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Genetics

DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*

(*din* gene/*recA*/*lexA* regulation/operon fusion/SOS functions/*uvrA*)

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ABSTRACT **Operon fusions in *Escherichia coli* were obtained that showed increased β-galactosidase expression in response to treatment with the DNA-damaging agent mitomycin C. These fusions were generated by using the Mud(Ap^R, *lac*) vector [Casadaban, M. J. & Cohen, S. N. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4530–4533] to insert the lactose structural genes randomly into the bacterial chromosome. Induction of β-galactosidase in these strains, which carried fusions of *lac* to these *din* (*d*amage-*in*ducible) loci, was (*i*) triggered by UV light as well as by mitomycin C and (*ii*) abolished by either a *recA*$^-$ or a *lexA*$^-$ mutation. Similar characteristics of induction were observed when the lactose genes were fused to a prophage λ promoter by using Mud(Ap^R, *lac*). These results indicate that *E. coli* contains a set of genes that, like prophage λ genes, are expressed in response to DNA-damaging agents and regulated by the *recA* and *lexA* gene products. These *din* genes map at five bacterial loci. One *din*::Mud(Ap^R, *lac*) insertion results in a UV-sensitive phenotype and may be within the *uvrA* transcriptional unit.**

When *Escherichia coli* is exposed to any of a number of agents that either damage DNA or interfere with its replication, a set of diverse cellular responses occurs (1). The *recA* protein is induced to high levels. Certain prophages, such as λ, are induced. The cell's capacity to repair and to mutate DNA increases. Normal cell division is altered in such a way that filamentous growth ensues. These and other responses elicited by DNA damage have been collectively termed the "SOS functions" (1, 2). These functions are coordinately regulated by a process that involves the *recA* and *lexA* gene products; they do not occur in cells that carry either a *recA*$^-$ or a *lexA*$^-$ mutation. Furthermore, they are expressed at high temperature in cells carrying a *tif* mutation (*tif* is an allele of *recA*).

The occurrence of two SOS functions—the induction of the *recA* gene product and the induction of prophage λ—requires the increased expression of specific genes. The crucial step in λ induction appears to be the proteolytic cleavage of the λ repressor by the *recA* protein (3), which allows expression of the prophage genome. The mechanism by which *recA* protein is induced is not as well understood. Like induction of other SOS functions, the process requires functional *recA* protein (4, 5). Current genetic evidence is consistent with the *lexA* protein's being a negative regulator of the *recA* gene (6). Relatively little is known about the mechanism of induction of the other SOS functions. In particular, it has not yet been determined whether their occurrence also results from the increased expression of certain bacterial genes.

We were interested in determining directly whether there is in fact a set of genes in *E. coli* whose expression is induced by DNA-damaging agents. Such an experiment was made possible by the recent (7) construction of a specialized transducing phage that can act as a single-step operon fusion vector. The phage, Mud(Ap^R, *lac*), is a derivative of the temperate bacteriophage Mu which integrates into the bacterial chromosome with no appreciable site specificity (8, 9). The phage carries the lactose (*lac*) structural genes but no promoter capable of initiating their transcription. However, when this phage integrates within a bacterial transcriptional unit, the *lac* genes can be expressed as a result of continued transcription into the phage genome. Such an insertion creates an operon fusion in which the synthesis of β-galactosidase has been placed under the control of a cellular regulatory locus.

This Mud(Ap^R, *lac*) phage has several features that make it particularly attractive for studying genetic regulatory networks. Specific loci involved in the network can be identified by generating random fusions and then screening directly for cells in which Mud(Ap^R, *lac*) has inserted such that the *lac* genes are expressed in response to a given treatment. In certain cases, the functions of such loci can be deduced because insertions of Mud(Ap^R, *lac*) that occur either within a gene or proximal in its transcriptional unit abolish the function of that gene. Furthermore, second-site mutants affecting *lac* gene expression in the fusion strains can be obtained subsequently as a means of studying the regulation of the particular system.

We have isolated a set of Mud(Ap^R, *lac*) insertions within the *E. coli* chromosome in which β-galactosidase (the product of the *lacZ* gene carried by the phage) is induced in response to treatment with the DNA-damaging agent mitomycin C (MC). The isolation and preliminary characterization of these strains is presented here.

MATERIALS AND METHODS

Strains. GW1000 is a *pro*$^+$, *lac*Δ(*U169*) derivative of the *tif sfi* strain GC3217 (10). The *sfi* allele suppresses MC-induced filamentation and permits continued growth in the presence of the chemical (10). Experiments were carried out at 30°C, which minimizes *tif* expression (10). *recA*$^-$ derivatives were constructed as described (11). *lexA*$^-$ derivatives were obtained by P1 transduction using a *malE*::Tn*5*, lexA$^-$ donor, strain RB800. λO_8 was obtained from E. Signer. pKB280 (12) was obtained from R. Sauer, and RB800 was from R. Brent.

Media. Bacteria were routinely propagated in Luria broth and LB agar (11). Supplemented minimal medium E plates (13) and M9 liquid media (14) were used for assays of β-galactosidase and measurements of survival after UV and MC treatments.

Isolation of Fusion Strains. Colonies of GW1000 containing random insertions of Mud(Ap^R, *lac*) were prepared as described by Casadaban and Cohen (1979). The screening procedure used to detect *din* fusions is outlined in *Results*. Putative λ-*lac* fusions (see text) were tested for cosegregation of Mu and λ immunity after transductions of the fusion strains to gal$^+$.

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Abbreviations: MC, mitomycin C; IPTG, isopropyl-1-thio-β-D-galactoside.

RESULTS

Isolation of Fusions. Our basic strategy for the isolation of fusions responding to MC has been to screen colonies of bacteria containing random Mud(Ap^R, *lac*) insertions for those that show increased β-galactosidase activity in the presence of MC. (Although it does induce certain other temperate phage, MC does not trigger Mu induction.) Colonies carrying Mud(Ap^R, *lac*) insertions were replica plated onto two lactose indicator plates, one of which contained MC (1 μg/ml). Those colonies that appeared to synthesize increased levels of β-galactosidase on the plate containing MC were then tested for MC induction in liquid media (see legend to Fig. 1). In addition, some colonies grew poorly on MC plates, presumably because Mud(Apr^R, *lac*) had inserted within transcriptional units necessary for normal MC-resistance levels. Because it was not possible to assess their levels of *lac* gene expression accurately on indicator plates, such isolates were screened directly for MC induction in liquid media.

One concern that we had prior to these experiments was that MC could have some nonspecific effect on an integrated Mud(Ap^R, *lac*) phage that would result in increased *lac* gene expression after treatment with MC. In fact, β-galactosidase activity was essentially unaffected by MC treatment in the vast majority of cells carrying Mud(Ap^R, *lac*) insertions.

Among 35,000 colonies carrying Mud(Ap^R, *lac*) insertions, however, we identified 22 (10 of independent origin) that displayed substantially greater β-galactosidase activity after treatment with MC (Table 1). The sites of Mu insertion in these isolates were termed *din* (*d*amage-*in*ducible) loci.

Before further investigating these *din*::Mud(Ap^R, *lac*) fusions, we chose to obtain a Mud(Ap^R, *lac*)-generated fusion of *lac* to a prophage λ promoter, because λ genes are known to be expressed in response to MC. Such a fusion would serve as a standard of comparison in studies of other MC-inducible fusions.

The isolation of the λ::Mud(Ap^R, *lac*) fusion involved a preliminary screening for Mud(Ap^R, *lac*) insertions that prevented the release of free λ from a λ lysogen. Of the 33 Mud(Ap^R, *lac*) insertions obtained that prevented λ release and thus were candidates for insertions in the λ genome, 13 synthesized β-galactosidase in response to MC treatment. Thus, it appears that about half of the insertions of Mud(Ap^R, *lac*) within putative *din* loci are oriented in the direction of transcription and could permit MC-inducible *lac* gene expression. The expression of β-galactosidase in these λ::Mud(Ap^R, *lac*) fusions was shown to be under the direct control of the λ regulatory circuitry by introducing a plasmid (pKB280) (12) that overproduces λ repressor. High levels of λ repressor are known to block the expression of λ genes even in the presence of SOS-inducing agents. High levels of λ repressor blocked MC-induction of β-galactosidase in a λ::Mud(Ap^R, *lac*) fusion (Fig. 1*A*). As expected, the plasmid did not significantly affect β-galactosidase induction in a *din*::Mud(Ap^R, *lac*) fusion strain.

Table 1. MC induction of β-galactosidase in *din*::Mud(Ap^R, *lac*) fusion strains

Strain	Fusion	β-Galactosidase, units/A_{600} No MC	β-Galactosidase, units/A_{600} With MC	Induction ratio
GW1010	*dinA1*	8	67	8.4
GW1020	*dinA2*	15	80	5.3
GW1030	*dinB1*	10	150	15
GW1040	*dinD1*	9	185	21
GW1050	*dinD2*	8	190	24
GW1060	*dinE1*	50	301	6.0
GW1070	*dinF1*	35	130	3.7
GW1080	*dinF2*	20	90	4.5
GW1090	*dinF3*	45	120	2.7
GW1100	*dinF4*	18	180	10
GW1200	λ	1	685*	685*

The kinetics of β-galactosidase induction were determined for each strain as described in the legend to Fig. 1. Activities of β-galactosidase in untreated and fully induced cultures were calculated from differential plots of the data.

* These values may be artificially high. Lysis of this fusion strain occurs upon induction, presumably as a result of a prophage λ gene expression.

MC Induction of β-Galactosidase. We measured the specific activity of β-galactosidase in each of the 10 *din*::Mud(Ap^R, *lac*) fusion strains and in the λ::Mud(Ap^R, *lac*) isolate during growth in medium with and without MC (Table 1). Constitutive β-galactosidase activity varied extensively between isolates, as did the magnitude of the induction.

The kinetics of β-galactosidase induction were determined for each of the *din–lac* fusions (representative results are shown in Fig. 1 *B–D*). Although treatment of growing cells with MC induced β-galactosidase, the time interval between the addition of MC and a detectable increase in β-galactosidase activity varied between isolates. For example, the *dinD* fusions (see mapping section) showed an appreciable lag prior to the onset of β-galactosidase induction (Fig. 1*D*), whereas other isolates such as the *dinA* and *dinB* fusions (Fig. 1 *B* and *C*) began to show increased *lac* gene expression more quickly.

Growth of the fusion strains was only slightly affected by MC during the course of this experiment. Two fusion strains (*dinE* and the λ::Mu isolate) were in fact more sensitive to MC (see below). However, at the relatively low MC concentrations used, their growth was impaired only after prolonged incubation.

MC induction of β-galactosidase in the fusion strain was a transient phenomenon. Dilution of induced cultures into fresh medium lacking MC followed by incubation led to a decline in β-galactosidase levels (Table 2). Because we have not yet measured the kinetics of this decay, we do not know whether the variation between different *din* fusions is significant. However, these results do indicate that the increased levels of β-galactosidase seen when these strains are grown in the presence of MC do not result from a permanent genetic or epigenetic change.

UV Induction. Because the induction of the SOS functions can be triggered by a diverse array of DNA-damaging treatments, we tested the effect of a second potent inducer, UV light, on induction of β-galactosidase in the *din*::Mud(Ap^R, *lac*) strains. Representative results are shown in Fig. 2. β-Galactosidase was UV-inducible in all the *din* fusion strains and, as predicted, in the λ::Mud(Ap^R, *lac*) isolate. Furthermore, at the doses used, the magnitude and kinetics of UV induction mimicked those of the MC response. This suggests that induction of the *din* gene products may occur as a result of a common signal that arises from both UV and MC treatments.

Preliminary Genetic Analysis. The induction of the SOS functions by DNA-damaging agents is dependent upon a *recA*$^+$, *lexA*$^+$ genotype. To determine whether the induction of *din* gene expression is subject to the same regulatory controls, we introduced *recA*$^-$ and *lexA*$^-$ mutations into the fusion strains by bacteriophage P1 transduction and examined the effects of these mutations on inducible *lac* gene expression. (In the case of multiple insertions mapping at a single locus, a representative fusion strain was tested.) Remarkably, induction was abolished by a *recA*$^-$ or *lexA*$^-$ mutation in every isolate examined. As expected, induction was abolished in the

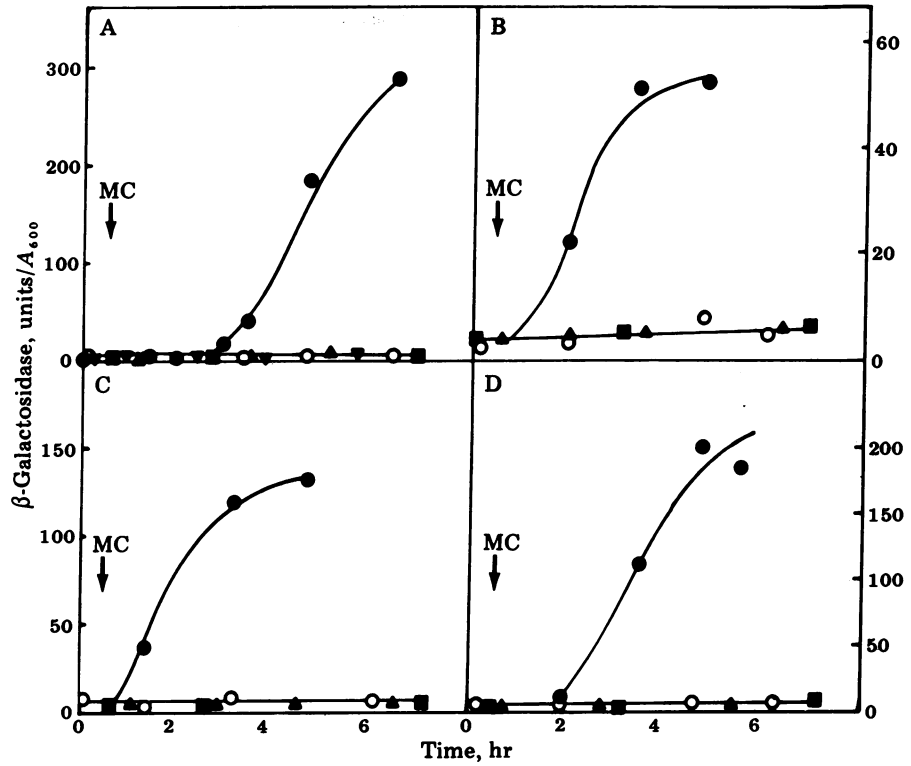

FIG. 1. Kinetics of MC induction of β-galactosidase in fusion strains. Cells were grown in supplemented minimal media at 30°C. MC (1 μg/ml) was added to exponential cultures as indicated. Aliquots (1 ml) were removed periodically, and the total activity of β-galactosidase present in the culture was determined essentially as described by Miller (14). Cell density was determined by measuring the absorbance at 600 nm. ○, Untreated fusion strains; ●, fusion strains + MC; ▲, *lexA*⁻ derivatives + MC; ■, *recA*⁻ derivatives + MC; ▼, λ::Mud(Ap^R, *lac*)/pKB280 + MC. (*A*) λ::Mud(Ap^R, *lac*); (*B*) *dinA*::Mud(Ap^R, *lac*); (*C*) *dinB*::Mud(Ap^R, *lac*); (*D*) *dinD2*::Mud(Ap^R, *lac*).

λ::Mud(Ap^R, *lac*) fusion strain. In the presence of MC, the growth rate of *recA*⁻ and *lexA*⁻ strains was decreased but the cells remained fully competent to induce expression of other genes. In particular, MC-treated *recA*⁻ cells could induce the expression of a wild-type *lac*⁺ operon. To show this, a *lac*⁺ strain (GC3217), which is closely related to the parent of the Mud(Ap^R, *lac*) fusion strains, was made *recA*⁻ by P1 transduction. β-Galactosidase synthesis was still triggered by isopropyl-1-thio-β-D-galactoside (IPTG), an inducer of the *lac* operon, even when the cells were grown in the presence of MC (Fig. 3).

These results indicate that expression of the *din* genes is controlled by the *recA* and *lexA* gene products. Furthermore, these gene products are required specifically for induction of increased *din* gene expression because uninduced β-galactosidase activity is not significantly affected by *recA*⁻ or *lexA*⁻ mutations (Fig. 1).

Table 2. Response of fusion strains to removal of inducing stimulus

Fusion	β-Galactosidase activity, units/A_{600}		
	Initial	After dilution into media	
		No MC	With MC
dinA1	43	7	15
dinB	125	7	173
dinD1	190	18	136
dinF1	175	93	288

Exponential cultures were treated with MC (1 μg/ml) and incubation was continued for 4 hr. Aliquots were then withdrawn and diluted 1:100 into fresh media with and without MC (1 μg/ml). Cultures were incubated for 7–10 generations and then assayed for β-galactosidase activity.

Functions of the *din* Genes. The *din* loci were initially identified by their ability to induce β-galactosidase in response to MC when fused to *lac* with Mud(Ap^R, *lac*). Because the insertion of the Mud(Ap^R, *lac*) phage is a mutational event, the biological processes in which the *din* genes are involved can be deduced by identifying particular mutant phenotype(s) associated with the various *din*::Mud(Ap^R, *lac*) strains.

So far, we have identified specific mutant phenotypes of one fusion strain, *dinE*. This mutant showed extreme MC and UV sensitivity (Fig. 4). The phenotype of the fusion strain is genetically linked to the integrated Mud(Ap^R, *lac*) phage because "removal" of Mud(Ap^R, *lac*) by P1 transduction (see next sec-

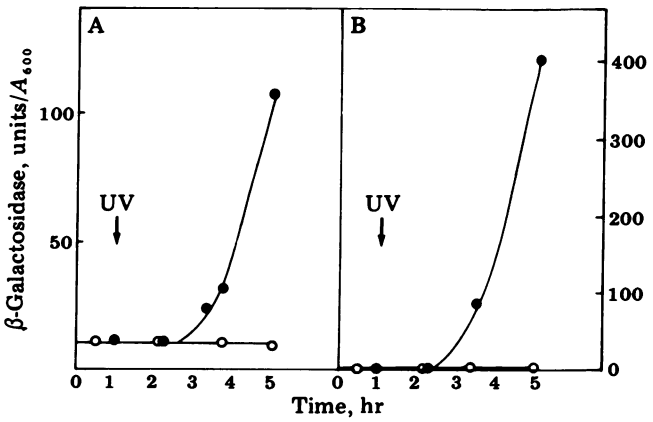

FIG. 2. Kinetics of UV induction of β-galactosidase. Experimental procedure was similar to that described in the legend to Fig. 1. Exponential cultures were exposed to UV light at 60 J/m^2 at the times indicated. ○, Unirradiated cultures; ●, irradiated cultures. (*A*) *dinD2*::Mud(Ap^R, *lac*); (*B*) λ::Mud(Ap^R, *lac*).

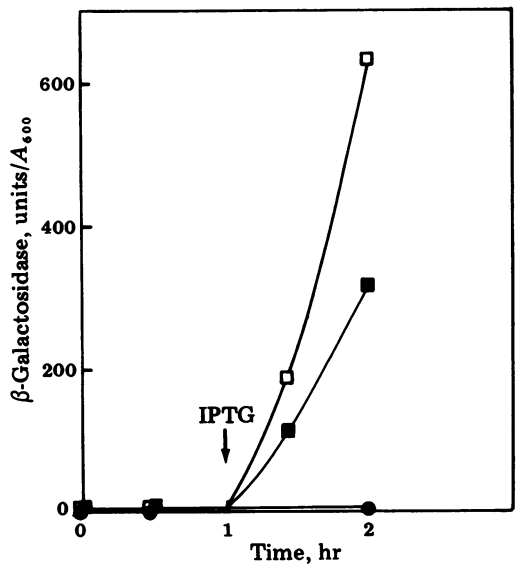

FIG. 3. Kinetics of β-galactosidase induction in *lac*$^+$ *recA*$^-$ cells. An exponential-phase culture of strain GW1002 growing in minimal medium at 30°C was divided, and MC (1 μg/ml) was added to one portion. Incubation was continued for 3 hr, and then IPTG (25 μM) was added. Generation time of the *recA*$^-$ cells was 110 min (no MC) and 210 min (with MC). □, No MC, with IPTG; ■, with MC, with IPTG; ●, with MC, no IPTG.

tion) simultaneously restored wild-type UV- and MC-resistance levels. Thus, the mutant phenotype of *dinE* most likely results from insertion of Mud(Ap^R, *lac*) within, or upstream from, a gene involved in UV or MC resistance (also see *Discussion*).

We have not yet identified specific mutant phenotypes of the other fusion strains. However, only mutagenesis and recombinational proficiency have been carefully examined so far.

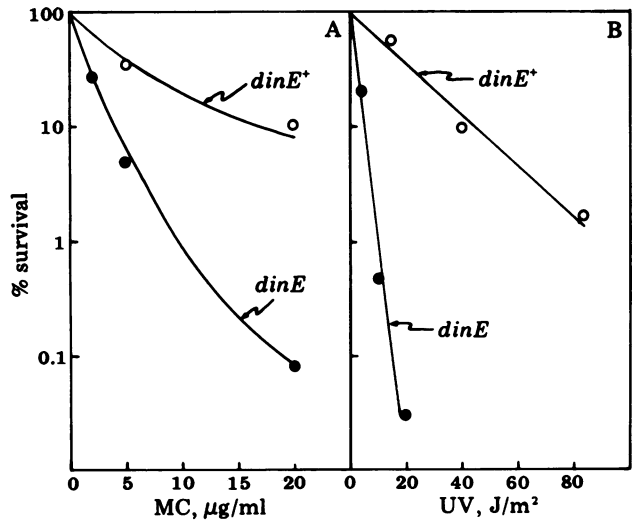

FIG. 4. Survival of the *dinE*::Mud(Ap^R, *lac*) fusion strain after MC (*A*) and UV (*B*) treatments. Strains GW1060 (●) and GW1000 (○) were grown in supplemented minimal media at 30°C. (*A*) Exponential cultures were divided into 1-ml portions and treated with the indicated amounts of MC. Cells were incubated for 30 min in the presence of MC and then diluted and plated as above. (*B*) Exponential cultures were irradiated as indicated, and appropriate dilutions were plated on minimal plates. A *dinD*::Mud(Ap^R, *lac*) fusion strain showed UV and MC survival levels similar to those of strain GW1000.

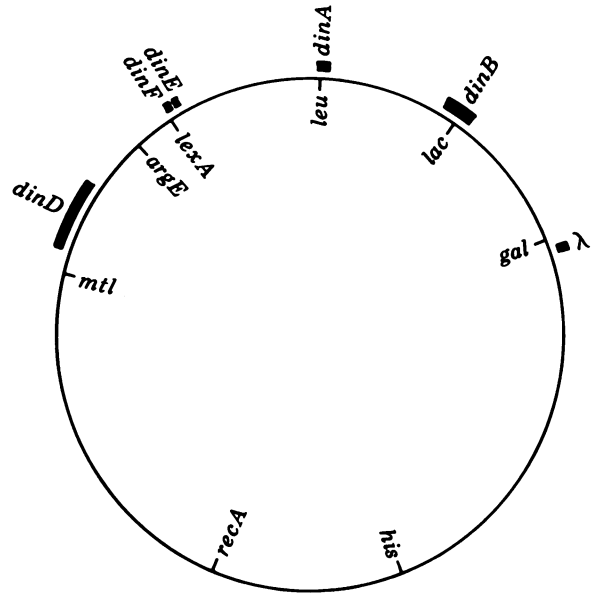

FIG. 5. Map positions of the *din*::Mud(Ap^R, *lac*) insertions. *dinA*, *-E*, and *-F* insertions are genetically linked to their respective adjacent bacterial markers by P1 transduction (see text). The approximate positions of the *dinB* and *dinD* insertions were determined by interrupted mating experiments between fusion strain recipients and donor strain KL266 (HfrC). Methods were essentially as described by Miller (14).

Locations of the *din*::Mud(Ap^R, *lac*) Insertions. The *din*::Mud(Ap^R, *lac*) insertions have been assigned to approximate map positions based upon the results of interrupted mating experiments and P1 transductions (Fig. 5). The insertions map at five distinguishable loci which we have termed *dinA*, *-B*, *-D*, *-E*, and *-F*.

The *dinA*, *-E*, and *-F* insertions were linked to known markers by P1 transduction. Transduction of the two *dinA* fusion strains to *leu*$^+$ led to the loss of Mud(Ap^R, *lac*) in approximately 50% of the recombinants. The four *dinF* insertions were very tightly linked to *lexA*. However because (*i*) the strains do not show the phenotype expected for complete loss of *lexA* function (Spr) (6) and (*ii*) we have recovered rare *lexA*$^-$, *dinF1*::Mud(Ap^R, *lac*) recombinants, the insertions are unlikely to be within the *lexA* structural gene itself. *dinE*$^+$ (Mu$^-$) is also cotransducible with *lexA* but at a lower frequency than *dinF*. Results of three factor crosses indicate that the *dinF* and *dinE* insertions are located clockwise from *lexA*.

Mud(Ap^R, *lac*) carries a temperature-sensitive repressor and thus kills the host at high temperature. This fact has allowed us to map the *dinB* and *dinD* loci by determining the time of entry of temperature-resistant (Mu$^-$) recombinants in interrupted mating experiments (Fig. 5). *dinB* maps near *lac* by this criterion. *dinD* is located between the reference markers *mtl* and *argE*.

Spontaneous temperature-resistant revertants occur with the same frequency in all the fusion strains. Thus, each strain most likely carries a single copy of Mud(Ap^R, *lac*).

DISCUSSION

DNA-damaging agents are known to give rise to various responses in prokaryotic and eukaryotic cells (1). Using *E. coli*, which is the only organism in which such an experiment is currently tractable, we have looked for direct evidence of a set of genes that are turned on as a result of DNA-damaging treatments. By utilizing the Mud(Ap^R, *lac*) fusion vector, we have isolated cells in which the *lacZ* gene carried by the phage has been inserted into the chromosome in such a way that

β-galactosidase is induced in response to MC treatment. The simplest explanation consistent with our results is that there exists a set of *din* (*d*amage-*in*ducible) genes in *E. coli* whose expression is increased in response to DNA damage and that we have fused the promoter-regulatory regions of some of these *din* genes to the phage *lac* gene.

Two of the most striking features of the induction of expression of this set of *din* genes are that (*i*) it is elicited by the DNA-damaging agents MC and UV and (*ii*) it is under the coordinate control of the *recA* and *lexA* gene products. In this respect the *din* genes resemble the two previously characterized examples of damage-inducible genes in *E. coli*—the *recA* gene (4, 5) and the λ prophage genes (1). These similarities suggest that the SOS functions, which have so far been characterized primarily at a phenomenological level, may be coded for by *din* genes. Although the *din* genes are coordinately regulated, it is possible that the expression of each may be individualized because fusions to various *din* loci can differ widely in their basal levels, lag times for expression, and final induced levels of β-galactosidase.

The *din* genes described here map at a minimum of five loci, four of which are widely separated. Because some of the loci are represented by only a single fusion, we probably have not yet obtained an insertion in all possible *din* loci. In addition, our screen precluded detection of certain possible classes of *din* loci. Autoregulated (like *recA*) or essential genes probably would have escaped detection.

The map positions of certain *din* genes raise interesting possibilities as to their identities. One particularly interesting finding was that the *dinE* insertion has the phenotype and map position of a *uvrA* mutant. This suggests that the *uvrA uvrB* endonuclease (15), which participates in excision repair and is normally regarded as being constitutively expressed, may in fact be induced to higher levels in response to DNA damage. The *dinA* insertions map very near *leu* and are thus candidates for insertion within the gene for polymerase II.

The four insertions at the *dinF* locus are very tightly linked to *lexA*. However, if the insertions are within the *lexA* transcriptional unit, they are positioned distal to the *lexA* structural gene as discussed above. Moreover, evidence has recently been obtained that indicates that the *lexA* protein is a negative regulator of its own synthesis (ref. 16; R. Brent, personal communication). This suggests that an insertion within the *lexA* structural gene would lead to constitutive *lac* gene expression and would not have been detected in our screen. Alternatively, the *dinF* insertions may be within a nearby transcriptional unit.

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