deoQ</i> <sup>+</sup>	Gly	0.3	1.3	— <sup>e</sup>
3	<i>deoR</i>	<i>deo(QKPX)</i> <sup>+</sup>	Gly	0.08	0.4	116
4	<i>deoR</i>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + dRib	1.3	5.8	—
5	<i>deoR</i>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + dT	0.05	0.5	—
6	<i>deoR</i>	Parental	Glu	1.2	8.7	—
7	<i>deoR</i> <sup>+</sup>	Parental	Gly	0.3	1.4	3.0
8	<i>deoR</i> <sup>+</sup>	Parental	Gly + dRib	—	—	3.3
9	<i>deoR</i> <sup>+</sup>	Parental	Gly + dT	5.8	6.1	145
10	<i>deoR</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly	0.08	0.4	3.2
11	<i>deoR</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + dRib	1.4	4.8	178
12	<i>deoR</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + dT	0.05	0.3	150

<sup>a</sup> MEC011 (*deoR*) or CSH26 (*deoR*<sup>+</sup>) lysogenized with phage  $\lambda$  carrying either the *deoK-lacZ* or the *deoQ-lacZ* translational fusion and the indicated plasmid was grown exponentially for at least three generations at 32°C in minimal medium with the indicated carbon sources. Cell extracts were assayed for  $\beta$ -galactosidase and thymidine phosphorylase activities.

<sup>b</sup> All plasmids were derivatives of pWSK29. Parental indicates the vector with no insert. The serovar Typhimurium genes carried on the plasmids are indicated in italics.

<sup>c</sup> All carbon sources were added at 0.2%.

<sup>d</sup> Thymidine phosphorylase activity was measured only in strains carrying the *deoQ-lacZ* fusion.

<sup>e</sup> —, not measured.

and the  $\beta$ -galactosidase activity levels were determined. As shown in Table 3, expression from the *deoK* promoter was 10-fold higher than from the *deoQ* promoter when cells were grown with glycerol as the carbon source (experiments 1 versus 3). In glucose medium, however, a significant repression of *deoK-lacZ* expression but not of *deoQ-lacZ* expression was observed (experiments 5 versus 3).

**Nature and specificity of the inducer.** Introduction of a second plasmid, carrying *deoQ* and various amounts of the *deoK* locus into MEC011/pFZY $\Phi$ (*deoK-lac*<sup>+</sup>), showed (Table 3) that the presence of *deoQ* resulted in strong repression (80- to 90-fold) and that induction by dRib was entirely dependent on the simultaneous presence of *deoK*. Neither *deoP*

nor *deoX* were required for induction to occur. This suggested that the endogenous inducer of *deoK* was a metabolite of deoxyribose, rather than the free sugar itself. Under no conditions did the induced level reach the fully constitutive levels.

The ability of L-fucose, D-xylose, L-arabinose, and D-ribose to induce the DeoQ-mediated repression of *deoK-lacZ* expression was investigated using cultures of MEC011/pFZY $\Phi$ (*deoK-lac*<sup>+</sup>)/pWSK*deoQKPX* grown in glycerol minimal medium in the presence of 0.2% of the putative inducer. Of the compounds tested, only D-ribose was capable of stimulating  $\beta$ -galactosidase synthesis (Table 3, experiment 14).

**Mapping of the transcriptional start sites.** The transcriptional start site for each of the two promoters within the intergenic region was established by primer extension analysis. Total RNA was isolated from log-phase cultures of MEC011/pWSK129*deoQKPX* grown in minimal medium with either 0.2% glycerol or 0.2% dRib as the carbon source. As shown in Fig. 3, initiation of dRib-inducible *deoK* transcription occurred with ATP, 39 bp upstream of the DeoK start codon (Fig. 4). A weaker signal, which also appeared to be dRib regulated, was observed 6 bp downstream of the strong signal. Upstream of both of these sites, with the correct spacing, sequences resembling the consensus extended -10 element (TGNTATAAT) of putative promoters were identified (22). A dRib-regulated transcriptional start site signal was also observed for *deoQ*, although about 20-fold-higher levels of the same total RNA was required to obtain intensities similar to those observed for the *deoK* transcripts. The start site corresponded to an ATP start, 72 bp upstream of the *deoQ* start codon, and is preceded by a correctly spaced -10 element.

**DeoQ binding to the intergenic DNA.** The binding of purified His-tagged DeoQ to the 324-bp *deoQ-deoK* intergenic region was determined using an electrophoretic gel mobility shift assay. The radiolabeled oligodeoxynucleotide was a

TABLE 3. Regulation of  $\beta$ -galactosidase synthesis from transcriptional *deoK-lacZ* and *deoQ-lacZ* fusions and specificity of induction<sup>a</sup>

Expt no.	Genotype of pFZY-plasmid	Relevant genotype of pWSK129-plasmid	Carbon source	$\beta$ -Galactosidase (units/OD <sub>436</sub> )
1	<i>deoQ-lacZ</i> <sup>+</sup>	Vector	Gly	8.2
2	<i>deoQ-lacZ</i> <sup>+</sup>	Vector	Glu	13
3	<i>deoK-lacZ</i> <sup>+</sup>	Vector	Gly	81
4	<i>deoK-lacZ</i> <sup>+</sup>	Vector	Gly + dRib	76
5	<i>deoK-lacZ</i> <sup>+</sup>	Vector	Glu	24
6	<i>deoK-lacZ</i> <sup>+</sup>	<i>deoQ</i> <sup>+</sup>	Gly	0.9
7	<i>deoK-lacZ</i> <sup>+</sup>	<i>deoQ</i> <sup>+</sup>	Gly + dRib	0.7
8	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QK)</i> <sup>+</sup>	Gly	0.9
9	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QK)</i> <sup>+</sup>	Gly + dRib	23
10	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QKP)</i> <sup>+</sup>	Gly	0.9
11	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QKP)</i> <sup>+</sup>	Gly + dRib	17
12	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly	1.0
13	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + dRib	15
14	<i>deoK-lacZ</i> <sup>+</sup>	<i>deo(QKPX)</i> <sup>+</sup>	Gly + ribose	13

<sup>a</sup> Cells of MEC011 carrying the transcriptional fusions pFZY $\Phi$ (*deoK-lacZ*<sup>+</sup>) or pFZY $\Phi$ (*deoQ-lacZ*<sup>+</sup>) and the indicated pWSK129-based plasmids were grown exponentially for at least three generations at 37°C in minimal medium with various carbon sources. Cell extracts were assayed for  $\beta$ -galactosidase activity.

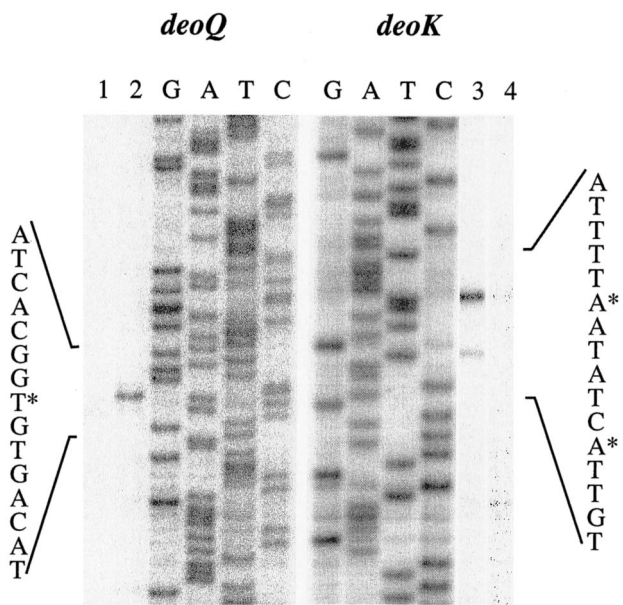


FIG. 3. Primer extension analysis with total RNA from cells carrying pWSK129*deoQKPX* and grown in the absence (lanes 1 and 4) and presence (lanes 2 and 3) of 0.2% dRib. For the *deoQ* analyses, 20-fold more total RNA was used. Transcriptional start points are indicated with asterisks.

386-bp fragment containing the intergenic region and the first few codons of DeoQ and DeoK. As shown in Fig. 5A, multiple species of DeoQ-DNA complexes were observed, suggesting more than one binding site on the DNA. By determining the percentages of total DNA bound at different DeoQ concentrations, an apparent  $K_d$  of 2.4 nM was determined from the binding curve depicted in Fig. 5B. To estimate the contribution

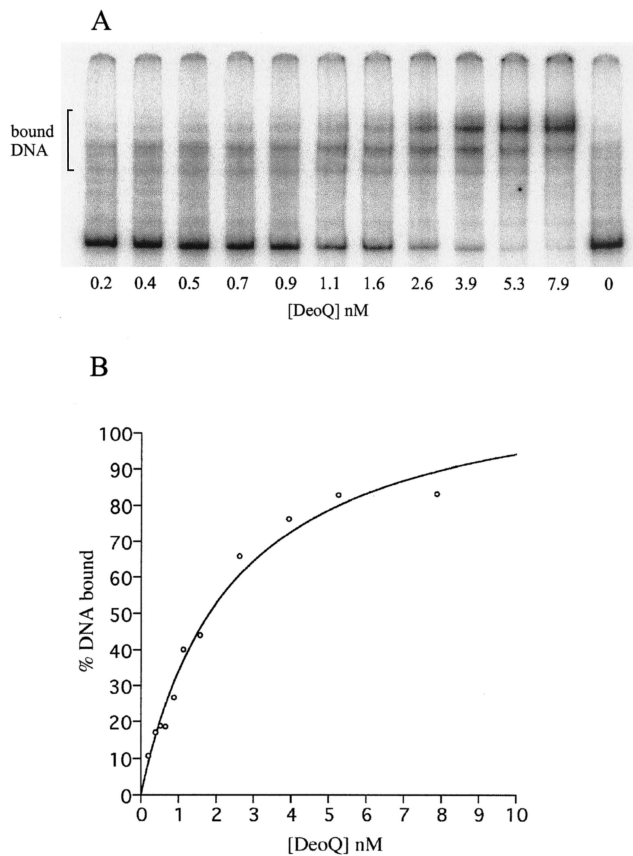


FIG. 5. Binding of the DeoQ repressor protein to the 324-bp *deoQ-deoK* intergenic DNA. (A) Profile of gel retardation assays using 200 to 400 pM labeled probe and increasing concentrations of DeoQ. (B) Calculated binding curve for DeoQ with the intergenic DNA.

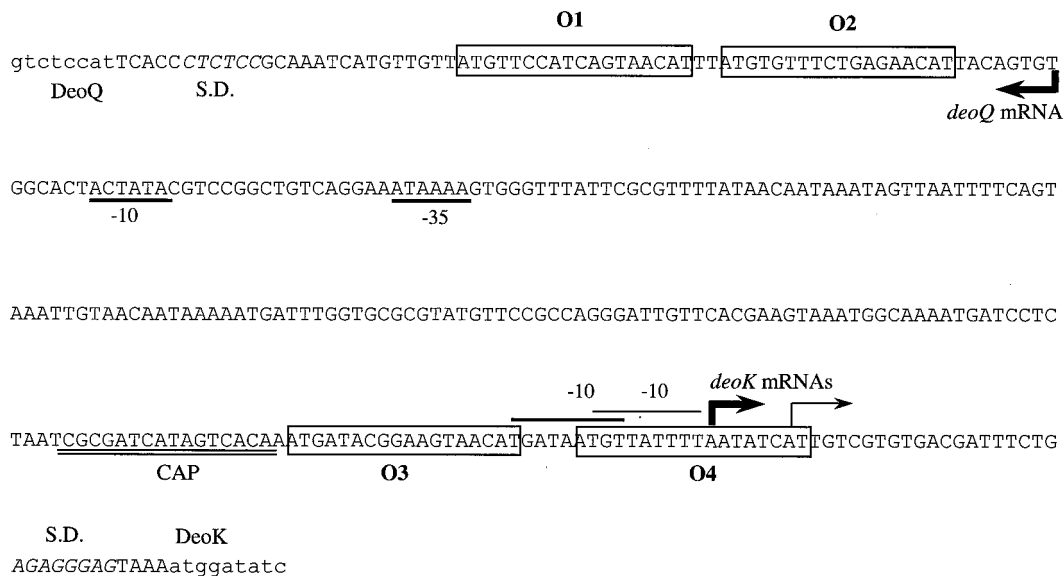


FIG. 4. Nucleotide sequence of the *deoQ-deoK* intercistronic region. Major transcriptional starts are indicated with heavy arrows, and secondary starts with thin arrows. Coding regions are indicated with lowercase lettering. Deduced -10 elements for *deoK* are indicated by a line above them, and -10 and -35 elements for *deoQ* are underlined. Putative operator sites are boxed and numbered O1 through O4, and the inferred cAMP-CRP binding site is labeled CAP. Shine-Dalgarno elements are in italics and indicated by S.D.

TABLE 4. Binding of purified DeoQ to fragments of the *deoQ*-*deoK* intergenic region<sup>a</sup>

Fragment <sup>b</sup>	$K_d$ (nM) <sup>c</sup>	[DeoQ]/[DNA] <sup>d</sup>
<div style="display: flex; justify-content: space-between;"> <div style="text-align: center;"> <p><i>deoQp</i></p> <p>386 bp</p> </div> <div style="text-align: center;"> <p><i>deoKp</i></p> <p>189 bp</p> </div> </div>	2.4	16
<div style="display: flex; justify-content: center;"> <div style="text-align: center;"> <p><i>deoQp</i></p> <p>206 bp</p> </div> </div>	54	ND <sup>e</sup>

<sup>a</sup> Binding was determined using electrophoretic mobility shift assays as described in Materials and Methods.

<sup>b</sup> DNA fragments used in the binding assays were prepared by PCR and labeled at the 5' ends with <sup>32</sup>P.

<sup>c</sup> The apparent  $K_d$  values were determined as the concentration of DeoQ that gave 50% saturation of the operator sites.

<sup>d</sup> [DeoQ], concentration of DeoQ based on the subunit size of 29 kDa.

<sup>e</sup> ND, not determined.

to this binding from each of the divergent promoter-operators, the binding of purified DeoQ to the individual promoter regions was determined, using radiolabeled PCR fragments of each half of the intergenic region, i.e., the 189-bp *deoKp* fragment and the 206-bp *deoQp* fragment (data not shown). The results (Table 4) indicated that the affinity of DeoQ to the *deoK* proximal operator was 18-fold higher than for the *deoQ* proximal operator. Inclusion of a 1 mM concentration of either dRib, dRib5P, or ribose in the mobility shift assays did not affect the percentage of DeoQ bound to the radiolabeled DNA probes (data not shown).

**Stoichiometry of DeoQ binding.** To determine the binding stoichiometry of DeoQ to the operator DNA, gel retardation assays were performed, using concentrations of operator DNA much higher than the  $K_d$  values. As shown for the *deoKp* fragment, containing only the *deoK* promoter/operator, approximately eight DeoQ subunits were bound per 189-bp fragment (Fig. 6). Similar titration experiments showed that approximately 16 DeoQ subunits were bound per 386-bp fragment when the full-length intergenic DNA was used as the probe (Table 4), suggesting that at saturation each promoter/operator is capable of binding one octameric DeoQ species.

Consistent with these findings, purified His-tagged DeoQ protein was observed to elute slightly ahead of the  $\beta$ -amylase (200 kDa) marker on a Superose 12HR gel filtration column, indicating that DeoQ in solution may exist as an octamer (8  $\times$  29 kDa) (data not shown).

**Two operator sites are required for efficient DeoQ binding.** Inspection of the nucleotide sequence of the intergenic region between *deoQ* and *deoK* revealed a pair of closely spaced putative DeoQ binding sites within each of the two transcription initiation regions (Fig. 4). All four sites showed significant similarity with the consensus DeoR binding site (NTGTN<sub>10</sub>ACAN [14]). One pair (O3-O4) included the entire *deoK* pro-

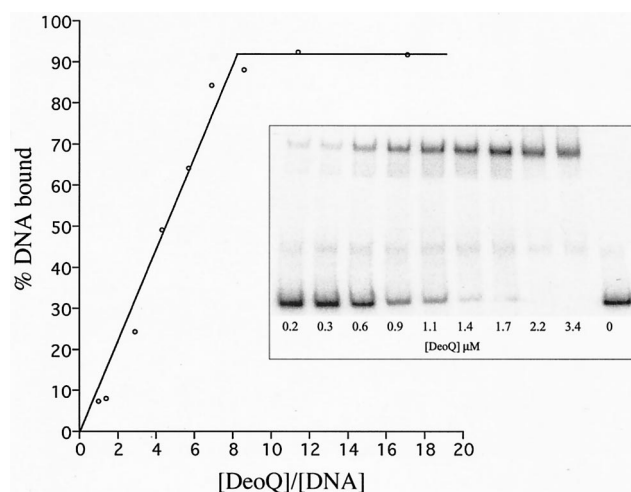


FIG. 6. DeoQ binding stoichiometry for the 189-bp *deoK* promoter/operator DNA. In each binding assay, a 200 nM nonradioactive 189-bp DNA fragment was present in addition to the radiolabeled DNA (200 pM).

motor and transcriptional start site, whereas the other (O1-O2) was located immediately downstream of the major transcriptional start site for *deoQ* mRNA. To determine whether O3 and O4 were involved in binding of DeoQ to the *deoKp* region, three 54-bp oligodeoxynucleotides were synthesized and used as competitor DNAs in gel retardation assays with radiolabeled *deoKp* and purified DeoQ. The unlabeled 54-mers contained either wild-type O3 and wild-type O4 (O3-O4), wild-type O3 and mutant O4 (O3-mutO4), or mutant O3 and wild-type O4 (mutO3-O4). The mutated operators harbored multiple base substitutions to destroy the palindromic nature of the sites (legend to Fig. 7). The concentration of DeoQ used in the experiment was chosen to give retardation of approxi-

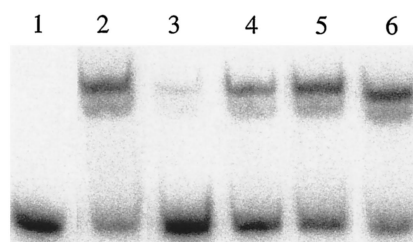


FIG. 7. Gel retardation assays of DeoQ binding to the 189-bp radiolabeled *deoK* promoter/operator DNA in the presence of synthetic, 54-bp, unlabeled operator DNA. All assays contained a 400 pM radioactive DNA fragment and an amount of DeoQ that gave an approximately 60% shift in the absence of competitor DNA. Lane 1, control with no DeoQ; lane 2, DeoQ with no competitor DNA; lane 3, DeoQ plus 1  $\mu$ M unlabeled O3-O4 DNA; lane 4, DeoQ plus 1  $\mu$ M unlabeled O3-mutO4 DNA; lane 5, DeoQ plus 1  $\mu$ M unlabeled mutO3-O4 DNA; lane 6, DeoQ plus 0.02  $A_{260}$  units of unlabeled poly(dI-dC). The 54-bp sequences of the competitor DNAs were as follows (the lowercase letters indicate mutations, and the putative operator sites are underlined): O3-O4, GTCACAAATGATACGGAAGTAACATGATAATGTTATTTTAATATCATTGTCGTG; O3-mutO4, GTCACAAATGATACGGAAGTAACATGATAAgaaTcgTTTTAAcgtTgTTGTCGTG; mutO3-O4, GTCACAAgaaAcgCGGAAGgcAtgTGATAATGTTATTAAATATCATTGTCGTG.

mately 60% of the labeled DNA in the absence of competitor DNA. As shown in Fig. 7, the presence of a large excess of unlabeled O3-O4 resulted in a large decrease in the proportion of labeled DNA in the shifted position, whereas only minor competition was observed with the oligonucleotides harboring either one of the two operators in a mutated form. This suggested that both O3 and O4 are required for optimal binding of the DeoQ repressor to its DNA target.

## DISCUSSION

The *deoQKPX* gene system, responsible for the ability of serovar Typhimurium to utilize dRib as the sole carbon and energy source, was cloned for the purpose of a genetic characterization and a study of expression of the involved genes. In silico analysis led to the predictions that *deoQ* encoded a regulatory protein of the *deoR-lacI* family and that *deoP* encoded an inner-membrane protein involved in dRib transport (38). Recent studies of the purified DeoX protein have shown that it possesses mutarotase activity with high specificity towards deoxyribose (O. Barzu and A.-M. Gilles, unpublished results).

In the current study, complementation of the dRib<sup>-</sup> phenotype of *E. coli* with various regions of the *deoQKPX* locus showed that neither *deoP* nor *deoX* was required for growth on 0.2% dRib as carbon source. However, at suboptimal concentrations of dRib (0.08%), the presence of the two genes resulted in cumulative stimulation of growth (Table 1). Thus, both DeoP and DeoX are involved in facilitating the conversion of exogenous dRib to the endogenous substrate of deoxyribokinase, i.e., 2-D-deoxyribofuranose. This involves transport across the membrane and eventual interconversion of different steric forms of dRib.

The divergent *deoQ* and *deoKPX* promoters, *deoQp* and *deoKp*, are located at opposite ends of the *deoQ-deoK* intercistronic region, with *deoKp* being about 10-fold stronger than *deoQp* (Table 3). Initiation of transcription from each of the promoters is regulated negatively both by the DeoQ repressor and by DeoR, the repressor of the deoxynucleoside catabolic *deoCABD* operon. It should be noted, however, that DeoQ is not capable of repressing expression of the *deoCABD* operon, as shown by the high constitutive levels of thymidine phosphorylase in a *deoR/pdeoQKPX* strain (Table 2). In a *deoR<sup>+</sup>/pdeoQKPX* strain, which expresses both repressor proteins, addition of dRib resulted in induction of both *deoQp* and *deoKp* as well as the *deoCABD* operon. Thymidine, on the other hand, induced only the *deoCABD* operon. This indicates that dRib5P, which is the true inducer of the DeoR regulon (15), is not the endogenous inducer of DeoQ-mediated repression. Since we further observed (Table 3) that induction of *deoKp* by dRib required the presence of the *deoK* gene, we propose that deoxyribokinase, in addition to its metabolic function of catalyzing the conversion of dRib to dRib5P, may catalyze alternative transformations (phosphorylations) of dRib or dRib5P to produce the true inducer. This may resemble the induction pattern observed for the *E. coli lac* operon, where a functional  $\beta$ -galactosidase (*lacZ*) is required not only for cleavage of the substrate lactose but also for converting lactose to the true inducer of the operon, allolactose (19, 29).

Gel retardation assays with purified DeoQ protein showed that the repressor binds to each of the promoter-proximal

halves of the *deoQ-deoK* intergenic DNA. In accordance with the in vivo results showing that DeoQ represses expression of *deoK* more effectively than expression of *deoQ*, the in vitro binding studies showed that the affinity of DeoQ for the *deoKp* region was much higher than for the *deoQp* region (Table 4). Although DeoQ is similar to DeoR from both *E. coli* and *Bacillus subtilis* in appearing to exist as an octamer in solution (28, 40) and containing a helix-turn-helix domain characteristic for DNA-binding proteins, it differs from the two DeoR proteins in that dRib5P is not the low-molecular-weight effector for the protein (see above).

Four putative operator sites resembling the *E. coli* DeoR-binding operators of the *deoCABD* operon were identified within the *deoQ-deoK* intergenic region (Fig. 4). They are organized in pairs, with one tandem operator, (O1-O2), located shortly after the transcriptional start point of *deoQ*, and the other (O3-O4) covering the entire *deoK* promoter. From competition experiments with synthetic mutant operator sequences (Fig. 7), we conclude that both the O3 and O4 wild-type operators are required for efficient binding of DeoQ to the *deoK* promoter. DeoR-mediated repression of the *deoCABD* operon, on the other hand, involves long-range cooperative binding to two or three operators located several hundred base pairs apart, resulting in looping of the interoperator sequences (6, 9). Thus, the observation that DeoR is an effective repressor of *deoKPX* expression suggests not only that the two repressor proteins may recognize the same operator sequences but also that DeoR binding may involve cooperation between operator sites from both *deoQp* and *deoKp*. The observation that DeoQ did not repress expression of the *deoCABD* operon may be explained simply by the lack of closely spaced tandem operators in the promoter region of the *deoCABD* operon.

In addition, transcription from *deoKp*, but not from *deoQp*, was observed to be subject to catabolite repression by glucose. Although direct evidence has not been obtained, the absence of a -35 element combined with the presence of a putative CAP binding site at the consensus position for a class II CAP-dependent promoter (centered at position -41 with respect to the transcription start) (Fig. 4) indicated that the glucose effect is likely to be mediated through the cAMP/CAP gene activation system (4, 10).

PSI-BLAST (1) searches were carried out to identify homologues of DeoX, DeoP, DeoK, and DeoQ. The entire *deoQKPX* locus, with the same organization as in *S. enterica*, was found in the genomes of serovar Enteritidis and the uropathogenic *E. coli* CFT073, whereas it is not present in the genome of the enterohemorrhagic *E. coli* O157:H7. *P. syringae* harbors a similar region except that the gene order is *deoQ-deoX-deoP-deoK*, whereas for *L. plantarum*, a bacterium known to ferment deoxyribose (11, 13), the four genes are found in a cluster together with the gene for deoxyriboaldolase (*deoC*). The gene order in *L. plantarum* is *deoQ-deoC-deoP-deoX-deoK*. The deoxyribose utilization genes are also organized together with *deoC* in the plant pathogen *A. tumefaciens*; however, in this bacterium the *deoP* gene is replaced by four genes encoding members of a putative ABC transporter system with specificity towards pentoses. The significance of the presence of the *deoQKPX* locus in only one of the three complete *E. coli* genomes is unknown, and the distribution of the operon in



various *E. coli* strains is presently under investigation (C. Bernier-Febreau et al.; L. du Merle et al., unpublished data).

#### ACKNOWLEDGMENTS

The expert technical assistance of Lisbeth Stauning is gratefully recognized.

This work was supported by a NATO International Collaborative research grant (to R.A.K. and J.N.) and grants from the Natural Sciences and Engineering Research Council of Canada (to R.A.K.), from the Centre National de la Recherche Scientifique (URA2185), and from Institut Pasteur (to O.B. and A.M.G.).

#### REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Bartolomé, B., J. Jubete, E. Martínez, and F. de la Cruz. 1991. Construction and properties of a family of pACYC184-derived cloning vectors compatible with pBR322 and its derivatives. *Gene* **102**:75–78.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513–1523.
- Busby, S., and R. H. Ebright. 1999. Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.* **293**:199–213.
- Clark, D. J., and O. Maaløe. 1967. DNA replication and the division cycle in *Escherichia coli*. *J. Mol. Biol.* **23**:99–112.
- Dandanell, G. 1992. DeoR repression at-a-distance only weakly responds to changes in interoperator separation and DNA topology. *Nucleic Acids Res.* **20**:5407–5412.
- Dandanell, G., and K. Hammer. 1985. Two operator sites separated by 599 base pairs are required for *deoR* repression of the *deo* operon of *Escherichia coli*. *EMBO J.* **4**:3333–3338.
- Dandanell, G., and K. Hammer. 1989. The *deoR* repressor from *E. coli* and its action in regulation-at-a-distance, p. 79–91. In F. Eckstein and D. M. J. Lilley (ed.), *Nucleic acids and molecular biology*, vol. 3. Springer-Verlag, Berlin, Germany.
- Dandanell, G., P. Valentin-Hansen, J. E. L. Larsen, and K. Hammer. 1987. Long-range cooperativity between gene regulatory sequences in a prokaryote. *Nature (London)* **325**:823–836.
- De Crombrughe, B., S. Busby, and H. Buc. 1984. Cyclic AMP receptor protein: role in transcription activation. *Science* **224**:831–838.
- Domagk, G. F., and B. L. Horecker. 1958. Pentose fermentation by *Lactobacillus plantarum*. V. Fermentation of 2-deoxy-D-ribose. *J. Biol. Chem.* **233**:283–286.
- Feiss, M., S. Adyha, and D. L. Court. 1972. Isolation of plaque-forming, galactose-transducing strains of phage lambda. *Genetics* **71**:189–206.
- Ginsberg, A. 1959. A deoxyribokinase from *Lactobacillus plantarum*. *J. Biol. Chem.* **235**:1292–1298.
- Hammer, K., L. Bech, P. Hobolth, and G. Dandanell. 1993. DNA specificity of *Escherichia coli deoP1* operator-DeoR repressor recognition. *Mol. Gen. Genet.* **237**:129–133.
- Hammer-Jespersen, K. 1983. Nucleoside catabolism, p. 203–258. In A. Munch-Petersen (ed.), *Metabolism of nucleotides, nucleosides and nucleobases in microorganisms*. Academic Press, Inc., Ltd., London, United Kingdom.
- Hmiel, S. P., M. D. Snavely, C. G. Miller, and M. E. Maguire. 1986. Magnesium transport in *Salmonella typhimurium*: characterization of magnesium influx and cloning of a transport gene. *J. Bacteriol.* **168**:1444–1450.
- Hoffee, P. 1968. 2-Deoxyribose gene-enzyme complex in *Salmonella typhimurium*. I. Isolation and enzymatic characterization of 2-deoxyribose-negative mutants. *J. Bacteriol.* **95**:449–457.
- Hove-Jensen, B., and M. Maigaard. 1993. *Escherichia coli rplA* gene encoding ribose phosphate isomerase A. *J. Bacteriol.* **175**:5628–5635.
- Jobe, A., and S. Bourgeois. 1972. *lac* repressor-operator interaction. VI. The natural inducer of the *lac* operon. *J. Mol. Biol.* **69**:397–408.
- Kelln, R. A., and J. Neuhard. 1988. Regulation of *pyrC* expression in *Salmonella typhimurium*: identification of a regulatory region. *Mol. Gen. Genet.* **212**:287–294.
- Koop, A. H., M. E. Hartley, and S. Bourgeois. 1987. A low-copy-number vector utilizing  $\beta$ -galactosidase for the analysis of gene control elements. *Gene* **521**:245–256.
- Kumar, A., R. A. Malloch, N. Fujita, D. A. Smillie, A. Ishihama, and R. S. Hayward. 1993. The minus 35-recognition region of *Escherichia coli* sigma 70 is inessential for initiation of transcription at an “extended minus 10” promoter. *J. Mol. Biol.* **232**:406–418.
- Mathews, D. H., T. C. Andre, J. Kim, D. H. Turner, and M. Zuker. 1998. An updated recursive algorithm for RNA secondary structure prediction with improved free energy parameters. *Am. Chem. Soc. Symp. Ser.* **682**:246–257.
- McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* **413**:852–856.
- McKenney, K., H. Shimatake, D. Court, U. Schmeissner, D. Brady, and M. Rosenberg. 1981. Analysis of nucleic acids by enzymatic methods, p. 383–415. In J. C. Chirikjian, and T. S. Papas (ed.), *Gene amplification and analysis*, vol. II. Elsevier-North Holland, Amsterdam, The Netherlands.
- Müller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Møllegaard, N. E., P. B. Rasmussen, P. Valentin-Hansen, and P. E. Nielsen. 1993. Characterization of promoter recognition complexes formed by CRP and CytR for repression and by CRP and RNA polymerase for activation of transcription on the *Escherichia coli deoP2* promoter. *J. Biol. Chem.* **268**:17471–17477.
- Mortensen, L., G. Dandanell, and K. Hammer. 1989. Purification and characterization of the *deoR* repressor of *Escherichia coli*. *EMBO J.* **8**:325–331.
- Müller-Hill, B., H. V. Rickenberg, and K. Wallenfels. 1964. Specificity of the induction of the enzymes of the *lac* operon in *Escherichia coli*. *J. Mol. Biol.* **10**:303–318.
- Rasmussen, M. A. 1993. Isolation and characterization of *Selenomonas rumantium* strains capable of 2-deoxyribose utilization. *Appl. Environ. Microbiol.* **59**:2077–2081.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
- Schwartz, M. 1978. Thymidine phosphorylase from *Escherichia coli*. *Methods Enzymol.* **51**:442–445.
- Shin, M., S. Kang, S. J. Hyun, N. Fujita, A. Ishihama, P. Valentin-Hansen, and H. E. Choy. 2001. Repression of *deoP2* in *Escherichia coli* by CytR: conversion of a transcription activator into a repressor. *EMBO J.* **20**:5392–5399.
- Sørensen, K. I., and J. Neuhard. 1991. Dual transcriptional initiation sites from the *pyrC* promoter control expression of the gene in *Salmonella typhimurium*. *Mol. Gen. Genet.* **225**:249–256.
- Stern, S., D. Moazed, and H. F. Noller. 1988. Structural analysis of RNA using chemical and enzymatic probing monitored by primer extension. *Methods Enzymol.* **164**:481–489.
- Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**:60–89.
- Tourneux, L., N. Bucurenci, C. Saveanu, P. A. Kaminski, M. Bouzon, E. Pistotnik, A. Namane, P. Marlière, O. Barzu, I. L. de la Sierra, J. Neuhard, and A.-M. Gilles. 2000. Genetic and biochemical characterization of *Salmonella enterica* serovar Typhi deoxyribokinase. *J. Bacteriol.* **182**:869–873.
- Wang, R. F., and S. R. Kushner. 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* **100**:195–199.
- Zeng, X., and H. H. Saxild. 1999. Identification and characterization of a DeoR-specific operator sequence essential for induction of *dra-nupC-pdp* operon expression in *Bacillus subtilis*. *J. Bacteriol.* **181**:1719–1727.