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Genetics

DNA-damage-inducible (*din*) loci are transcriptionally activated in competent *Bacillus subtilis*

(*din/cin* locus/operon fusion/SOB system/*recE*/competence)

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ABSTRACT **DNA damage-inducible (*din*) operon fusions were generated in *Bacillus subtilis* by transpositional mutagenesis. These YB886(*din*::Tn*917-lacZ*) fusion isolates produced increased β-galactosidase when exposed to mitomycin C, UV radiation, or ethyl methanesulfonate, indicating that the *lacZ* structural gene had inserted into host transcriptional units that are induced by a variety of DNA-damaging agents. One of the fusion strains was DNA-repair deficient and phenotypically resembled a UV-sensitive mutant of *B. subtilis*. Induction of β-galactosidase also occurred in the competent subpopulation of each of the *din* fusion strains, independent of exposure to DNA-damaging agents. Both the DNA-damage-inducible and competence-inducible components of β-galactosidase expression were abolished by the *recE4* mutation, which inhibits SOS-like (SOB) induction but does not interfere with the development of the competent state. The results indicate that gene expression is stimulated at specific loci within the *B. subtilis* chromosome both by DNA-damaging agents and by the development of competence and that this response is under the control of the SOB regulatory system. Furthermore, they demonstrate that at the molecular level SOB induction and the development of competence are interrelated cellular events.**

The study of DNA repair in *Bacillus subtilis* has led to the identification of an inducible response, the SOB system (1, 2), which appears similar in several respects to the more extensively characterized SOS response of *Escherichia coli*. The SOB response consists of a set of coordinately induced functions such as enhanced capacity for DNA repair and mutagenesis of bacterial genes, Weigle reactivation of UV-damaged bacteriophage, filamentation, and prophage induction. As in *E. coli*, the expression of these functions is elicited by DNA damage or inhibition of DNA replication and is under the control of several genes that are involved in DNA repair, recombination, or both (1, 2).

Nevertheless, there are significant differences that distinguish the inducible DNA repair system of *B. subtilis* from that of *E. coli*. These include the specific types of DNA damage that are repaired (3), the regulation of the inducible response (2), and, in the case of *B. subtilis*, the apparent relationship between SOB induction and the development of the competent state (the physiological state in which the cells are capable of binding and taking up exogenous DNA) (1, 2). This information has been obtained primarily through genetic analyses aimed at determining the effects of DNA repair or recombination-deficient mutations on the expression of SOB phenomena (1, 2, 4).

The recent construction of Tn*917*-mediated operon fusion elements by Youngman *et al.* (5, 6), has enabled the study of gene expression at specific loci in *B. subtilis*. One such vector, Tn*917-lacZ* (6), is capable of generating chromosomal insertions that place the *lacZ* structural gene under the transcriptional control of contiguous operons. Utilizing a similar insertion element to generate random gene fusions, Kenyon and Walker (7) were able to identify a set of DNA-damage-inducible (*din*) operons in *E. coli*. Following the same methodology, we employed the Tn*917-lacZ* vector in an attempt to determine whether *din* loci could be identified in *B. subtilis*.

In this report, we describe the isolation and initial characterization of *din* operon fusions in *B. subtilis*. The phenotypic properties of these isolates suggest that *din* gene expression is regulated by the *B. subtilis* SOB system and provide definitive evidence for a relationship between SOB induction and the development of competence in this microorganism.

MATERIALS AND METHODS

Strains

B. subtilis strain YB886 (*metB5, trpC2, xin-1*, SPβ⁻), a naturally competent derivative of *B. subtilis* 168, was used as the host for transpositional mutagenesis (8). Strain PY258(pTV32) was obtained from P. Youngman. Plasmid pTV32 carries the Tn*917-lacZ* insertion element, which codes for resistance to macrolide, lincosamide, and streptogramin B antibiotics (6).

Media

Luria–Bertani (LB) medium (5) was used to propagate cultures of YB886(pTV32) for the generation of operon fusions. Screening for putative *din*::Tn*917-lacZ* insertions was done on nutrient broth agar (NBA) medium plus erythromycin at 1 μg/ml, lincomycin at 25 μg/ml, and 4-methylumbelliferyl β-D-galactoside (Sigma), a β-galactosidase indicator that generates a fluorescent hydrolysis product at 20 μg/ml (6). NBA medium consists of 25 g of Oxoid nutrient broth no. 2 and 15 g of Oxoid purified agar per liter (Oxoid, Hants, England). Competence media (GM1 and GM2) and minimal medium were prepared as described previously (9). β-Galactosidase assays were performed on cultures of fusion strains grown in supplemented GM1 plus erythromycin and lincomycin at the concentrations given above. The β-galactosidase assay protocol was essentially as described by Miller (10).

Experimental Procedures

Mechanisms of Genetic Exchange. Plasmid pTV32 DNA was purified by cesium chloride gradient centrifugation using

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Abbreviations: SOB system, SOS-like system of *Bacillus subtilis*; Met⁺, methionine prototrophy; MC, mitomycin C.

the method of Gryczan *et al.* (11). YB886(*din*::Tn*917-lacZ*), *recE4* strains were constructed by congression (12) with chromosomal DNA isolated from strain YB1259 (*metB5*, *recE4*, *xin-1*, SPβ^-) (2). The procedure of Yasbin *et al.* (8) was used to maximize competence in cultures of fusion strains. Competent subpopulations of *B. subtilis* cultures were separated from noncompetent cells in Renografin block gradients (Renografin-60, Squibb methylglucamine diatrizoate) by the procedure of Hadden and Nester (13). Fusion strains were transformed to methionine prototrophy (Met$^+$) with DNA isolated from strain YB1011 (*xin-1*, SPβ^-) (2) or to resistance to chloramphenicol with plasmid pMK4 DNA (14) at 5 μg/ml. After a 30-min incubation, cultures were treated with DNase I (100 μg/ml) for 15 min. β-Galactosidase activity was measured in cells isolated from Renografin gradient bands after they had been pelleted and resuspended in GM1. Numbers of viable cells in each gradient fraction were determined on NBA medium. Transformation frequency was determined on minimal medium supplemented with tryptophan (for Met$^+$ transformants) or NBA medium plus chloramphenicol at 5 μg/ml (for pMK4 transformants). UV irradiation of bacteria and bacteriophage was performed as described previously (1).

Isolation of *din*::Tn*917-lacZ* Fusions. The protocol for generating Tn*917-lacZ* chromosomal insertions and curing fusion strains of plasmid pTV32, described in detail by Youngman *et al.* (5), was duplicated without change in these experiments except in proportion (a 10-liter fermentation rather than a 100-liter one). Aliquots (4 ml) of strain YB886 containing random insertions of Tn*917-lacZ* were stored at −70°C in 10% (vol/vol) sterile glycerol and thawed as needed. Screening for putative *din* fusions was essentially as described by Kenyon and Walker (7) and is detailed below.

There were several considerations that prefaced the large-scale screening experiment. Prior experience had indicated that certain types of media routinely used for maintaining *B. subtilis* cultures would stimulate the induction of the SOB system (2). For this reason, NBA medium, which contains highly purified agar, was employed in these experiments. Second, it was necessary to determine whether the β-galactosidase indicator (4-methylumbelliferyl β-D-galactoside) would itself cause the induction of the SOB system. Accordingly, colonies of YB886 lysogenic for bacteriophage ϕ105 (1) were transferred to NBA plates with or without the indicator that had been overlayed with nonlysogenic (ϕ105-sensitive) bacteria. Since plaque sizes on the two plates were similar, we concluded that 4-methylumbelliferyl β-D-galactoside does not induce SOB phenomena. An identical approach was employed to determine the appropriate concentration of mitomycin C (MC) (25 ng/ml) to use in the screening experiments (i.e., one that would result in the induction of prophage, indicated by larger zones of lysis, but only minimally affect the viability of YB886). A final concern was whether 4-methylumbelliferyl β-D-galactoside could be used as an effective indicator of *din* gene expression in *B. subtilis*. This substrate is conventionally applied to fully grown colonies; detection of β-galactosidase expression by this method might be extremely difficult if it occurred very early in cell growth and was temporally related or if the turnover rate of β-galactosidase were rapid. For these reasons, the indicator was incorporated into the growth medium so that β-galactosidase expression occurring during any stage of growth would be detected. Diffusion of the fluorescent hydrolysis product was effectively minimized by incubating the indicator plates at 32°C and by prompt screening of colonies after incubation.

The screening procedure consisted of plating aliquots of YB886 containing random insertions of Tn*917-lacZ* onto NBA medium containing erythromycin and lincomycin at a cell density of about 100 colonies per plate. After overnight incubation the colonies were replica plated onto otherwise identical medium containing 4-methylumbelliferyl β-D-galactoside indicator with or without MC (25 ng/ml). Plates were incubated for 12–24 hr at 32°C and then examined in the dark for fluorescence excited by a long-wave UV light source. Colonies that appeared to synthesize increased levels of β-galactosidase in the presence of MC were picked and tested quantitatively for β-galactosidase expression in liquid medium with or without MC (50 ng/ml). Of approximately 36,000 colonies screened in this manner, 114 putative *din* fusion colonies were isolated, and 15 of these were confirmed by β-galactosidase assay (see Fig. 1 for assays of representative isolates).

RESULTS

Response of Fusion Strains to DNA-Damaging Agents

Liquid cultures of each of the 15 YB886(*din*::Tn*917-lacZ*) fusion strains were challenged with MC during mid-exponential growth and then assayed at various times to determine the kinetics of β-galactosidase induction. Representative graphs are shown in Fig. 1 *B–F*. The response of *din* fusion strains to MC was dose dependent, with higher concentrations of MC stimulating increased synthesis of β-galactosidase. Furthermore, MC induction of β-galactosidase was essentially linear with respect to time. Variations in both the constitutive

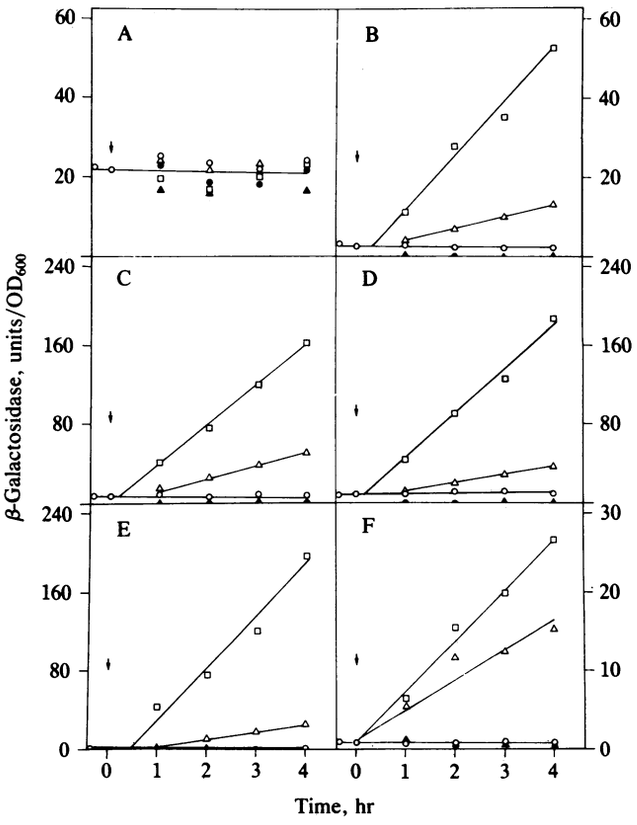

FIG. 1. Induction of β-galactosidase in representative YB886-(*din*::Tn*917-lacZ*) fusion strains after exposure to MC. Plots shown are indicative of the results of three separate experiments. During exponential growth in liquid culture, fusion strains were challenged (at time indicated by arrow) with MC at either 50 (△, ▲) or 500 (□) ng/ml. ○ and ●, untreated cultures. At 1-hr intervals, 1-ml aliquots of each culture were assayed for β-galactosidase activity (10). Open symbols, *recE*$^+$ strains; closed symbols, *recE4* strains. (*A*) YB886::Tn*917-lacZ*(C+) (see text); (*B*) *din-17*; (*C*) *din-18*; (*D*) *din-22*; (*E*) *din-23*; (*F*) *din-76*.

and induced levels of β-galactosidase production (together with MC sensitivity in the case of *din-76*) suggested that several of the *din* isolates might be genetically distinct.

The differences in induction kinetics between certain isolates were even more apparent when the response of fusion strains to UV radiation was determined (Fig. 2). In this case, *din* strains differed from each other not only in the magnitude of β-galactosidase induction but also in the kinetics of induction. For example, *din-18* reached its highest level of β-galactosidase expression 2–3 hr after irradiation, whereas *din-22* was maximally activated after only 1 hr (Fig. 2 *C* and *D*). Interestingly, the kinetic response of *din-76* (the MC-sensitive isolate) to UV radiation was linear (Fig. 2*F*) and resembled the kinetics of MC induction (Fig. 1*F*).

Preliminary linkage analyses suggest that the *din* strains do not contain multiple insertions of the Tn*917-lacZ* element since the Din phenotype and resistance to macrolide, lincosamide, and streptogramin B (and sensitivity to MC in the case of *din-76*) cotransformed with a frequency of 1. Further support for this assertion has been obtained from Southern hybridization experiments, in which singular hybridization bands were observed when *Eco*RI-digested chromosomal DNA from each of the *din* strains was probed with labeled pTV32 (data not shown).

It was conceivable that DNA-damaging agents might directly affect the synthesis of β-galactosidase in some manner that was independent of the chromosomal location of the Tn*917-lacZ* element. To determine whether this could be occurring, several colonies were selected at random from the original test plates and assayed for β-galactosidase expression. None of these strains, including the parental strain YB886(pTV32), exhibited a discernable increase in β-galactosidase production in response to treatment with DNA-damaging agents. The results obtained with one of these control strains are shown in Figs. 1*A* and 2*A*. We have designated this strain YB886::Tn*917-lacZ*(C+) since it apparently contains a Tn*917-lacZ* insertion in an operon that is active constitutively.

MC and UV radiation were potent inducers of *din* gene expression, whereas ethyl methanesulfonate only weakly stimulated the production of β-galactosidase when added to growing cultures (Figs. 1 and 2). The relative ineffectiveness of ethyl methanesulfonate as a *din* gene inducing agent is apparently due to the nature of the DNA lesion produced by this chemical rather than its lethal effects, since concentrations of ethyl methanesulfonate ranging from 0.2% to 2% gave the same low levels of β-galactosidase expression (data not shown).

To determine whether *din* gene expression was under the control of the *B. subtilis* SOB system, the *recE4* mutation was introduced into each of the five representative *din* fusion strains by congression. The *recE4* mutation abolishes homologous recombination and the expression of all but one of the SOB phenomena (filamentation) (2). The induced expression of β-galactosidase was completely inhibited in each of the *din, recE4* strains examined (Figs. 1 and 2). Constitutive β-galactosidase production was also markedly reduced in every strain save *din-76, recE4*. The effect of the *recE4* mutation on *din* gene expression was regulatory rather than direct, since synthesis of β-galactosidase in the control strain was not altered significantly by this mutation (Fig. 1*A*).

The *din, recE4* strains grew slower than corresponding *din, rec*$^+$ strains in the presence of DNA-damaging agents, and some lysis was observed in cultures exposed to high concentrations of MC (500 ng/ml) after prolonged incubation. Nevertheless, the sensitivity of *recE4* strains to DNA-damaging agents could not account for the suppressed β-galactosidase production since (*i*) the control strain continued to synthesize β-galactosidase when exposed to UV and MC (Fig. 1*A*), (*ii*) lower doses of DNA-damaging agents also failed to induce β-galactosidase expression, and (*iii*) protein synthesis (measured by uptake and incorporation of ^{3}H-labeled amino acids) continued at a fixed ratio for up to 4 hr after MC treatment in corresponding *din, recE4* and *din, rec*$^+$ strains (unpublished data).

Induction of β-Galactosidase in Competent Subpopulations of *din* Fusion Strains

It has been known for some time that certain SOB phenomena, specifically prophage induction and enhanced mutagenesis of bacterial genes, are expressed in *B. subtilis* cells as they become competent (2, 15). Recent work has suggested that SOB induction and competence may be associated

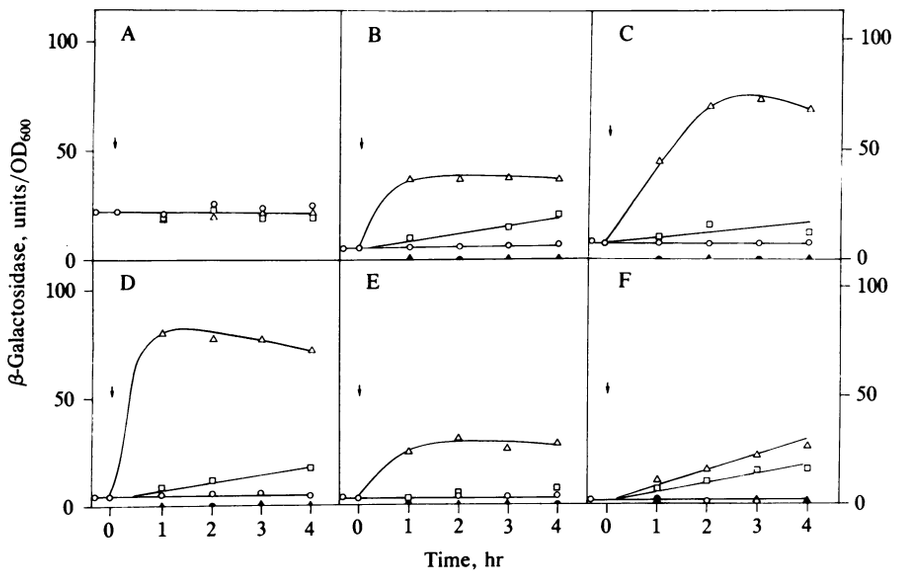

FIG. 2. Induction of β-galactosidase in representative *din* fusion strains after exposure to UV radiation or ethyl methanesulfonate. The experimental procedure was similar to that described for Fig. 1. At the time indicated by the arrows the cultures were exposed to 2% (vol/vol) ethyl methanesulfonate (□) or UV radiation (50 J/m^2) (△, ▲). ○ and ●, untreated cultures. Open symbols, *recE*$^+$ strains; closed symbols, *recE4* strains. (*A*) YB886::Tn*917-lacZ*(C+); (*B*) *din-17*; (*C*) *din-18*; (*D*) *din-22*; (*E*) *din-23*; (*F*) *din-76*.

cellular events, perhaps sharing common elements in their regulatory pathways (2). The availability of *din* gene fusions provided an opportunity to test this hypothesis at the molecular level by determining whether gene expression is enhanced in these isolates during the development of competence.

Initially, β-galactosidase activity was assayed at various times in *din* fusion strains as they were grown to competence. The results (data not shown) indicated that β-galactosidase expression increased as the strains reached maximal competence. However, since only about 1–20% of a *B. subtilis* population becomes competent, even with optimal growth conditions (16), β-galactosidase expression in this subpopulation was masked by the preponderance of noncompetent cells in the culture. To circumvent this problem, competent cells were separated from the noncompetent majority in Renografin density gradients (13). The results of these experiments are shown in Table 1. The data obtained with each of the 5 representative strains exemplified the range observed for all 15 *din* fusion isolates. In every case, cells from the top band (T) of the gradient (predominantly competent) produced significantly greater amounts of β-galactosidase than those isolated from the bottom band (B, predominantly noncompetent). As expected, the β-galactosidase activity was intermediate in the unfractionated cells. With the exception of *din-23*, which exhibited a consistently lowered transformation frequency (Table 1), none of the *din*::Tn*917-lacZ* insertions appeared to interfere markedly with competence, transformation efficiency, or homologous recombination, and, in every strain except the control, β-galactosidase expression paralleled transformation frequency. Interestingly, the relative magnitudes (or efficiencies) of β-galactosidase induction in the *din* fusion strains were similar for competent and MC-treated cultures (see Fig. 1 and Table 1), suggesting that gene expression at these loci may be controlled by a single regulatory mechanism.

Induction of *din* gene expression in competent bacteria could conceivably be due either to the activation of the SOB system or through some independent mechanism. To determine how *cin* (competence-*in*ducible) gene expression is regulated, we assayed β-galactosidase activity in competent cultures of each of the five *din, recE4* strains. Remarkably, the competence-associated induction of β-galactosidase was abolished in the presence of the *recE4* mutation even though the capacity of these strains to undergo plasmid DNA-mediated transformation indicated that they were in fact competent (Table 2).

Table 1. Induction of β-galactosidase in competent subpopulations of *din*::Tn*917-lacZ* fusion strains

Fusion strain	Fraction	Met$^+$, cfu/ml	Transformation frequency	β-Galactosidase, units/OD_{600}
din-17	U	4.8×10^5	9.2×10^{-3}	7.0
	T	7.8×10^5	3.1×10^{-2}	15.0
	B	2.8×10^3	4.5×10^{-5}	4.5
din-18	U	6.8×10^5	1.1×10^{-2}	51.0
	T	3.1×10^6	7.9×10^{-2}	90.0
	B	8.9×10^3	1.6×10^{-4}	5.5
din-22	U	4.1×10^5	1.3×10^{-2}	46.0
	T	8.4×10^5	4.2×10^{-2}	101.0
	B	3.2×10^3	5.0×10^{-5}	7.0
din-23	U	4.0×10^3	4.0×10^{-5}	9.0
	T	1.0×10^4	1.5×10^{-3}	49.0
	B	1.0×10^2	5.0×10^{-6}	2.3
din-76	U	2.9×10^5	8.5×10^{-4}	0.7
	T	1.9×10^5	1.9×10^{-2}	5.0
	B	1.7×10^3	1.5×10^{-5}	0.1
C+	U	9.3×10^5	2.9×10^{-3}	26.0
	T	6.0×10^5	2.4×10^{-2}	23.5
	B	1.0×10^2	2.2×10^{-6}	20.0

Fusion strains were grown to competence, transformed with *met*$^+$ DNA, and fractionated in Renografin density gradients. U, unfractionated cells; T, top band of Renografin gradient, containing predominantly competent cells; B, bottom band of Renografin gradient, containing predominantly noncompetent cells. Transformation frequency is the number of colony-forming units (cfu) of Met$^+$ transformants divided by the total number of viable cells. The results shown are representative of three separate experiments.

Table 2. Induction of β-galactosidase in competent subpopulations of *din*::Tn*917-lacZ*, *recE4* fusion strains

Fusion strain (*recE4*)	Cm^r, cfu/ml	pMK4 transformation frequency	β-Galactosidase, units/OD_{600}		
			U	T	B
din-17	3.7×10^4	2.5×10^{-4}	<0.2	<0.2	<0.2
din-18	6.8×10^4	5.1×10^{-4}	<0.2	<0.2	<0.2
din-22	6.4×10^4	1.2×10^{-3}	<0.2	0.3	<0.2
din-23	2.4×10^4	6.3×10^{-4}	<0.2	<0.2	<0.2
din-76	2.6×10^4	2.6×10^{-4}	0.9	1.0	0.7
C+	3.1×10^4	6.1×10^{-4}	17.0	25.0	10.0

Experimental procedures were as for Table 1. Colony-forming units (cfu) of chloramphenicol-resistant (Cm^r) (5 μg/ml) cells and transformation frequencies were determined for unfractionated cells only. Plasmid pMK4 transformants are provided as an indication of competence. The number of Met$^+$ transformants in each of the *din, recE4* strains was $<10^2$/ml. U, unfractionated cells; T, top band of Renografin gradient; B, bottom band of Renografin gradient.

The possibility of chromosomal rearrangements during the construction of the *din, recE4* strains raised a concern about whether these *din/cin* fusions were still capable of expressing β-galactosidase. Therefore, DNA was extracted from each of the five *din, recE4* strains and used to transform strain YB886 to erythromycin and lincomycin resistance at limiting concentrations. All of the resulting recombination-proficient transformants were inducible for β-galactosidase and exhibited the same kinetic response as the corresponding parental *din, rec*$^+$ fusion isolates (unpublished data).

Isolation of a DNA-Repair-Deficient *din* Fusion Strain

Preliminary investigation of *din-76* revealed that this mutant was sensitive to UV radiation and was impaired in its ability to reactivate UV-damaged bacteriophage (Fig. 3). These data, in conjunction with further investigation of *din-76* (unpublished data), suggest that this strain is deficient in excision repair capacity and that it probably contains a gene

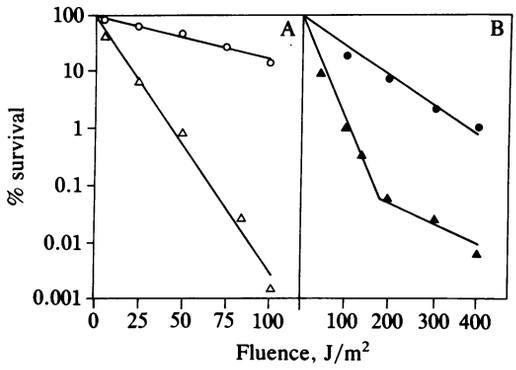

FIG. 3. DNA repair capacity of *din-76*. (*A*) Survival of strains YB886::Tn*917-lacZ*(C+) (○), and YB886(*din-76*::Tn*917-lacZ*) (△) after exposure to UV. (*B*) Survival of UV-irradiated bacteriophage φ105 on indicator fusion strains (C+) (●) and *din-76* (▲).

fusion that has interrupted one of the *uvr*$^+$ loci in *B. subtilis*. In addition, the *din-76* operon fusion is both DNA damage inducible and competence inducible, and its expression is regulated by the *recE* gene product (Figs. 1 and 2 and Tables 1 and 2). Interestingly, constitutive β-galactosidase activity was not suppressed measurably by the *recE4* mutation in this isolate, suggesting that the *din-76* operon contains both constitutive and inducible promoter regions (Fig. 1*F* and Tables 1 and 2).

CONCLUSION

Fifteen *B. subtilis* YB886 (*din*::Tn*917-lacZ*) gene fusion isolates were obtained that produced increased β-galactosidase in response to a variety of DNA-damaging agents. On the basis of differences in the kinetics of this induction response and their sensitivity to DNA-damaging agents, these isolates appear to fall into several phenotypically distinct groups. To characterize the *din* fusions more fully, it will be necessary to determine the location of each of the *din* genes on the *B. subtilis* chromosome.

Clearly, the most interesting properties of these isolates are that *din* gene expression is stimulated during competence and is abolished in a *recE4* host. From these observations it appears that (*i*) the RecE protein of *B. subtilis*, like RecA in *E. coli*, plays a primary role in the regulation of the SOS-like response; and (*ii*) although not triggered by the same initial event, both *din*- and *cin*-associated enhancement of gene expression are controlled by the SOB regulatory system. These conclusions are reinforced by the fact that one of the isolates (*din-76*) contained a Tn*917-lacZ* fusion in a locus that is directly involved in DNA repair.

With the exception of *din-76* (see Fig. 3), none of the *din* gene fusions markedly effect the organisms' ability to carry out DNA repair, homologous recombination, or the development or maintenance of competence. This does not preclude the possibility that one or more of the *din* genes may be involved in the aforementioned processes, since they could conceivably contain transposon insertions that do not abolish the expression or function of their gene products. This may, in fact, be the case for *din-23* (see Table 1), which was consistently reduced in chromosomal transformation efficiency.

The SOB response of *B. subtilis* has been shown to parallel the SOS response of *E. coli* in many respects (1, 2, 15). Evidently, the similarities between these two systems extend to the molecular level, since both organisms contain a set of specific loci that become transcriptionally activated by a "Rec"-dependent mechanism after treatment with DNA-damaging agents. Our results are consistent with those obtained by Lovett and Roberts (17), which demonstrated that *E. coli* RecA protein and a Rec protein in *B. subtilis* (presumably the *recE*$^+$ gene product) perform similar functions.

The *B. subtilis* model system offers an opportunity to investigate the relationship between complex cellular processes such as competence, sporulation, and the SOB response. These experiments have demonstrated that the inducible expression of *din* loci occurs as a consequence of, but not a prerequisite to, the development or maintenance of the competent state (see *Results*).

Competence-inducible genes have been described in a number of organisms (18–22), but to date, no correlation between competence and an SOS-like response has been established in any genus other than *Bacillus*. It is conceivable that the induction of the SOB system in competent cells is merely a consequential event, resulting from the generation of inducing signal(s) prior to or during homologous recombination. Another hypothesis is that the enhanced capacity for DNA repair (and recombination) that results from the induction and expression of the SOB system serves a functional and protective role during competence—a period of relative genetic vulnerability for the cell. Indeed, it appears that SOS-like systems exhibit a spectrum of inducible phenomena that are involved in maintaining cellular integrity in response to a variety of stress conditions. For example, while the SOS response in *E. coli* is activated by DNA damage or the inhibition of DNA replication, this system also plays some role in the organisms' capacity to deal with temperature variations (23) and parasitic infection (24).

The data presented here indicate that the inducible response may play a role in handling stress conditions resulting from cellular differentiation events such as the development of competence. Further characterization of the *din/cin* fusions described in this report should aid in the elucidation of the regulatory mechanism controlling the SOB response and in the determination of its role in cellular differentiation.

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