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Characterization of *dinY*, a New *Escherichia coli* DNA Repair Gene Whose Products Are Damage Inducible Even in a *lexA*(Def) Background

CLAUDE PETIT,[1,2] CAROLINE CAYROL,[1] CLAIRE LESCA,[1] PETER KAISER,[3] CHARLES THOMPSON,[3] AND MARTINE DEFAIS[1]*

Laboratoire de Pharmacologie et Toxicologie Fondamentales du Centre National de la Recherche Scientifique, 205 route de Narbonne, 31077 Toulouse Cedex,[1] Département de Biologie Moléculaire, Ecole Nationale Vétérinaire, 31076 Toulouse Cedex,[2] and Unité de Génie Microbiologique, Institut Pasteur, 75724 Paris Cedex 15,[3] France

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Bacteriophage Mu dX(Ap *lac*) was used to isolate a mutation in an *Escherichia coli lexA*(Def) strain representing a previously undescribed gene (*dinY*) which does not seem to be under the direct control of LexA. The insertion created a *dinY*::*lacZ* fusion in which β-galactosidase expression required a DNA-damaging treatment (UV irradiation or mitomycin) and activable RecA protein. This strain showed a decreased Weigle reactivation of bacteriophage lambda. However, it was fully inducible for UV mutagenesis. Two-dimensional gel electrophoresis analysis identified two spots absent in the mutant which were both UV inducible only in the presence of activated RecA protein (RecA*). This finding suggests that the *dinY*::*lacZ* fusion lies in a gene either that is under the direct control of activated RecA or whose product undergoes RecA*-dependent posttranscriptional/posttranslational modification(s). The *dinY* gene may also control the expression of some other gene(s) and/or lie in an operon. The fusion was mapped at a position between 41 and 41.5 min on the *E. coli* chromosome, in the vicinity of the *ruv* operon.

DNA repair following the exposure of *Escherichia coli* to agents that damage the chromosome or interfere with its replication requires induction of genes which define the SOS regulon (for reviews, see references 30 and 36). Genetic analyses reported to date have been consistent with the notion that except for prophage induction, all genes of the SOS regulon are repressed by LexA and induced when LexA is inactivated by RecA protein (21). To carry out this regulatory role, RecA must itself be activated by an unknown modification catalyzed in vitro by single-stranded DNA and nucleotides. Activated RecA (RecA*), probably generated at a replication fork stalled at a region of damaged, single-stranded DNA, may interact directly with the replication complex to allow mutagenesis (23). In addition, RecA* allows cleavage of the LexA repressor, leading to the expression of the SOS functions which include error-free and error-prone repair systems (35).

Point mutations or insertions in the *lexA* gene lead to the constitutive expression of most known SOS genes. Even though these strains are somewhat more resistant to UV or mitomycin, they do not show high rates of spontaneous mutagenesis (2, 17, 26). The mutator phenotype in a *lexA*-(Def) background requires the *umuD* gene and RecA* for activation of the UmuD protein (2–4, 10, 28, 32, 37). In addition to its regulatory role, mediated through cleavage of the LexA repressor, evidence now suggests that RecA could play another role in mutagenesis (9, 28, 34). We have shown previously that mutagenic repair of bacteriophage lambda requires RecA* and de novo protein synthesis even in *lexA*(Def) strains (5, 6). The implication that a subset of RecA-dependent genes may not be under *lexA* control is consistent with our preliminary observations of proteins induced after UV irradiation of *lexA*(Def) strains (19). Here, we report the isolation and characterization of a new repair-defective mutant which falls into this class and analyze UV induction of the related proteins in *lexA*(Def) bacteria, using two-dimensional gels and automated methods of data analysis.

MATERIALS AND METHODS

Chemicals. Mitomycin was from Boehringer. Ampicillin, *o*-nitrophenyl-β-D-galactopyranoside, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were from Sigma. [^{35}S]methionine (about 1,000 Ci/mmol) was from Amersham. Urea, *N*,*N*,*N*′,*N*′-tetramethylenediamine, and sodium persulfate were from Bio-Rad. Acrylamide and bisacrylamide were from Fluka, and ampholines of pH 5 to 7 and pH 3.5 to 10 from LKB.

***E. coli* strains and growth conditions.** The strains used are listed in Table 1. Some of these strains were constructed by bacteriophage P1 transduction as described previously (6). Unless stated otherwise, bacteria were grown at 37°C in tryptone broth medium (25).

Isolation and characterization of mutants. Exponentially growing cultures of strain CP152 were infected with bacteriophage Mu dX(Ap *lac*) as described previously (25). Mu dX is a derivative of Mu *cts62* containing a temperature-sensitive mutation in the Mu repressor (1). An insertion in the Mu *B* gene prevents the expression of both *B* and *kil* genes, allowing growth of the fusion-containing strains at 37°C. In addition, this X mutation in the *B* gene reduces secondary transposition, since the *B* gene product is necessary for Mu replication. Lysis, however, is still observed when the strains are grown continuously at 37°C (16). To prevent this from occurring, fusion experiments were done in the *lexA*(Def) strain CP152, which carries a Mu c^+::*trp* insertion. Ampicillin-resistant transductants were plated on LB agar at 30°C and then further replica plated to the same

* Corresponding author.

TABLE 1. *E. coli* strains used

Strain	Relevant genotype	Reference or source
AB1157	F⁻ *thr-1 leuB6 proA2 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mrt-1 supE44 tsx-33 rpsL31*	5
CAG5050	F′ *pro lacZ8305*::Mu *cts62*/Mu dX Δ(*pro lac*) *his met tyr rpsL nalA*	1
DM1420	Same as AB1157 but *lexA51*(Def) *sfiA11*	26
GE152	Same as DM1420 but Δ*lacU169*	G. Weinstock
CP152	Same as GE152 but Mu *c*⁺::*trp*	This work
CP225	Same as CP152 but *dinY*::*lacZ*	This work
CA23	Same as CP225 but *recA430*	This work
CA88	Same as CP225 but Δ*recA*	This work
CA10	Same as CP225 but *lexA*(Ind⁻)	This work
PC1424	Same as DM1420 but *recA430*	5
PC1421	Same as DM1420 but Δ*recA*	5
CAG5054	λ⁻ *trp*::Tn*10 relA1 spoT1 thi-1*	M. Faelen
BW5660	λ⁻ Δ(*gpt lac*)*5 supE44 srlC300*::Tn*10 thi*	M. Faelen
HRS1004	Same as AB1157 but *ruvAB*::Tet^r	G. Maenhaut
LN2101	Same as AB1157 but *manX*::Tn*10 thy leu thi*	J. M. Louarn

medium containing X-Gal, in the presence or absence of mitomycin (0.3 μM). Colonies that were dark blue on the mitomycin-containing plates were tested for UV inducibility of β-galactosidase (5). These strains were then tested for SOS-induced DNA repair by Weigle reactivation (5) and reversion of a *his* auxotrophic mutation (25).

Labeling and two-dimensional gel electrophoresis of proteins. Cultures were grown at 37°C to a concentration of 2 × 10^8 cells ml^{-1} in M63 minimal medium lacking methionine (6). Five milliliters of culture was irradiated in minimal medium at 60 J/m^2. Aliquots (1 ml) of irradiated and nonirradiated cultures were then incubated again at 37°C with agitation for 10 min. They were then pulse-labeled for 5 min with 50 μCi of [^{35}S]methionine (specific activity, 1,000 Ci/mmol; 1 Ci = 37 GBq) and chased by the addition of 160 μl of 0.2 M cold methionine as described previously (19). Techniques for extraction of proteins and resolution on two-dimensional polyacrylamide gels (19) were modified from O'Farrell (29) and Hochstrasser et al. (13).

Five different labelings were prepared for each strain (control and UV irradiated). At least five sets of two-dimensional gels from each extract were run.

Quantitation of protein induction. Labeled proteins in two-dimensional polyacrylamide gels were recorded by exposure to films (Amersham hyperfilm Betamax) for 2, 5, and 7 days to ensure accurate quantitation and linearity between optical density and incorporated label. Autoradiograms were laser scanned and analyzed by using the PDQuest system for computer analysis of two-dimensional gel images (11).

Genetic mapping. Genetic mapping of the fusion was performed by using defined Hfr strains according to Miller (25). Generalized P1 transduction was also used as described previously (33). The amount of ampicillin-sensitive clones among the recombinants was measured.

RESULTS

Isolation of a damage-inducible fusion, independent of LexA repressor control. Phage Mu dX(Ap *lac*) was used to isolate stable mutations containing *lac* gene fusions in strain CP152 *lexA*(Def) (1). About 20,000 ampicillin-resistant mutants were isolated. Among these, about 600 produced blue colonies on X-Gal plates in the presence of mitomycin. Colonies were reisolated on the same medium, and only 10% that were strongly blue in the presence of mitomycin but only pale blue without treatment were then analyzed for UV induction of the fusion product, using a β-galactosidase assay. We chose to further characterize a strain (CP225) containing a mutation defining a gene which we call *dinY*, according to the nomenclature of Kenyon and Walker (15), since it was UV inducible. CP225 showed a 3.5-fold increase of β-galactosidase expression after a 75-J/m^2 irradiation (Fig. 1), which is consistent with the amount of induction described for damage-inducible genes with use of *lacZ* fusions (3, 15).

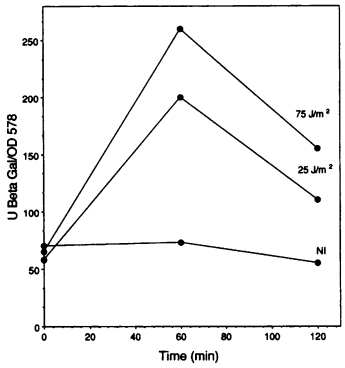

FIG. 1. β-Galactosidase (Beta Gal) induction after UV irradiation of CP225 (for conditions, see Materials and Methods). OD 578, optical density at 578 nm; NI, not irradiated.

Two-dimensional gel electrophoresis analysis of induced proteins in *lexA*(Def) bacteria. To determine whether the Mu d-*lac* fusion lead to disappearance of protein spots, proteins induced by UV irradiation in *lexA*(Def) strains were examined by two-dimensional electrophoresis and then subjected to computer analysis (PDQuest). About 1,800 proteins were detected on each gel. UV irradiation of the *lexA*(Def) strain DM1420 led to induction of many proteins, some of which required the presence of an activable RecA protein to be induced (19) (not shown).

Strain CP225, bearing the *dinY* fusion, lacked spots 1 and 2 (Fig. 2). These proteins had pIs of 6.2 and 5.9, respectively, and a molecular mass of around 27,000 Da. Protein 1 was induced 8-fold by UV irradiation of 60 J/m^2, while protein 2 increased 65-fold under the same conditions in the *lexA*(Def) strain. These data from PDQuest analysis differ from those obtained in β-galactosidase induction, possibly because of posttranslational modifications such as phosphorylation, which are likely different in the fusion protein and in the natural gene product. However, the full levels of expression of the two spots in UV-induced bacteria were similar as measured by PDQuest quantification. Both proteins belong to the most abundant proteins in irradiated bacteria (not shown). These two proteins, which were absent in CP225, were present but not inducible in the *lexA*(Def) *recA430* (not shown) and *lexA*(Def) Δ*recA* strains (Fig. 2). Thus, the *dinY*::*lacZ* insertion is responsible for the disappearance of two spots which are expressed (or undergo some posttranscriptional and/or posttranslational modifications) only in the presence of an activable RecA. These results show that although not repressed by LexA, the expression or posttranscriptional/posttranslational modification of the *dinY* fusion was dependent on activable RecA.

Regulation of the *dinY* fusion by the RecA and LexA

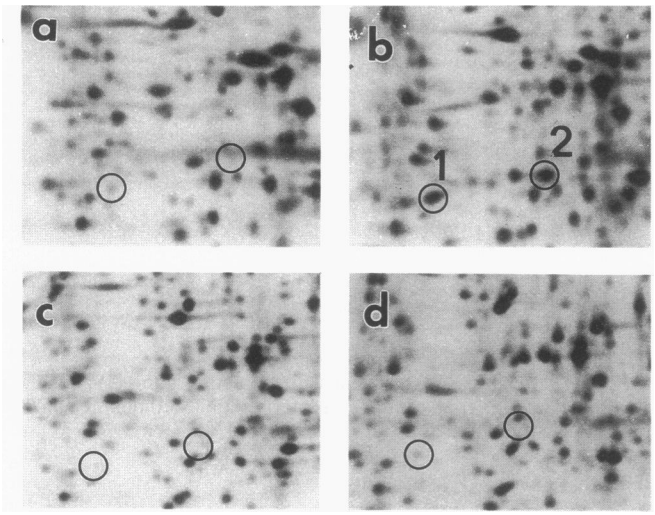

FIG. 2. Two-dimensional gel analysis of UV-induced proteins. Bacteria were irradiated at 60 J/m^2. (a) DM1420 [*lexA*(Def)] control; (b) UV-irradiated DM1420; (c) UV-irradiated CP225; (d) UV-irradiated PC1421 [*lexA*(Def) Δ*recA*]. Circles represent spots 1 and 2 that disappeared in *dinY*::Mu dX strains. Results are representative of the analysis of several sets of gels. The results for strains CP152 (DM1420 Mu *c*$^+$::*trp*) and PC1424 [*lexA*(Def) *recA430*] were identical to those obtained for strains DM1420 and PC1421, respectively (data not shown).

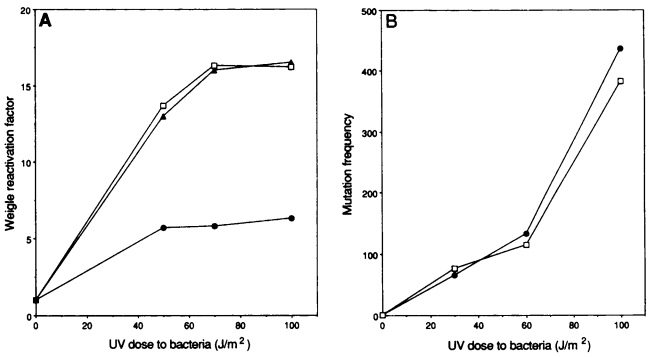

FIG. 3. (A) Weigle reactivation factor of lambda phage as a function of irradiation of strains DM1420 [*lexA*(Def); ▲), CP152 (DM1420 Mu *c*$^+$::*trp*; □), and CP225 (CP152 *dinY*; ●). Bacteriophage were irradiated at 400 J/m^2 before infection, leading to a survival of 10^{-3} phage ml^{-1}. (B) Mutation frequency (reversion of *his* to *his*$^+$) as a function of UV fluence in strains CP152 (□) and CP225 (●). Spontaneous mutation frequency was 0.1/10^8 bacteria ml^{-1}.

proteins. Table 2 shows that when RecA was mutated in its coprotease activity (*recA430*), UV-induced β-galactosidase expression from the *dinY*::*lacZ* fusion was almost totally suppressed. A complete deletion of the *recA* gene affected *dinY* expression to the same extent. A noncleavable LexA protein [*lexA*(Ind$^-$)] permitted a significant amount of induction, though less than in the *lexA*(Def) mutant, after identical inducing treatment. A *recA730* mutation giving a constitutive RecA* phenotype did not lead to a constitutive induction of *dinY*; the basal level of β-galactosidase was around 50 Miller units, not different from the levels obtained for other strains. Furthermore, in this strain, *dinY* was not inducible.

Role of the *dinY* fusion in DNA repair. The capacity to repair UV-irradiated bacteriophage lambda was compared in CP152 and its *dinY*-defective derivative (CP225). Irradiation of CP152 with increasing UV fluences allowed a 15-fold enhancement of bacteriophage survival (Weigle reactivation) as previously observed (5). However, CP225 presented only a fivefold increase in Weigle reactivation (Fig. 3). Nevertheless, the two strains when not irradiated were equally efficient with regard to bacteriophage survival, indicating that CP225 was deficient in a gene which is expressed, or whose product undergoes posttranscriptional and/or posttranslational modifications, only when *lexA*(Def) strains are damaged, allowing activation of RecA. In addition, in a *uvrC* background, there was no difference in Weigle reactivation between CP225 and its parent strain CP152; in this case, Weigle reactivation factors represented only a twofold increase (data not shown). This result confirms that *dinY* is involved in DNA repair.

Bacterial mutagenesis was measured in both strains. UV mutagenesis results for the two strains were identical, as shown in Fig. 3. Weigle mutagenesis was also identical in CP152 and CP225 (data not shown). Thus, the protein(s) absent in CP225 does not seem to be part of the error-prone replicative complex.

Genetic mapping. The *dinY* fusion was mapped using several Hfr strains. As shown in Table 3, Hfr CAG5054, which introduces DNA clockwise from 45 min, gave rise to 98% recombinants which had lost ampicillin resistance and acquired tetracycline resistance. Hfr BW5659, introducing DNA in the same direction from 51 min, gave 25% recombinants. However, Hfr BW5660, which introduces DNA counterclockwise from 42.5 min, did not give any recombinant. This result indicates that the *dinY* fusion is located between 42.5 and 38 min on the *E. coli* chromosome.

The map position was located more precisely by generalized P1 transduction. Table 4 shows that the *ruvAB* operon was 65% cotransduced with *dinY*, while the *manX* gene gave only 5% cotransduction with the fusion. It can therefore be

TABLE 2. UV induction of β-galactosidase in various strains containing a *dinY* fusion

Strain (genotype)	UV dose (J/m^2) to bacteria	Factor of induction at time (min)[a]:				
		0	30	60	90	120
CA88 [*lexA*(Def) *dinY* Δ*recA*]	3	1	1	1	1.2	1.3
	5	1	1	1.2	1.2	1.3
	10	1	1	1.3	1.3	1.5
CA23 [*lexA*(Def) *dinY recA430*]	25	1	ND	1.3	ND	1.3
	75	1	ND	1.4	ND	1.4
CA10 [*dinY lexA*(Ind$^-$)]	15	1	ND	1.8	2.1	2.1
CP225 (*dinY*)	15	1	ND	3	2.7	2.4

[a] Expressed with respect to the value for nonirradiated controls. Results are means of at least three experiments. ND, not determined. β-galactosidase was measured as a control in CP225 irradiated at 15 J/m^2.

TABLE 3. Hfr recombination

Cross	Point of origin (min)	Tn*10* position (min)	No. of recombinants		
			Tet^r Sm^r	Ap^s	% Ap^s
CAG5054 × CP225	45	27	145	143	98.6
BW5660 × CP225	42.5	58	115	0	0
BW 5659 × CP225	51	37	126	31	24.6

TABLE 4. Location of the *dinY* fusion by phage P1 transduction

Strain	Transduced gene	Gene position (min)	Number of recombinants[a]		
			Tet^r	Ap^s	% Cotransduction
P1.HRS1004	*ruvAB*::Tet^r	41	27	18	65
P1.LN2001	*manX*::Tn*10*	40.2	42	2	5

[a] Results are means of three experiments.

concluded that the *dinY* fusion is located between 41 and 41.5 min on the *E. coli* chromosome.

DISCUSSION

Until now, it was thought that except for prophage induction, which involves a different kind of repressor, all genes of the SOS regulon were regulated by the *recA*/*lexA* control circuit (35). The SOS regulon was thought to include more than 17 genes (26). However, it is generally accepted that RecA has at least another role in SOS repair aside from catalyzing LexA or UmuD cleavage (6, 9, 34). The *dinY* fusion that we isolated was inducible after irradiation of *lexA*(Def) bacteria carrying either a point mutation or a Tn*5* insertion (not shown). The increased amount of β-galactosidase was also confirmed by the increased label of the two spots observed by two-dimensional electrophoresis analysis. PDQuest analysis of the electrophoresis supported the fact that a great number of protein spots can be induced by UV in a strain which is defective for LexA (not shown).

The *dinY*::*lacZ* insertion eliminated two spots found in the parental strain. Since these spots have the same molecular weight, they may be the product of the same gene at different stages of posttranslational processing, for example, phosphorylation. Also, the fusion may lie in a operon; one of the spots may represent the *dinY* product, and the other may represent a *dinY*-controlled protein of the same molecular weight. Finally, the disappearance of the spots may be due to a control by *dinY* of some posttranscriptional and/or posttranslational events.

It can be speculated that some of the induced proteins are associated with prophages, since these are generally not under the LexA repressor control (31). It is thus conceivable that RecA inactivates not only LexA but also another repressor(s).

In a *lexA*-noninducible strain [*lexA*(Ind$^-$)], *dinY* was still UV induced but to lower extent than in the *lexA*(Def) strain. In addition, in a *lexA*$^+$ background, *dinY* was inducible to the same extent as in the *lexA*(Def) strain, and the basal level of expression was not lower than that observed when the fusion was in *lexA*(Def) bacteria (data not shown). The residual induction of *dinY* observed in the *lexA*(Ind$^-$) strain is comparable to prophage induction (7, 31). The limited amount of activable RecA protein would be responsible for it, since in such a strain LexA wild-type protein can be cleaved (20). Another explanation for the residual *dinY* induction in a *lexA*(Ind$^-$) strain could be that some *lexA*-dependent gene product(s) is required for activation of the *dinY* transcription.

The *recA* dependence of *dinY* induction was analyzed in classical *recA* mutants. In the *recA430* and Δ*recA* backgrounds, only some residual inducibility of *dinY* seemed to be detectable by β-galactosidase activity measurement, but it was not measurable in two-dimensional electrophoresis by computer analysis. A *recA730* mutation which brings a constitutive activated configuration to RecA protein behaved like the *recA430* mutation and was unable to induce the *dinY*::*lacZ* fusion. This finding suggests that the induction of the *dinY* fusion requires an active conformational change of RecA protein which is absent in mutants carrying point mutations in *recA*; in these cases, RecA protein is already modified by the mutations. The fact that *recA730* did not reproduce all of the features of an activable RecA protein for some SOS genes has been documented by Maenhaut-Michel and Caillet-Fauquet (24). Our result suggests that *dinY* belongs to a family of genes that are not induced in a *recA730* background comparable to those involved in the untargeted mutagenesis of phage λ (24).

Some proteins of the heat shock regulon belong to a class called chaperonins, which are known to interact with other multisubunit proteins (12). Among these are the heat shock GroEL and GroES proteins, which are necessary for mutagenesis probably by stabilizing the UmuDC complex (8, 22). Even though *dinY* is not involved in mutagenesis, it could be a member of the chaperonin family. However, it was inducible neither by heat shock nor by 4% ethanol, as measured both by the β-galactosidase assay and by two-dimensional electrophoresis analysis (data not shown).

UV doses of 60 to 75 J/m^2 were classically used to measure SOS induction (14, 15, 27), but it has been demonstrated that these UV fluences are able to induce heat shock proteins in a *lexA*(Def) background (18). However, the *dinY* fusion was already significantly expressed at 15 J/m^2.

Although *dinY* induction does not require the cleavage of the LexA repressor, it may be considered a bona fide member of the SOS regulon, since its induction requires DNA damage and activable RecA protein, being thus somehow LexA dependent and behaving like a prophage. Although *dinY* is involved in Weigle reactivation of bacteriophage lambda, the Mu d-*lac* insertion had no effect on mutagenesis. However, when the excision pathway was blocked, there was no difference between *dinY* and its parent, indicating that *dinY* belongs to an error-free repair pathway.

The *dinY*::*lacZ* insertion is unique in the genome of CP225, as confirmed by Southern blotting of restricted genomic DNA and hybridization with a *lacZ* probe (not shown). The fusion is located between 41 and 41.5 min on the chromosomal map. Around this region, the only known repair gene is the *ruvAB* operon, which is under *lexA* control. The results of P1 cotransduction shows that *dinY* is different from *ruvAB*. The *dinY* fusion therefore determines a new locus involved in DNA repair. Further characterization of the locus will require cloning of the gene(s) implicated in this fusion, which is currently being done.

It can be concluded that *E. coli* has developed a complex system under RecA protein control in addition to the known *rec*/*lex* response to deal with bulky lesions to DNA. This system appears to comprise several additional genes, one of which, *dinY*, has been identified by Mu d-*lac* insertion.

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