DNA Repair and the Evolution of Transformation in the Bacterium *Bacillus subtilis*

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ABSTRACT

The purpose of the work reported here is to test the hypothesis that natural genetic transformation in the bacterium *Bacillus subtilis* has evolved as a DNA repair system. Specifically, tests were made to determine whether transformation functions to provide DNA template for the bacterial cell to use in recombinational repair. The survivorship and the homologous transformation rate as a function of dose of ultraviolet irradiation (UV) was studied in two experimental treatments, in which cells were either transformed before (DNA-UV), or after (UV-DNA), treatment with UV. The results show that there is a qualitative difference in the relationship between the survival of transformed cells (sexual cells) and total cells (primarily asexual cells) in the two treatments. As predicted by the repair hypothesis, in the UV-DNA treatment, transformed cells had greater average survivorship than total cells, while in the DNA-UV treatment this relationship was reversed. There was also a consistent and qualitative difference between the UV-DNA and DNA-UV treatments in the relationship between the homologous transformation rate (transformed cells/total cells) and UV dosage. As predicted by the repair hypothesis, the homologous transformation rate increases with UV dose in the UV-DNA experiments but decreases with UV dose in the DNA-UV treatments. However, the transformation rate for plasmid DNA does not increase in a UV-DNA treatment. These results support the DNA repair hypothesis for the evolution of transformation in particular, and sex generally.

SEXUAL systems in both eukaryotes and prokaryotes involve two basic components, (1) recombination, in the sense of the breakage and reunion of two homologous DNA molecules, and (2) outcrossing, in the sense that the two homologous DNA molecules involved in recombination come from two different individuals (for discussion, see MICHOD and LEVIN 1987). Both recombination and outcrossing occur in natural transformation, a process in which some bacteria take up, and recombine into their own genome, DNA released from other bacteria or provided experimentally.

The purpose of the work reported here is to test the DNA repair hypothesis (BERNSTEIN, BYERS and MICHOD 1981; BERNSTEIN *et al.* 1984, 1985a–c; BERNSTEIN, HOPF and MICHOD 1987a, b) as an explanation for the evolution of natural transformation in the eubacterium *Bacillus subtilis*. Because many of the molecular and cellular details of transformation (for review, see DUBNAU 1982) and DNA repair (for review, see YASBIN 1985) have been elucidated, *B. subtilis* provides an ideal organism to experimentally address predictions of the repair hypothesis. From what is known about the mechanisms involved, natural transformation in *B. subtilis* appears to be a highly evolved trait. It results from a complex, energy-requiring, developmental process and not passive entry of DNA into the cell or artificial manipulation of the cell. Its genetic control involves genes involved in both homologous recombination and DNA repair. However, although much is known about the mechanisms involved, the evolutionary function of natural transformation is poorly understood.

The repair hypothesis argues that the evolutionary function of transformation lies in its role in providing DNA template for recombinational repair of genetic damages. To study this hypothesis, we have measured the densities of transformed cells and total cells at different DNA concentrations in the presence of increasing levels of UV radiation. Under common laboratory conditions, a culture of *B. subtilis* grown to competence is a mixture of predominately noncompetent (asexual) cells and a minority of approximately 10–20% competent (sexual) cells (SOMMA and POLSINELLI 1970; DUBNAU 1982). Competence refers to the physiological state of the cell in which it can be transformed, that is, in which it can bind, take up and recombine free DNA which may have been released from another cell or provided experimentally. Because the total population of a competent culture is predominately composed of asexual cells, we view comparisons of transformed cells with total cells as indicative of comparisons of sexual and asexual cells. However, because the population of total cells is a mixture of asexual and sexual cells, the differences we observe between the transformed cells and the total

cells presumably underestimate the actual differences between sexual and asexual cells.

MATERIALS AND METHODS

Bacterial strains, plasmid and media: We have studied transformation and DNA repair in *B. subtilis* strain YB886 (*trpC2, metB5, amyE, sigB, xin-1, SPβ⁻*). This strain is a derivative of the naturally competent, recombination- and repair-proficient (*rec⁺*) strain *B. subtilis* 168, and has been cured of bacteriophage SPβ and rendered noninducible for the endogenous prophage PBSX (YASBIN, FIELDS and ANDERSEN 1980). Strain YB1011 (*xin-1*, SPβ⁻), a prototrophic (Trp⁺, Met⁺) derivative of strain YB886 (B. FRIEDMAN and R. YASBIN, personal commmunication) was used as a source of homologous chromosomal DNA (donor) for the transformation experiments. Chromosomal DNA was isolated from strain YB1011 using a modification of the method of SILHAVY, BERMAN and ENQUIST (1984).

Plasmid pMK4, an ampicillin-resistant and chloramphenicol-resistant bifunctional plasmid which replicates in both *B. subtilis* and *Escherichia coli* (SULLIVAN, YASBIN and YOUNG 1984), was obtained from P. E. LOVE. Plasmid DNA (form I) was isolated from host strain *E. coli* JM83 (VIEIRA and MESSING 1982) by a modified alkaline lysis procedure and purified by CsCl-ethidium bromide density gradient centrifugation as described by MANIATIS, FRITSCH and SAMBROOK (1982) (propagation of plasmid DNA in *E. coli* was done to increase the frequency of multimeric forms in the DNA preparation).

Dilutions and suspension of cells were done in SPIZIZEN (1958) minimal salts (1×SS) unless otherwise stated. SPIZIZEN 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.

Genetic procedures: Liquid cultures of *B. subtilis* YB886 were grown to maximize competence for chromosomal or plasmid DNA transformation essentially as described by BOYLAN *et al.* (1972). An overnight (12–15 hr) plate culture of the recipient strain, grown on TBAB medium (Tryptose blood agar base, Difco Laboratories, Detroit, MI) at 37°, was used as inoculum for GM1 medium. Cultures of recipient cells were grown in GM1 at 37° with vigorous shaking until 90 min following the end of exponential growth (designated as T_0, growth was monitored using a Klett-Summerson colorimeter, No. 66 filter). At T_0 + 90 min the cells were then diluted tenfold into warm GM2 medium and incubated for 60 min with aeration before UV irradiation or the addition of transforming DNA. The type and amount(s) of DNA added for transformation of competent cells was as follows.

Chromosomal DNA from *B. subtilis* YB1011 was added to competent cultures at concentrations ranging from 10 ng/ml to 2 μg/ml. After a 30-min incubation at 37° with aeration, DNase I (Sigma, St. Louis, MO) was added to 100 μg/ml and the cultures were incubated an additional 10 min. Cells were then diluted appropriately and plated immediately. Total numbers of viable cells were determined on both TBAB medium and MG minimal medium supplemented with methionine and tryptophan (denoted as MG⁺ medium below), since there have been reports of different survival on the two media used (YASBIN 1977). TBAB medium is a rich medium and gives more consistent results, however, MG⁺ medium is closer to the medium used to screen for the transformed cells as it differs from it only by the addition of the amino acid tryptophan which allows cells with the *trpC2* mutation to survive. The numbers of Trp⁺ revertants were determined on MG medium supplemented only with methionine. Trp⁺ revertants are unlikely since the *trpC2* mutation is believed to be a small deletion (R. YASBIN, personal communication). Nevertheless, Trp⁺ revertants were assayed for in each experiment by plating a sample of the culture which had no DNA added on MG medium supplemented only with methionine. Mutation rates were always less than 10^{-7}. Transformation at the *metB5* locus was studied in a similar manner.

Plasmid pMK4 DNA was added to competent cultures at a concentration of either 0.5 or 2.0 μg/ml, and the cultures were incubated with aeration for 10 min prior to the addition of DNase I. Cells transformed with pMK4 DNA (for chloramphenicol-resistant transformants) were diluted accordingly, and poured onto 20–25 ml LB plates (MILLER 1972) in a 2.5-ml semisolid LB agar overlay. Following a subsequent 90 min incubation at 37° these cells were challenged by a second LB semisolid overlay containing 125 μg of chloramphenicol. Numbers of total viable cells were determined on LB medium. All plates were incubated at 37° for 12–48 hr before being counted.

UV irradiation of competent cultures: For the experiments in which competent cells were UV-irradiated before the addition of transforming DNA (termed UV-DNA experiments), the following protocol was used. Samples (8–10 ml) of the competent culture (2–7 × 10^7 CFU/ml) were treated with UV light (254 nm) in a 100-mm glass petri dish with constant stirring. Immediately after irradiation, the cells were centrifuged (5000 × *g*, 10 min, 25°), suspended in the same volume of warm (37°) GM2 and transformed with either YB1011 DNA or pMK4 plasmid DNA as described above. Reverse experiments (termed DNA-UV experiments), in which cells were transformed with DNA prior to UV irradiation, were performed in the following manner. A competent culture was transformed with YB1011 DNA at the concentrations described above. Following the incubation with DNase I, the cells were collected by centrifugation (5000 × *g*, 10 min, 4°), resuspended, irradiated as just described, and plated on the appropriate media.

The UV light source used in these experiments was a 15-W germicidal lamp (Sylvania G8T5). UV light intensities were determined using a Blak-Ray UV meter (UltraViolet Products, number J225).

RESULTS

Our primary results involve comparing survivorships and transformation rates in DNA-UV and UV-DNA treatments. In these treatments cells were transformed with homologous chromosomal DNA either before (DNA-UV), or after (UV-DNA), treatment with UV. In the UV-DNA treatment the repair hypothesis predicts an increasing frequency of sexual cells relative to asexual cells with increasing dose of UV, either as a result of induction of transformation by UV or as a result of greater survivorship of sexual cells stemming from "transformational repair." We define transformational repair as the use of homologous DNA brought into the cell during competence as template in recombinational repair of chromosomal damages. In the DNA-UV treatment we do not predict an increase in the transformation rate with increasing UV, since transformation should have al-

ready taken place before exposure to UV. All previous work involving DNA repair and transformation in *B. subtilis* that we are aware of (*e.g.*, MITA, SADIE and KADA 1983; YASBIN 1977) has dealt with competent cells which were transformed prior to treatment with UV, as was done in our DNA-UV treatments.

Log transformations were taken of all data in Figures 1, 2, and 4 for the purpose of statistical analysis since this transformation made the variances more homoscedastic. Means of the raw data were compared graphically to the means of transformed data and in all cases, the means of the transformed data differed very slightly from the means of the untransformed data. In addition to the means and standard errors, regression curves are also plotted in the figures. Coefficients for the regression curves and measures of fit are provided in Table 1. When regression lines are compared to each other in the following paragraphs, they were compared using the tests given in NETER and WASSERMAN (1974, pp. 160–167). Regression and analysis of covariance analyses were performed using the GLM and REG procedures of the SAS (Statistical Analysis System) computer package.

Cell survivorship in homologous DNA experiments: Survival data for the three classes of cells and for the two treatments is given in Figures 1 and 2 for experiments in which homologous chromosomal DNA was added to cells grown to competence. The two treatments are UV-DNA and DNA-UV and the three classes of cells are total cells, plated either on TBAB or MG^+, and Trp^+ transformants. The survival points are given as the average of $\log(N_{UV}/N_0)$, where N_{UV} is the number of cells surviving a given UV dose and N_0 is the number at zero UV dose. It is important to realize that "survivorship" calculated in this manner could be influenced by other factors in addition to mortality such as induction of transformation by UV.

Survival data for the three classes of cells are plotted for the UV-DNA treatment in Figure 1A and for the DNA-UV treatment in Figure 1B. The data from experiments using different DNA concentrations (0.01, 0.10, 1.0 and 2.0 μg/ml) are pooled in Figure 1, A and B. As detailed in Table 1, all linear regression lines have an estimated slope which is significantly different from zero whereas the estimated intercept is not different from zero. A linear model for the MG^+ data in the UV-DNA treatment (Figure 1A) did not fit the data and a quadratic function with no intercept term was fit to the data. This gave estimated coefficients which were both statistically different from zero (Table 1).

The results in Figure 1, A and B, show that there is a qualitative difference in the relationship between the survival of the transformed cells and total cells in the UV-DNA and DNA-UV treatments. As predicted by the repair hypothesis, in the UV-DNA treatment

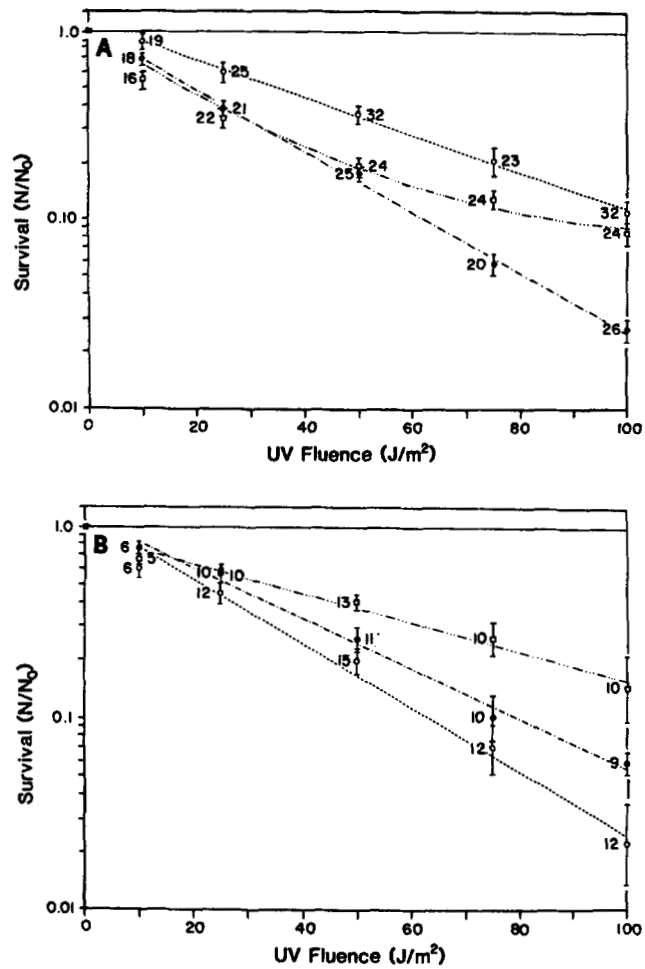

FIGURE 1.—Survival curves for YB886. Survival curves are given for three classes of cells subjected to the two treatments UV-DNA and DNA-UV. Points plotted are averages of $\log(N_{UV}/N_0)$ over multiple experiments. The data from experiments using different DNA concentrations (0.01, 0.1, 1.0 and 2.0 μg/ml) are pooled. Error bars give mean ± the standard error. Sample size is given near each mean. Regressions are graphed for each set of data. See text for further explanation. See Table 1 for information on regressions. Figure 1a gives results for UV-DNA treatments. Figure 1B gives results for DNA-UV treatments. Key for means: Total cells plated on TBAB (●), total cells plated on MG^+ (□); Trp^+ cells transformed with homologous DNA (○). Key for regression: Total cells plated on TBAB (·–·–), total cells plated on MG^+ (· · ·–); Trp^+ cells (——).

(Figure 1A), transformed cells had greater average survivorship than total cells, while in the DNA-UV treatment (Figure 1B) this relationship was reversed. The three regression lines in Figure 1A, and the three regression lines in Figure 1B, are different from one another at the $P < 0.001$ level. The average survival of total cells was greater at higher UV doses when plated on MG^+ than when plated on TBAB (Figure 1; $P < 0.0001$). In addition, the survivorship curves for total cells (TBAB and MG^+) in the DNA-UV treatment (Figure 1B) are higher than the corresponding curves in the UV-DNA treatment (Figure 1A). This is because the UV treatment is administered after

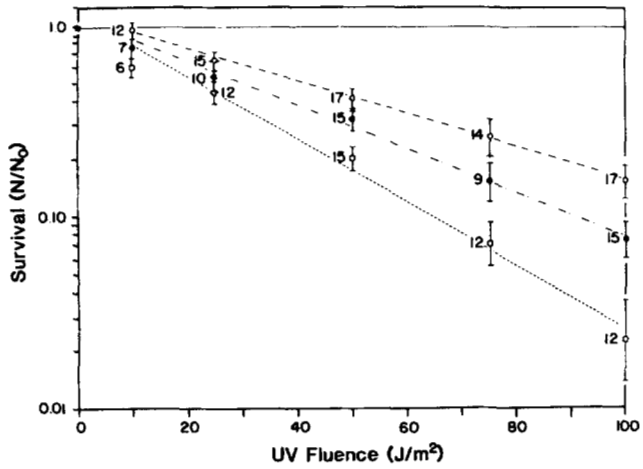

FIGURE 2.—Survival curves for YB886 Trp$^+$ transformants for homologous transformation. Points plotted are averages of $\log(N_{UV}/N_0)$ over multiple experiments. Error bars give mean ± standard error. Sample size is given by each mean. See text for further explanation and discussion of statistical tests. In the UV-DNA treatment, results are pooled for 0.01 and 0.1 μg/ml and for 1.0 and 2.0 μg/ml. All DNA concentrations are pooled for DNA-UV treatment. For information on plotted regressions see Table 1. Key for means: UV-DNA, Trp$^+$, 0.01 and 0.1 μg/ml pooled (○); UV-DNA, Trp$^+$, 1.0 and 2.0 μg/ml pooled (●); DNA-UV, Trp$^+$, 0.1 and 1.0 and 2.0 μg/ml pooled (□). Key for regressions: UV-DNA, Trp$^+$, 0.01 and 0.1 μg/ml pooled (– – –); UV-DNA, Trp$^+$, 1.0 and 2.0 μg/ml pooled (· · · –); DNA-UV, Trp$^+$, 0.1 and 1.0 and 2.0 μg/ml pooled (——).

an additional 40 min of growth in transformation medium in the DNA-UV treatment as compared to the UV-DNA treatment. This additional time results in higher cell densities and hence lower effective killing in the DNA-UV treatments (data not shown). In spite of this fact that there is less killing in the DNA-UV treatment for a specified UV dosage, the Trp$^+$ cells did worse at each UV dosage in the DNA-UV treatments than in the UV-DNA treatments (Figure 1, A and B; see also Figure 2). The two regression lines for Trp$^+$ cells in Figure 1, A and B (UV-DNA and DNA-UV treatments respectively), are statistically different in slope at the $P < 0.001$ level. In addition, using an analysis of covariance, we found that the interaction of treatment (UV-DNA and DNA-UV) with UV was statistically significant for Trp$^+$ cells at the $P < 0.0001$ level.

In Figure 2, the survival of Trp$^+$ cells only are plotted for the UV-DNA and DNA-UV treatments (same data as in Figure 1). The two highest (1.0 and 2.0 μg/ml) and two lowest (0.01 and 0.10 μg/ml) DNA concentrations are pooled separately for the UV-DNA treatments, whereas all DNA concentrations are pooled for the DNA-UV treatment. There was no detectable effect of DNA concentration on cell survival except for the Trp$^+$ cells in the UV-DNA treatments (Figure 2). As detailed in Table 1, all regressions have an estimated slope which is significantly different from zero while the estimated intercept did

TABLE 1

Regression analyses for data in figures 1, 2, 4 and 5

	Coefficients	r^2
Survivorship		
UV-DNA		
MG$^+$ [a]	−0.0180****, 0.000081****	0.91
TBAB	−0.0160****	0.84
TRP$^+$ (all)[b]	−0.0099****	0.54
TRP$^+$ (low)[c]	−0.0086****	0.50
TRP$^+$ (high)[d]	−0.0110****	0.64
DNA-UV		
MG$^+$	−0.0070****	0.39
TBAB	−0.0130****	0.77
TRP$^+$ (all)	−0.0170****	0.62
Transformation Rate		
UV-DNA		
TBAB (high)	0.0035**	0.23
TBAB (low)	0.0077****	0.43
MG$^+$ (high)[e]		
MG$^+$ (low)[e]		
Plasmid DNA	0.0014	0.04
DNA-UV		
TBAB (all)	−0.0028****	0.43
MG$^+$ (all)	−0.0083****	0.52

Data are for homologous DNA experiments except where noted otherwise. Tests for differences between regressions are discussed in the text. In all cases the estimated intercept term of the regression was not different from zero. ** $P < 0.01$; **** $P < 0.0001$.

[a] The coefficients correspond to the x and x^2 terms, respectively.
[b] All DNA concentrations are pooled.
[c] Low DNA concentrations, 0.1 and 0.01 μg/ml, pooled.
[d] High DNA concentrations, 1.0 and 2.0 μg/ml pooled.
[e] No regressions could be fit with coefficients significantly different from zero.

not differ significantly from zero (1.0 on a log scale). As shown in Figure 2, the concentration of DNA affected a transformed cell's survival in the UV-DNA treatment, with increased survival in the face of UV at lower DNA concentrations (see also Figure 4). In an analysis of covariance, this interaction effect of DNA concentration and UV in the UV-DNA treatment is statistically significant at the $P < 0.0001$ level. The two regression lines for the two pooled DNA concentrations in the UV-DNA treatments are statistically different in slope at the $P < 0.001$ level. Therefore the greater survivorship of Trp$^+$ cells as compared to total cells shown in Figure 1a obscures the even greater differences which exist at lower DNA concentrations.

Transformation rates for homologous DNA experiments: Figure 3 shows that the absolute transformation rates increased with the concentration of homologous DNA over the range of DNA concentrations from 0.01, μg/ml to 2.0 μg/ml, with saturation at levels greater than 1.0 μg/ml. Although not shown here, transformation rates at the *metB5* locus were similar.

It is informative to plot the relationship between

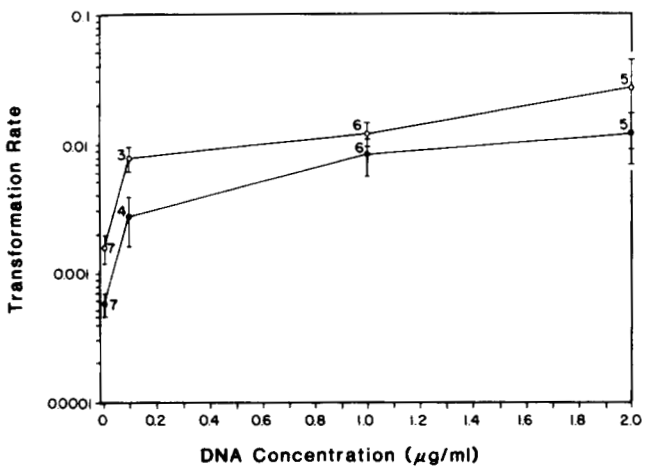

FIGURE 3.—Transformation rate as a function of homologous DNA concentration. Results are given for total viable cells plated on MG$^+$. Similar results were obtained on TBAB. Error bars give mean ± standard error. Sample size is given by each mean. See text for further explanation. Key: strain YB886 at 0 J/m^2 (●); strain YB886 at 50 J/m^2 (○) in UV-DNA treatment.

the transformation rate and UV dosage for the same experiments plotted in Figures 1 and 2, since the transformation rate preserves the relationship between the numbers of transformed cells and the numbers of total cells at each UV dose for each particular experiment. This relationship is lost when only the survivorships of cells are compared (Figures 1 and 2). The transformation rate at a given UV dosage is the following ratio,

$$\frac{N_{\mathrm{Trp}^+,\mathrm{UV}}}{N_{\mathrm{Tot,UV}}},$$

in which $N_{\mathrm{Trp}^+,\mathrm{UV}}$ and $N_{\mathrm{Tot,UV}}$ are the numbers of transformants and total cells (either on MG$^+$ or TBAB), respectively, which survive a given UV dose. Because of the difference in the absolute magnitude of the transformation rate at different concentrations of transforming DNA (Figure 3), we calculated for each experiment the ratio of the transformation rate at each UV dose to the transformation rate at 0 J/m^2 UV dose. Averages of the log of this ratio over multiple experiments are termed "average factor increase" in Figure 4. Values greater than 1.0 indicate an increase, and values less than 1.0 indicate a decrease, in the transformation rate after UV treatment from what it was with no UV (0 J/m^2).

Figure 4, A and B, plots the average factor increase for the two kinds of media used to determine total cells. As indicated in Figure 4, there is a consistent and qualitative difference between the UV-DNA and DNA-UV treatments in the relationship between the transformation rate and UV dosage for the *rec*$^+$ strain (YB886). In this strain, the transformation rate increases with UV dose in the UV-DNA experiments but decreases with UV dose in the DNA-UV treatments. This explains the positive association of com-

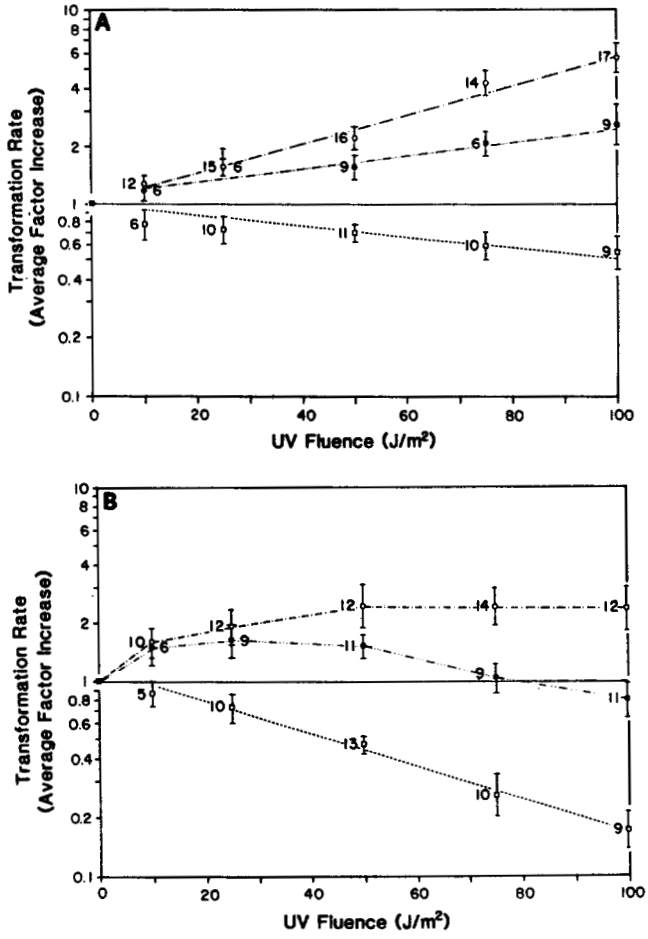

FIGURE 4.—Change in transformation rate as a function of UV dosage for YB886 for homologous DNA transformation. Points given are averages of the log of the ratio of the transformation rate at the specified UV dose divided by the transformation rate at the no UV dose for the same experiment. Transformation rate at a given UV dose is defined as $N_{\mathrm{Trp}^+,\mathrm{UV}}/N_{\mathrm{Tot,UV}}$. Total cells counted on TBAB (A) and total cells counted on MG$^+$ (B). Error bars give mean ± standard error. Sample size is given by each mean. See text for further explanation and discussion of statistical tests. See Table 1 for information on regression equations. Key for means: UV-DNA treatment, 0.01 and 0.1 μg/ml pooled (○); UV-DNA treatment, 1.0 and 2.0 μg/ml pooled (●); DNA-UV treatment, 0.1 and 1.0 and 2.0 μg/ml pooled (□). Key for regressions: UV-DNA treatment, 0.01 and 0.1 μg/ml pooled (·–·–); UV-DNA treatment, 1.0 and 2.0 μg/ml pooled (· · · –); DNA-UV treatment, 0.1 and 1.0 and 2.0 μg/ml pooled (——).

petence and damage demonstrated by YASBIN (1977), LOVE and YASBIN (1984) and LOVE, LYLE and YASBIN (1985) by showing that damage causes increased transformation rather than transformation causing increased repair.

In the UV-DNA treatments, the concentration of DNA affects the magnitude of this increase with lower DNA concentrations giving a steeper rise in the average factor increase with increasing UV dose (Figure 4, Table 1), even though the absolute transformation rate increases with DNA concentration for a fixed UV dose (Figure 3). On TBAB (Figure 4A), the two

UV-DNA regressions have an estimated slope which is different from zero and an estimated intercept which is not significantly different from zero (one on a log scale). These two regressions for high DNA concentration (1.0 and 2.0 μg/ml) and low DNA concentration (0.01 and 0.10 μg/ml) are statistically different from each other in slope ($P < 0.001$). The regressions for 0.01 and 0.10 μg/ml were not statistically different from one another, nor were the regressions for 1.0 and 2.0 μg/ml. For this reason the data in the UV-DNA treatment were pooled as indicated. In the DNA-UV treatment on TBAB (Figure 4A), the slope differs from zero at the $P < 0.001$ level (Table 1). For MG^+ medium in the DNA-UV treatment the slope is statistically different from zero at the $P < 0.0001$ level and the intercept does not differ from one. On MG^+ medium in the UV-DNA treatment no statistical model could be found which fits the data in the sense that the estimated coefficients were all significantly different from zero. Consequently, the means are simply connected by straight lines in Figure 4B. In Figure 4B, there appears to be a maximum transformation rate in the 25–50 J/m^2 range depending on DNA concentration. It is worth noting that UV induction of the major Rec protein (*recE* gene product) in *B. subtilis* is maximal in this UV range (P. LOVE and C. LOVETT, personal communication).

Upon comparing transformation rates between UV-DNA and DNA-UV experiments (Figure 4) one result is consistent. The transformation rate in UV-DNA experiments increases with increasing UV, while in the DNA-UV experiments the transformation rate decreases with increasing UV. Similar results were obtained for transformation at the *metB5* locus (unpublished results). We have attributed this result to the difference between the order of UV irradiation and addition of DNA in the two treatments. However, there is another difference between the two treatments. In the UV-DNA experiments, cells were given 30 min incubation in GM2 medium after UV irradiation to allow for transformation, whereas in the DNA-UV experiments the cells were transformed first then plated immediately after UV irradiation. It is possible that in the UV-DNA treatment, the 30-min postirradiation incubation period might allow time for some process, such as excision repair, to occur preferentially in competent cells. This could result in the differential survival of competent cells relative to the noncompetent or total population (R. YASBIN, personal communication). To determine whether this could account for our results, several sets of DNA-UV experiments, similar to those described in the experimental procedures, were performed. YB886 cells were grown to competence in GM1-GM2, transformed with 1 μg/ml of YB1011 DNA for 30 min and then exposed to UV doses of 25, 50, 75 and 100 J/m^2. A sample of these cells were then diluted and plated. A second sample was resuspended in GM2 and incubated with aeration for 30 min before plating. We find no detectable difference in the survival of transformed cells between those plated immediately after UV damage and those given a 30 min incubation in growth medium before plating (data not shown). These results indicate that the difference in survival of transformed cells (*i.e.*, competent cells), as well as total cells, in the UV-DNA and DNA-UV experiments was not due to this aspect of the experimental procedure.

In the DNA-UV treatments, it is possible that transforming DNA was damaged by the UV irradiation. We think that this is an unlikely possibility, since studies on the kinetics of transformation indicate that transformation takes only a few minutes to be completed (DUBNAU 1982). Consequently, we expect transformation to be completed at the time of the UV treatment. Nevertheless, it is conceivable that UV-damaged DNA could be taken up by competent cells and integrated thereby resulting in a decrease in the transformation rate in our DNA-UV treatment. To test this hypothesis, competent cells were incubated with YB1011 DNA at 1 μg/ml, UV-irradiated at 50 J/m^2 at various times from 10 to 120 min after the addition of transforming DNA, and then plated as described previously. Transformation frequencies were maximal when cells were incubated with transforming DNA for 30–45 min prior to UV irradiation (data not shown) as was done in our standard DNA-UV treatment. This same period of time also resulted in maximal transformation rates in the un-irradiated controls. Thus, under the conditions employed in our standard DNA-UV treatment, the transformation rate was not decreased as a result of the incoming DNA being subject to UV-induced damage prior to its incorporation into the recipient chromosome.

Transformation rates in plasmid DNA experiments: The increase in homologous (chromosomal) DNA transformation rates with increasing UV dose observed in our UV-DNA treatments prompted us to further investigate the nature of this effect. First, competent cells might benefit from homologous DNA brought into the cell from transformational repair, resulting in higher survivorship in a damaging environment than noncompetent cells. As a consequence, transformation rates would increase with increasing dose of UV irradiation. Second, UV irradiation might induce higher rates of transformation, either because DNA lesions are recombinogenic or because of induction of enzymes involved in homologous recombination, binding or uptake of DNA. To examine these alternative hypotheses, competent YB886 (*rec*$^+$) cells were treated with UV and transformed with limiting (0.5 μg/ml) as well as saturating (2.0 μg/ml) levels of plasmid pMK4 DNA, as was done in the chromosomal

UV-DNA experiments. In *B. subtilis*, plasmid DNA-mediated transformation proceeds by the same pathways of binding, uptake, and processing as linear duplex chromosomal DNA, but does not require homologous recombination (*rec*) functions to achieve transformation, since the transforming DNA is not recombined into the bacterial chromosome but simply resides in the cell as an extrachromosomal DNA element (DUBNAU 1982). Therefore, transforming plasmid DNA should not result in any repair benefit to a competent cell which depends upon homology nor should plasmid transformation rates increase as a direct result of increased synthesis of proteins involved in homologous recombination.

There is no statistically significant effect of UV dose on plasmid transformation rates (Table 1). A plot of the means over ten experiments suggests a trend of increasing transformation rate with increasing UV (data not shown). However, this trend is not even close to significance at the $P = 0.05$ level.

DISCUSSION

The central result of our experiments is that an increased level of UV radiation increased the homologous DNA transformation rate in the case of the UV-DNA experiments and decreased the transformation rate in the case of the DNA-UV treatments. We suggest three hypotheses for why the transformation rate might change with increasing prior dose of a damaging agent. (1) Transformation may be inducible by DNA damage. For example, the transformation rate might be higher at a higher UV dose because cells bind, take up or recombine more DNA at the higher dose than at a lower dose. (2) Competent cells and noncompetent cells may have different survivals in a damaging environment. For example, competent cells might have a lower survival than noncompetent cells because competence is costly (this appears to be a factor) (NESTER and STOCKER 1963; M. A. HOELZER, unpublished data). On the other hand, competent cells might have a higher survival than noncompetent cells either (2a) because of transformational repair or (2b) because competent cells have more effectively induced SOS-associated DNA repair (not involving transformational repair) than non-competent cells. This induced SOS-associated repair could result from the competence-specific amplification of cellular levels of the major recombination protein in *B. subtilis* (*recA* analog) that is independent of any DNA damage (LOVE 1986; P. LOVE and C. LOVETT, personal communication). (3) The cells which become competent may be more hardy cells to begin with and so survive a challenge like UV radiation better than noncompetent cells.

We think that the third hypothesis is an unlikely explanation for our results. It is difficult to see how differences in the initial health of cells could explain the qualitative difference between the DNA-UV and UV-DNA results, since these differences should be present in both treatments.

It is also difficult to see how hypothesis (2b) could explain the difference between the DNA-UV and UV-DNA treatments. To explain the difference, in particular the decreasing transformation rate in the DNA-UV experiments, hypothesis (2b) would have to postulate that transformation of competent cells results in an inhibition of the UV-inducible SOS response and/or normal levels of excision repair in competent cells. However, just the opposite is more likely to be the case, because of the higher levels of the major recombination protein with concomitant induction of the SOS response (P. LOVE and C. LOVETT, personal communication) and higher levels of single-stranded gapped regions (HARRIS and BARR 1971) in competent cells.

This leaves transformation induction by UV (hypothesis 1) or direct fitness differences due to transformational repair (hypothesis 2a) as likely explanations for our results. Note that there is the possibility of transformation induction or a fitness benefit from transformational repair in the UV-DNA treatments but not in the DNA-UV treatments. As already pointed out either hypothesis supports the view that transformation functions in DNA repair. In the case of transformation induction by damage, cells are inducing sex in precisely the environment predicted by the repair hypothesis.

The lack of any significant effect of prior UV dosage on the plasmid transformation rate (Table 1) rules out UV induction mediated through increased binding and/or uptake of DNA as the explanation for our homologous DNA results. However, it does not rule out UV induction mediated through a homologous recombination pathway, since plasmid transformation does not depend on homologous recombination functions. The plasmid result is also consistent with hypothesis (2a), since there should be no fitness benefit from plasmid DNA, and, indeed, the effect of UV dose on transformation, which is so apparent in the homologous UV-DNA experiments, goes away in the plasmid UV-DNA experiment. Thus, although our empirical results do not conclusively distinguish between transformation induction or transformation repair, they clearly support the repair hypothesis.

There is an *a priori* reason to favor transformation induction over fitness differences as the explanation for our results, since the differences in frequencies observed here are larger than we expect based solely on a simple mechanistic model of the fitness effects of transformational repair. If we assume that a damaged site is the same size as the site of the *trpC2* mutation, then, all other things being equal, the probability of

transformational repair of a particular lethal damage should approximately equal the transformation rate. Assuming that transformational repair benefits a cell by repairing a single lethal damage and that the distribution of damages is Poisson, a simple calculation shows the benefit of transformational repair to be only $1+\mu x$, where μ is the mean number of lethal DNA damages per cell and x is the transformation rate. Since x is 10^{-2}–10^{-4} (Figure 3) and μ can be calculated to be between 1 and 3 in our experiments, the calculated benefit on these assumptions is small. However, there are several factors which are ignored by this calculation. First, competent cells may take in more DNA after they are damaged then when they are undamaged, although this appears unlikely in light of our plasmid DNA results discussed above. Second, there may be targeted uptake of DNA homologous to damaged sites. Nevertheless, it seems unlikely based on first principles that fitness differences *directly* explain the differences in transformation rates observed here in the DNA-UV and UV-DNA experiments. However, such small fitness differences may be *indirectly* responsible for our results. When operative over long periods of time, small fitness effects can select for large phenotypic responses, such as induction of transformation in damaging environments.

Since competent cells bind DNA, it is not surprising that there is an effect of the level of DNA concentration on the change in transformation rate with prior UV. It appears that saturating amounts of transforming DNA (1 and 2 μg/ml experiments) are less beneficial, or possibly deleterious, to competent cells when they are in a DNA-damaging environment. It can be estimated that these saturating levels of DNA correspond to the equivalent of four to eight chromosomes (*ca.* 5000 kilobase pairs of DNA per chromosome) on a per cell basis, whereas, in our 0.01 and 0.1 μg/ml treatments there are approximately 0.025 to 0.25 chromosome equivalents for every cell. We believe that the lower DNA concentrations more closely approximate natural and physiologically relevant levels of DNA, however the exact nature of the DNA effect remains unresolved by our experiments.

We have used a strain of *B. subtilis* which has no DNA damage-inducible prophages. Such phages occur in laboratory strains (168 and derivatives) and in natural strains and are induced by UV more efficiently in competent than in noncompetent cells (YASBIN 1977; F. COHAN, unpublished data). Consequently they represent an additional cost of sex which is not addressed in the work reported here. We have chosen to ignore this effect in the present work for two reasons. First, we wish to test whether there is a benefit and/or induction of transformation in a damaging environment. It is another matter, one which we intend to address in subsequent work, whether any benefit and/or induction of transformation is overcome by the cellular costs of transformation. Second, although prophage induction appears to be a cost of competence in this particular organism and perhaps in other species of naturally competent bacteria, we do not see it as a cost of sex in general. By the repair hypothesis the benefit of repair should be a general one and, in an initial study, we do not wish to study its effects when they are confounded by factors which do not generalize in a similar manner.

We have chosen UV radiation as a damaging agent for several reasons. First, it occurs naturally and presumably is an important factor in the survival of *B. subtilis*, which has evolved a variety of mechanisms to cope with it (for review see YASBIN 1985). Second, UV radiation is easy to work with and pulse dosage is possible. Finally, although UV induced damages are primarily of the single-strand variety (HARM 1980), there appears to be sufficient numbers of double-strand damages introduced by UV for them to be a major source of cell mortality. For example, we expect (R. E. MICHOD, unpublished calculations) an average of 2–3.3 cross-links per genome for the lower UV fluences used in our experiments. Only a single unrepaired interstrand cross-link is sufficient to kill an *E. coli* cell (MAGANA-SCHWENCKE *et al.* 1982). Consequently, double-strand damages introduced by UV are expected to be an important source of mortality in our populations.

The work reported here was primarily undertaken with the aim of testing predictions of the repair hypothesis and was not intended to conclusively distinguish between the repair hypothesis and variation hypotheses (see appropriate chapters in MICHOD and LEVIN 1987) or food hypotheses (STEWART and CARLSON 1986) as explanations for natural transformation. However, it is worth noting that a variation based hypothesis could not explain the differences observed here, since the cultures used were clonal derivatives of a common ancestor and differed at few loci. Likewise it is difficult to see how the hypothesis that transformation functions to take in exogenous DNA for use as "food" could explain the difference observed between our UV-DNA and DNA-UV treatments.

In summary, our results support the hypothesis that the evolutionary function of transformation is to provide template for recombinational repair of genetic damage. Because we were interested in an evolutionary hypothesis, we concentrated on measuring effects at the level of cell numbers. It would not have been sufficient for our purpose to solely demonstrate on the molecular level that DNA brought into the cell is used for recombinational repair, since this would leave open the issue of an effect at the whole cell level. Nevertheless, such a confirmation at the molecular

level is needed to put our interpretation of our results on firm ground.

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