The Bacillus subtilis dinR Gene Codes for the Analogue of Escherichia coli LexA

PURIFICATION AND CHARACTERIZATION OF THE DinR PROTEIN*

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The Bacillus subtilis dinR gene encodes a 23-kDa protein that shares about 34% homology with the Escherichia coli LexA protein. We have purified the dinR gene product to near homogeneity, and we describe its activities. The purified DinR protein binds specifically to the SOS box, CTGTN8ACAG, the upstream sequences of the known SOS genes (2) has led to LexA binding to the lexA operator mutants (6), quantitative footprinting studies of inhibits their transcription in vitro, recA (25, 26). B. subtilis RecA protein is structurally and functionally analogous to the E. coli LexA protein, and accordingly, we propose renaming the protein B. subtilis LexA.

The SOS regulatory system, as characterized in the bacterium Escherichia coli, comprises a set of DNA repair and cellular survival genes that are coordinately induced by DNA damage (1–3). The expression of these DNA damage-inducible (din) genes, or SOS genes, is controlled by two proteins (which are themselves products of SOS genes): the LexA protein, which represses SOS gene expression, and the RecA protein, which is activated by a metabolic signal to cause the proteolytic inactivation of LexA.

LexA binds to the operator sequences of SOS genes and inhibits their transcription in vitro (3–5). Analyses of recA and lexA operator mutants (6), quantitative footprinting studies of LexA binding to the recA operator (7, 8), and a comparison of the upstream sequences of the known SOS genes (2) has led to the identification of the so-called SOS box, CTGTN8ACAG, which appears to be the principal recognition site for a LexA dimer. Induction of the SOS genes following DNA damage occurs when RecA, activated by an inducing signal, promotes the proteolytic destruction of LexA. Corresponding to the requirement for an activating signal, RecA is activated for repression of the SOS operon and certain phage repressors cleavage in vitro when it binds single-stranded DNA and nucleoside triphosphate to form a ternary complex (9–11). There is strong evidence that for certain DNA damaging treatments, the inducing signal is single-stranded DNA exposed by either the processing or replication of damaged DNA (12, 13).

RecA-mediated cleavage of the 22.3 kDa LexA protein occurs at a single site between Ala-84 and Gly-85 (14). This site is part of a flexible hinge region that separates two functional domains (15), the amino-terminal domain that binds to the SOS operon and the carboxyl-terminal domain that is involved in dimerization (16, 17). A significant feature of the cleavage reaction is that RecA cleaves LexA from the observation that LexA, in the absence of RecA, undergoes autodigestion at alkaline pH at the same Ala–Gly bond that is cleaved in the RecA-promoted reaction (18). Thus, contrary to early descriptions of RecA as a highly specific protease, RecA functions as a coprotease to facilitate the cleavage catalyzed by repressor functional groups. Kinetic analysis of the autodigestion reaction indicates that repressor cleavage is intramolecular and involves the deprotonation of an amino acid residue with a pKₐ of 9.8 (19). A model for the cleavage mechanism, similar to that for serine proteases, has been proposed in which Ser-119 is activated as a nucleophile by Lys-156 (20–22).

The bacterium Bacillus subtilis responds to agents that damage DNA or block DNA replication by inducing an SOS-like system (23, 24), and several components of the B. subtilis SOS-like regulon have been identified. The B. subtilis RecA protein, which cross-reacts strongly with E. coli RecA antibody, is induced by mitomycin C, UV radiation, and nalidixic acid (25, 26). B. subtilis RecA promotes the cleavage of E. coli LexA repressor in vitro (25) and in vivo (27, 28). A set of B. subtilis din genes was identified whose induction by DNA damaging treatments is blocked in recA mutants (29). Inspection of the DNA sequences upstream of the B. subtilis din genes revealed a consensus sequence, 5′-GAACN₄GTTC-3′, overlapping the dinA, dinB, dinC, and recA promoters (30). This sequence was proposed as a possible binding site for the B. subtilis SOS repressor and has since been found overlapping the promoters of the recM13, dnaX (31), and dinR (32) genes. Using the din promoter regions to search for the putative SOS repressor, a 23-kDa DNA binding protein was purified that binds specifically to the consensus site and whose binding activity was destroyed by activated B. subtilis RecA protein in vivo and in vitro (33).

The B. subtilis dinR gene encodes a 22.8-kDa polypeptide having 34% identity and 47% similarity with the E. coli LexA protein (32). Thus, it has been considered the likely candidate for the B. subtilis lexA analogue. We report here the cloning,
overexpression, and purification of the DinR protein, and we show that it has all of the activities of _E. coli_ LexA _in vitro_: specific binding to _din_ promoters and inhibition of RNA polymerase binding and transcription, autodigestion at high pH, and RecA-mediated cleavage. We also show that the _dinR_ gene codes for the protein previously identified as the SOS repressor (33).

**EXPERIMENTAL PROCEDURES**

**Materials**—Plasmid pET21a and BL21(DE3) _E. coli_ cells were purchased from Novagen. The _B. subtilis_ RecA (25) protein was purified as described previously. Polyclonal _B. subtilis_ DinR antiserum was prepared by the subcutaneous injection of New Zealand White rabbits with DinR protein of greater than 95% purity. Initially 100 μg of DinR protein suspended in 1 ml of complete Freund’s adjuvant was injected, and two boosts of 100 μg of DinR suspended in 1 ml of incomplete Freund’s adjuvant were injected at 3-week intervals. The antiserum was collected 10 days after the third injection. Oligonucleotides were synthesized on a Milligen Cyclone Plus DNA synthesizer. Restriction enzymes, DNA-modifying enzymes, and _Taq_ polymerase were purchased from New England Biolabs Inc. and Promega and used as recommended by the manufacturers. Affinity-purified goat anti-rabbit horseradish peroxidase conjugate and heparin-agarose were purchased from Bio-Rad. Mitomycin C was purchased from Sigma. Nitrocellulose filters were purchased from Schleicher & Schuell. RNA polymerase was provided by Leendert Hamoen (University of Groningen). The _dinR_ deletion strain 8G5 (pLGW3) was provided by Bert Jan Hajema (University of Groningen). The coding portion of the _dinR_ gene was introduced into an _E. coli_ plasmid pET21a such that _Nde_I restriction site was introduced at the initial ATG codon (GAGGTGCGACATATGACGAAGCTATC); the 3’-primer (CCCAAAA-GTACGGCGCCTGGGCGCCTGTCGGCCGGCT) corresponded to a downstream of the structural gene (+715) modified to contain a_Sau_I restriction site. After digestion with _Nde_I and _Sau_I, the pyrene chain reaction product was ligated in _Nde_I-Sau_I-digested pET21a, and the ligation mixture was used to transform DH5α cells. Plasmid DNA obtained from DH5α transformants was then transformed into competent BL21(DE3) cells.

**Cloning of the _dinR_ Gene**—The coding portion of _dinR_ was amplified from _B. subtilis_ chromosomal DNA using a 5’-primer modified such that an _Nde_I restriction site was introduced at the initial ATG codon (GAGGTGCGACATATGACGAAGCTATC); the 3’-primer (CCCAAAA-GTACGGCGCCTGGGCGCCTGTCGGCCGGCT) corresponded to a downstream of the structural gene (+715) modified to contain a _Sau_I restriction site. After digestion with _Nde_I and _Sau_I, the pyrene chain reaction product was ligated in _Nde_I-Sau_I-digested pET21a, and the ligation mixture was used to transform DH5α cells. Plasmid DNA obtained from DH5α transformants was then transformed into competent BL21(DE3) cells.

**Purification of DinR Protein**—_E. coli_ strain BL21(DE3) containing pET21a- _dinR_ was grown in 1 liter of LB broth containing carbenicillin (50 μg/ml) with selection by 30 μg/ml of ampicillin. The culture was then induced with 10 ml of IPTG (100 μM) and grown for an additional 3 h. Cells were harvested by centrifugation at 4°C, 5000 ×g for 20 min, and then resuspended in 5 ml of 20 mM Tris, pH 7.5, 10% (w/v) sucrose, 1 mM EDTA. Cells were lysed by lysozyme (0.2 mg/ml) by incubation on ice for 30 min followed by a 15-min incubation at 37°C. After selected times, aliquots of the reaction were removed, added to 6 × SDS-PAGE sample buffer to stop the reaction, and then subjected to SDS-PAGE (13%). After staining with Coomassie Brilliant Blue, the cleavage activity was analyzed by SDS-PAGE analysis using reagents from the Promega core footprinting kit as per manufacturer instructions. Samples (50 μl) for hydroxyl radical footprinting were incubated as described for mobility shift assays, without glycerol in the binding buffer, followed by addition of 6 μl of a freshly made mixture of (NH₄)₂Fe(SO₄)₆·6H₂O (0.9 mM), EDTA (1.9 mM), _H₂O₂ (11.1%), and _H₂O (45.6%). After incubation for an empirically determined time period, reactions were stopped by the addition of 44 μl of stop solution (0.7 mM sodium acetate, pH 5.2, yeast RNA (0.14 mg/ml), 73 mM thio urea). Samples were prepared for electrophoresis by phenol extraction, ethanol precipitation, and resuspension in 4 μl of loading buffer (35). Samples were subjected to electrophoresis in a 6% acrylamide-urea gel and autoradiography.

**In Vivo Transcription**—In _in vitro_ transcription was analyzed using the “run-off” assay as described (36). Reaction mixtures contained 40 μM Tris, pH 8.0, 0.5 mM EDTA, 1 mM dithiothreitol, 5 mM MgCl₂, 100 mM KC1, bovine serum albumin (0.1 mg/ml), and indicated amounts of _B. subtilis_ RNA polymerase, restriction-digested plasmid DNA, and DinR protein. After incubation at 37°C for 15 min, a nucleotide mixture containing [³²P]CTP was added to give final concentrations of 0.1 mM ATP, 0.1 mM GTP, and 0.1 mM UTP. After incubation at 37°C for 1 min, samples were treated with 1.0 μg of DNase I and 1.0 μg of RNase A, and the reaction solution was incubated for an additional 10 min before adding CTP (0.1 mM). Reaction was assayed at 37°C for 10 min after CTP addition and then stopped by the addition of stop solution (0.05% bromphenol blue and 0.05% xylene blue in formaldehyde). The samples were subjected to electrophoresis on a 6% acrylamide-urea gel and autoradiography. Template DNA was a HindIII-ClaI fragment from plasmid pBT1 containing the recA promoter region and ending at +292 of the _recA_ structural gene; the _recA_ run-off transcript was identified by comparison with ethidium bromide-stained molecular weight markers (not shown) run alongside the transcription reactions.

**RESULTS**

**Purification of _B. subtilis_ DinR Protein**—Using the polymerase chain reaction, we produced the _dinR_ gene with unique _Nde_I and _Sal_I sites located at the beginning and end of the structural gene, respectively. Expression from the _B. subtilis_ _recA_ promoter was used. We inserted the promoterless _Nde_I-Sal_I-digested _dinR_ gene in plasmid pET21a such that _dinR_ expression is controlled by bacteriophage T7 transcription and translation signals. The T7
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Fig. 1. Purification of B. subtilis DinR protein. Samples from purifications were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. Columns represent total cellular proteins from uninduced cells (1); total cellular proteins from cells induced by IPTG (2); pooled peak from heparin-agarose column (3); pooled peak from Mono S column (4); 29 kDa marker (M).

Fig. 2. Binding of a B. subtilis protein to din promoter regions. A, mobility shift assays were conducted with the dinC (a, b, c), dinB (d, e, f), and recA (g–i) radiolabeled probes. Reactions contained 5 nM DNA, mobility shift buffer as described under “Experimental Procedures,” and no protein (a, d, g), 1.0 μg YB1015 (recA4) crude extract (b, e, h), 200 nM DinR protein (c, f), or 50 nM DinR protein (i–l). Competing DNA was added as described under “Experimental Procedures.” B, mobility shift assays with radiolabeled dinC promoter DNA (5 nM) and no protein (a), 0.1 μg YB1015 (recA4) crude extract (b), 1.0 μl affinity-purified repressor (c) (33), 50 nM DinR protein (d), 200 nM DinR protein (e), and 5 μl SGS1(pLGW3) (dinC) crude extract (f).

Fig. 3. Footprinting analysis of DinR bound to the dinC operator. Purified DinR (+) was incubated with a radiolabeled 199-base pair dinC promoter fragment (~130 to +69) and subjected to either hydroxyl radical cleavage (lanes 1 and 2) or DNase digestion (lanes 3 and 4) as described under “Experimental Procedures.” Radiolabeled dinC DNA was similarly cleaved in the absence (−) of DinR protein. Experiments were done with either strand labeled as indicated and run alongside G sequencing reactions with dinC DNA. The left and right panels correspond to radiolabeled coding and template strands, respectively.

promoter also contains the lac operator to minimize expression in the absence of an inducer. After transfer to an E. coli host containing a chromosomal copy of the T7 RNA polymerase, under the control of lacUV5 promoter, the DinR protein was induced by IPTG to comprise more than 40% of the total cellular protein (Fig. 1).

We found that the DinR protein binds tightly to heparin-agarose, which is commercially available as prepacked columns from Bio-Rad or Pharmacia, either of which can be used with an FPLC system. Crude extract from lysed cells was applied directly to a heparin-agarose column and eluted with an NaCl gradient. As illustrated in Fig. 1, DinR protein is purified to near homogeneity by heparin-agarose chromatography, eluting at about 250 mM NaCl. Subsequent chromatography on either Mono S or Superose 12 FPLC columns does not provide any further purification as judged by the specific activity of the protein (DNA binding activity per total protein).

Fig. 1 shows that DinR migrates in an SDS-polyacrylamide gel with an apparent Mr of about 27,000; however, amino acid analysis of the purified protein indicates an Mr of 22,823, consistent with the primary structure predicted from the DNA sequence (32). It is noteworthy that the Mr determined by SDS-PAGE for B. subtilis RecA is also significantly larger than the actual size (25). This behavior is not observed for the E. coli RecA and LexA proteins that are about the same size as the corresponding B. subtilis proteins.

dinR Protein Binds Specifically to B. subtilis SOS Operators—The putative SOS repressor, previously purified by affinity chromatography, binds specifically to the SOS boxes (GAACN4GTTC) of the B. subtilis dinA, dinB, dinC, and recA genes (33). We conducted mobility shift assays with purified DinR protein and the promoter regions of B. subtilis dinB, dinC, and recA genes. Fig. 2A shows that the DinR protein retarded the mobility of DNA fragments containing recA (~77 to +34), dinB (~155 to +70), and dinC (~130 to +69) promoter regions. To determine if additional cellular proteins (not present in the E. coli strain from which DinR was purified) might be involved in din promoter binding in B. subtilis, we ran samples containing purified DinR alongside samples containing crude extract from YB1015 (recA4) B. subtilis cells (it was previously shown that the putative SOS repressor produces mobility shifts with din promoters that are identical to those produced by these crude extracts (33)). In every case, DinR causes mobility shifts that are visually indistinguishable from those produced by crude extract. By contrast, a B. subtilis strain, SGS1(pLGW3), in which the dinR gene has been deleted, does not cause a detectable shift (Fig. 2B).

The mobility shifts caused by DinR with each of the din promoters are abolished by the addition of excess unlabeled DNA containing any of the other din promoters. Fig. 2A shows...
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mobility shift assays using the radiolabeled recA promoter fragment and subsaturating amounts of DinR. In the presence of a 20-fold molar excess of either dinB or dinC promoter DNA, the recA promoter fragment shift is not detectable; however, a 20-fold molar excess of the recA promoter region lacking the SOS box has no inhibitory effect.

The dinC promoter contains two SOS boxes that result in two shifted bands at sub-saturating concentrations of either crude extract or purified repressor (33). Fig. 2B shows that purified DinR causes two distinct bands at subsaturating concentrations that are visually indistinguishable from those produced by B. subtilis crude extract or repressor that has been affinity purified from B. subtilis; at higher concentrations of DinR, only the larger complex is formed.

We conducted DNase and hydroxyl radical footprinting analyses on the binding of DinR to the dinC operator (Fig. 3). The DNase results show protection of the two consensus sequences as well as hypersensitivities to DNase digestion at sites flanking these sequences. Hydroxyl radical footprinting indicates specific contacts along the DNA backbone; all of the sites protected by hydroxyl radical coincide with the DNase footprints.

To determine where DinR interacts with the DNA closely enough to preclude penetration by hydroxyl radicals, we mapped graphically the protected DNA sites on a DNA model using SYBYL software (Tripos Associates Inc.) on a Silicon Graphics Indigo 2 workstation. The modeling results indicate that all of the protected sites are on one face of the helix as shown for one of the two dinC boxes in Fig. 4. Inspection of the protected face reveals that the base pairs corresponding to the entire consensus site are accessible in two adjacent major grooves of the DNA.

**DinR Protein Inhibits Transcription from din Promoters in Vitro**—To confirm that DinR protein acts as a transcriptional repressor we tested the ability of DinR to prevent binding of B. subtilis RNA polymerase to din promoters and inhibit their transcription in vitro. Fig. 5A shows a mobility shift assay using the recA promoter fragment and B. subtilis RNA polymerase in the absence and presence of DinR protein. The addition of RNA polymerase holoenzyme retards significantly the mobility of the recA promoter fragment. When a subsaturating amount of DinR is included, the amount of RNA polymerase bound to the DNA is reduced, and a shift corresponding to the DinR-DNA complex appears. Thus, DinR inhibits the binding of RNA polymerase to the promoter. The fact that no other shifts are visible indicates that the two proteins do not bind simultaneously to the promoter fragment. Consistent with its ability to inhibit binding of RNA polymerase to the recA promoter, DinR protein inhibits the in vitro transcription of DNA from the recA promoter (Fig. 5B).

**DinR Protein Undergoes Autodigestion at High pH**—Although the degree of sequence homology is limited, the LexA catalytic residues (Ser-119 and Lys-156) are conserved in DinR (Ser-127 and Lys-165), suggesting that the cleavage mechanism has been conserved. We conducted autodigestion assays with DinR over a range of pH values essentially as described by Littler (18). Fig. 6 shows typical autodigestion assays in which DinR was incubated at 37°C. The reaction is first order in repressor concentration, and the apparent rate constants are independent of initial repressor concentration. Amino acid sequence analyses of the cleaved protein fragments revealed the cleavage site to be the specific peptide bond between Ala-91 and Gly-92 (corresponding to the LexA site between Ala-84 and Gly-85).

**Fig. 5. Inhibition of RNA polymerase binding to the recA promoter by DinR protein.** A, mobility shift assays with radiolabeled recA promoter DNA (5 nM) and no protein (a), 1 µg B. subtilis RNA polymerase holoenzyme (b), 1 µg B. subtilis RNA polymerase holoenzyme + 50 nM DinR protein (c), and 50 nM DinR protein (d). B, in vitro transcription assays with DNA containing recA promoter as described under “Experimental Procedures” in the presence (+) or absence (-) of 50 nM DinR protein.
and 2.5 × 10⁻³ s⁻¹, respectively (19). It is noteworthy that the pKₐ for λ repressor autodigestion is also 9.8, while the rate is 40-fold slower than for LexA (19). Although we have not made direct comparisons of LexA and DinR autodigestion reactions under identical conditions, the 10-fold slower rate and the difference in pKₐ values seem significant.

B. subtilis RecA Promotes DinR Cleavage—According to the E. coli model, RecA protein, when activated by an inducing signal, promotes the cleavage of LexA repressor at physiological pH. There is evidence that in both E. coli and B. subtilis, the inducing signal is single-stranded DNA generated by the processing of damaged DNA (12, 13, 28). Correspondingly, the RecA-mediated cleavage reaction in vitro requires that RecA be activated by binding single-stranded DNA and nucleoside triphosphate (9, 10, 25). Fig. 8 shows that B. subtilis RecA, in the presence of single-stranded DNA and either dATP or ATPγS promotes the cleavage of DinR protein. Analyses of cleavage rates by densitometric scanning of stained gels gives first order rate constants of 2.3 × 10⁻⁴ s⁻¹ for ATPγS and 2.0 × 10⁻⁴ s⁻¹ for dATP at a RecA concentration of 2 µM (Table I). As with the autodigestion reaction, these values are about 10-fold lower than the corresponding reaction with LexA and E. coli RecA (37). Moreover, the rate of B. subtilis RecA-mediated DinR cleavage is about 3-fold lower than the cleavage of LexA by B. subtilis RecA (25). Under otherwise identical conditions, there is no detectable cleavage when DinR is incubated without either RecA or a nucleoside triphosphate. There is also no detectable cleavage when RecA alone is incubated with DinR (data not shown). Surprisingly, we detected a low level of cleavage activity (k = 6.3 × 10⁻⁵ s⁻¹) when RecA and dATP are incubated with DinR in the absence of single-stranded DNA.

**DISCUSSION**

We have shown that the dinR gene codes for the B. subtilis SOS repressor and that the DinR protein is the functional analogue of E. coli LexA in every respect. This report presents...
is about 3-fold slower than the rate promoted by *E. coli* RecA (25, 39), indicating that the interaction between RecA and repressor is not unimportant in determining the cleavage rate. It is possible that the reduced rate of LexA cleavage with *B. subtilis* RecA simply reflects a lower affinity for the foreign repressor; alternatively, the foreign RecA may not be as effective in stabilizing the catalytically active repressor conformation.

The reduced rate of SOS repressor cleavage in *B. subtilis* relative to *E. coli* is consistent with studies showing that the rates of repressor inactivation and SOS gene induction are slower in *B. subtilis* (26, 33) than in *E. coli* (13). Although we have not yet determined the *in vivo* cleavage rate, we conducted a qualitative comparison between the rates of DinR and LexA disappearance in the same cells. After a short lag period, the rate of LexA cleavage in *B. subtilis* cells (which is slower than the cleavage rate in induced *E. coli* cells (13)) appears to be faster than the rate of DinR cleavage between 5 and 15 min, whereas the rate of DinR disappearance is greater between 15 and 30 min. Although a more quantitative analysis is needed, a reasonable interpretation of this discrepancy is that *B. subtilis* RecA has a higher affinity for the *B. subtilis* repressor than for the *E. coli* repressor. In any case, our results are consistent with previous studies indicating that the time course for SOS induction in *B. subtilis* is considerably slower than it is in *E. coli*. Moreover, the evidence presented here suggests that differences in the rate of SOS induction may ultimately be due to a lower rate constant for repressor autodigestion.

A significant physiological difference between *E. coli* and *B. subtilis* makes the investigation of SOS regulation in *B. subtilis* particularly interesting. *B. subtilis* naturally differentiates to a state of competence, which is characterized by the ability of the cell to bind and take up exogenous DNA (42); incoming DNA can be integrated into the host chromosome via general genetic recombination, which presumably requires the DNA strand exchange activity of the RecA protein (25). Corresponding to this requirement, competence development in *B. subtilis* is accompanied by the induction of the *recA* gene as well as other SOS genes (43, 44). However, the induction of the *recA* gene in *recA*− competent cells indicates that RecA is induced by a competence-specific mechanism that does not require activated RecA protein; by contrast, induction of other SOS genes during competence development requires RecA (43). In light of the results presented here, we infer that RecA can be induced in competent cells despite an inability to inactivate the DinR repressor. Since it has been shown that the *recA* gene is transcribed in *recA*− competent cells from the same promoter used during DNA damage induction (45), *recA* induction during competence development must involve the displacement of DinR repressor from the *recA* operator. The likely candidate for this is the ComK protein, a transcriptional activator induced during competence development that binds to a site overlapping the *recA* operator *in vitro* (42, 46). If this is the case, we predict that ComK should be able to displace DinR from the *recA* promoter, but not other *din* promoters.

How are the other *B. subtilis* SOS genes induced during competence development in the absence of an inducing signal generated by DNA damage? Since RecA is required for induction, the simplest explanation is that RecA causes DinR cleavage. It has been suggested (43) that RecA could be activated in competent cells by single-stranded gaps present in chromosomal DNA (47) or by incoming donor DNA molecules that are single-stranded (42). Alternatively, our detection of DinR cleavage activity with dATP, in the absence of single-stranded DNA, suggests that *B. subtilis* RecA may be activated for DinR cleav-

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**TABLE I**

First order rate constants* for cleavage of *B. subtilis* DinR and *E. coli* LexA at 37 °C

|            | High pH | *E. coli* RecA + ATP | *B. subtilis* RecA + ATP | *B. subtilis* RecA + dATP |
|------------|---------|----------------------|--------------------------|---------------------------|
| LexA       | 2.5 × 10⁻³⁶ | 3.1 × 10⁻³⁶ | 6.9 × 10⁻³⁶ | 6.1 × 10⁻³⁶ |
| DinR       | 1.8 × 10⁻⁴  | ND¹                  | 2.3 × 10⁻⁴  | 2.0 × 10⁻⁴  |

* All rate constants are relative to repressor concentration and given in units of s⁻¹.

¹ Taken from Ref. 19.
² Value for 1 µM RecA taken from Ref. 37.
³ Values for 0.5 µM RecA taken from Ref. 25.
⁴ ND, not determined.

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**FIG. 9.** Destruction of DinR protein in cells treated with mitomycin C. Extracts from equivalent numbers of cells, harvested at indicated times after treatment with mitomycin C (1.0 µg/ml), were subjected to Western analysis as described under “Experimental Procedures” with antisera raised against *B. subtilis* DinR (upper band) and *E. coli* LexA (lower band). Samples on left were from wild-type strain YB886, and samples on right were from YB886 cells containing plasmid pPL608-lexA.

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definitive evidence that the SOS repressor has been functionally conserved in *B. subtilis*. Although *lexA*-like genes have been characterized from a variety of enterobacteria (37, 38), this is the first characterization of a LexA-like protein whose structure has diverged significantly from the *E. coli* protein. As such, this is an important step in the continued characterization of an SOS DNA repair system from a phylogenetically distant organism.

The *B. subtilis* SOS box does not resemble the *E. coli* SOS box in either sequence or spacing between the two half sites (by contrast, the SOS boxes identified in other bacterial species are identical to the *E. coli* consensus site (38)). Although we do not yet have any definitive evidence for the stoichiometry of DinR binding to the SOS operator, the palindromic nature of the site suggests that at least two monomers bind to each 8-base pair site. Since LexA binds its operator site as a dimer (8, 16), it is reasonable to assume that DinR also binds as a dimer. Analyses of DinR binding to various din operators reveal sigmoidal binding curves (indicative of highly cooperative binding), suggesting that the protein binds as a multimer.² Moreover, the extent of retardation in mobility shift experiments is similar to that with *E. coli* LexA and operator fragments of similar sizes suggesting that, like LexA, DinR binds its operator as a dimer (16, 33). However, we have not ruled out the possibility that it binds as a larger complex.

The rate constants determined for DinR cleavage at 37 °C are about the same for reactions promoted either by *B. subtilis* RecA or by alkaline pH and, in each case, about 10-fold lower than the corresponding rate constants for LexA cleavage (19, 39). This suggests that the rate of RecA-promoted cleavage (in both *E. coli* and *B. subtilis*) is primarily dependent on the intrinsic rate constant associated with autodigestion and not influenced significantly by the specific interaction between RecA and repressor (although it is possible that the *in vitro* reaction conditions are not optimal for DinR cleavage). On the other hand, the rate of *E. coli* LexA cleavage by *B. subtilis* RecA

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² P. Banks and C. M. Lovett, unpublished results.
age in competent cells by an altogether different metabolic signal.

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