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Identification of *dinR*, a DNA Damage-Inducible Regulator Gene of *Bacillus subtilis*

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A *Bacillus subtilis* strain deficient in homologous recombination was isolated from a library of Tn*917lac* insertion mutants. The interrupted locus consists of an open reading frame encoding a 22,823-dalton polypeptide. Analysis of the deduced amino acid sequence revealed 34% identity and 47.3% similarity with the LexA protein from *Escherichia coli*. The gene was designated *dinR*. It is located between the *recA* and *thyA* genetic markers, at 162° on the *B. subtilis* chromosome. The *dinR* gene was shown to be expressed during the entire *B. subtilis* cellular cycle with at least a threefold increase when cells develop competence. In addition, the use of a merodiploid strain, in which a copy of the wild-type *dinR* gene coexists with a *dinR-lacZ* transcriptional fusion, demonstrated that *dinR* is an SOS gene and that the SOS-induced expression of *dinR* occurred only when a wild-type copy of *dinR* was present. In addition, DinR seems to regulate the expression of *dinC*, another SOS gene.

When exposed to DNA-damaging agents, prokaryotic cells induce the SOS DNA repair pathway, leading to the expression of multiple genes. In *Escherichia coli*, the transcriptional induction of SOS genes results from activation of the RecA protein, which promotes cleavage and thus inactivation of both LexA, the repressor of the SOS genes, and the repressor of temperate phage genes (for reviews, see references 26 and 27). The *E. coli* and *Bacillus subtilis* SOS systems are similar. The induction of *B. subtilis recA* (formerly *recE*) and damage inducible (*din*) genes by DNA damage is controlled by an analogous RecA-dependent mechanism (17). Proteolytic cleavage of the *E. coli* LexA protein in vitro is facilitated by the *B. subtilis* RecA protein (15). In addition, *B. subtilis recA* mutants that are unable to induce the SOS response have been partially *trans*-complemented by a plasmid expressing the *E. coli recA* gene (3, 14). Finally, comparison of the deduced amino acid sequences of the two RecA proteins demonstrates several regions of similarity, and the immunoreactivity of *B. subtilis* RecA to antibodies raised against *E. coli* RecA suggests conservation of structural domains (17, 25).

While many similarities exist between the *E. coli* and *B. subtilis* SOS systems, one notable difference is observed during genetic transformation, which is an efficient means of homologous recombination in *B. subtilis*. During the first step of transformation, cells develop competence, which confers on them the capacity to bind and take up exogenous DNA. Genetic exchange then occurs by integration of the donor DNA, which has been converted to single-stranded DNA molecules, into the recipient chromosome. Several other phenomena, such as enhanced mutagenesis (23), prophage induction (28), and transcriptional activation of *din* genes (13), occur during the development of competence. These observations have led to the proposal that the SOS response is induced during transformation, even in the absence of a known DNA-damaging agent.

To initiate the process of genetic transformation, specific transcription of competence (*com*) genes is necessary. Some of these genes are expressed throughout exponential growth in a variety of media; others are expressed during the transition from the exponential to the stationary phase of growth in a specific competence medium (4). In the latter condition, a fourfold overexpression of the *recA* gene (16) has also been observed. At present, this increased synthesis of RecA is poorly understood. Nevertheless, it appears to be controlled by a competence-specific mechanism that is distinct from the classical SOS response, since this stimulation does not require the presence of a functional RecA protein. The competence-related stimulation of *recA* expression is observed in a *recA4* (formerly *recE4*) mutant, whereas the overexpression of *recA* after DNA damage is inhibited (16). However, the induction of other *din* genes during competence is observed only in the presence of wild-type RecA activity (13). Although RecA accounts for most recombinational events during transformation, transduction, and protoplast fusion in *B. subtilis* (5, 9), other Rec$^-$ mutants have been isolated that are sensitive to radiation or chemicals that damage DNA (9). Therefore, the identification of genes involved in genetic recombination and the characterization of their activities during the SOS response might establish a molecular basis for the relationship between these two processes.

By using Tn*917lac* insertional mutagenesis, we have identified a new gene in *B. subtilis* that is involved in genetic recombination. Molecular characterization of this gene (*dinR*) revealed that it encodes a protein that is similar in sequence to that of LexA. The transcription of the *B. subtilis dinR* gene occurs during all stages of the cellular cycle and appears to be controlled by a self-regulatory mechanism. Comparison of *dinR* expression during the SOS response with *dinR* expression during the development of competence leads to the conclusion that an SOS-independent regulation of *dinR* occurs during genetic transformation.

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophages, and growth media. *B. subtilis* strains were derivatives of wild-type strain 168 and are listed in Table 1. To construct a *dinR* merodip-

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TABLE 1. *B. subtilis* strains used in this study

Strains	Genotype or relevant characteristics	Reference, source, or comments
168Θ$^+$	Wild type, prototrophic	Our collection
MO503	*purB ura-1 trpF7 acf-1 rfm-486*	Our collection
MO518	*purB ura-1 trpF7 acf-1 comB*::*cat*[pBR322][a]	Campbell recombinant[b] in *comB*; our laboratory
MO534	*dinR*::Tn*917lac*	This work
MO542	*dinR*::Tn*917lac*::pTV21Δ21	This work
MO556	168Θ$^+$[pB16]	This work; Campbell recombinant in *dinR*
MO575	*dinR*::pTV55Δ2	This work
MO576	*dinC18*::Tn*917lac*	This work
MO577	*dinR*::pTV55Δ2 *dinC18*::Tn*917lac*	This work
YB5018	*dinC18*::Tn*917lac metB5 trpC2 xin-1* SPβ$^-$ *amyE*$^+$	13

[a] Brackets indicate an integrated plasmid molecule.
[b] Campbell recombinant refers to a strain that was made by transformation with a DNA circular molecule.

loid strain, the plasmid carrying a promoter-proximal 2.4-kb *Bgl*II fragment (pB16 obtained as described below; see Fig. 1) was used to transform wild-type (*dinR*$^+$) *B. subtilis*, with selection for chloramphenicol resistance (Cm^r). This resulted in integration of the plasmid by a single reciprocal recombination event (Campbell-like) with duplication of the 2.4-kb fragment, generating a disrupted copy of *dinR* as well as an intact copy. To construct the MO576 and MO577 strains, the *dinC18* was moved into the wild type and MO575 by PBS1 transduction. The strain YB5018 harboring the *dinC18* mutation was obtained from R. E. Yasbin (13).

To construct the *dinR1* Δ*lac* mutant strain, we used pTV55Δ2 (*lac*, tetracycline resistant [Tc^r], Cm^r). This plasmid is a pTV55 derivative with a 2.4-kb deletion within the *lacZ* gene (6). Strain MO534 was transduced with a PBS1 lysate carrying pTV55Δ2. All of the transductants obtained were Cm^r as well as erythromycin resistant (Em^r), presumably as a result of Campbell-like integration of the plasmid into the chromosome. This single-crossover event of recombination was not expected to result in the loss of the transposon and yielded chromosomal duplication. The resultant strains were unstable since the duplicated region could recombine, allowing the loss of genetic material by looping out. pTV55Δ2 plasmids replicate in *B. subtilis* by using the temperature-sensitive replication of pE194. Therefore, upon passage at 50°C to allow the loss of plasmid recombinant DNA after looping out, a Cm^r Em^s Lac$^-$ colony was obtained. This recombinant was a replacement derivative in which the original *dinR1*::Tn*917lac* insertion was replaced by pTV55Δ2. The strain obtained (MO575) exhibits the mitomycin (MC)-sensitive phenotype, and the structure of the recombinant was verified by Southern blot analysis.

E. coli JM109 (18) was used as the host strain for M13 recombinant molecules. *E. coli* HB101 (18) or SURE (Stratagene) strains were used as the host strains for recombinant plasmids constructed during cloning experiments. Bacteriophages used in this study were the wild-type strain of PBS1 (5) and the M13mp19 vector (18). The following plasmids were used: pTV32 (30) was used to make a Tn*917lac* insertion library, pTV21Δ2 (30) was used to clone the chromosomal *dinR*::Tn*917* gene fusion, and pB16 was derived by cloning the *Bgl*II fragment containing the *dinR*::*lacZ* fusion from MO542 (*dinR*::Tn*917*::pTV21Δ2). Media used for growth of *B. subtilis* cells or phages have been described previously (5). Erythromycin was utilized at 20 μg/ml, lincomycin was used at 40 μg/ml, MC was used at 50 or 150 ng/ml, rifamycin was used at 5 μg/ml, and chloramphenicol was used at 5 μg/ml. *E. coli* cells were grown in LB medium (18) with ampicillin at 50 μg/ml and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 40 μg/ml when required. M13 phage stocks were grown in 2× YT medium (18).

Genetic, physiological, and molecular procedures. Genetic transformation, transduction, development of competence, chromosomal and plasmid DNA purification, Southern blotting, and hybridization were performed as described previously (5). β-Galactosidase assays were conducted by using bacteria grown in Schaeffer's medium or in Oxoid rich medium; no significant differences relating to a particular medium were observed. For measuring β-galactosidase during competence, a one-step protocol was used as described previously (1). In particular experiments, MC was added during the exponential phase of growth at 150 ng/ml. The values for β-galactosidase activity correspond to the means of three experiments in similar conditions.

Construction of a Tn*917lac* insertion library and isolation of Rec-deficient strains. To obtain an insertion library with the transposon Tn*917lac*, which confers resistance to macrolides-lincosamides-streptogramin B (MLS^r), the plasmid pTV32, containing also a chloramphenicol resistance marker, was introduced by transformation into the wild-type strain. Colonies resistant to MLS and chloramphenicol were selected. One of these clones was mutagenized in 10 liters of culture by using the protocol of Youngman et al. (30). Tn*917lac*-mutagenized cells were plated to yield about 200 colonies per plate in Schaeffer's medium containing MLS. After overnight growth at 37°C, 37,000 of these colonies were replica plated onto both rich medium with MLS and rich medium containing 50 ng of MC per ml. MC sensitivity was verified by restreaking colonies on the same media.

Inverse PCR. The inverse polymerase chain reaction (PCR) (20) was performed on chromosomal DNA purified from the *B. subtilis* wild-type strain. Because the Southern analysis had revealed an *Eco*RV-*Pvu*II fragment into which the transposon was inserted (see Fig. 1), genomic DNA was cleaved with the restriction enzymes *Eco*RV and *Pvu*II. Digested DNA was circularized by ligation with T4 DNA ligase (New England Biolabs). The ligated DNA was then digested with the restriction enzyme *Acc*I, in order to relinearize the target fragment such that the *Eco*RV-*Pvu*II fusion joint was located in the fragment. Two oligonucleotide primers were synthesized by use of an Applied Biosystems automated oligonucleotide synthesizer. One primer corresponded to nucleotides 847 through 867 (5′ TTGTAGT TGCGATGACAGAAG 3′); the other corresponded to nucleotides 514 through 494 (5′ GAACTGGACGCAAGCCCG ACA 3′) (see Fig. 2). Primers were used at 20 pmol each, and the target DNA was used at 1 ng. DNA amplification was performed in 60 mM Tris-HCl–17 mM ammonium sulfate–6.7 mM $MgCl_2$–10 mM β-mercaptoethanol–5 mM EDTA with 30 cycles of denaturation at 92°C for 2 min, primer annealing at 55°C for 5 min, and extension by *Taq* polymerase (Perkin-Elmer Cetus) at 72°C for 5 min. At the end of the amplification reaction period, a supplementary incubation of 9.9 min at 72°C was performed to allow the extension of all of the initiated molecules. The DNA products from the PCR were resolved on 0.8% agarose gels. To synthesize DNA for sequencing, the target DNA was asymmetrically amplified under the same conditions by using primers in unequal

concentrations (1:50). The PCR products were purified on a Qiagen column (U.S. Biochemicals) before being used as templates in classical DNA sequencing reactions.

Cloning into M13 phage and DNA sequencing. DNA restriction fragments were submitted, after purification, to a random double-stranded breakage by sonication (three times for 30 s each time). Recessed 3′ ends of the resulting DNA fragments were filled in by the Klenow enzyme, and the fragments were then cloned into M13mp19 by using published procedures (18). Sequencing of DNA was performed by the dideoxy nucleotide chain termination method (18) with [α-^{35}S]dATP (specific activity, 400 Ci/mmol; Amersham) by using the Pharmacia T7 DNA polymerase sequencing kit. Universal primer was used for M13 sequencing, and oligonucleotides 1 (5′ TTGTAGTTGCGATGACAGAAG 3′), 3 (5′ GCAGAATGTCAGCATTCTAC 3′), and 4 (5′ ATGGTGTAAACATGATGTTC 3′) (Fig. 2) were used for single-stranded PCR sequencing.

Nucleotide sequence accession number. The nucleotide sequence of *dinR* was submitted to the Genetic Sequence Data Bank (GenBank) and was assigned the accession number M64684.

RESULTS

Isolation of the recombination-deficient strain MO534 (*dinR*::Tn*917lac*). A library of random insertions of the transposon Tn*917lac* into the *B. subtilis* chromosome was constructed as described in Materials and Methods. Since all *B. subtilis* Rec$^-$ mutants described so far are sensitive to low doses of MC (9), 37,000 independent Tn*917lac* mutants were screened for the inability to grow on media containing a low dose of MC. Several colonies that were resistant to MLS and sensitive to MC were identified. Three of these colonies were shown to maintain stably both the MLS^r and the MC^s phenotypes. One was a slow grower on a variety of media and was not studied further. The others, MO534 and MO535, grew at the same rate as did the wild-type strain. Southern analysis (data not shown) indicated that the sites of the transposon insertion in these two strains were located very close to each other; strain MO534 was studied in detail.

To confirm that only one copy of the transposon was inserted into the chromosome of strain MO534, and that this insertion was responsible for the MC^s phenotype, the linkage of these markers was determined in a backcross transformation experiment. Competent cells of the wild-type strain were transformed with DNA purified from MO534. MLS^r transformants were selected and restreaked on rich medium containing MC. All MLS^r colonies were sensitive to MC, indicating 100% linkage between the transposon insertion and the MC^s phenotype. The ability of strain MO534 to undergo genetic recombination was assessed by transformation and transduction of chromosomal markers. In transformation experiments, a residual activity corresponding to 1% of the activity of the wild-type strain was observed when MO534 cells were used as recipients. Transformation was performed by using 1 μg of donor DNA purified from strain MO518 (Cm^r). Competent cells were prepared as described in Materials and Methods. The recipient cells were at a concentration of 2×10^8 bacteria ml^{-1}. No reversion of the selected marker was observed among 10^7 recipient cells tested. Transformants were selected by the acquisition of chloramphenicol resistance. When the wild type was used as the recipient strain, 2×10^5 transformants per ml resulted; when MO534 was used, 2×10^3 transformants per ml resulted.

Recombination during transformation should be affected both by variations in competence and in the rate of DNA integration into the recipient chromosome. To distinguish between these possibilities, the recombinational capacities of MO534 cells were also tested by transduction. Transduction was performed with PBS1 transducing phage grown on MO503 (Rif^r), with selection for rifamycin resistance. Nonrevertants were observed in 10^7 recipient cells. When the wild type was used as the recipient strain, 10^4 transductants per ml resulted; when MO534 was used, 5×10^2 transductants per ml resulted. When a Cm^r marker was transferred by transduction, MO534 exhibited a recombinational activity that was reduced to 5% of that of the wild-type strain.

Thus, both transformation and transduction experiments demonstrated a diminution of the efficiency of genetic exchanges in MO534 cells, indicating that the locus in which the transposon was inserted is involved in recombination. The transposon insertion in MO534 was mapped between the *thyA* and *recA* markers, at 162° in the *B. subtilis* chromosome (data not shown), in a region where no *rec* mutation had been previously identified. In experiments described below, we show that the product of the locus mutated in strain MO534 is required for induction of a *din* gene by MC. We therefore designated this locus *dinR*, and the Tn*917lac* insertion mutation in strain MO534 was given the allele number *dinR1*.

Cloning the *dinR*::*lacZ* fusion from strain MO534. To clone the *dinR*::*lacZ* fusion, we took advantage of the residual recombinational activity in strain MO534 to replace the internal DNA sequences of Tn*917* by the plasmid pTV21Δ2 which contains both ends of the transposon, the pBR322 replicon, and a chloramphenicol resistance marker (30). This plasmid was introduced into MO534 by transformation, and the recombinants in which the plasmid had been crossed onto the chromosome by homologous recombination were selected by plating bacteria on media containing chloramphenicol. The DNA of the resulting strain MO542 (MLS sensitive and chloramphenicol resistant) was purified and digested by the restriction enzyme *Bgl*II. Digested DNA was subsequently circularized by ligation with T4 DNA ligase and introduced by transformation into competent *E. coli* HB101 cells, with selection for ampicillin resistance (Amp^r) on X-Gal-containing plates. The resulting plasmid of 11.5 kb (named pB16) contained the gene fusion from MO542 with 2.4 kb corresponding to the region upstream from the transposon insertion; its restriction map is shown in Fig. 1.

To ensure that no additional chromosomal rearrangements had occurred during the transposon mutagenesis or during the replacement of Tn*917* internal sequences by pTV21Δ2, we used pB16 DNA as a probe in Southern blot experiments to determine the physical map of the *dinR* locus in wild-type and MO534 strains. As shown in Fig. 1, the resulting physical map of the chromosomal region affected by the Tn*917lac* insertion reveals that the transposon insertion is located in a 5-kb *Eco*RI fragment. This fragment also contains one *Bgl*II, one *Pst*I, two *Sph*I, one *Pvu*II, and two *Eco*RV sites. The same restriction sites were observed in corresponding positions on the wild-type and MO534 chromosomal DNAs as well as in pB16. These data confirmed that the *B. subtilis* DNA present in pB16 indeed corresponds to the chromosomal DNA located upstream from the transposon in MO534.

Nucleotide sequence of the *dinR* gene. To determine the nucleotide sequence of the gene affected by the transposon insertion in strain MO534, multiple attempts were made to clone the intact gene either by cloning from the merodiploid

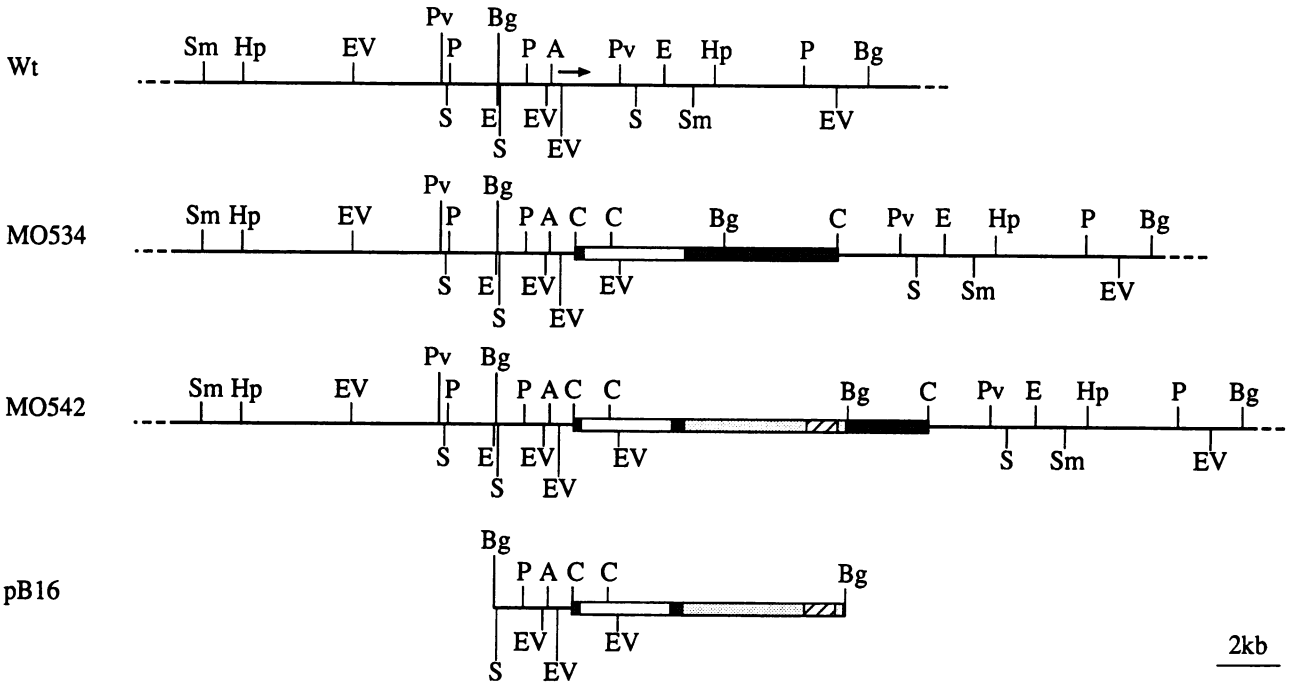

FIG. 1. Physical map of the Tn*917lac* insertion region of the *B. subtilis* chromosome and of plasmid pB16. DNA from the wild-type (Wt) strain and from strain MO534 was purified, digested by several enzymes, and fractionated on a 0.8% agarose gel. DNA fragments were blotted to hybond-N membranes and probed with radioactively labelled plasmid pB16. The resulting physical map corresponds to a 22-kb region around the transposon insertion. The direction of transcription is indicated by an arrow. The plasmid pB16 was obtained from *Bgl*II-digested chromosomal DNA from strain MO542 as described in the text. Abbreviations: A, *Acc*I; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; Hp, *Hpa*I; Sm, *Sma*I; S, *Sph*I; P, *Pst*I; Pv, *Pvu*II. Symbols: ▦, Tn917; □, *lacZ*; ▩, pBR322; ▨, *cat*.

strain MO556 as described in published methodology (29) or by cloning from chromosomal DNA by a shotgun approach. These strategies were unfruitful, suggesting that the presence of the intact *B. subtilis dinR* gene on a multicopy plasmid was toxic for *E. coli*.

Because strategies to clone the intact gene were unsuccessful, nucleotide sequencing was carried out on restriction fragments of the chromosomal insert in pB16 (see Fig. 4). The 460-bp *Eco*RV-*Cla*I and 420-bp *Eco*RV-*Eco*RV fragments from pB16 (Fig. 1) were purified, sonicated, and cloned in M13. To obtain clones overlapping the junctions between these two fragments, the 1.6-kb *Pst*I-*Cla*I fragment was also sonicated and cloned in M13. Recombinant phages that hybridized with the ^{32}P-labelled *Eco*RV-*Cla*I fragment were recovered. These strategies allowed us to determine the double-stranded nucleotide sequence of the 900-bp fragment located immediately upstream from the transposon insertion. To sequence the distal region of the gene, the inverse PCR technique was used. The core DNA sequence chosen for this procedure was the fragment between the *Eco*RV site nearest to the transposon insertion point and the upstream end of the transposon. Inverse PCR and nucleotide sequence determination were performed as described in Materials and Methods.

Overall, the nucleotide sequence of 1,100 bp corresponding to the area into which the transposon was inserted was determined for both strands of DNA (Fig. 2). Analysis of the DNA sequence revealed an open reading frame (ORF) that extends from nucleotide 369 to nucleotide 1008. Within this ORF, the first ATG at nucleotide 393 is preceded by a potential ribosome-binding site (5′ GAGGTG 3′). This ORF encodes a 205-amino-acid protein, which we designate DinR, with a predicted molecular weight of 22,823. The Tn*917lac* insertion in strain MO534 was located after the nucleotide at position 899. The mutant strain should therefore express a polypeptide lacking the last 36 residues.

Comparison of the amino acid sequence deduced from this ORF with the NBRF protein data base, by using the computer program FASTP (11), showed significant similarity between this protein and (i) the sequence from the repressor LexA from *E. coli* (7) and (ii) the sequence of the UmuD protein from *E. coli* (21). By using the RDF program (11) to randomize comparisons, this homology was found to be highly significant: the comparison of the *B. subtilis* ORF with the *E. coli* LexA or UmuD proteins yielded similarity values corresponding to 21.86 and 14.86 standard deviations above the mean of random sequences, respectively.

The alignment of the ORF sequence with that of UmuD indicated that the similarity was restricted to areas concerned with RecA-mediated cleavage. These regions are also conserved in the sequences of the other proteins that undergo RecA-mediated cleavage (Fig. 3B). On the other hand, alignment with the LexA sequence was found to extend over the entire length of the proteins (Fig. 3A), showing 33.6% identity and 47.3% functional similarity.

Analysis of the *dinR* upstream region (Fig. 2) shows a possible promoter region defined by a putative −10 TATAAT box at nucleotide position 355 and a −35 TTGTAC box at nucleotide position 332. These sequences are similar to the consensus sequences of the σ^{43} promoters (19). In addition, three putative *B. subtilis* SOS boxes (GAAC-N_4-GTTC) (2) were found at −40, −60, and −100 nucleotides upstream of the proposed transcription initiation site. A potential transcription termination site with dyad symmetry

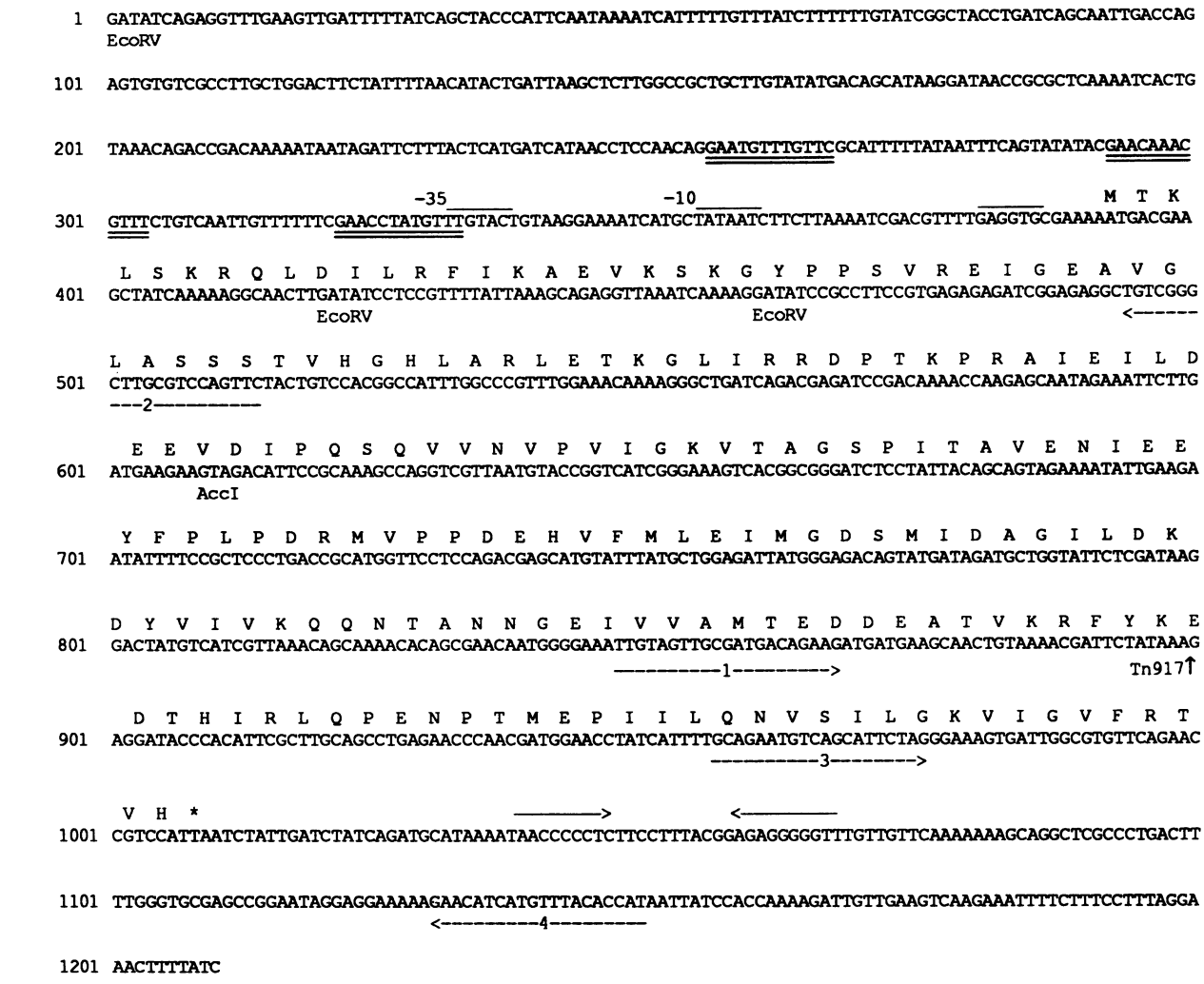

FIG. 2. Nucleotide sequence of the *dinR* gene from *B. subtilis*. The DNA sequence begins at the *Eco*RV site most upstream from the transposon insertion. The deduced amino acid sequence is shown in one-letter code. The proposed start of the ORF corresponds to the ATG codon located at nucleotide 892. The stop codon is indicated by an asterisk. The putative −35 (nucleotides 332 to 338) and −10 (nucleotides 355 to 360) regions as well as the ribosomal binding site (nucleotides 380 to 385) are overlined. The proposed *B. subtilis* SOS boxes (nucleotides −40, −60, and −100) are underlined. The location of the Tn*917lac* insertion after nucleotide 899 is indicated by an arrow. The region of symmetry corresponding to a putative transcription terminator is indicated by converging arrows. The dotted arrows show the sequences used to synthesize the oligonucleotide primers for PCR or sequencing reactions.

was located downstream from *dinR* at nucleotide position 1038.

DNA damage-related expression of *dinR*. The transposon insertion in mutant MO534 resulted in a transcriptional fusion between the *lacZ* gene carried by Tn*917* and *dinR*. β-Galactosidase activity was measured during growth in rich medium with or without the addition of MC (Fig. 4). β-Galactosidase activity was the same during all phases of bacterial growth and in the presence and absence of MC. This result indicates that, in the *dinR1* background, *dinR* is expressed during growth and is not induced by DNA-damaging agents.

To determine if functional DinR protein was necessary for efficient induction of *dinR* during SOS response, we constructed the strain MO556, a merodiploid that contains both the *dinR-lacZ* fusion and a wild-type copy of *dinR* (see Materials and Methods). β-Galactosidase activity was measured in the merodiploid strain MO556 grown in rich medium with and without the addition of MC. The β-galactosidase activity in MO556 cells grown in rich medium was four times higher than that in MO534 cells. Moreover, immediately after the addition of MC, β-galactosidase activity increased fourfold in the merodiploid strain (Fig. 4). These results indicate that the induction of *dinR* transcription by MC requires a wild-type DinR protein.

Competence-related expression of *dinR*. Transcriptional activation of *din* and *recA* genes occurs in competent cells in the absence of any known DNA-damaging agent. We quantified the influence of the development of competence on the expression of the *dinR* gene by measuring β-galactosidase activity in the MO534 and MO556 strains during this physiological state. Both the mutant and merodiploid strains, which had produced constant levels of β-galactosidase activity through all phases of growth in rich medium, were grown in a competence-developing medium. In both strains, β-galactosidase activity increased threefold over a period of 3 h (Fig. 5). Since only 10 to 20% of the total cell population becomes competent (4), the threefold increase in *dinR* tran-

A

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LexA_EC  MKALTARQQEVFDLIRDHISQTGMPPTRAEIAQRLGFRSPNAAEEHLKAL  50
DinR     MTKLSKRQLDILRFIKAEVKSKGYPPSVREIGEAVGLASSSTVHGHLARL  50

LexA_EC  ARKGVIEIVSGASRGIRLLQEEEEG-------LPLVGRVAAGEPLLAQQH  93
DinR     ETKGLIRRDPTKPRAIEILDEEVDIPQSQVVNVPVIGKVTAGSPITAVEN 100

LexA_EC  IEGHYQVDPSLFQPNAD-FLLRVSGMSMKDIGIMDGDLLAVHKTKDVRNG 142
DinR     IEEYFPLPDRMVPPDEHVFMLEIMGDSMIDAGILDKDYVIVKQQNTANNG 150

LexA_EC  QVVVARI-DDEVTVKRLKKQGNKVELLPENSEFKPIVVDLRQQSFTIEGL 191
DinR     EIVVAMTEDDEATVKRFYKEDTHIRLQPENPTMEPIIL----QNVSILGK 196

LexA_EC  AVGVIRNGDWL                                        202
DinR     VIGVFRTVH                                          205
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B

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                77        111             152
LexA_EC  ... PLVG-RVAAG.... FLLRVSGMSM.... EVTVKRL...
P22      ... PLIS-WVSAG.... FWLDVQGDSM.... EATFKKL...
434      ... PLIS-MVRAG.... FWLKVEGDSM.... EATFKKL...
CI(λ)    ... PVFS-HVNAG.... FWLEVEGNSM.... EFTFKKL...
UmuD     ... PLFSDLVQCG.... YFVKASGDSM.... EFTVKKL...
DinR     ... PVIG-KVTAG.... FMLEIMGDSM.... EATVKRF...
              84              119             161
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FIG. 3. (A) Alignment of the *B. subtilis* DinR and *E. coli* LexA amino acid sequences. Symbols: =, amino acid identity; –, functional homology. (B) Homology at the RecA-mediated cleavable sites of the DinR protein to other RecA cleavable proteins. Numbering of residues corresponds to the amino acid positions in the LexA or DinR protein.

scription represents a minimal estimate of *dinR* induction in competent cells. This result shows that *dinR*, like *recA* and other *din* genes, is overexpressed during competence without the intervention of any known DNA-damaging agent. This competence-related increase in transcription does not appear to require DinR.

Effect of DinR on expression of another *din* gene after MC treatment. The similarities between the DinR and LexA proteins suggest that DinR might be the regulator of the expression of other *din* genes. To test such a hypothesis, the effect of the *dinR1* mutation on *dinC* gene expression was measured. We first constructed a derivative of strain MO534 which no longer expresses β-galactosidase. The MO575 strain (*dinR1* Lac$^-$ Cm^r) was created by transduction with a PBS1 lysate carrying plasmid pTV55Δ2 (see Materials and Methods). This resulted in the replacement of Tn*917lac* with DNA sequences deleted for *lacZ*. The *dinC18*::Tn*917lac* fusion was then introduced into strains MO575 and 168$^{\oplus+}$ by PBS1 transduction. β-Galactosidase activity driven by the *dinC* promoter was measured in both MO576 (*dinC18*::Tn*917lac*) and MO577 (*dinR1*::pTV55Δ2 *dinC18*::Tn*917-lac*) strains during growth in rich medium with or without the addition of MC (Fig. 6). The expression of *dinC* in the MO576 strain, which contains the wild-type allele of *dinR*, was induced 25-fold 1 h after MC treatment. This induction of *dinC* expression was not observed in the MO577 mutant that carries the *dinR1* allele. This result suggests that the wild-type *dinR* gene product is required for induction of the

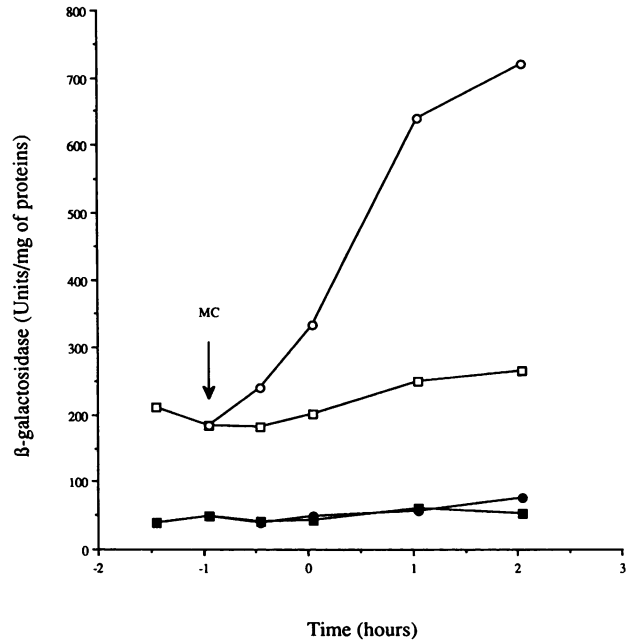

FIG. 4. Expression of the *dinR*::Tn*917lac* fusion during growth in rich medium with or without the addition of MC. During exponential growth of strains MO534 and MO556, 1-ml aliquots of each culture were assayed for β-galactosidase activity at the indicated times. One hour before the onset of the stationary phase of growth, 150 ng of MC was added per ml to one half of the culture. Symbols: ■, MO534 without added MC; ●, MO534 in the presence of MC; □, MO556 without added MC; ○, MO556 in the presence of MC.

dinC gene after DNA damage. An alternative view is that DinR is normally a repressor of *din* genes and that the *dinR1*::pTV55Δ2 mutation creates a repressor that is always in the active form.

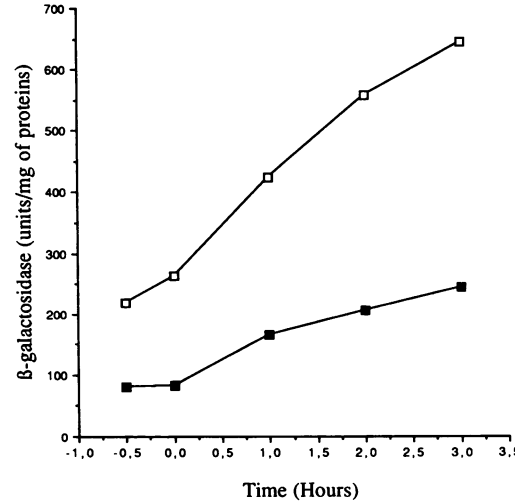

FIG. 5. Expression of *dinR*::Tn*917lac* during the development of competence. MO534 and MO556 strains were grown according to a one-step competence protocol. At the indicated times, β-galactosidase activity was assayed for 1 ml of culture. Time zero indicates the end of the exponential growth of strain MO556 (□) and strain MO534 (●).

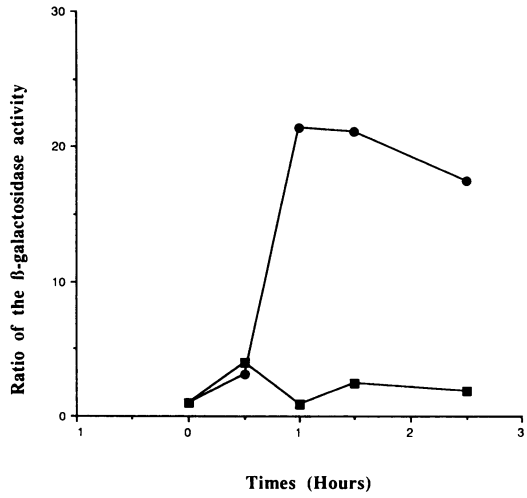

FIG. 6. Effect of *dinR* on the expression of the *dinC*::Tn*917lac* fusion during growth in rich medium with or without the addition of MC. During exponential growth of strains MO575 and MO576, 1-ml aliquots of each culture were assayed for β-galactosidase activity at the indicated times. At time zero, MC was added to one half of the culture. Values represent the ratio of β-galactosidase activity found in cells from the culture with or without MC. Symbols: ●, MO576; ■, MO577.

DISCUSSION

By using Tn*917lac* insertional mutagenesis in *B. subtilis*, we have identified a new locus involved in genetic recombination and in the SOS response. Molecular and genetic characterization of the mutated locus has permitted its identification as the *B. subtilis dinR* gene. The transposon library constructed in this study was prepared in 10 liters of culture. Only three independent insertions conferring the MC-sensitive phenotype were isolated, indicating that this library is probably incomplete. In addition, such a low number of isolated MC^s mutants might be a consequence of the criteria used for the screening, since total inhibition of growth by a 50-ng/ml concentration of MC was demanded.

The *B. subtilis dinR* gene encodes a 205-amino-acid polypeptide of molecular weight 22,823, which shares 34% amino acid identity with the LexA protein of *E. coli*. The amino acid sequences important for RecA-mediated cleavage of LexA are conserved in DinR (Fig. 3B), which suggests that DinR is inactivated proteolytically by a mechanism similar to that for repressors cleaved by RecA in *E. coli*. The DNA-binding site of LexA has been located in the N-terminal region of the protein that is organized in a configuration of three α-helixes (10). A similar organization can be predicted for the N-terminal region of the DinR protein. Differences in the N-terminal sequences of LexA and DinR, however, suggest that the DNA-binding sites of the two proteins might be different. This hypothesis is supported by recent findings regarding the identification of the *B. subtilis* SOS boxes (2); in *E. coli*, the consensus DNA sequence for LexA binding corresponds to a 5′ CTG-N_{10}-CAG 3′ target sequence that is present in all SOS-regulated genes that have been identified so far (26). In *B. subtilis*, a different motif, consisting of the sequence 5′ GAAC-N_4-GTTC 3′, has been identified in the regulatory regions of all *din* genes, the *recA* gene, and, in this study, the *dinR* gene. It has been proposed that this consensus sequence functions as an SOS operator site in the regulation of the expression of the mentioned loci.

The specific cleavage of LexA produces two peptides, the first of which is 84 amino acids long and is sufficient to confer the DNA-binding capacities of the protein (8). The second, carboxy-terminal peptide is involved in the dimerization of the protein, which reinforces the binding of LexA to the operator (24). The *dinR1* mutant isolated during this work should synthesize a protein which conserves 80% of the wild-type DinR; 36 amino acids of the carboxy end of this protein are missing and are replaced by 24 amino acids encoded by the Tn*917* sequence. Therefore, the DNA-binding region and the RecA-mediated cleavage site should be conserved in this truncated polypeptide. The fact that addition of MC did not induce *dinR* expression in MO534 suggests that the truncated DinR protein synthesized in the mutant is inactive as a positive regulator or is an uncleavable repressor. Therefore, the phenotype of the *dinR1* mutant (Rec deficient and SOS repressed) might be the result of the maintenance of the RecA protein at a concentration lower than that of the wild-type strain. Such a hypothesis is supported by the observation that a noncleavable *lexA3* (Ind^-) mutant of *E. coli* displays a fourfold-lower rate of transcription of *recA* as compared to the wild type (12). In addition, recent experiments have confirmed the presence of a decreased level of RecA in MO534 cells (22).

When β-galactosidase activity was measured in the merodiploid strain MO556, in which a wild-type DinR protein is produced along with the mutant protein, the basal level of expression of the *dinR-lacZ* fusion was four times higher than that of the mutant (Fig. 4). Moreover, the addition of MC led to induction of expression of the *dinR-lacZ* fusion in the merodiploid strain, indicating that *dinR* is indeed an SOS gene. We conclude from these results that the *dinR* wild-type allele is dominant over the *dinR1* allele and that the *dinR* expression in *B. subtilis* is self-regulated. The low-level expression of the *dinR-lacZ* fusion in strain MO534 suggests that the truncated DinR1 protein is still able to bind to its DNA target, perhaps in a dimer conformation as was previously suggested for LexA (8). The dominance of the *dinR* wild-type allele over the *dinR1* allele might be the result of either a lower rate of formation of a DinR1 homodimer or a weaker interaction with the DNA target sequence.

The *dinR* gene is expressed at a constant rate throughout the cellular cycle of *B. subtilis*, as judged by the β-galactosidase activity assayed in the *dinR-lacZ* fusion strains MO534 and MO556. Overexpression of this gene is observed in both strains when cells are grown in a competence-developing medium in the absence of an external signal of DNA damage. The induction of *dinR* under these conditions is also observed for the other *din* genes and for *recA*. Previous work has demonstrated that the inducible expression of *din* loci as a consequence of the development of competence occurs by a RecA-dependent mechanism. Nevertheless, cells carrying *din* mutations are able to develop competence and are recombination proficient (17). In contrast, *recA* is induced by a competence-specific regulation in a RecA-independent way. The competence-related overexpression of *dinR* is independent of the presence of a *dinR* wild-type gene (Fig. 4). This suggests that *dinR* is induced during competence by a mechanism different from the SOS response; the relationship of such an induction with the activity of RecA is still to be elucidated. These data extend the idea of the existence of a dual system of regulation for some SOS-inducible genes as postulated in earlier works (16), one mediated by the repressor activity of DinR and the other mediated by a mechanism related to the competence phenomenon.

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