DNA Repair and the Evolution of Transformation in *Bacillus subtilis*. III. Sex With Damaged DNA

Mary A. Hoelzer and Richard E. Michod

*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721*

Manuscript received April 29, 1990
Accepted for publication January 28, 1991

**ABSTRACT**

Natural genetic transformation in the bacterium *Bacillus subtilis* provides an experimental system for studying the evolutionary function of sexual recombination. The repair hypothesis proposes that during transformation the exogenous DNA taken up by cells is used as template for recombinational repair of damages in the recipient cell's genome. Earlier results demonstrated that the population density of transformed cells (i.e., sexual cells) increases, relative to nontransformed cells (primarily asexual cells), with increasing dosage of ultraviolet irradiation, provided that the cells are transformed with undamaged homologous DNA after they have become damaged. In nature, however, donor DNA for transformation is likely to come from cells that are as damaged as the recipient cells. In order to better simulate the effects of transformation in natural populations we conducted similar experiments as those just described using damaged donor DNA. We document in this report that transformants continue to increase in relative density even if they are transformed with damaged donor DNA. These results suggest that sites of transformation are often damaged sites in the recipient cell's genome.

DNA damaging agents abound in nature, producing lesions that interfere with replication and transcription (Walker 1984). These lesions have significant effects on individual fitness as testified to by the diverse systems of DNA repair found in all living organisms, from the simplest virus to the most complicated metazoan (Bernstein, Hoff and Michod 1987, 1988). Any unrepaired double strand lesion severely reduces the changes of passing on a healthy genome and, as a result, has significant effects on fitness. Such double strand lesions are common and are repaired by recombination between homologous chromosomes. During phases of rapid cell division, bacteria, like *Bacillus subtilis* and *Escherichia coli*, have on average more than one copy of the chromosome present in each cell and so recombinational repair can occur between daughter chromosomes. However, during phases of slow growth most cells contain only a single copy of the chromosome, and, as a result, should have reduced opportunity for recombinational repair. Thus, it is significant that the bacterium *B. subtilis* becomes competent at taking in exogeneous DNA for transformation at precisely this stage when intracellular redundancy, and the opportunity for recombinational repair, is otherwise reduced.

We have previously undertaken tests of the idea that transformation in *B. subtilis* functions to provide DNA template for use in recombinational repair (Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989). In a *B. subtilis* culture grown to competence, approximately 10–20% of the cells in the culture actually become competent at transformation. The remainder of the population consists of noncompetent cells. Since the majority of cells in a culture are noncompetent at transformation, the transformation rate (population density of transformants divided by population density of total cells) provides an estimate of the ratio of transformants to what are primarily noncompetent (nonsexual) cells. In our previous work, competent cultures of cells were either first exposed to damaging UV radiation and subsequently given exogenous DNA for transformation (UV-DNA experiments), or given exogenous DNA for transformation first and then UV irradiated (DNA-UV). In previous work, the donor DNA was isolated from undamaged log-phase cells and so was, presumably, itself undamaged.

Comparison of the transformation rates in UV-DNA experiments and DNA-UV experiments revealed a statistically significant pattern, consistent with the hypothesis that transformation functions in recombinational repair (Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989). In UV-DNA experiments with homologous chromosomal DNA, the transformation rate increased with increasing dosage of UV. However, in DNA-UV experiments, in which transformation is complete before UV treatment, the transformation rate did not increase with increasing dosage of UV. With nonhomologous DNA, such as plasmid DNA, transformation can occur for certain markers carried on the plasmid. Plasmid DNA is brought into
the cell by a similar pathway as a homologous DNA, but it is not recombined into the recipient’s chromosome and, instead, resides in the cell as an extrachromosomal element. Plasmid DNA transformation does not depend on homologous recombination. Significantly, transformation rates with plasmid DNA did not increase with UV in UV-DNA experiments, whereas DNA-UV experiments behaved similarly to transformation with chromosomal DNA.

Although these previous experimental results support the hypothesis that competence and transformation functions in DNA repair, the relevant evolutionary question entails the behavior of natural populations. These previous experiments all shared the common protocol that the recipient cells were damaged by UV irradiation, whereas the exogenous donor DNA used for transformation had not been exposed to UV radiation and so was relatively undamaged in comparison to the recipient’s DNA. In addition the recipient cells were grown to competence, whereas the donor DNA was isolated from early-log phase cells. Thus, the DNA of donor and recipient differed in a number of significant ways that bear on the hypothesis under test.

Since the recipient’s DNA was damaged but the donor DNA was relatively undamaged, recipient cells might benefit from recombining undamaged DNA, regardless of whether transformation functions as a repair system in nature. In nature, donor DNA is likely to be as damaged as the recipient’s DNA. Even if recombination (transformation) is random with regard to sites of damage in the recipient’s genome, one might expect there to be an advantage to transformation under our experimental circumstances. By chance undamaged donor DNA would sometimes replace damages in the recipient’s DNA. The question then arises as to whether transformation is beneficial if both donor and recipient are equally damaged.

To address this question, we report here UV-DNA and DNA-UV experiments in which the donor DNA came from competent cells that had been exposed to damaging conditions similar to the recipient cells. Results from new experiments using DNA isolated from undamaged competent cells, were used for comparison of results with DNA isolated from damaged competent cells.

MATERIALS AND METHODS

Transformation and DNA repair were examined using the *B. subtilis* strain YB1011 (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 that has been cured of bacteriophage SPβ and rendered non-inducible for the endogenous prophage PBSX (YSABIN, FIELDS and ANDERSON 1980). Strain YB1011 (xin-1, SPβ†), a prototrophic (Trp+, Met+) derivative of strain YB886 was used as a source of homologous chromosomal DNA for the transformation experiments. Chromosomal DNA was isolated from strain YB1011 using a modification of the method of SILHAVY, BERNAN and ENQUIST (1984).

SPIZZEN (1958) minimal medium plus 0.5 glucose (MG) and competence media GM1 and GM2 were prepared as described by YAETIN, WILSON and YOUNG (1975), and supplemented with the appropriate amino acids at a final concentration of 50 μg/ml. Liquid cultures of recipient cells were grown to maximize competence for chromosomal DNA transformation as described by BOYLAN et al. (1972). An overnight plate culture of the recipient strain, grown on TBA medium (Tryptose broth agar base, Difco Laboratories, Detroit, MI) at 37° was used as inoculum for GM1 medium. Cells were grown in GM1 at 37° with vigorous shaking for 90 min after cessation of exponential growth (T0). At T0 + 90 minutes the cells were diluted tenfold into warm GM2 medium and incubated for 60 minutes with aeration prior to UV irradiation.

Damaged and undamaged DNA from *B. subtilis* YB1011 was added to competent cultures at concentrations ranging from 10 ng/ml to 2 μg/ml, depending on the experiment, and incubated for 30 min at 37° with aeration. Suspensions and serial dilutions of the cells were done in SPIZIZEN (1958) minimal salts (1 X SS), immediately prior to plating. The total number of viable cells was determined on MG minimal medium supplemented with methionine and tryptophan. Met+ transformants were counted on MG medium supplemented only with tryptophan.

UV irradiation of competent cultures: In the UV-DNA experiments 10 ml of competent culture (2-7 × 107 CFU/ml) were exposed to UV light (254 nm) in a 100-mm glass Petri dish with constant stirring. Immediately after irradiation the cells were centrifuged (500 g, 10 min, 25°) and resuspended in an equal volume of warm GM2. Cells were then transformed for 30 min at 37° with 100 ng of YB1011 DNA (0.025 to 0.25 chromosome/cell), which was either undamaged or damaged by UV irradiation. After incubation, DNaseI was added at 100 μg/ml and cultures were incubated for an additional 10 min. In the DNA-UV experiments competent cells were transformed with undamaged or damaged YB1011 DNA prior to exposure to UV irradiation. Thus, UV irradiation was administered approximately 45 min later in a DNA-UV treatment than in a UV-DNA treatment.

Cells in the UV-DNA treatments have approximately 45 min in GM2 media following UV irradiation and prior to plating during which transformation occurs. This 45-min time period after UV but before plating is an intrinsic feature of UV-DNA treatments. So as to make UV-DNA and DNA-UV treatments as similar as possible (except for the order of administration of DNA and UV) all DNA-UV treatments included a 45-min delay in GM2 following the UV treatment and prior to plating.

**UV irradiation of donor DNA:** Two methods were used to damage the donor DNA. First YB1011 DNA, at a concentration of 10 μg/ml (SETLOW and SETLOW 1962) was exposed to UV fluences of 25, or 100 J/m² with constant stirring. Second YB1011 cells were grown to competence and exposed to 50 J/m² of UV irradiation. Then DNA was isolated from these cells (using a modified SILHAVY, BERNMAN and ENQUIST (1984) protocol) to use as donor DNA in subsequent UV-DNA and DNA-UV experiments.

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

\[ N_{TOT} X N_{TRX} X N_{TRX} \]
\[ N_{TOT} X N_{TRX} X N_{TRX} \]

\[ N_{TOT} X N_{TRX} X N_{TRX} \]
respectively, \( N_{\text{TOT,X}} \) and \( N_{\text{TRANS,X}} \) are the numbers of total and transformed cells which survive a UV treatment of \( X \, \text{J/m}^2 \). 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 \( X \, \text{J/m}^2 \) is defined as the transformation rate at \( X \, \text{J/m}^2 \) divided by the transformation rate at 0 J/m². In addition to the means and standard errors, regression curves are also plotted in the Figures. When regression 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), order of the UV and DNA treatments (UV-DNA and DNA-UV), or state of donor DNA (damaged or undamaged). To test whether these factors had significant effects on survivorship and transformation rate, an \( F \) statistic was constructed using the error sum of squares for the full model (SSE₀) and for a reduced model (SSEᵣ). 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

\[
\frac{\text{SSE}_0 - \text{SSE}_r}{\text{df}_0 - \text{df}_r} < \frac{\text{SSE}_r}{\text{df}_r}
\]

where \( \text{SSE}_0 \) and \( \text{SSE}_r \) are the numbers of total and transformed cells which survived a UV treatment of \( X \, \text{J/m}^2 \). 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 \( X \, \text{J/m}^2 \) is defined as the transformation rate at \( X \, \text{J/m}^2 \) divided by the transformation rate at 0 J/m². In addition to the means and standard errors, regression curves are also plotted in the Figures. When regression 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), order of the UV and DNA treatments (UV-DNA and DNA-UV), or state of donor DNA (damaged or undamaged). To test whether these factors had significant effects on survivorship and transformation rate, an \( F \) statistic was constructed using the error sum of squares for the full model (SSE₀) and for a reduced model (SSEᵣ). 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

\[
\frac{\text{SSE}_0 - \text{SSE}_r}{\text{df}_0 - \text{df}_r} < \frac{\text{SSE}_r}{\text{df}_r}
\]

in which \( \text{df}_0 \) and \( \text{df}_r \) 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

Transformation using donor DNA damaged outside the cell: Interest in damaging naked donor DNA, prior to transformation, goes back more than 20 years. Some of the earlier results showed that UV irradiation decreases (inactivates) the transforming ability of donor DNA (Bresler, Kalinin and Perumov 1964). Subsequent work has confirmed that damage to donor DNA inactivates its transforming ability both in B. subtilis (Bresler, Kalinin and Perumov 1967; Bron and Venema 1972; Hadd 1981; Harm 1980) and Haemophilus influenzae (Rupert and Goodal 1960; Setlow 1977). Inactivation of transforming DNA does not follow first order kinetics of a Poisson distribution as is commonly observed for UV survivorship curves of damaged bacterial cells. Rather the inactivation of transforming DNA by UV irradiation has been shown to follow an inverse square law (Bresler, Kalinin and Perumov 1964; Rupert and Goodal; Hadden 1981; Bron and Venema 1972; Setlow 1977),

\[
\frac{N_0}{N_X} = 1 + bX
\]

where \( N_0 \) and \( N_X \) are the number of transformants from unirradiated and irradiated DNA respectively. \( X \) is the UV fluence used to irradiate donor DNA and \( b \) is a constant that depends on the concentration of DNA, the selection marker locus, and the genotype of the recipient cells. In order to confirm this relationship in our system, we transformed competent cells with donor DNA (isolated from log phase cells) that had been irradiated with UV fluences ranging from 25 to 500 J/m² (Figure 1). Our results (Figure 1) closely resemble those previously reported (Bron and Venema 1972; Hadden 1981) and, as shown, are well described by the inverse square equation (1).

Transformation with damaged donor DNA also involves a DNA concentration effect, in which there is a decrease in the transformation rate as the concentration of donor DNA. This concentration effect is thought to be caused by the simultaneous integration of damaged DNA at sites other than the locus of selection (the metB5 locus) (Hadden 1981; Harm 1970; Bresler, Kalinin and Perumov 1968). This DNA concentration effect is shown in Figure 2 and, when viewed in conjunction with the work of Hadden (1981), indicates that damages in the donor DNA are being integrated into the recipient's genome in our experiments. In Figure 2, \( N_{100} \) is the number of trans-
formants when naked donor DNA has been irradiated with 100 J/m². \( N_0 \) is the number of transformants resulting from donor DNA that has not been irradiated. The amount of damaged donor DNA (100 J/m²) increases along the X axis from 10 ng to 1000 ng/ml.

**UV-DNA, DNA-UV experiments with donor DNA isolated from damaged or undamaged competent cells:** We conducted UV-DNA and DNA-UV experiments with donor DNA isolated from damaged or undamaged competent cells. We first ran a comparison of DNA dose curves using conventionally isolated DNA as well as DNA isolated from competent cells. Maximal transformation at the metB5 locus in recipient strain YB886 occurred between 10 and 100 ng/ml for undamaged DNA extracted from donor strain YB1011 according to the methods of SILHAVY, HERMAN and ENQUIST (1984) as well as for undamaged DNA isolated from competent cells (data not shown). Therefore, a concentration of 100 ng/ml donor DNA was used in all subsequent transformation experiments.

The donor DNA used in all UV-DNA and DNA-UV experiments was isolated from a competent culture that was either damaged with UV radiation at a fluence of 50 J/m² or not (0 J/m²). Transformation rates for these two kinds of donor DNA are shown in Figure 3 for UV-DNA (A) and DNA-UV (B) experiments. In UV-DNA experiments the transformation rate was uniformly lower at all UV dosages (to the recipient cells) when the donor DNA was from damaged cells than when it was from undamaged cells (Figure 3A). The two regressions graphed in Figure 3A are significantly different from each other \( (P = 0.044) \). Coefficients and parameters of fit for all regressions are given in Table 1. We expect transformation rates to be lower when the donor DNA is from damaged cells based on the experiments using donor DNA that was damaged after it had been extracted from cells (Figures 1 and 2). The lower transformation rate in both UV-DNA and DNA-UV experiments at low UV dosage to recipients indicates that the donor DNA isolated from damaged cells itself contains damages. For this reason we refer to the DNA isolated from damaged and undamaged competent cells as “damaged” and “undamaged” DNA, respectively. In DNA-UV experiments the transformation rates behave similarly to UV-DNA experiments at low UV dosages to the recipient cells (in other words, rates using damaged donor are lower than those using undamaged donor), but, at higher UV fluences to the recipient cells (>50 J/m²), the curves appear to cross. However, the two regressions given in Figure 3B are not significantly different \( (P = 0.23) \).

Survivorship curves are shown in Figure 4 for UV-
**TABLE 1**

Regression analyses, YB886 recipient, donor DNA from competent cells

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Intercept</th>
<th>Coefficients</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-DNA</td>
<td>SURV, Met, pooled</td>
<td>-0.0777</td>
<td>-0.0084**</td>
</tr>
<tr>
<td></td>
<td>SURV, Tot, pooled</td>
<td>-0.0483</td>
<td>-0.0466****</td>
</tr>
<tr>
<td></td>
<td>TR, 50J to donor</td>
<td>-2.9036*****</td>
<td>0.0094*</td>
</tr>
<tr>
<td></td>
<td>TR, 0J to donor</td>
<td>-2.7068*****</td>
<td>0.0117**</td>
</tr>
<tr>
<td></td>
<td>DTR, 50J to donor</td>
<td>0.1132</td>
<td>0.0137**</td>
</tr>
<tr>
<td></td>
<td>DTR, 0J to donor</td>
<td>0.0532</td>
<td>0.0179****</td>
</tr>
<tr>
<td>DNA-UV</td>
<td>SURV, Met, pooled</td>
<td>0.0183</td>
<td>-0.0360*****</td>
</tr>
<tr>
<td></td>
<td>SURV, Tot, pooled</td>
<td>0.0217</td>
<td>-0.0370*****</td>
</tr>
<tr>
<td></td>
<td>TR, 50J to donor</td>
<td>-2.8863****</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>TR, 0J to donor</td>
<td>-2.7028*****</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>DTR, 50J to donor</td>
<td>-0.0057</td>
<td>-0.0102*****</td>
</tr>
<tr>
<td></td>
<td>DTR, 0J to donor</td>
<td>0.0458</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Tests for differences between regressions are discussed in the text and the figure legends.

1. **SURV** = survivorship, **TR** = absolute transformation rate, **DTR** = deviation (factor change) in transformation rate.
2. Intercept given as log value
3. The coefficients correspond to the X, \( X^2 \) and \( X^3 \) 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.
4. Damaged and undamaged donor DNA pooled.

DNA (Figure 4A) and DNA-UV (Figure 4B) experiments. In Figure 4 the results for the two kinds of donor DNA (either isolated from 50 J/m² or 0 J/m² cells) have been pooled, since there was no statistically significant difference between the survivorship curves for cultures treated with the two kinds of donor DNA (\( P = 0.12 \) for DNA-UV transformants, \( P = 0.75 \) for DNA-UV total cells, \( P = 0.23 \) for UV-DNA transformants, \( P = 0.16 \) for UV-DNA total cells). In UV-DNA experiments transformants survived significantly better relative to total cells, at least during the first two logs of killing (Figure 4A). However, in DNA-UV experiments, the transformants did significantly worse that total cells (Figure 4B). The two regression coefficients for total cells and transformants are significantly different at the \( P = 0.0009 \) level in UV-DNA experiments (Figure 4A) and \( P = 0.0001 \) level in DNA-UV experiments (Figure 4B).

It is important to realize that cell densities in the two treatments (UV-DNA and DNA-UV) are different at the time of exposure to UV radiation. In the DNA-UV treatment the culture has had an additional 45 min of growth prior to exposure to UV radiation. Cell division during this additional period results in cell densities at the time of UV that are approximately two fold higher in a DNA-UV experiment than they are in a UV-DNA experiment. This higher cell density translates into proportionately less killing in the DNA-UV experiment than in the UV-DNA experiment. As a result, cells in the DNA-UV treatment have higher survivorship than total cells in a UV-DNA treatment (compare total cells in panel (a) and (b) of Figure 4). However, this is not true for the transformed cells. Even though transformed cells in a UV-DNA experiment receive more effective killing at each UV dosage, they survive as well or better than those from the DNA-UV experiment.

The average factor change in the transformation rates as a function of UV dosage to the recipient cells is shown in Figure 5 for UV-DNA and DNA-UV experiments for DNA isolated from damaged cells (Figure 5A) and damaged cells (Figure 5B). Although there is not any statistical difference between the curves for undamaged or damaged donor DNA (\( P = 0.80 \) for UV-DNA experiments and \( P = 0.28 \) for DNA-UV experiments), they are presented separately for visual inspection of our conclusion that similar results are obtained for both kinds of donor DNA. These results indicate clearly that donor DNA isolated from cells that are damaged has a similar effect on the response of the transformation rate to UV as does DNA isolated from undamaged cells. In the UV-DNA experiments for both kinds of DNA the transformation rate increased with increasing UV dose to a maximum of about two fold at 50 J/m². In contrast to the UV-DNA results, the transformation rate generally decreased with increasing UV dose in DNA-UV experiments for both kinds of DNA. The difference between the UV-DNA and the DNA-UV regressions was significant in both data sets at the \( P = 0.0001 \) level. Similar results have been obtained previously for DNA isolated from undamaged, log-phase cells (Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989).

**DISCUSSION**

Partners in sex are likely to have been exposed to similar environments. For example, competent cells
in natural populations must utilize for recombination DNA available in their immediate vicinity. DNA in the immediate vicinity is likely to be provided by other cells exposed to equivalent levels of damage as the recipient cells. Strains of *B. subtilis* exchange genes when grown together in soil, a common natural habitat for *B. subtilis* (GRAHAM and ISTOCK 1979; DUNCAN et al. 1989). Our results indicate that the response of transformation rate to UV dose and the higher relative survivorship in the UV-DNA treatment remains even when the donor DNA is as damaged as the recipient’s DNA (Figure 5).

It is difficult to reconcile these results with the idea that sites of recombination bear no relation to sites of damage in the genome. If donor DNA were recombined at random, there should be no net advantage to transformation with damaged donor DNA. This is because, if donor DNA were recombined at random with respect to sites of damage, sometimes undamaged donor DNA would be swapped for damaged recipient DNA but sometimes just the opposite might happen with damaged donor DNA being swapped for undamaged recipient DNA. Thus, there should be no net advantage to recombination, if it occurred at random with respect to sites of damage. However, if recombination is targeted to damaged sites in the recipient cell's genome, damaged donor DNA should provide a benefit equivalent to undamaged donor DNA. This follows because it is highly unlikely that the donor DNA would be damaged at the same site as is the recipient’s chromosome. Alternatively, transformed cells could also benefit if the sites of recombination were random but the cell could screen available exogenous DNA fragments and omit damaged DNA before use. However, previous work reviewed above (see also Figures 1, 2, and 3) shows that damaged
DNA is brought into the cell and recombined into the recipient's genome.

One might wonder why the cells go to the trouble to bring in damaged donor DNA if transformation evolved for the function of repairing damages in the recipient's DNA. For the reasons just discussed, if recombination is targeted to damaged sites in the recipient's genome, it does not matter whether the donor DNA is damaged or not (damages in the donor DNA will likely be at different sites than damages in the recipient's DNA).

In UV-DNA experiments there was a clear difference in the magnitude of transformation between donor DNA isolated from damaged cells or from undamaged cells (Figure 3a), whereas this was not so clear in DNA-UV experiments (Figure 3b). In DNA-UV experiments the transformation rate with damaged DNA began lower than with undamaged DNA, but as the recipient cells received more damage this difference went away. Presumably, in all DNA-UV experiments the initial transformation rate with damaged donor DNA (that existing before the recipient cells were damaged) was lower than with undamaged donor DNA. Why in DNA-UV experiments does the transformation rate appear to drop off more slowly when the donor DNA is damaged than when it is undamaged (Figure 3b) while this doesn't occur in UV-DNA experiments (Figure 3a)? We consider two possible explanations.

First, in DNA-UV experiments, the transformation rate decreases as a result of damage to the recipient cells, whether or not the donor DNA is damaged (Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989; Figure 5 above). Therefore with increasing levels of damage to the recipient cell, the latter effect could overwhelm the effect of the damages present in the donor DNA, resulting in approximately equal transformation rates with damaged or undamaged donor DNA at higher UV dosages to the recipient cells (≥50 J/m²).

Second, in DNA-UV experiments uptake of damaged donor DNA could induce the SOS system (Yassin 1977; Love, Lyle and Yassin 1985) in the recipient cells. This induction could allow the recipient cells to more effectively cope with subsequent damages. Thus, in DNA-UV experiments, cells transformed with damaged DNA, as opposed to those transformed with undamaged DNA, may be preadapted to cope with the subsequent UV treatment.

The amount of killing at a given UV dose depends on cell density in the culture at the time of UV irradiation. Cells in a DNA-UV treatment have an additional 45 minutes of growth prior to UV irradiation resulting in higher cell density at the time of exposure to UV light. This higher cell density translates into relatively less killing in a DNA-UV experiment (compared to a UV-DNA experiment), as shown by the total cell survivorship curves in Figure 4. If competent and non-competent cells responded to damage in the same manner the survivorship curve for transformed cells in a UV-DNA treatment should fall below that of a DNA-UV treatment (as do the total cells). However, they do not. As shown in Figure 4, competent cells in UV-DNA treatment survive as well or better than competent cells in a DNA-UV treatment even though they are subjected to higher killing.

It is important to recognize that comparing survivorship curves between UV-DNA and DNA-UV experiments for a given kind of cell (transformed or total) does not yield all available information in the data. It is the relative survivorship within a UV-DNA or DNA-UV experiment that demonstrates the effect of transformation. In a UV-DNA treatment the survivorship of transformed cells is higher relative to total cells whereas this is not the case in a DNA-UV treatment (Figure 4).

The increase in transformation rate found with increasing UV in UV-DNA experiments and the lack of increase in DNA-UV experiments (Figure 5 above, as well as Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989) could be due to several factors. First, damage to recipient cells may directly increase transformation if damaged sites directly stimulate recombination. This factor depends on homologous recombination. Second, damage to recipient cells may stimulate binding and/or uptake of transforming DNA. This factor could operate in the absence of homologous recombination, but evidence against it has already been presented by Wojciechowski, Hoelzer and Michod (1989) and so this factor will not be discussed further here. Third, competent cells may have higher fitness as a result of the benefit of recombinational repair involving transforming donor DNA as template. Fourth, there may be some difference between competent and non-competent cells, other than their different capacities for recombinational repair, that leads to the observed results.

There are numerous possible differences between competent and non-competent cells. For example, transformed cells are known to divide more slowly than nontransformed cells and this could allow more time for repair before cell division. Or, nonrecombinational (SOS or excision) repair processes may be enhanced in competent cells over their levels in non-competent cells. In a previous paper (Wojciechowski, Hoelzer and Michod 1989), we have provided evidence against these explanations for our results. There are other possible explanations in this fourth category that we have not directly tested for. For example, homologous recombination might promote
recovery from UV damage for reasons other than its role in recombinational repair, perhaps by slowing down cell division or by inducing repair systems (suggested by an anonymous reviewer).

However, to satisfactorily explain our results a hypothesis must explain not only the increase in transformation rate in a UV-DNA experiment but the lack of increase in a DNA-UV experiment. It is difficult to see why homologous recombination should give rise to the qualitatively different outcomes in the two kinds of experiments, except for the reason that it is involved in recombinational repair of prior damage. If homologous recombination slowed down cell division or if it induced non-recombinational repair systems, these factors should operate similarly in both UV-DNA and DNA-UV experiments. For this reason we do not believe that these factors can explain the qualitative differences between UV-DNA and DNA-UV experiments.

This leaves either direct stimulation of recombination by damage or fitness differences between competent and non-competent cells due to recombinational repair or prior damage as the most likely explanations for our results. As discussed in more detail elsewhere (Michod, Wojciechowski and Hoelzer 1988; Wojciechowski, Hoelzer and Michod 1989), either of these factors may contribute to the results we have observed and support the view that transformation has been selected because of its ability to repair DNA damage. In our previous papers [p. 421 of Wojciechowski, Hoelzer and Michod (1989) and pp. 37–38 of Michod, Wojciechowski and Hoelzer (1988)], we argued that direct stimulation of recombination by damage was the most likely reason for our results. The basis of our argument was the assumption that the probability of transformation (and hence recombinational repair) at damaged sites was the same as it is at marker loci (approximately $10^{-5}$). If this were the case, the attendant fitness benefit of transformation should be small. However, targeting of recombination to damaged sites (such as has been observed in yeast, Orr-Weaver, Szostak and Rothstein 1981) means that transformation frequencies at marker loci likely underestimate the frequencies of transformation at damaged sites. Whether this occurs in our experiments is in need of further work.

The purpose of the present work is to more closely simulate natural conditions in which donor DNA is in the same state (derived from the same environment) as is the DNA in the recipient cells. By so doing, we have demonstrated that the increase in transformation frequency with increased UV to the recipient cell does not depend on the donor DNA being in a relatively undamaged state.

This work was supported by National Institutes of Health grant GM 36410. We are grateful to Steve Abedon, Harris Bernstein, Rick Hudson and Marty Wojciechowski for helpful criticism and comment on this work. We thank Marty Wojciechowski and Paul Love for the gift of strains used in this study.

LITERATURE CITED


Evolution of Transformation


Communicating editor: J. R. Roth