DNA Repair and the Evolution of Transformation in *Bacillus subtilis*. II. Role of Inducible Repair

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ABSTRACT

In *Bacillus subtilis*, DNA repair and recombination are intimately associated with competence, the physiological state in which the bacterium can bind, take up and recombine exogenous DNA. Previously, we have shown that the homologous DNA transformation rate (ratio of transformants to total cells) increases with increasing UV dosage if cells are transformed after exposure to UV radiation (UV-DNA), whereas the transformation rate decreases if cells are transformed before exposure to UV (DNA-UV). In this report, by using different DNA repair-deficient mutants, we show that the greater increase in transformation rate in UV-DNA experiments than in DNA-UV experiments does not depend upon excision repair or inducible SOS-like repair, although certain quantitative aspects of the response do depend upon these repair systems. We also show that there is no increase in the transformation rate in a UV-DNA experiment when repair and recombination proficient cells are transformed with nonhomologous plasmid DNA, although the results in a DNA-UV experiment are essentially unchanged by using plasmid DNA. We have used *din* operon fusions as a sensitive means of assaying for the expression of genes under the control of the SOS-like regulon in both competent and noncompetent cell subpopulations as a consequence of competence development and our subsequent experimental treatments. Results indicate that the SOS-like system is induced in both competent and noncompetent subpopulations in our treatments and so should not be a major factor in the differential response in transformation rate observed in UV-DNA and DNA-UV treatments. These results provide further support to the hypothesis that the evolutionary function of competence is to bring DNA into the cell for use as template in the repair of DNA damage.

THE processes of DNA repair and recombination play a fundamental role in maintaining the genetic integrity of all living systems. In the eubacterium *Bacillus subtilis* these processes are intimately associated with competence, a distinct physiological state that is characterized by the ability of cells to bind and take up exogenous DNA from the environment, and thereby undergo genetic transformation (DUBNAU 1982). The development of competence in *B. subtilis* is accompanied by the induction of the SOS-like system for DNA repair and mutagenesis (YASBIN 1977b; LOVE, LYLE and YASBIN 1985). The SOS-like response in *B. subtilis* is similar in many respects to the more extensively characterized SOS response of *Escherichia coli* (LITTLE and MOUNT 1982; WALKER 1984), and consists of a set of coordinately induced cellular phenomena such as enhanced DNA repair capacity (LOVE and YASBIN 1984; MIEHL-LESTER 1985; YASBIN 1977a) which ultimately augment the cell's ability to survive. The SOS response is elicited by DNA damage or an inhibition of DNA replication and results from the specific derepression of diverse genes involved primarily in DNA repair and mutagenesis. In *B. subtilis* the SOS-like response is also elicited as the cells become competent, without subjecting the cells to any exogenous source of DNA damage.

As in *E. coli*, the expression of SOS functions in *B. subtilis* following DNA damage depends primarily upon the activities of a single multifunctional enzyme, the major recombination protein (designated RecbS by LOVE and YASBIN 1986), which is presumably the product of the *recE* gene in *B. subtilis* (DEVOS and VENEMA 1983; LOVETT and ROBERTS 1985; MARRERO and YASBIN 1988). RecbS, itself a DNA damage-inducible protein, functions in general homologous recombination, postreplication or recombinational repair, the regulation of the SOS response to DNA damage (LOVE and YASBIN 1984; LOVETT and ROBERTS 1985; LOVETT *et al.* 1988) and also in the induction of SOS functions during competence (LOVE, LYLE and YASBIN 1985; LOVE and YASBIN 1986). The expression of SOS-like phenomena during competence results from the *recE*-dependent transcriptional activation of specific chromosomal loci (DNA damage-inducible or *din* genes) which ensues following the substantial amplification of cellular levels of RecbS during competence development. Recent evidence indicates this induction of RecbS protein operates by a competence-specific mechanism that is independent of the SOS regulatory pathway and exogenous sources of DNA damage (LOVE 1986; LOVETT, LOVE and YASBIN 1989).

The relationship between inducible repair and competence in *B. subtilis* takes on special significance when viewed in the context of the role of DNA repair in the evolution of sex, a topic in which there is considerable interest [see MICHOD and LEVIN (1988) for review]. At the molecular level, sex can be defined as the breakage and rejoining of homologous DNA molecules (recombination) which come from different individuals (outcrossing). Both recombination and outcrossing are essential components of natural genetic transformation in bacteria. The evolution of transformation has come to be viewed as a case problem in the evolution of sex (see, for example, LEVIN 1988; MICHOD, WOJCIECHOWSKI and HOELZER 1988; REDFIELD 1988). The DNA repair hypothesis [see BERNSTEIN, HOPF and MICHOD (1988) for review] argues that the primary function of sex is to repair DNA damage. To test this hypothesis in the case of bacterial transformation, we previously studied the relative densities of transformed and total cells in competent cultures of *B. subtilis* as a function of dose of UV radiation. We did so under conditions in which cells are either transformed before (DNA-UV) or after (UV-DNA) ultraviolet (UV) irradiation (MICHOD, WOJCIECHOWSKI and HOELZER 1988). Our results demonstrated that the transformation rate (ratio of the numbers of transformants to total cells) increased significantly in UV-DNA treatments but decreased in DNA-UV treatments (see Figure 1). We interpreted these results as support for the hypothesis that DNA repair is the evolutionary function of natural genetic transformation.

According to the repair hypothesis, the primary function of competence in *B. subtilis* is to bring DNA into the cell for the recombinational repair of genetic damage. The high levels of Recbs protein present in competent cells, but not in noncompetent cells, are consistent with this hypothesis, since the increased capacity for recombinational repair using transforming homologous DNA as template should contribute to cell survival. However, as mentioned above, other nonrecombinational repair capacities are also enhanced in competent cells as a direct consequence of induction of the SOS system by elevated amounts of Recbs protein. Whether these processes play a significant role in cell survival during competence, and thereby contribute to the results we have obtained previously, is unknown.

To resolve this issue, we report here the results of experiments aimed at determining the effects of two repair mutations on the relative densities of transformed and total cells in competent culture. While strains carrying these mutations are able to undergo homologous recombination (see Table 2 below), they are deficient either in excision repair capacity (Uvr^-, *uvrA42*), or, in the ability to induce the SOS-like system (including excision repair) during competence and the Recbs protein and the SOS-like system in response to DNA damage (Rec^-, *recA1*) (HADDEN 1979; LOVE 1986; YASBIN 1977a). Further, we have used *din* operon fusions as a sensitive means of assaying for the expression of genes under the control of the SOS-like regulon in both competent and noncompetent cell subpopulations as a consequence of competence development and our subsequent experimental treatments. We report results of nonhomologous plasmid DNA-mediated transformation in a recombination and repair-proficient strain as a way to determine whether the observed effect of homologous DNA is derived from the integration of this donor DNA via genetic recombination ("transformational repair") or due to some generalized effect or factor(s) present in competent cells. Preliminary results with plasmid DNA were reported in MICHOD, WOJCIECHOWSKI and HOELZER (1988). Finally, we report results of "delay experiments" in which our standard UV-DNA and DNA-UV protocols have been modified to control for differences in the timing of UV treatment and differences in the time before plating after UV treatment which exists in our standard protocols.

MATERIALS AND METHODS

Bacterial strains and plasmid: The *B. subtilis* strains used in this study and their relevant genotypes are listed in Table 1. Strain YB886 (and the derivatives of YB886 used here) is a derivative of the naturally competent, recombination- and repair-proficient *B. subtilis* strain 168, and has been cured of bacteriophage SPβ and rendered noninducible for the endogenous prophage PBSX (YASBIN, FIELDS and ANDERSEN 1980). The isolation and characterization of the *din*::Tn*917-lacZ* operon fusions in *B. subtilis* YB886 has been described (LOVE, LYLE and YASBIN 1985). The Tn*917-lacZ* insertion element in these transcriptional fusion strains codes for resistance to macrolide, lincosamide, and streptogramin B antibiotics (YOUNGMAN, PERKINS and LOSICK 1983). Plasmid pMK4, which confers resistance to chloramphenicol in *B. subtilis*, replicates in both *B. subtilis* and *E. coli* (SULLIVAN, YASBIN and YOUNG 1984). Plasmid DNA was isolated from YB886 (pMK4) (GRYCZAN, CONTENTE and DUBNAU 1978) and propagated in a $recA^+$ strain of *E. coli* to increase the frequency of multimeric forms in the CCC DNA (covalently closed circular) preparation as described previously (MICHOD, WOJCIECHOWSKI and HOELZER 1988).

Media: SPIZIZEN (1958) minimal medium plus 0.5% glucose (MG) and competence media (GM1 and GM2) were prepared as described by YASBIN, WILSON and YOUNG (1975), and supplemented with the appropriate amino acids at a final concentration of 50 μg/ml. Dilutions and suspensions of cells were done in SPIZIZEN (1958) minimal salts unless otherwise indicated. *din* operon fusion strains were maintained on LB medium (MILLER 1972) containing erythromycin at 0.1 μg/ml and lincomycin at 25 μg/ml.

Genetic procedures: The method of BOYLAN *et al.* (1972) was used to maximize competence in liquid cultures of *B. subtilis* strains. Essentially, at 90 min following the end of exponential growth (designated as "T_{90}") at 37° with aera-

TABLE 1

***Bacillus subtilis* strains**

Strain	Relevant genotype	Source or reference
YB886	*metB5, trpC2, xin-1*, SPβ^-	YASBIN, FIELDS and ANDERSEN (1980)
YB1005	*metB5, trpC2, uvrA42, xin-1*, SPβ^-	FRIEDMAN and YASBIN (1983)
YB1260	*metB5, trpC2, recA1, xin-1*, SPβ^-	LOVE and YASBIN (1984)
YB886/*din22*	*din22*::Tn*917-lacZ, recE*$^+$	LOVE, LYLE and YASBIN (1985)
YB886/*din76*	*din76*::Tn*917-lacZ, recE*$^+$	LOVE, LYLE and YASBIN (1985)
YB1260/*din22*	*din22*::Tn*917-lacZ, recA1*	This study[a]
YB1260/*din76*	*din76*::Tn*917-lacZ, recA1*	This study[a]

[a] Strains constructed by transformation of strain YB1260 with DNA isolated from YB886/*din* strains, with selection for Tn*917* encoded erythromycin and lincomycin resistance (YOUNGMAN, PERKINS and LOSICK 1983) as described in MATERIALS AND METHODS.

tion in GM1 medium, cells were diluted 10^{-1} into warm GM2 medium and incubated for 60 min with aeration before the addition of transforming DNA or UV irradiation. Competent cultures were incubated with transforming DNA for 30 min, after which DNase I (Sigma, St. Louis, MO) was added to 100 μg/ml and the incubation was continued an additional 10 min. The UV irradiation of competent cultures was performed as described previously (MICHOD, WOJCIECHOWSKI and HOELZER 1988). In our "standard" experiments, competent cultures were either UV irradiated at T_{90}+ 60 min before the addition of transforming DNA (UV-DNA), or incubated with transforming DNA at T_{90}+ 60 min and UV irradiated after transformation at approximately T_{90}+ 100 min. Thus in these standard experiments cells were given transforming DNA at approximately the same time in both UV-DNA and DNA-UV treatments. In these standard experiments, cells were plated immediately after either transformation (UV-DNA) or UV irradiation (DNA-UV), so that UV-DNA and DNA-UV experiments were of equal duration. As a consequence, cells had approximately 40 min in growth medium (GM2) after UV irradiation before plating in a UV-DNA experiment but were plated immediately after UV irradiation in a DNA-UV experiment. In the "delay" experiments described in this report, we have incorporated the following changes in the standard protocol; (1) in delayed UV-DNA experiments, cells were incubated an additional 40 min (*i.e.*, to T_{90}+ 100 min) before UV treatment and subsequent transformation so that the culture was UV irradiated at the same time of growth (and thus same density) as in DNA-UV cultures; (2) in delayed DNA-UV experiments, cultures were incubated with transforming DNA and then UV irradiated as in standard experiments, but following UV irradiation cells were collected by centrifugation, resuspended in warm GM2 medium and incubated with aeration for 40 min before plating. These delays control for any differences in both timing of UV and growth period after UV before plating. These differences are present in our standard experiments in which cells are transformed at the same time but UV irradiated at different times.

Cells were transformed to methionine prototrophy (Met$^+$), or tryptophan prototrophy (Trp$^+$), with high molecular weight homologous chromosomal DNA isolated from the prototrophic strain YB1011 (*xin-1*, SPβ^-) or to resistance to chloramphenicol with plasmid pMK4 DNA as described previously (MICHOD, WOJCIECHOWSKI and HOELZER 1988). Numbers of total viable cells were determined on MG medium supplemented with methionine and tryptophan or on LB medium. Transformation frequencies for chromosomal markers were determined by selection of transformed cells on MG medium supplemented with either tryptophan or methionine. Cells transformed with pMK4 DNA were selected for chloramphenicol resistance on LB medium using an antibiotic overlay method described previously (MICHOD, WOJCIECHOWSKI and HOELZER 1988).

Competent cultures of *B. subtilis din*::Tn*917-lacZ* fusion strains were fractionated in Renografin (RENO-M-60, diatrizoate meglumine, Squibb Diagnostics, New Brunswick, NJ) block gradients by a modification of the procedure of HADDEN and NESTER (1968) as described by LOVE (1986). β-Galactosidase assays were performed on aliquots of unfractionated cultures of fusion strains and on separate competent and noncompetent cell fractions isolated from Renografin gradients using a modification of the MILLER (1972) procedure. Samples of cells (0.5–1 ml) were harvested by centrifugation (2 min at 16,000 × *g* in a microcentrifuge) and stored at −20°. Cell pellets were resuspended in 1 ml Z buffer containing lysozyme at 200 μg/ml, incubated on ice for 30 min, and assayed for β-galactosidase activity as described by MILLER (1972).

Statistical analysis of data: At a given UV dosage, *X*, survivorship of total cells, survivorship of transformed cells and transformation rate are defined as,

$$\frac{N_{TOT,X}}{N_{TOT,0}}, \frac{N_{TRA,X}}{N_{TRA,0}}, \frac{N_{TRA,X}}{N_{TOT,X}},$$

respectively. $N_{TOT,X}$ and $N_{TRA,X}$ are the numbers of total and transformed cells which survive a UV treatment of *X* J/m^2. Log transformations were taken of the survivorships, transformation rates, and factor changes in transformation rate for the purpose of statistical analysis, since this made the variances more homoscedastic. The factor change in transformation rate at *X* J/m^2 is defined as the transformation rate at *X* J/m^2 divided by the transformation rate at 0 J/m^2. In addition to the means and standard errors, regression curves are also plotted in the figures. Coefficients for the regressions are given in Tables 3 and 4. When regression and survival curves are compared to each other in the following discussion, they were compared using the indicator variable technique discussed in NETER, WASSERMAN and KUTNER (1985, Chapter 10). This technique involves defining binary variables for each of the following qualitative variables: cell type (total cell or transformed cell), DNA concentration (1.0 or 0.01 μg/ml for strain YB1005 (*uvrA42*); 1.0 and 0.1 μg/ml for strain YB1260 (*recA1*); high DNA (1.0 and 2.0 μg/ml pooled) and low DNA (0.10 and 0.01 μg/ml pooled) for strain YB886 (*rec*$^+$ *uvr*$^+$)), order of the UV and DNA treatments (UV-DNA and DNA-UV), and, in the case of strain YB1260 (*recA1*), UV dosage (≤ or > 10 J/m^2). To test whether the qualitative variables just mentioned had significant effects on survivorship and transformation rate, an *F* statistic was constructed using the error sum of squares for the full model (SSF_F) and for a reduced model (SSE_R). The full model contains the indicator variable

and its interactions with UV, whereas the reduced model is obtained by deleting the indicator variable and its interactions with UV from the full model. Thus the reduced model pools the data in a way which ignores the qualitative variable of interest. The F statistic used in the test is

$$\frac{SSE_R - SSE_F}{df_R - df_F} \div \frac{SSE_F}{df_F},$$

in which df_F and df_R are the degrees of freedom for the full and reduced models, respectively. Regression analyses were performed using the REG procedure of the SAS (Statistical Analysis System) computer package.

RESULTS

Overview: Our primary results involve the change in transformation rate as a function of UV fluence in two treatments, in which cells were given exogenous DNA either before (DNA-UV) or after (UV-DNA) treatment with UV. Survivorship curves were also obtained and analyzed as discussed in MATERIALS AND METHODS. However, for the purposes of the present paper, the transformation rate curves are sufficient and so we have not presented the survivorship curves here (these curves are available from the authors). For comparison to our previous work with the repair and recombination proficient strain YB886 (MICHOD, WOJCIECHOWSKI and HOELZER 1988; Figure 1), the UV fluences administered for each of the two repair deficient strains were chosen to give approximately two logs of killing at the highest UV dose which was the range of killing used in our previous work.

We view the survivorships and ratios of transformed cells and total cells presented below as indicative of that of sexual and asexual cells, respectively. Cells which have been transformed with homologous DNA at chromosomal loci (either *trpC2* or *metB5*) are, by definition, competent cells which have taken up homologous exogenous DNA and recombined it into their genome. Cells transformed with nonhomologous plasmid DNA are competent cells which have taken up plasmid DNA containing the plasmid marker for chloramphenicol resistance. This plasmid DNA is not recombined into the cell's genome, rather it resides in the cell as an extrachromosomal element. Total cells are primarily made up of noncompetent (asexual) cells, since in *B. subtilis* only about 10% of the cells in a population grown to maximize competence actually become competent (DUBNAU 1982). Cells transformed at a marker locus are only a small percentage, approximately 1.0%, of the competent subpopulation. We assume that these transformed cells provide us with an unbiased "window" into the behavior of the larger subpopulation of competent (sexual) cells.

The homologous DNA transformation rates at 0 J/m^2 for the different strains are given in Table 2. Since the transformation rate varies with strain and kind and amount of DNA, the factor change in the transformation rate for each UV dose, is studied in what follows. The factor change at X J/m^2 is defined as the transformation rate at X J/m^2 divided by the transformation rate at 0 J/m^2. A factor change greater than one indicates an increase, and a factor change less than one indicates a decrease, in the transformation rate with UV dose. In the figures below the average of many experiments of the log of the factor change is graphed.

TABLE 2

Chromosomal DNA transformation rates[a]

Strain	Relevant genotype	Transformation frequency[b] Trp$^+$	Met$^+$
YB886	*rec*$^+$ *uvr*$^+$	1.35×10^{-3}	1.21×10^{-2}
YB1005	*uvrA42*	3.35×10^{-3}	4.70×10^{-3}
YB1260	*recA1*	3.67×10^{-4}	1.23×10^{-3}

[a] Strains were grown to competence, transformed with *met*$^+$, *trp*$^+$ DNA (isolated from YB1011) at 1 μg/ml, and plated for both Met$^+$ and Trp$^+$ transformants, as described in MATERIALS AND METHODS.
[b] Transformation frequency defined in text. Results are averages of several experiments.

Plasmid DNA transformation of strain YB886 (*rec*$^+$, *uvr*$^+$): To begin investigating whether the observed increase in homologous DNA transformation frequency results from a more generalized effect present in competent but not in noncompetent cells, the effects of UV irradiation on nonhomologous plasmid DNA-mediated transformation were studied in strain YB886 (*rec*$^+$, *uvr*$^+$). Although the efficiency of plasmid DNA-mediated transformation as a function of DNA added is quite low compared to that for homologous chromosomal DNA, competence for plasmid and chromosomal DNA transformation in *B. subtilis* develops with similar kinetics. That plasmid DNA (CCC form) and chromosomal DNA compete for uptake further indicates that plasmid transformation generally proceeds by the same mechanism of uptake and processing as linear duplex chromosomal DNA, although the process is *rec*-independent since plasmid DNA is not integrated into the bacterial chromosome [see DUBNAU (1982) for review]. Since transforming plasmid DNA should provide no repair benefit to a competent cell which depends upon homology, plasmid transformation serves as a control for the effect of DNA damage on the process of transformation in competent cells.

For strain YB886 the mean plasmid transformation frequency was 8.70×10^{-4} at 0 J/m^2 UV for a saturating amount (2 μg/ml) of pMK4 DNA. In contrast to the experiments with homologous chromosomal DNA transformation in this strain (Figure 1), we find no statistically significant effect of UV dose on the rate of plasmid DNA-mediated transformation when cells are UV irradiated prior to the addition of transforming DNA (see Figure 2 and Table 3). However, this rate decreases significantly ($P < 0.001$) with UV

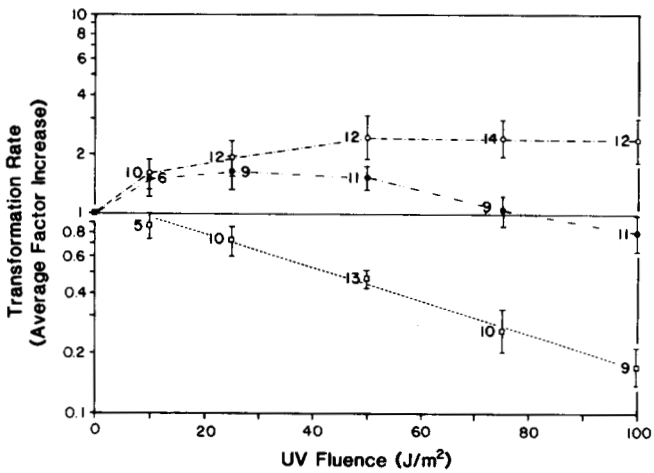

FIGURE 1.—Factor change in chromosomal DNA transformation rates for YB886 (*rec*$^+$ *uvr*$^+$) as a function of UV dosage for UV-DNA and DNA-UV treatments. Taken from MICHOD, WOJCIECHOWSKI and HOELZER (1988). Points plotted are averages of the log factor change for each experiment. Error bars give average ± the standard error. Sample size is given near each mean. Key: UV-DNA 0.01 and 0.10 μg/ml pooled (○, ·–·–·–·–); UV-DNA 1.0 and 2.0 μg/ml pooled (●, –···–···–···); DNA-UV (□, -------).

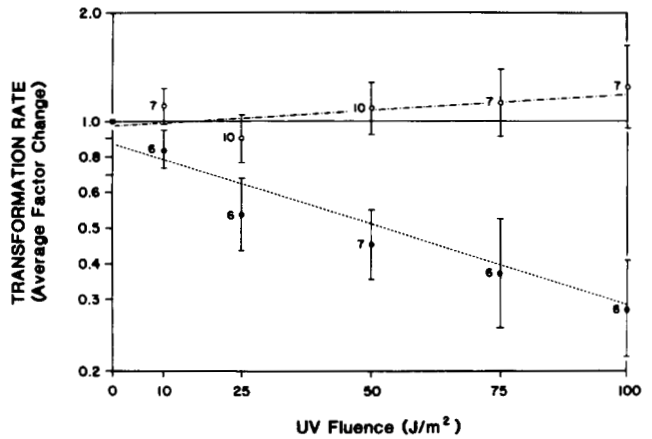

FIGURE 2.—Factor change in plasmid DNA transformation rates for YB886 (*rec*$^+$ *uvr*$^+$) as a function of UV dosage for UV-DNA and DNA-UV treatments. See text for discussion. Points plotted are averages of the log factor change for each experiment. Error bars give average ± the standard error. Sample size is given near each mean. Regressions (see Table 3) are graphed for each data set. Key for means and regressions: UV-DNA (○, ·–·–·–·–); DNA-UV (●, -------).

dose when cells are transformed with plasmid DNA prior to UV irradiation (DNA-UV), and, decreases in a manner that is quantitatively similar to our results with chromosomal DNA-mediated transformation in YB886 (*rec*$^+$, *uvr*$^+$) in DNA-UV experiments (Figure 1).

Homologous DNA transformation in the excision repair-deficient strain YB1005: The effects of the Uvr$^-$ mutation *uvrA42* (MUNAKATA 1977) on chromosomal DNA transformation in *B. subtilis* cells were studied in both UV-DNA and DNA-UV experiments. The *uvrA42* mutation blocks the initial steps essential for the excision repair of UV-induced DNA damage (HADDEN 1979). This mutation makes strain YB1005 very sensitive to UV radiation (FRIEDMAN and YASBIN 1983) but does not affect the ability of cells to carry out recombination or postreplication repair (DODSON and HADDEN 1980) or affect genetic exchange (see Table 2) (LOVE and YASBIN 1984).

As is the case with strain YB886 (Figure 1), transformation rates for strain YB1005 (*uvrA42*) increase significantly with UV dose in the UV-DNA experiments (Figure 3, Table 3). In DNA-UV experiments the transformation rates decrease, but not significantly so (Figure 3, Table 3). DNA-UV experiments with strain YB886 (*rec*$^+$, *uvr*$^+$) showed a significant decrease in transformation rate with UV dose (Figure 1). For both sets of experiments in Figure 3, data for the two DNA amounts used, 0.01 and 1.0 μg/ml, are pooled. When the data for the two DNA concentrations are kept separate, the 0.01 μg/ml curve appears to show a steeper increase than the 1.0 μg/ml curve, as is the case for YB886 (Figure 1). However, the curves for the two DNA concentrations are not statistically different for our YB1005 data set. Since DNA concentration has never affected the qualitative nature of the outcome, we do not view this as a major issue in the present work and have not pursued it further here.

Homologous DNA transformation in the DNA repair-deficient strain YB1260 (*recA1*): Considerable genetic evidence suggests that the *recA1* mutation, like the *recE4* mutation (DUBNAU and CIRIGLIANO 1974), resides in the gene that encodes the major recombination protein (RecbS) of *B. subtilis* (DEVOS and VENEMA 1983; LOVE 1986; LOVE and YASBIN 1986; LOVETT, LOVE and YASBIN 1989; LOVETT *et al.* 1988; MARRERO and YASBIN 1988). Like *recE4*, the *recA1* mutation inhibits markedly the expression of the SOS-like response (YASBIN 1977a) and decreases DNA repair capacity, making strains carrying these mutations extremely sensitive to DNA damaging agents (LOVE 1986). However, while both mutations impair the formation of donor-recipient heteroduplex DNA molecules during chromosomal DNA-mediated transformation (DUBNAU *et al.* 1973), *recA1* mutants are only moderately reduced in their capacity for genetic exchange (LOVE and YASBIN 1984) (Table 2). Since the *recA1* mutation profoundly affects the induction of RecbS and/or impairs the SOS-inducing and DNA repair activities of the protein without completely abolishing its' recombination functions (in contrast to the *recE4* mutation), we decided to characterize the effects of this mutation on chromosomal DNA transformation in the two experimental treatments.

Transformation rates for the two DNA concentrations used, 0.1 and 1.0 μg/ml, both increase with increasing UV in a qualitatively similar manner. However, the two DNA concentrations show significant

TABLE 3

Regression analyses of factor change in transformation rate for strains YB886 (*rec*$^+$ *uvr*$^+$), YB1005 (*uvrA42*), and delay experiments for YB1260 (*recA1*)

	Intercept[a]	Coefficients[b]		r^2
Strain YB886 (*rec*$^+$ *uvr*$^+$), plasmid DNA				
UV-DNA	−0.0129	0.0009		0.03
DNA-UV	−0.0644	−0.0045****		0.30
Strain YB1005 (*uvrA42*), homologous DNA[c]				
UV-DNA	−0.0087	0.0305****	−0.0007**	0.54
DNA-UV	−0.0171	−0.0029		0.02
Strain YB1260 (*recA1*), homologous DNA, delay experiment				
UV-DNA	0.1568*	0.0680*****	−0.0009****	0.85
DNA-UV	−0.0175	0.0441****	−0.0005**	0.87
Strain YB886, (*rec*$^+$ *uvr*$^+$), homologous DNA				
UV-DNA high DNA[d]	0.04810	0.00709***	−0.00009****	0.22
UV-DNA low DNA[d]	0.4418	0.0172***	−0.00008**	0.16
DNA-UV delay	−0.00181	0.00463	−0.00010*	0.38

Tests for differences between regressions are discussed in the text.

[a] Intercept given as log.

[b] The coefficients correspond to the X and X^2 terms, respectively, where X is UV dosage. Levels of significance that coefficients are different from zero: * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; ***** $P < 0.0001$.

[c] DNA concentrations 1.0 and 0.01 μg/ml pooled.

[d] High DNA = 1.0 and 2.0 μg/ml pooled. Low DNA = 0.01 and 0.10 μg/ml pooled. UV-DNA experiments reported in MICHOD, WOJCIECHOWSKI and HOELZER (1988).

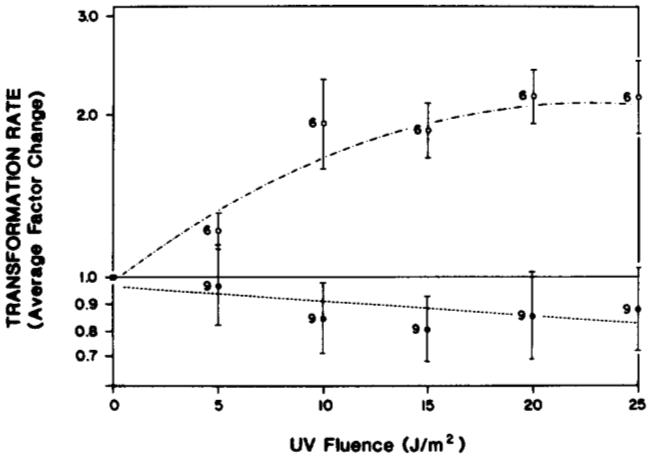

FIGURE 3.—Factor change in homologous chromosomal DNA transformation rates for YB1005 (*uvrA42*) as a function of UV dosage for UV-DNA and DNA-UV treatments. Points plotted are averages of the log factor change for each experiment. See text for discussion. DNA concentrations 0.01 and 1.0 μg/ml are pooled. Error bars give average ± the standard error. Sample size is given near each mean. Regressions (see Table 3) are graphed for each data set. Key for means and regressions: UV-DNA (O, ·–·–·–·–); DNA-UV (●, -------).

differences in the quantitative nature of the response. Only the data for the saturating level of DNA, 1.0 μg/ml, is presented in graphed form since the same qualitative relationships were observed for the lower DNA concentration (0.1 μg/ml) data (regression curves for both data sets are presented in Table 4). The data were analyzed by a variety of regression techniques including linear, polynomial, and piecewise linear. Two-piecewise linear regressions for the UV doses ≤ or > 10 J/m^2, which worked best in those cases in which linear regression was inappropriate, were generated and compared using the indicator variable technique described above (NETER, WASSERMAN and KUTNER 1985, Chapter 10).

Rates of chromosomal DNA transformation in the *recA1* mutant (Figure 4) increase significantly as a function of the UV dose in both the UV-DNA and DNA-UV treatments, but do so to a much greater extent in the UV-DNA experiments. Furthermore, the increase in the rate of chromosomal DNA transformation with UV dose in UV-DNA experiments in the *recA1* mutant strain is approximately five times greater (increasing over tenfold total) than that observed with either strain YB1005 (*uvrA42*, Figure 3) or strain YB886 (*rec*$^+$, *uvr*$^+$, Figure 1). Regressions in Figure 4 for each of the two UV dose ranges, ≤ or > 10 J/m^2, are significantly different from each other ($P < 0.0001$; second order polynomial regressions fit to the data were also different at this level but they did not describe the data as well).

Delay DNA-UV experiments using homologous DNA: For the strains YB1260, YB1005 and YB886, we performed a series of delay experiments described in the MATERIALS AND METHODS section in which the UV treatment and plating are conducted at the same time in both UV-DNA and DNA-UV treatments (the DNA is administered 40 min later in the UV-DNA treatment than in the DNA-UV treatment in these delay experiments). The results of these experiments are presented in Figures 5 and 6 and Table 3 for YB1260 and YB886. Preliminary results for strain YB1005 were similar to those for YB1260 and YB886 described below.

As can be seen in Figure 5 for YB1260 (*recA1*), the

TABLE 4

Piecewise linear regression analysis of factor change in transformation rate for YB1260 (*recA1*)

	UV ≤ 10 J/m²			UV > 10 J/m²		
	Intercept[a]	Coefficient	r^2	Intercept	Coefficient	r^2
UV-DNA						
1.0 μg*****[b]	0.04218	0.10314*****	0.77	1.07677***	0.00210	0.01
0.1 μg****	0.01275	0.06553*****	0.66	0.71694****	0.00147	0.00
DNA-UV						
1.0 μg	0.04905[c]	0.00352***	0.24			
0.1 μg*	0.00413	0.03300*****	0.82	0.19560	0.00304	0.03

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$; ***** $P < 0.0001$.

[a] Intercept given as log.

[b] Asterisks in this column give the level of significance for the F test comparing the two regressions (UV ≤ or > 10 J/m²). See text for explanation of test.

[c] In cases in which the two regressions (UV ≤ or > 10 J/m²) are not significantly different, a single regression is fit for all UV doses. This single regression is given in the UV ≤ 10 J/m² column.

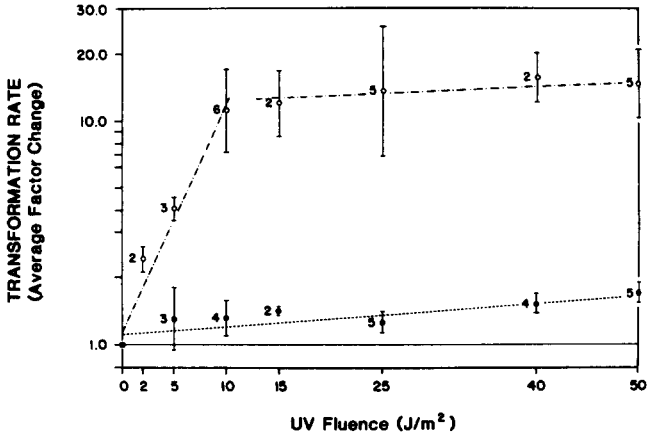

FIGURE 4.—Factor change in homologous chromosomal DNA (1.0 μg/ml) transformation rate for YB1260 (*recA1*) as a function of UV dosage for UV-DNA and DNA-UV treatments. Points plotted are averages of the log factor change for each experiment. See text for discussion. Error bars give average ± the standard error. Sample size is given near each mean. Regressions are graphed for each data set. The two regressions for each of the two UV dose ranges are significantly different from each other at the $P = 0.0001$ level. See text and Table 4 for discussion of regressions. Key for means and regressions: UV-DNA (O, ·–·–·–·–); DNA-UV (●, -------).

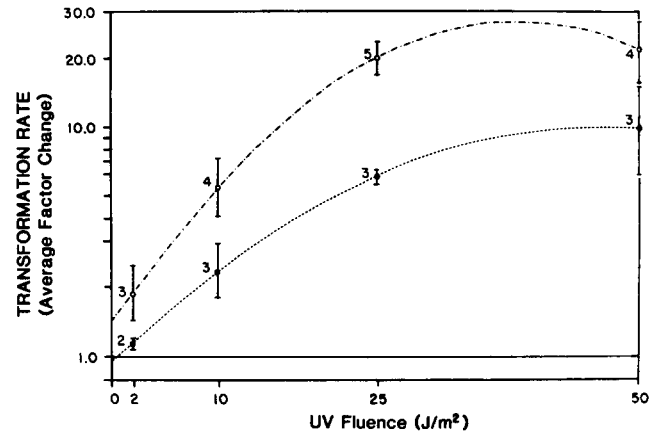

FIGURE 5.—Factor change in homologous chromosomal DNA (1.0 μg/ml) transformation rate for YB1260 (*recA1*) as a function of UV dosage for UV-DNA and DNA-UV treatments for delay experiments. Points plotted are averages of the log factor change for each experiment. See text for discussion. Error bars give average ± the standard error. Sample size is given near each mean. Regressions are graphed for each data set. The quadratic regressions are significantly different from each other at the $P = 0.0005$ level. See text and Table 3 for discussion of regressions. Key for means and regressions: UV-DNA (O, ·–·–·–·–); DNA-UV (●, -------).

delay of UV by 40 min in a UV-DNA experiment had little effect, apart from making the response curve more continuous in the delay experiment. However, in the DNA-UV treatment, delaying plating for 40 min following UV had the effect of raising the response curve to more closely resemble the UV-DNA treatment. Nevertheless, the two curves remain statistically different ($P = 0.0005$).

Delay DNA-UV experiments were conducted for YB886 (*rec*$^+$, *uvr*$^+$) and compared in Figure 6 with the standard UV-DNA experiments reported in Figure 1. Both regressions for the UV-DNA experiments (Table 3) are significantly higher than the delay DNA-UV regressions (Table 3) [$P = 0.0099$ for UV-DNA experiments done at high DNA concentrations (1.0 and 2.0 μg/ml pooled) and $P = 0.0001$ for UV-DNA experiments done at low DNA concentrations (0.01 and 0.10 μg/ml pooled)]. By comparing the DNA-UV curves in Figures 1 and 6, we see that the transformation rate in delay DNA-UV experiments does not drop off as rapidly when cells are allowed to grow after the UV treatment. In fact, the first order effect of UV dosage on the change in transformation rate in delay DNA-UV experiments is not significantly different from zero (Table 3).

Survival curves for transformants and total cells (not presented here) show that the primary effect of the delay for both strains YB886 (*rec*$^+$, *uvr*$^+$) and YB1260 (*recA1*) was on total cells in the DNA-UV treatment. Survival regression curves were compared to one another by the indicator variable technique described in

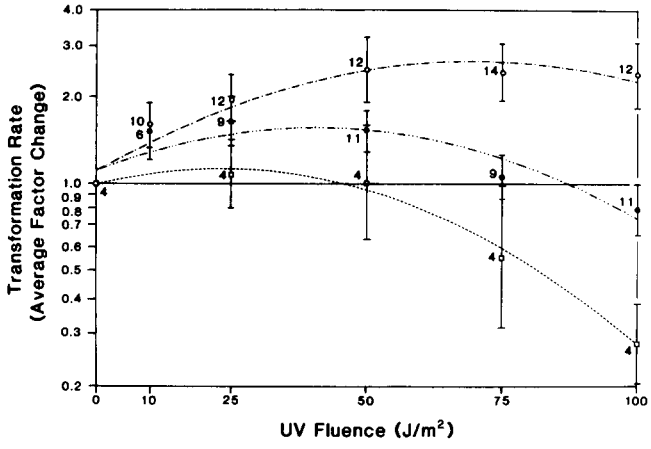

FIGURE 6.—Factor change in homologous chromosomal DNA transformation rate for YB886 (*rec*$^+$ *uvr*$^+$) as a function of UV dosage for the standard UV-DNA experiments presented in Figure 1 and delay DNA-UV experiments. Points plotted are averages of the log factor change for each experiment. See text for discussion. Error bars give average ± the standard error. Samples size is given near each mean. Regressions are graphed for each data set. The UV-DNA quadratic regressions are significantly different from the delay DNA-UV regressions as described in the text. See text and Table 3 for discussion of regressions. Key for means and regressions: UV-DNA 0.10 and 0.01 μg/ml pooled (○, ·–·–·–·–); UV-DNA 1.0 and 2.0 μg/ml pooled (●, ···–···–···–); delay DNA-UV 1.0 μg/ml (□, -------).

MATERIALS AND METHODS. The survival curves for transformants were not statistically different in delay and standard experiments for both strain YB886 (DNA-UV, P = 0.5341) and strain YB1260 (UV-DNA, P = 0.1509; DNA-UV, P = 0.1789). Survival curves for transformants were significantly higher in UV-DNA than in delay DNA-UV experiments for both strain YB886 (all DNA pooled in UV-DNA, P = 0.0049) and strain YB1260 (P = 0.0008). Survival curves for total cells in standard and delay UV-DNA experiments were not statistically different in strain YB1260 (P = 0.8925). However, survival curves for total cells in delay DNA-UV experiments were significantly lower than in standard DNA-UV experiments in both strain YB886 (P = 0.0890) and stain YB1260 (P = 0.0001). As discussed in more detail in the DISCUSSION, this probably results from growth in rich medium (GM2) before plating in the delay DNA-UV experiments, during which time damages can interfere with replication and kill the cell. Once cells are plated, cell division is slower and there is more opportunity for repair before damages interfere with replication. This appears not to be an important factor with transformed cells, probably since they are dividing more slowly than noncompetent cells. Nevertheless when this factor is controlled for, the transformation rates in UV-DNA experiments still increase to a greater extent than in the delay DNA-UV experiments (Figures 5 and 6).

Induction of *din* operon expression by DNA damage in competent and noncompetent cells: The rate of homologous DNA transformation may increase with UV dose because competent cells are surviving DNA damage better than noncompetent cells. It is possible that a survival difference results from the inability of the noncompetent subpopulation of cells to induce the expression of SOS-associated repair genes following UV irradiation and not from, as the repair hypothesis predicts, a benefit of enhanced recombinational repair in the competent subpopulation. Furthermore, as already mentioned, the transformation rate may increase in a UV-DNA experiment as a result of induction of recombination by the SOS-like system in *B. subtilis*.

To examine these possibilities, we have quantitated the induction of *din* gene expression (β-galactosidase activity) in both competent and noncompetent cell subpopulations of two *din*::Tn*917-lacZ* fusion strains (as representatives of the set of DNA damage-inducible loci or SOS genes in this bacterium) following exposure to UV radiation. These *din* fusion strains produce increased levels of β-galactosidase (*lacZ* gene product) when exposed to a variety of DNA damaging agents such as mitomycin C or UV radiation (LOVE, LYLE and YASBIN 1985). In addition, β-galactosidase induction occurs in the competent subpopulation of each *din* fusion strain during the development of competence in a culture, independent of any DNA damage (LOVE, LYLE and YASBIN 1985). The *B. subtilis* strains we have employed contained a *din*::Tn*917-lacZ* operon fusion that either does not affect DNA repair capacity (*din22*) or that causes a marked deficiency in excision repair (*din76*) (LOVE, LYLE and YASBIN 1985; LOVE and YASBIN 1986). Genetic mapping analyses indicate the Tn*917-lacZ* insertion element in the *din76* fusion strain is located in or near the *uvrA* locus (GILLESPIE and YASBIN 1987).

As shown in Table 5, when competent cultures of the *din*, *recE*$^+$ fusion strains were UV-irradiated (UV-DNA treatment) and fractionated on Renografin gradients to separate the competent and noncompetent subpopulations of cells, β-galactosidase production increased substantially in the noncompetent cells (bottom band isolated from Renografin gradients) to a level comparable to that measured in the competent cell fraction (top band isolated from gradients). The results indicate that *din* operons are induced in noncompetent cells in response to DNA damage, and suggest that the relative expression of SOS-associated repair functions in the two subpopulations of cells are not markedly different in a UV-DNA experiment. Compared with the *din*, *recE*$^+$ strains, induction of β-galactosidase activity in the respective fractions of unirradiated competent cultures of *din*, *recA1* fusion strains was reduced about 50%. However, we find no significant induction or difference in *din* gene expression as measured by β-galactosidase activity in either

TABLE 5

Induction of β-galactosidase in competent and noncompetent subpopulations of *Bacillus subtilis recE*$^+$ and *recA1 din*::Tn*917-lacZ* fusion strains following UV irradiation

Fusion strain	Fraction	Transformation frequency −UV	Transformation frequency +UV[a]	β-Galactosidase, units/A_{600} −UV	β-Galactosidase, units/A_{600} +UV[a]
YB886/*din22*	Un	6.5×10^{-3}	3.0×10^{-2}	23.6	58.2
	T	1.4×10^{-2}	3.7×10^{-2}	80.2	66.9
	B	6.9×10^{-5}	5.6×10^{-4}	10.8	46.4
YB886/*din76*	Un	1.0×10^{-3}	9.1×10^{-3}	2.2	36.4
	T	2.4×10^{-2}	3.0×10^{-2}	8.9	43.5
	B	5.6×10^{-6}	1.6×10^{-4}	1.7	23.6
YB1260/*din22*	Un	1.1×10^{-3}	4.3×10^{-3}	20.5	22.0
	T	1.5×10^{-3}	3.7×10^{-3}	31.8	32.0
	B	1.2×10^{-5}	2.7×10^{-5}	3.2	7.4
YB1260/*din76*	Un	4.0×10^{-4}	2.7×10^{-3}	1.4	2.7
	T	1.4×10^{-3}	1.9×10^{-3}	3.1	4.1
	B	2.6×10^{-6}	8.3×10^{-5}	0.9	2.1

Cultures of *recE*$^+$ and *recA1* strains containing the *din22*::Tn*917-lacZ* and *din76*::Tn*917-lacZ* operon fusions were grown to maximize competence, UV irradiated (UV-DNA treatment) at doses indicated below, resuspended in fresh GM2 medium, transformed with *met*$^+$ DNA (YB1011) at 1 μg/ml, and fractionated in Renografin density gradients (LOVE 1986). β-galactosidase assays were performed on aliquots of unfractionated cultures and on samples of cells collected from each gradient band, washed once in 1× SPIZIZEN (1958) salts, and resuspended in GM1 medium. Met$^+$ transformation frequencies were determined by dividing the number of Met$^+$ transformants by the number of total viable cells from each fraction. Un, unfractionated cells; T, top band of Renografin gradients, contains predominantly competent cells; B, bottom band of Renografin gradients, containing predominantly noncompetent cells. Results shown are representative of two to three separate experiments.

[a] YB886/*din22*, UV fluence of 50 J/m^2. YB886/*din76*, UV fluence of 25 J/m^2. YB1260/*din22* and *din76*, UV fluence of 10 J/m^2.

the competent or noncompetent cell subpopulations of the *din*, *recA1* fusion strains following UV irradiation, consistent with the known effects of the *recA1* mutation on the *B. subtilis* SOS-like system and induction of *din* operons (SOS genes) in competent cells and following DNA damage (LOVETT, LOVE and YASBIN 1989; LOVETT *et al.* 1988).

It is worth noting that the transformation frequencies increase with UV irradiation in all strains in Table 5. This result confirms the results of Figures 1, 4 and 5 and MICHOD, WOJCIECHOWSKI and HOELZER (1988) and supports our interpretation that the observed increase in transformation rate with UV cannot be explained by the induction of SOS-associated repair by UV.

DISCUSSION

Overview: The repair hypothesis predicts that the survival of competent cells should increase relative to noncompetent cells in a UV-DNA experiment, either because they are more resistant to UV as a result of their heightened recombinational repair, or because prior DNA damage directly increases levels of transformation. The latter may occur as a result of the increased efficiency of binding, uptake and/or recombination of DNA in damaged cells or because DNA damages themselves stimulate recombination. Consequently, the repair hypothesis predicts that the transformation rate should increase with UV fluence in UV-DNA experiments, at least over the range of UV fluences to which the cell's repair systems can respond. In DNA-UV experiments, there can be no repair benefit from the uptake and integration of homologous DNA, since this occurs prior to DNA damage. Similarly, in DNA-UV experiments there could be no induction of transformation, if such a phenomenon occurs.

However, other factors, not directly related to the transformation process, such as excision repair and other inducible SOS functions, may influence the relative densities of transformed and total cells. To explain our previous results (MICHOD, WOJCIECHOWSKI and HOELZER 1988), especially the differences mentioned above between UV-DNA and DNA-UV treatments, on the basis of SOS repair or some other difference between competent and noncompetent cells, one must postulate that the hypothesized factor operates differently in the two treatments. Although we do not expect excision repair or other SOS repair functions to operate differently in the UV-DNA and DNA-UV treatments, there is more time (approximately 40 min) after the UV treatment before plating in our standard experiments for these processes to operate in a UV-DNA experiment as compared to a DNA-UV experiment (see MATERIALS AND METHODS). In addition, as discussed in the introduction, *din* loci, and thus SOS repair functions, are known to be induced in competent cells (but not in noncompetent cells) as a secondary consequence of RecBs protein amplification, without subjecting the culture to a damaging agent. The experiments conducted here were designed to determine the effect of these other, nontransformational, repair processes on the relative densities of competent and noncompetent cells in our treatments.

General result: In both of the repair-deficient strains studied here (Figures 3, 4 and 5), the transformation rate increases more in UV-DNA experiments than in DNA-UV experiments, as was the case with YB886 (*rec*$^+$, *uvr*$^+$; Figures 1 and 6). Thus, excision repair, inducible SOS repair, or protocol differences between UV-DNA and DNA-UV experiments cannot explain the general qualitative result that the transformation rate increases with UV dosage more in UV-DNA experiments than in DNA-UV experiments. There appears to be some effect of homologous chromosomal DNA if it is added after the cells are exposed to UV.

Plasmid transformation. Plasmid transformation "tags" competent cells without providing any additional benefits of homologous DNA, such as template for recombinational repair. If some general property of competent cells was primarily responsible for the above-mentioned increase in homologous transformation rates in UV-DNA experiments, or, if prior UV, induced increased binding or uptake of DNA, similar increases should be observed with plasmid transformation rates. However, as shown in Figure 2, this is not so for the UV-DNA experiments, even though, in DNA-UV experiments, plasmid transformation rates behaved similarly to homologous DNA transformation rates (compare Figure 2 and Table 3 with Figure 1 above and Table 1 of MICHOD, WOJCIECHOWSKI and HOELZER 1988).

Delay Experiments: There are several differences between the UV-DNA and DNA-UV experiments presented in Figures 1, 2, 3 and 4 other than the order of administration of UV and DNA. In these "standard" experiments, we have transformed the cells at equivalent densities and time during growth of the culture (T_{90} + 60 min). As a result, the UV radiation was administered at different times in the experimental procedure, approximately 40 min later in a DNA-UV experiment. This additional time for growth creates higher cell densities in the DNA-UV treatments. This is not as significant a problem as it may seem, since our comparisons between the two treatments do not involve absolute densities of cells, but, rather, we compare for each treatment the relative response of the transformation rate to UV.

The time after administration of the UV but before plating also varies between the standard UV-DNA and DNA-UV treatments. In the standard experiments, the overall duration of UV-DNA and DNA-UV treatments was kept constant so that plating occurred immediately after treatment with DNase in the UV-DNA experiments and immediately after treatment with UV in the DNA-UV experiments. Cells have approximately 40 min in growth medium after UV but before plating in a UV-DNA experiment but no time in growth medium after UV treatment in standard DNA-UV experiments. Consequently, UV-DNA and standard DNA-UV experiments differ in the opportunity for DNA replication and cell division after UV treatment.

The different opportunities for cell division after UV treatment in UV-DNA and standard DNA-UV experiments are potentially important. Unrepaired DNA lesions interfere with DNA replication causing cell death. Cells may have longer to repair DNA lesions caused by the UV treatment in standard DNA-UV experiments than in UV-DNA experiments, since cell division is slower on agar plates than in broth. This factor may be especially pronounced in the noncompetent cells, since competent cells are in a state of biosynthetic latency and are not dividing as rapidly as noncompetent cells. As a result of these factors, transformation rates may be higher in UV-DNA experiments and in standard DNA-UV experiments.

As discussed in MATERIALS AND METHODS, the UV-DNA and DNA-UV protocols were adjusted in "delay experiments" in which cells were administered UV at the same time, and given the same amount of time after UV before plating. It should be realized that in these delay experiments, cells are UV irradiated at equivalent densities and times but receive DNA at different times in the UV-DNA and DNA-UV treatments. Results from such delay experiments indicate that the time in growth media after UV treatment does explain some of the difference between the change in the transformation rate in standard UV-DNA and DNA-UV experiments. However, UV-DNA experiments still exhibit higher transformation rates than do delay DNA-UV experiments by a factor of about two (see Figures 5 and 6).

DNA-UV results in YB886 (*rec*$^+$, *uvr*$^+$): There are several possible reasons why both homologous DNA and plasmid DNA transformation rates decrease dramatically in standard DNA-UV experiments with strain YB886. First, as just discussed, cells are plated immediately in a standard DNA-UV experiment whereas they have 40 min of growth in rich medium in a UV-DNA experiment. The delay experiments indicate that this factor contributes to the decrease, however, transformation rates eventually decrease even in delay DNA-UV experiments (Figure 6). Second, competent cells may be recombining damaged DNA. However, results discussed in MICHOD, WOJCIECHOWSKI and HOELZER (1988) indicate that this is an unlikely explanation, although they do not completely preclude this possibility. Third, transformed cells may be more sensitive to stress encountered immediately after transformation due to the physiological costs of competence development, DNA uptake and transformation in DNA-UV experiments. In the mutant strains these costs may be overcome (Figures 3, 4 and 5) by the intrinsic disadvantages of noncompetent cells discussed in the next section.

Quantitative aspects of results: Although we have shown that the qualitative result, that transformation rates increase more in UV-DNA experiments than in DNA-UV experiments, cannot be attributed to increased survival brought on by SOS or excision repair, certain quantitative aspects of the response of transformation rate probably reflect these repair processes. The mutant strains differ in the magnitude of the increase in homologous transformation rate in the UV-DNA experiments and, in their response in the DNA-UV experiments. Strain YB1260 (*recA1*) showed the highest increase in transformation rate in

the standard UV-DNA experiments, greater than tenfold increase over the range 10–50 J/m^2 (Figures 4 and 5), whereas both strains YB1005 (*uvrA42*, Figure 3) and YB886 (*rec*$^+$, *uvr*$^+$; Figure 1) exhibited approximately a twofold increase. In the DNA-UV experiments, UV has no significant effect on the transformation rate in YB1005 (*uvrA42*) and causes a slight increase in YB1260 (*recA1*), whereas in YB886 (*rec*$^+$, *uvr*$^+$) both plasmid and homologous DNA transformation rates decreased dramatically in the DNA-UV experiments (Figure 2 above and Figure 4b of MICHOD, WOJCIECHOWSKI and HOELZER 1988).

These results can be understood on the basis of what is known about induction of the major recombination protein and repair loci in *B. subtilis* after UV irradiation and during competence. In the Uvr$^-$ mutant, both competent and noncompetent cells lack excision repair, while they both retain the ability to induce other aspects of the SOS-like system (including Recbs induction) and competent cells presumably retain the ability to undergo transformational repair. This lack of a functional excision repair system appears to put the noncompetent cells at an intrinsic disadvantage when compared to the competent cells, and may explain our result that the DNA-UV experiments with this strain do not show a decrease in transformation rate with increasing UV dose as was the case with YB886 (*rec*$^+$, *uvr*$^+$).

The dramatic increase in transformation rate observed in the *rec* mutant, YB1260 (*recA1*), in the UV-DNA experiments, and the less dramatic but still significant increase in the DNA-UV experiments, probably reflect an even larger intrinsic survival difference between competent and noncompetent cells in this strain as compared to strains YB1005 (*uvrA42*) and YB886 (*rec*$^+$, *uvr*$^+$). While the Recbs protein produced in the *recA1* mutant strain is reasonably proficient in recombination (Table 2) and, thus, should be able to perform transformational repair, it is deficient in its ability to induce the SOS-like response following DNA damage or during competence (LOVE 1986; LOVETT, LOVE and YASBIN 1989; LOVETT *et al.* 1988). Further, while strain YB1005 lacks a functional excision repair system, it is still capable of making increased amounts of *fully functional* Recbs protein in response to DNA damage and during competence, while the *recA1* mutant cannot. However, in contrast to the marked deficiency in induction of the SOS-like response after DNA damage or during competence, the *recA1* mutation does not appear to effect significantly Recbs protein amplification in competent cells (LOVETT, LOVE and YASBIN 1989; Table 5). Thus, at the time of DNA damage, competent *recA1* cells have levels of Recbs that are comparable to wild-type (YB886; *rec*$^+$, *uvr*$^+$) competent cells in either UV-DNA and DNA-UV experiments. On the other hand, noncompetent *recA1* cells have neither the amplified levels of Recbs present in the competent cells, or the ability to induce Recbs protein and other SOS-like repair functions in response to UV irradiation, thereby putting them at a significant disadvantage in both treatments. This intrinsic disadvantage of noncompetent cells operates in both UV-DNA and DNA-UV treatments and may explain the larger increases observed in this strain. Nevertheless, there remains the significant difference between UV-DNA and DNA-UV experiments as shown in Figures 4 and 5.

***A priori* considerations:** In our previous paper (MICHOD, WOJCIECHOWSKI and HOELZER 1988), based on *a priori* considerations, we estimated the fitness benefit of transformational repair to be approximately $1+\mu x$, where μ is the number of lethal hits (a number around 2 or 3) and x the transformation rate at damaged sites. If we assume that the transformation rate at damaged sites is of the same order as the transformation rate at our marker loci, this estimated benefit is small. For this reason we felt that induction of transformation by UV played a significant role in our results. However, results of the present study indicate that UV induction of the SOS-like system, increased binding and uptake of DNA after UV treatment, excision repair, or differences in timing between experiments cannot explain the general result that transformed cells increase in relative density with increasing dosage of UV more in a UV-DNA experiment than in a DNA-UV experiment. Comparisons between these treatments in the most controlled case of the *recA1* mutant in delay experiments (Figure 5) indicates a fitness advantage of transformational repair to be around two, similar to the difference observed in YB886 (*rec*$^+$, *uvr*$^+$; Figure 6). Although there still remains a possibility of induction contributing to this effect, this possibility should be small in the *recA1* strain. One way to reconcile this effect with the *a priori* estimate of $1+\mu x$ is to assume that transformation at damaged sites is much greater than that measured at marker loci. This could be the case, because more DNA is taken into a damaged cell (although this conflicts with our plasmid DNA result), uptake and/or recombination (transformational repair) is targeted to damaged sites or because of an error-prone recombination process by which DNA which is not exactly homologous to the damaged site is used as template for recombinational repair. Any of these alternatives would tend to increase x in the above equation. Which, if any, of these alternatives is correct is presently unknown, although we are in the process of testing whether transformation is targeted to damaged sites in *B. subtilis*.

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