Regulation of Damage-inducible Genes in Escherichia coli

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The expression of the dinA, dinB, dinD and dinF genes of Escherichia coli is stimulated by certain agents that either damage DNA or block DNA replication. These agents induce the complex SOS response. Genetic evidence is presented to show that the expression of each of these genes is blocked in uninduced cells by the same repressor, the lexA protein. In addition, the regulatory regions of the dinA and dinB genes were cloned, and the approximate positions of the promoters were determined. Purified lexA protein was found to block transcription of these genes in vitro.

1. Introduction

There are instances in many different biological systems in which a particular environmental signal elicits a complex response that involves multiple physiological changes. One of the best-studied examples of such a co-ordinated cellular response occurs when the DNA of Escherichia coli is damaged or DNA replication is blocked by any of a variety of agents (for reviews, see Witkin, 1976; Gottesman, 1981). Under these conditions, several different DNA repair systems are induced as well as a system that is necessary for mutagenesis by ultraviolet irradiation and many types of chemicals. In addition, cell division is blocked, and the cells begin to grow filamentously, accumulating up to 16 chromosomes. Also, certain integrated bacteriophages are induced and begin to grow lytically. These and other inducible functions appear to be an adaptive response to a (dangerous) environmental condition. Because some responses at least appear to promote cell survival, they have been called collectively the SOS functions (Radman, 1975; Witkin, 1976).

The mechanism by which bacterial cells can regulate the expression of these metabolically unrelated processes has been studied intensively in a number of laboratories, and a fairly detailed picture has begun to emerge. The SOS functions are controlled co-ordinately by two regulatory elements, the recA and lexA
proteins; mutations that alter either protein can prevent the expression of these responses. The *lexA* protein appears to function exclusively as a regulator of the SOS response, while the *recA* protein also functions to promote homologous recombination. In response to SOS-inducing treatments, the expression of a number of genes is stimulated. These include the *recA* (McEntee, 1977; Gudas & Mount, 1977; Emmerson & West, 1977), *lexA* (Brent & Ptashne, 1980; Little & Harper, 1979), *umuC* (Bagg et al., 1981), *wrb* (Kenyon & Walker, 1981; Fogliano & Schendel, 1981), *sfiA* (Huisman & D’Ari, 1981) and *himA* (H. J. Miller et al., 1981) genes. In addition, we have identified a number of damage-inducible (*din*) genes on the basis of their inducibility by the DNA-damaging agent mitomycin C (Kenyon & Walker, 1981). These include the *dinA*, *dinB*, *dinD*, *dinF* and *wrbA* genes. Although the functions of some of these genes have not yet been determined, others are known to code for products that are necessary for the occurrence of particular SOS processes. Thus, it has been possible to study the regulation of the physiologically complex SOS response by focusing on the regulation of the inducible genes.

The induction of each *din* gene identified has been shown to involve the two SOS regulatory elements, the *recA* and *lexA* proteins. The mechanism by which these two proteins regulate the expression of three transcriptional units (the *recA*, *lexA* and prophage operons) has been deduced from a series of genetic and biochemical experiments. We have shown by genetic and biochemical techniques that the *recA* and *lexA* genes are repressed by the *lexA* gene product (Brent & Ptashne, 1981; also Little et al., 1981). Lysogenic bacteriophages, such as prophage λ, are repressed by phage-coded repressors. The initial event that leads to the derepression of these genes appears to be the activation of the *recA* protease function, which occurs in response to an unidentified signal arising from SOS inducing treatments. The *recA* protease specifically cleaves prophage repressors (Roberts et al., 1978), as well as the bacterial *lexA* protein (Little et al., 1981). Thus the way in which DNA damage leads to the expression of these genes is by stimulating the *recA* protein to inactivate their repressors.

In this paper we report a series of genetic and biochemical studies designed to determine more precisely the way in which the various *din* genes are controlled. We present evidence indicating that each gene is repressed by the same regulatory molecule, the *lexA* protein.

### 2. Materials and Methods

#### (a) Bacterial and plasmid strains

Bacterial strains and plasmids are listed in Table 1. The *din*: Mud fusions were isolated in strain GW1000, which carries a deletion of the lactose operon, a *sfiA* mutation to reduce filamentation in the presence of SOS-inducing agents, and the *recA* (his-1) mutation. In *lexA* cells, the *recA* mutation causes expression of the SOS response at high temperature (42°C), but not at 30°C, where all genetic manipulations were performed.

#### (b) Strain constructions

Bacteriophage P1 transductions were essentially as described by Miller (1972). The *spr-51* mutation was introduced into derivatives of the fusion strains carrying *recA* (his-1), *umuC*: Tn5 and *lexA* mutations (see Kenyon & Walker, 1981). Mal1 transductants were selected

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*Abbreviations:

- *recA*: Recombinase A
- *lexA*: Leucine zipper autoregulatory protein
- *din*: Damage-inducible genes
- *umuC*: Umu D dammaging protein
- *wrb*: White colony reversion
- *sfiA*: Specified by intA
- *himA*: Hybrid inversion mobilization

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*Strains used in the following experiments appear in Table 1 and are maintained in the author’s laboratory.*

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*The amplification of the fusion strains was confirmed by the isolation of the *recA* gene in a P1 transduction.*

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*Following the isolation of the *recA* gene, the strain was tested for the presence of the *recA* gene by restriction endonuclease digestion.*

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*The *recA* gene was then cloned into a suitable plasmid vector.*

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*The plasmid carrying the *recA* gene was then introduced into various bacterial strains.*

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*The expression of the *recA* gene in these strains was then monitored.*

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*The results of these experiments confirmed the role of the *recA* gene in the SOS response.*

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Damage-inducible genes

Table I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant markers</th>
<th>Source or Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW1000</td>
<td>lexA(T169), recA441</td>
<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
<td>GW1010</td>
<td>GW1000, but din11 :: Mud</td>
<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
<td>GW1011</td>
<td>GW1000, but din11 :: Mud, recA56, srl :: Tn10</td>
<td>Text</td>
</tr>
<tr>
<td>GW1012</td>
<td>GW1000, but din11 :: Mud, lexA3, mod6 :: Tn5</td>
<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
<td>GW1013</td>
<td>GW1000, but din11 :: Mud, spr-51</td>
<td>Text</td>
</tr>
<tr>
<td>GW1014</td>
<td>GW1000, but din11 :: Mud, spr-51, recA56, srl :: Tn10</td>
<td>Text</td>
</tr>
<tr>
<td>GW1030</td>
<td>GW1000, but din11 :: Mud</td>
<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
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<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
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<td>GW1000, but din11 :: Mud, lexA3, mod6 :: Tn5</td>
<td>Kenyon &amp; Walker (1981)</td>
</tr>
<tr>
<td>DM6659</td>
<td>recA56, srl :: Tn10</td>
<td>D. Botstein</td>
</tr>
<tr>
<td>DM1187</td>
<td>spr-51</td>
<td>D. Mount</td>
</tr>
<tr>
<td>GW1001</td>
<td>GW1000/pGW10(dinA-lac)</td>
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</tr>
<tr>
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<td>GW1000/pGW111 (Pmod)</td>
<td>Text</td>
</tr>
<tr>
<td>GW1003</td>
<td>GW1000/pGW220 (dinB-lac)</td>
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</tr>
<tr>
<td>GW1004</td>
<td>GW1000/pGW221 (Pmod)</td>
<td>Text</td>
</tr>
</tbody>
</table>

Strains were constructed as described in Materials and Methods, or in the legend to Fig. 2. Derivatives of the fusion strains carrying recA56, lexA3, spr-51; and spr-51 and recA56 mutations were obtained (and are numbered) as indicated for the din1 fusion strain derivatives.

Following infection with phage P1 grown on the spr-51 mutant, DM1187. The strains were tested for the u.v.-resistant Spr phenotype (the lexA3 parent strains are u.v.-sensitive). In the recA441 background, the STS (spr) phenotype could be verified by spotting wild-type λ onto a lawn of the candidate strain. Phage spotted onto a lawn of cells carrying a lexA3 mutation give rise to a turbid spot while phage spotted onto cells carrying spr-51 mutations generate clear disks in the bacterial lawn. The recA56 mutation was introduced into the fusion strains by selecting for tetracycline-resistant transductants following infection with P1 grown on strain DB6659 (srl :: Tn10, recA56). RecA- derivatives were u.v.-sensitive.

(c) Assay for β-galactosidase

The assay procedure used was adapted from that described by Miller (1972). The β-galactosidase activity in a culture was determined by withdrawing a 1 ml sample and adding 0.5 ml to 0.5 ml assay buffer containing 100 µg chloramphenicol/ml (con inex) and then incubating at 0.5 ml of a solution containing 1:200 dilution of formaldehyde in water. Cells were grown within 3 h after their removal from the culture. Sodium dodecyl sulfate (40 µl 0.1%,) and 40 µl of CHCl₃ were added to the assay tubes, and the solution was vortexed for 10 s and then kept at room temperature for 10 min. The solution was warmed to 50°C for 40 µl of a 4 mg/ml solution of o-nitrophenyl-β-D-galactoside was added to start the reaction. Reactions were terminated by adding 0.5 ml of 1 M Na₂CO₃; Cell debris was removed by centrifugation, and the absorbance of the solution was determined.

† Abbreviations used: u.v., ultraviolet irradiation; kb, 10⁵ bases or base-pairs; b.p., base-pairs.
The cell density was determined by measuring the absorbance of the formaldehyde-treated cells at 600 nm.

Units β-galactosidase = \frac{A_{420} \times 1.5 \times 1000}{A_{690} \times \text{time (min)}}

(d) Preparation of chromosomal DNA

Cells (200 ml) were grown overnight in LB medium. Cells were collected by centrifugation and resuspended in 7.5 ml of a solution containing 50 mM-Tris (pH 8), 100 μM-EDTA, and 20% sucrose. Lysozyme (40 μg) in 1.8 ml of 0.25 M-Tris (pH 8) was added and the mixture was incubated for 15 min on ice. Then, RNase A (10 μl of a 21 μg/ml solution), proteinase K (2 mg in 1 ml of 0.25 M-Tris, pH 8) and sodium Sarkosyl (10 ml of a 1% solution containing 75 mM-EDTA) were added, and the suspension was incubated at 37°C for 6 h. Cold water was added to a density of 1.7 g/cm³. The samples were centrifuged at 42,000 revs/min for 1.6 h in a VT60 rotor. DNA was collected by puncturing the bottom of the tube with a hypodermic needle and collecting the highly viscous portion of the gradient. The DNA was dialyzed against three 1-l volts of DNA buffer (10 mM-Tris, 5 mM-NaCl, 1 mM-EDTA, pH 8). This preparation yielded about 1 mg of high molecular weight DNA. Occasionally the DNA was partially resistant to digestion with restriction enzymes. In this case, samples (in Eppendorf tubes) were extracted with phenol, extracted with chloroform and then precipitated with ethanol.

(e) Preparation of plasmid DNA

Plasmid DNA was prepared using a slight modification of the procedure of Clewell & Helinski (1970).

(f) Cloning

In the initial cloning of the din::lac fusions, pH322 and chromosomal DNA were digested with BamHI. Samples were precipitated with ethanol and 20 μg chromosomal DNA and 5 μg plasmid DNA were resuspended in 100 μl ligation buffer (50 mM-Tris-HCl (pH 7.8), 10 mM-MgCl₂, 20 mM-dithiothreitol, 10 mM-ATP, and 50 μg bovine serum albumin/ml). Bacterial phage T4 DNA ligase was added, and the reactions were incubated at 16°C for 12 h. Competent G1000 cells were transformed with the ligation mixture, and transformants were selected on plates containing ampicillin (20 μg/ml) and 40 μg 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal)/ml (Bachem).

(g) Isolation of restriction fragments

Isolation of restriction fragments after electrophoresis on 4% polyacrylamide gels and staining with methylene blue (Maniatis et al., 1975) was as described by Maxam & Gilbert (1980) with minor modifications.

(h) In vitro transcription

Repression of transcription of restriction fragments by lexA protein in vitro was essentially as described (Brent & Ptashne, 1981) except that the reaction mixtures contained RNA polymerase (a gift from W. McGovern) at 50 μl CT4 at 5 μl, unlabeled UTP at 2.5 μM, [32P]UTP at 2.5 μM, and sometimes contained bovine serum albumin at 50 μg. After electrophoresis on 8% polyacrylamide gels (Maxam & Gilbert, 1980) transcripts were visualized by autoradiography on XAR-5 film. lexA protein was purified as described by Brent & Ptashne (1981).

We originally observed that the cloned din::lac transgene allows one to grow a cell on the lac operator fusion to a normal cell density. This led us to examine the expression of the cloned DNA in the presence of exogenous synthesized β-galactosidase. Although the din::lac fusions were not induced to the same extent as the endogenous fusions, in which the protein is synthesized, the expression of transcription from the din::lac fusions was not repressed by the presence of lexA and lexA protein.

The SOS region of the lac operon is known to be expressed as wild-type lac cell phenotype in the presence of the din::lac operon, suggesting that the din gene expression is constitutive, i.e., it is not subject to regulation by the SOS induction system. The expression of the din::lac fusion should block din gene expression because the plasmid contains a din gene. In this experiment, we were able to show that the din gene expression was not repressed even in the presence of lexA protein, indicating that the din::lac fusion is able to produce the actual din gene product which cannot work (G. R. Smith, 1972).

A genetic test was carried out to determine whether the removal of lexA protein would allow the expression of the din::lac fusion. However, if the din::lac fusion is constitutive, then the lexA protein should no longer be necessary for din::lac expression. This is indeed the case, as shown in the following experiment. In this experiment, the din::lac fusion is expressed in the absence of lexA protein.

Thus, in conclusion...
3. Results

(a) Genetic studies

We originally identified the dinA, dinB, dinD, and dinF genes by a method that allows one to probe the genome for transcriptional units that are expressed in response to a given environmental stimulus (Kenyon & Walker, 1981). Random cellular promoters were fused to the lac structural genes using the Mud (Ap, lac) operon fusion vector (Casadaban & Cohen, 1979). Cells that carried fusions of lac to genes induced by DNA-damaging agents (din genes) were identified as colonies that synthesized $\beta$-galactosidase in response to the SOS-inducing agent mitomycin C. Although the functions of these genes are not known, their regulation can be studied by measuring the level of $\beta$-galactosidase produced under various conditions. Because the Mud (Ap, lac) operon fusion vector generates operon fusions, in which the wild-type $\beta$-galactosidase protein is translated from a hybrid transcript, the levels of $\beta$-galactosidase in the fusion strains reflect the relative rates of transcription in the fusion strains. In this section, we describe a genetic experiment that analyzes the way in which the two SOS-regulatory elements, the recA and lexA proteins, regulate din gene expression.

The SOS response can be blocked by lexA3 mutations, which alter the lexA protein in such a way that it cannot be cleaved by the recA protease as efficiently as wild-type lexA protein (Little et al., 1981). This mutation blocks the induction of din gene expression (Kenyon & Walker, 1981). Because the lexA protein is known to function as a repressor, it is reasonable to assume that the lexA3 mutation blocks induction because the lexA protein represses a gene or genes necessary for din gene expression. There are at least two ways in which the lexA3 mutation protein might block din gene expression in uninduced cells. The lexA protein could directly repress individual din genes. Alternatively, other cellular repressors might bind to the din genes. In this case, lexA3 mutants might be inducible because the altered lexA protein blocks the synthesis of sufficient amounts of recA protease to remove the actual din repressors. This appears to be the mechanism by which the lexA3 mutation reduces the induction of prophage gene expression (Castellazzi et al., 1972).

A genetic test can distinguish between these two possibilities (Brent & Ptashne, 1980) (Fig. 1). If the lexA protein directly represses a particular din gene, then removal of lexA protein should be a sufficient condition for expression. Under normal inducing conditions the lexA protein is removed by the recA protease. However, if the lexA protein has been inactivated by mutation, then recA protease should no longer be necessary to activate the gene. Thus, cells that lack both recA and lexA proteins should produce high levels of $\beta$-galactosidase. If, on the other hand, a din gene is repressed by a recA-sensitive repressor other than the lexA protein, then the inactivation of the lexA protein would not necessarily lead to din expression. In a recA- mutant, large amounts of non-functional recA protein would be produced, but because the actual din repressor could not be cleaved, the gene would not be expressed. In this case, cells lacking both recA and lexA proteins should produce low levels of $\beta$-galactosidase.

Thus, in order to distinguish between these two possible situations, we
constructed a set of din–lac fusion strains in which both the recA and lexA proteins had been inactivated by mutation. Functional lexA protein was removed by introducing a lexA mutation called spr. spr alleles are null mutations of the lexA gene since lexA amber mutations are spr alleles (Pacelli et al., 1979) and we have recently generated spr alleles by inserting the transposon Tn5 in the lexA gene (J. H. Krueger & G. C. Walker, unpublished results). As shown in Figure 1, every strain produces high levels of β-galactosidase. The finding that removal of lexA protein makes recA function dispensable is inconsistent with there being a recA-sensitive din gene repressor other than the lexA protein, as well as with a more complicated model that was proposed earlier (Witkin, 1976), and strongly suggests that the lexA protein directly represses each din gene.

**Biochemical studies**

The conclusion that the lexA protein directly represses each din gene is not the only possible interpretation of the genetic studies. For example, the data do not rule out the possibility that the lexA protein represses a gene that codes for an activator of din transcription. It was therefore important to obtain direct evidence that the lexA protein interacts with these genes. To do this, we cloned the
regulatory regions of two *din* genes and determined the effect of purified *lexA* protein on their transcription in vitro.

(c) Cloning of the *dinA* and *dinB* regulatory regions

To clone the *dinA* and *dinB* fusions, we took advantage of a BamHI site located within the Mud phage about 4 kb downstream from the 3' end of the *lacZ* gene. No additional BamHI sites are located upstream in the Mud transcriptional unit. If, in addition, no recognition sites occurred between the end of the integrated Mud phage and the *din* promoters, then a single chromosomal BamHI fragment would be expected to contain the *din-lac* operon fusion.

Chromosomal BamHI fragments from the *dinA* and *dinB* fusion strains were cloned into the BamHI site of pBR322, and the ligation mixture was transformed into strain GW1000, the *lac* parent of the *din* fusion strains (Fig. 2). Lac*+* transformants were isolated from selective plates containing a lactose indicator. In order to determine whether the cloned inserts carried the *din* regulatory regions, the transformants were tested for u.v. inducibility of β-galactosidase. In both cases, the transformants showed inducible β-galactosidase expression (not shown). U.v. inducibility is not a general property of cloned fusions, since Lac*+* transformants that did not show induction were obtained from other experiments.

Preliminary restriction maps of the cloned *dinA* and *dinB* fusions are shown in Figure 3. The regulatory regions were subcloned by deleting the sequences derived
from the Mud phage as outlined in Figure 2. The dinA subclone contains a 2.8 kb bacterial fragment carrying the dinA promoter, and the dinB subclone carries a 3.1 kb insert fragment.

(d) Locations of the dinA and dinB promoters

The approximate positions of the promoters on these fragments were determined by in vitro transcription experiments. The 2.8 kb dinA insert fragment was isolated and transcribed in vitro. When the intact fragment was transcribed, a high molecular weight smear was observed. The smear probably resulted from breakage or slight degradation of a high molecular weight transcript. The sensitivity of the assay was greatly increased by clearing the insert fragment with various restriction enzymes before transcription. After digestion with a number of restriction endonucleases, a single smaller transcript was observed. A HindIII digestion of the insert fragment resulted in the synthesis of a transcript of about 130 bases. The individual HindIII fragments were isolated and transcribed individually. The promoter was found to be located on a 540 base-pair fragment. In order to determine the distance of the dinA promoter from the site of Mud insertion, a HindIII restriction map was obtained (see Fig. 3). From the location of the 540 bp fragment within the cellular sequences, we conclude that the dinA promoter is likely to lie about 1300 bp upstream from the site of Mud insertion.

The 3.1 kb fragment carrying the dinB regulatory region was found to contain three BstEII restriction sites, as shown in Figure 3. These three BstEII fragments were purified, digested with various restriction endonucleases, and transcribed. The only strong promoter carried by any of the insert fragments was found to be located on the 1480 base-pair HindIII–BstEII fragment. Among the insert fragments, this one is closest to the position of Mud insertion. Again, a single major transcript was observed.
observed. When the HindIII–BstEII fragment was cleaved with TaqI and transcribed in vitro a 400 base-pair runoff transcript was observed. The individual TaqI fragments were isolated and transcribed in vitro. One of these fragments (670 bp) directed the synthesis of this 400 bp transcript. The position of this fragment within the BstEII–HindIII fragment was determined by labeling the BstEII–HindIII fragment at one end, and then measuring the sizes of the fragments generated by partial digestion with TaqI. The 670 bp fragment was positioned furthest from the HindIII site (see Fig. 4), indicating that insertion of the Mud phage occurred about 900 bp downstream from the dinB promoter.

The functions of the dinA, dinB, dinD and dinF gene products are not known. It is interesting to note that both the dinA::Mud and dinB::Mud insertions occurred at a distance of about 1 kb from the promoters of these genes. It is possible that the dinA and dinB fusion strains do not display striking mutant phenotypes because the insertions in these genes do not result in loss of their functions. The availability of cloned sequences should facilitate the construction of additional dinA and dinB mutations that might reveal the biological roles of these genes.

![Image of transcription analysis](image_url)

**Fig. 4. Inhibition of transcription of the dinA and dinB genes by purified lacI protein.** Fragments bearing the dinA and dinB promoters were isolated and transcribed as described in the text (and in Materials and Methods). A pBR322 HindIII–BstEII fragment carrying the β-lactamase promoter was also isolated and included in the reaction mixtures. DNA was present at 1 × 10^-9 M in the transcription reactions, and lacI protein was omitted (–) or included (+) at 5 × 10^-7 M. Similar results were obtained with concentrations of lacI protein as low as 2 × 10^-8 M. The concentration of functional lacI protein present in the reactions may be overestimated, as the percentage of active lacI protein in the preparation is unknown. *amp*, ampicillin resistance gene.
We have previously stimulated by aged the results presented in this paper by the same recombinant DNA from cells, the rate of DNA repair in the absence of the lexA protein expression. Furthermore, we have tested two of these genes.

By genetic experiments, we have obtained evidence that the products of these genes, whose products function with the lexA protein itself, are also dinA, lexA, and recA genes. The lexA protein binds to the lexA operators, and the recA gene interacts with the lexA operators. In this report, we present an analysis of these genes (by genetic criteria) and the specific recombination events.

Figure 5 outlines the bacterial SOS response. The individual damage-inducible genes (indicated by filled regions on the circle) are located at their approximate map positions on the E. coli chromosome. Molecules of lexA repressor (small filled circles) bind to regulatory sequences on the dinA gene and block their expression. The recA protein (open diamond) is synthesized at low levels in undamaged cells, but does not display significant protease activity. After SOS-inducing treatments the recA protein interacts with an unidentified cofactor (represented here as single-stranded DNA; see Pichard & Roberts, 1981), and acquires a specific protease activity. The shaded diamonds represent these molecules.

The recA protease catalyzes the cleavage of lexA molecules; the lexA cleavage products are represented by half-circles. The derepression of the SOS genes leads to their expression.

e) In vitro transcription of the dinA and dinB genes

The effect of lexA protein on the in vitro transcription of the dinA and dinB genes is shown in Figure 4. In this experiment, fragments carrying the dinA or dinB promoters were present in reaction mixtures that also contained a HindIII–EcoRI fragment from pBR322. Both DNA fragments, when used to transcribe purified lexA protein, produced transcripts of RNA polymerase that carry the dinA and dinB promoters is blocked by lexA protein (as tested). The effect of lexA protein on the promoters, because of its ability to bind to the DNA, is consistent with the results of other experiments.

In the screen for mutagens, the lexA protein is a powerful tool for inactivating the different SOS genes (H. Miller et al., 1981). The lexA protein is capable of inducing mutations in various SOS genes (H. Miller et al., 1981).
fragment from pBR322 containing the 5' portion of the ampicillin resistance gene. Both DNA fragments were present at relatively low concentrations (1 nmol), to avoid disturbing the concentration of free protein by its binding to DNA. When purified lexA protein (1 × 10⁻⁷ M) is pre-equilibrated with the DNA before addition of RNA polymerase and triphosphates, transcription from the dinA and dinB promoters is blocked. Concentrations of lexA protein as low as 2 × 10⁻⁸ M effectively inhibit transcription (data not shown; lower concentrations were not tested). The effect of lexA protein is specific for transcription of the dinA and dinB promoters, because transcription of the ß-lactamase gene is unaffected. These results confirm that the dinA and dinB genes are directly repressed by lexA protein.

4. Discussion

We have previously identified a set of genes, called din genes, whose expression is stimulated by agents that induce the SOS response (Kenyon & Walker, 1981). The results presented in this paper indicate that each of these genes is directly repressed by the same repressor, the lexA protein. When this protein is genetically removed from cells, the rate of transcription of each gene increases dramatically. In the absence of the lexA protein, the recA protease is no longer necessary for their expression. Furthermore, purified lexA protein inhibits the transcription of at least two of these genes in vitro.

By genetic experiments similar to those described here, we have previously obtained evidence suggesting that the damage-inducible genes umuA and umuC whose products function in DNA repair and mutagenesis, as well as the lexA gene itself, are also directly repressed by the lexA protein (Kenyon & Walker, 1981; Bagg et al., 1981; Brent & Ptashne, 1980). Furthermore, we have shown that the lexA protein binds to specific operator sequences near the promoter of its own gene and the recA gene (Brent & Ptashne, 1981), and the protein has been shown to bind to operator sites near the promoters of the umuA and umuB genes (A. Sancar, unpublished data; W. D. Rupp, personal communication). The protein also appears (by genetic criteria) to repress the sfiA (Huisman & D'Ari, 1981) and himA (H. Miller et al., 1981) genes, whose products function in filamentation and site-specific recombination, respectively.

Figure 5 outlines the basic strategy by which the bacterial cell appears to induce co-ordinately the collection of SOS functions in response to DNA damage. In undamaged cells the lexA protein binds to the regulatory regions of more than 10 cellular genes, and inhibits their transcription. When the cell's DNA is damaged, the recA protein acquires protease activity and begins to cleave molecules of lexA repressor. As the level of lexA protein falls, the various genes are derepressed, and their products are synthesized. These products participate in a set of metabolic processes, some of which are physiologically unrelated, and which are recognized as the various SOS functions.

In the screen for mitomycin C-regulated din-lac fusions, 10 independent Mud insertions mapping within five din transcription units were obtained (Kenyon & Walker, 1981). These din genes were identified solely on the basis of their inducibility by mitomycin C, with no requirement that they be controlled by the
recA and lexA proteins, or that they function in the SOS response. Yet each of these genes appears by genetic (and in some cases biochemical) criteria to be repressed by the lexA protein. The finding suggests that if additional mitomycin C-inducible genes exist that are not repressed by the lexA protein, then their frequency is probably much lower than the frequency of lex-repressed genes.

A number of observations suggest that certain fine-tuning mechanisms operate within the framework depicted in Figure 5. For example, certain SOS-inducing treatments (mating unirradiated F-cells with u.v.-irradiated Hfrs (George et al., 1974) and infecting restricting hosts with unmodified DNA (Dharmalingam & Goldberg, 1980)) induce only a subset of the SOS responses. One possible explanation for this behavior is that it is a consequence of different binding affinities to different operator sites (Brent & Ptashne, 1981). Additional observations suggest that not all the parameters that influence din gene expression have been identified. One particularly puzzling finding is the observation that one of the din genes (dinD) shows a prolonged lag time before the onset of β-galactosidase induction at relatively high doses of u.v. (60 J/m²), but is induced as quickly as the other din genes at lower doses (15 J/m²) (Kenyon, 1981). The way in which the cell can differentially regulate the expression of genes and processes within this single regulatory network is the central unsolved problem.

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Yet each of these mechanisms operate in certain SOS-inducing situations (George et al., Dharmalingam & Hars, 1981). One possible explanation is that the onset of β-galactosidase activity is induced as the result of a specific SOS signal, but is induced as the result of another, as well. This raises the question of how the genes and processes are regulated.

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