Evolution of Natural Transformation: Testing the DNA Repair Hypothesis in *Bacillus subtilis* and *Haemophilus influenzae*

Rosemary J. Redfield[1]

Department of Biochemistry, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

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ABSTRACT

The hypothesis that the primary function of bacterial transformation is DNA repair was tested in the naturally transformable bacteria *Bacillus subtilis* and *Haemophilus influenzae* by determining whether competence for transformation is regulated by DNA damage. Accordingly, DNA damage was induced by mitomycin C and by ultraviolet radiation at doses that efficiently induced a known damage-inducible gene fusion, and the ability of the damaged cultures to transform was monitored. Experiments were carried out both under conditions where cells do not normally become competent and under competence-inducing conditions. No induction or enhancement of competence by damage was seen in either organism. These experiments strongly suggest that the regulation of competence does not involve a response to DNA damage, and thus that explanations other than DNA repair must be sought for the evolutionary functions of natural transformation systems.

MANY groups of bacteria have evolved mechanisms of natural transformation which enable them to take up free DNA fragments from their environments. If this DNA is homologous and genetically marked, recombinant pregnancy are often produced (STEWART and CARLSON 1986). Most researchers have assumed that transformation evolved for genetic exchange, but several recent reports have addressed a possible role for transformation in DNA repair (MICHOD, WOJCIECHOWSKI and HOELZER 1988; WOJCIECHOWSKI, HOELZER and MICHOD 1989; HOELZER and MICHOD 1991). These experiments were suggested by the DNA repair hypothesis, which postulates that transforming bacteria can use the DNA they take up for recombinational repair of DNA damage, and that the advantages of this repair have been responsible for the evolution of natural transformation systems. This proposal is not unreasonable, because DNA damage is ubiquitous and can have very high costs: damage that spans both DNA strands is lethal unless repaired and irreparable unless a homologous DNA strand is available to recombine across the damaged segment and serve as a template for repair synthesis (KUSHNER 1987).

Previous experiments have tested the prediction that DNA uptake will help cells survive DNA damage (MICHOD, WOJCIECHOWSKI and HOELZER 1988; WOJCIECHOWSKI, HOELZER and MICHOD 1989; HOELZER and MICHOD 1991). The initial experiments showed that when *Bacillus subtilis* cultures competent for transformation (containing both competent and noncompetent cells) are provided with homologous DNA after the cell's own DNA has been damaged by UV irradiation, the proportion of transformants is increased among the survivors (MICHOD, WOJCIECHOWSKI and HOELZER 1988). The favored interpretation was that the cells that took up DNA survived damage better than the cells that did not. Subsequent experiments have confirmed and extended the observations (WOJCIECHOWSKI, HOELZER and MICHOD 1989; HOELZER and MICHOD 1991), but several anomalies remain unexplained, and the increased frequency of transformants may be due to causes other than repair by recombination with transforming DNA.

Here I report experiments testing a different prediction of the DNA repair hypothesis: that the developmental regulation of competence should reflect the need for DNA repair. I have monitored changes in the competence of the two best characterized naturally transformable bacteria, *B. subtilis* and *Haemophilus influenzae*, in response to treatment with UV irradiation and with mitomycin C. Both of these organisms have SOS-like regulatory systems that induce DNA repair pathways in response to DNA damage (NOTANI and SETLOW 1980; YASBIN, CHEO and BAYLES 1991), and in both competence is known to be tightly regulated (DUBNAU 1991; REDFIELD 1991). Thus the prerequisite cellular regulatory pathways are in place: the cells can recognize and respond to DNA damage, and they can turn competence on and off in response to metabolic or environmental changes. The DNA repair hypothesis predicts that cells will induce competence in response to DNA damage.

[1] Present address: Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4 Canada.

MATERIALS AND METHODS

***B. subtilis* strains and culture conditions:** Cells were grown in Difco antibiotic medium #3 (PAB) and in the competence induction media GM1 and GM2 supplemented with tryptophan and methionine at 50 μg/ml (BOYLAN *et al.* 1972). Cells were plated on LB or minimal glucose (MG) agar, supplemented with tryptophan and/or methionine where appropriate. Damage and transformation experiments were carried out in strain YB886 (*metB5 trpC2 xin-1* SPβ⁻) using transforming DNA from strain WB101 (*xin-1* SPβ⁻).

***B. subtilis* DNA damage protocols:** Cells grown to OD_{600} = 0.3 in PAB (log-phase cultures), or to stationary phase (T_0 + 90) in GM1 (pre-competent cultures; BOYLAN *et al.* 1972), were damaged as follows.

Mitomycin C: Cells were incubated for 10 min at room temperature with the specified concentration of mitomycin C, washed once, and resuspended in the original volume of PAB or GM2.

UV irradiation: Cells were pelleted and resuspended in Spizizen minimal salts (SPIZIZEN 1958) at room temperature. Aliquots (1 ml) were irradiated in plastic Petri dishes with constant agitation under a small 256 nm UV lamp (intensity at a distance of 15 inches was 0.4 J/m²/sec). Cells were then pelleted and resuspended in the original volume of PAB or GM2.

After damage with either agent, 1 ml samples were taken immediately to tubes with and without transforming DNA for t = 0 survival and transformation assays, and the remainder of each culture was diluted 20-fold in prewarmed PAB or 10-fold in prewarmed GM2 and shaken at 37°. Samples were taken for transformation assays at intervals over the next 4 hr.

***B. subtilis* assays:** *Transformation assays:* Culture aliquots (1 ml) were incubated with or without 1 μg WB101 DNA at 37° for 15 min, 1 μg of DNase I was added for an additional 5 min, and cells were diluted and plated on LB to determine survival and growth, and on MG plates with and without tryptophan and/or methionine to determine transformation frequencies.

β-Galactosidase assays: Cultures of a YB886 derivative containing the *din-22*::Tn*917-lacZ* insertion (LOVE, LYLE and YASBIN 1985), were damaged and incubated as described above, and β-galactosidase activity was assayed in toluenized cells as described by MILLER (1972).

***H. influenzae* strains and culture conditions:** DNA damage experiments were carried out using strain BC200 (SETLOW *et al.* 1973). Cells were grown in Difco brain heart infusion supplemented with 10 μg/ml hemin and 2 μg/ml NAD (sBHI), and competence was induced by transfer of mid-log cultures to MIV starvation medium (HERRIOTT, MEYER and VOGT 1970).

***H. influenzae* DNA damage protocols:** These were similar to those used for *B. subtilis*. All began with exponentially growing cells at OD_{600} = 0.2–0.25 in sBHI. After incubation with mitomycin C, cells were washed in sBHI (for noncompetent cultures) or MIV (for competent cultures) and resuspended in an equal volume of sBHI or MIV. Cells were UV irradiated in MIV, and pelleted and resuspended in the original volume of sBHI or MIV. Samples were then taken for t = 0 survival and transformation assays, and the remainder diluted in prewarmed sBHI or MIV and shaken at 37°.

***H. influenzae* assays:** *Transformation assays:* One milliliter of cells was incubated at 37° with 1 μg chromosomal DNA from strain MAP7 [*nov*^{R} *str*^{R} *kan*^{R} *stv*^{R} *spc*^{R} *nal*^{R} *vio*^{R}; BARCAK *et al.* (1991)] for 15 min, 1 μg of DNase I was added for an additional 5 min, and cells were diluted and plated on sBHI agar with or without novobiocin at 2.5 μg/ml.

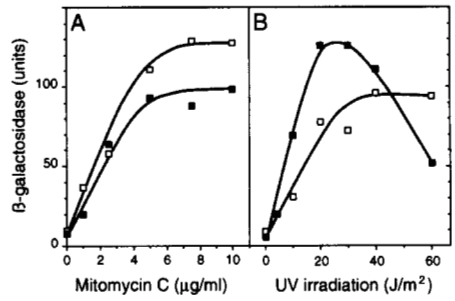

FIGURE 1.—Induction of the *din22 lacZ* fusion by DNA damaging agents. Samples were assayed 1 hr after exposure to (A) mitomycin C or (B) UV irradiation. Solid squares represent cultures growing exponentially in PAB; open squares represent competent cultures.

DNA uptake assays: ³H-Labeled DNA was prepared from strain KW26 (*thy str*^{R}) as described (TOMB *et al.* 1989). 1 ml samples of competent *H. influenzae* cultures were incubated with 1 μg of ³H-labeled KW26 DNA and with DNase I, as described above. The cells were then pelleted, washed three times with cold MIV, and the final pellet was counted in Aquasol. The OD_{600} was measured when each sample was taken, and uptake was expressed as μg DNA/OD_{600}.

RESULTS

B. subtilis

How much damage is required to induce damage-inducible gene expression? To ensure that the damaging agents UV and mitomycin C were used at doses sufficient to induce damage-regulated genes, I measured induction of β-galactosidase expression from the damage-inducible *lacZ* fusion *din-22* (LOVE, LYLE and YASBIN 1985), using the same damage protocols that would be tested for competence induction. The data in Figure 1 show that exposure to 20 J/m² of UV or to 6 μg/ml of mitomycin C for 10 min induced maximal β-galactosidase expression in both competent and exponentially growing cells. Survivals were very low at these doses (no more than 2% for YB886; see Figures 2 and 3). Accordingly, in addition to a maximally inducing dose of each agent, two lower doses were used, one giving at least 50% survival of YB886 and about 10% induction of *din-22*, and one giving about 20% survival of YB886 and about 50% induction of *din-22*.

Effects of DNA damage on noncompetent *B. subtilis* cultures: *B. subtilis* cells do not normally become competent while growing in PAB or other rich media. The effect of DNA damage on competence was therefore first examined by exposing cultures of strain YB886 growing exponentially in PAB to various doses of mitomycin C and UV, and then testing aliquots over the ensuing 4 hr (Figure 2). Control cultures were mock-irradiated or incubated for 10 min without mitomycin. To measure the extent of induction of

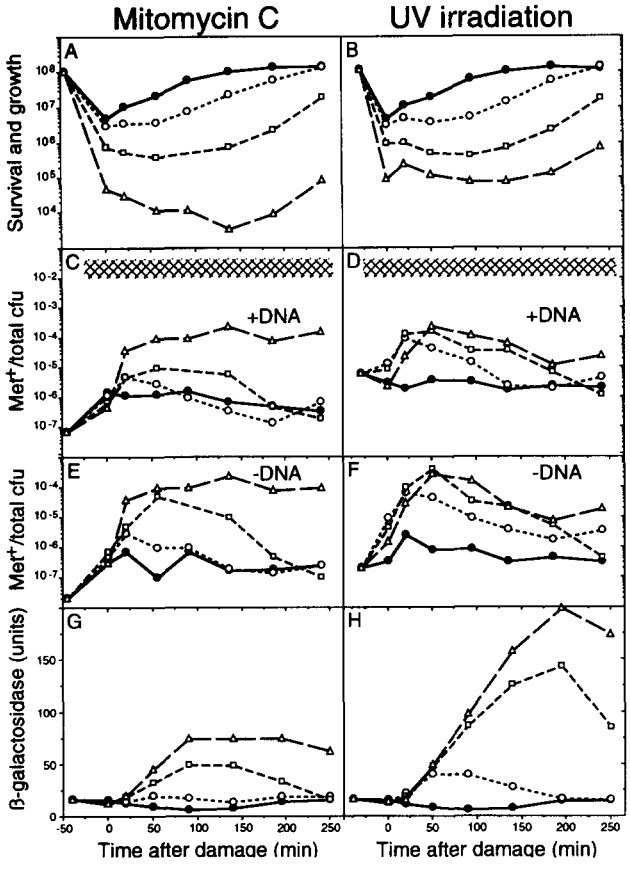

FIGURE 2.—Effect of DNA damaging agents on noncompetent *B. subtilis* cultures. Cells growing exponentially in PAB were damaged at room temperature, diluted 20-fold in PAB, and returned to culture (time = 0). Panels A-F: YB886. Panels G and H: *din-22*. Panels A, C, E, G: cells damaged with mitomycin C: solid circles undamaged, open circles 1 μg/ml, open squares 3 μg/ml, open triangles 6 μg/ml. Panels B, D, F and H: cells damaged with UV irradiation: solid circles undamaged, open circles 8 J/m², open squares 16 J/m², open triangles 24 J/m². Panels A and B: cfu on LB. Panels C, D, E, F: ratio of cfu on MG + tryptophan/MG + tryptophan + methionine. The shaded regions in panels C and D indicate the typical range of transformation frequencies of competent cultures. Panels A-D are samples incubated with WB101 DNA at the indicated times. Panels E and F are samples incubated without DNA. Panels G and H: β-galactosidase activity (Miller units) of *din22* cultures treated as above. The following data points are upper limits, because no methionine⁺ (Met⁺) colonies were detected from 1 ml of culture. Panel C, 6 μg/ml dose: 50, 90, 135 min. Panel E, 3 μg/ml dose: 20, 240 min. Panel E, 6 μg/ml dose: 50, 90 min.

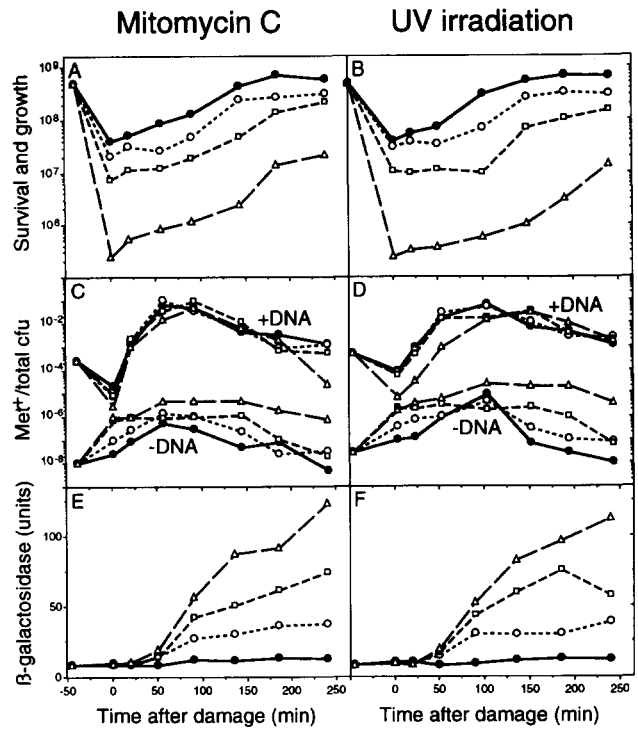

FIGURE 3.—Effect of DNA damaging agents on competent *B. subtilis* cultures. Cells at T_0+90 in GM1 were damaged at room temperature, diluted tenfold in GM2, and returned to culture (time = 0). Panels A, C and E: cells damaged with mitomycin C: solid circles undamaged, open circles 1 μg/ml, open squares 3 μg/ml, open triangles 7.5 μg/ml. Panels B, D and F: cells damaged with UV irradiation: solid circles undamaged, open circles 10 J/m², open squares 30 J/m², open triangles 60 J/m². Panels A-D: YB886. Panels A and B: cfu on LB. Panels C and D: ratio of cfu on MG + tryptophan/MG + tryptophan + methionine. The upper sets of curves in Panels C and D are from samples incubated with WB101 DNA at the indicated times; the lower sets of curves are from samples incubated without DNA. Panels E and F: β-galactoside activity (Miller units) of *din22* cultures treated as above. The following data points in the lower set of curves (−DNA) of Panel C are upper limits, because no methionine⁺ (Met⁺) colonies were detected from 1 ml of culture; undamaged, 0 min; 6 μg/ml dose, 0, 20 min.

damage-inducible operons, the *din-22 lacZ* fusion strain was similarly damaged and its β-galactosidase activity was measured over the same time period.

Figure 2, A and B, shows the effect of damage on the ability of cells to form colonies. The initial drop in colony-forming units (cfu) of the undamaged cultures (solid lines and circles) was due to the initial 1/20 dilution; the lower cfu of damaged cultures indicates the lethality of the DNA damage. (Although the $t = 0$ samples were actually taken just *before* dilution of the rest of the culture, to facilitate comparisons of survival and growth rates the $t = 0$ cfu values in panels A and B of Figures 2, 3, 4 and 5 have been divided by the appropriate dilution factor before graphing.) The three doses of mitomycin C and of UV used reduced survivals at $t = 0$ to 59%, 15% and 0.9%, and 69%, 21% and 2.0%, respectively. At the end of the experiments the undamaged cultures had entered stationary phase, while the heavily damaged cultures were still growing exponentially. Damage-induced β-galactosidase activity in the *din-22 lacZ* fusion strain had reached maximal levels and begun to fall by this time (Figure 2, G and H), indicating that 4 hr allowed sufficient time for expression of damage-induced changes.

The solid lines in Figure 2, C and D, confirm that the transformation frequencies of undamaged cells under these conditions were only slightly higher than the spontaneous mutation rate to Met⁺ (between 10^{-6}

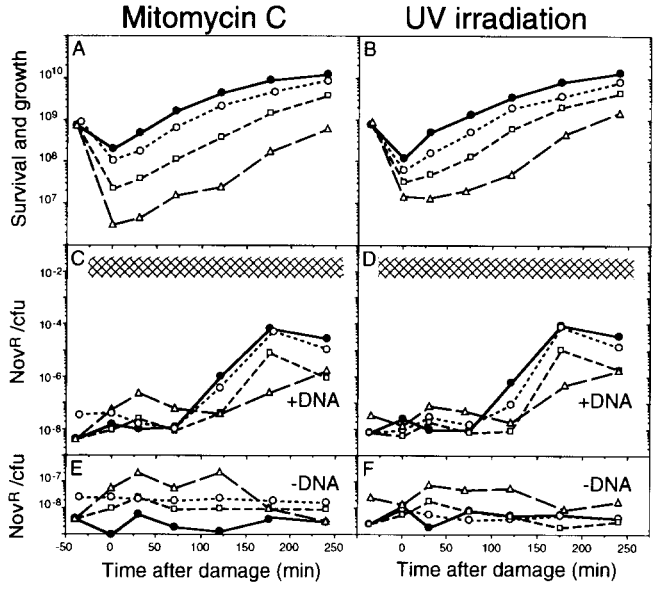

FIGURE 4.—Effect of DNA damaging agents on non-competent *H. influenzae* cultures. BC200 cells growing exponentially in sBHI were damaged at room temperature, diluted in fresh sBHI, and returned to culture at time 0. Panels A, C and E: cells damaged with mitomycin C and diluted fivefold: closed circles undamaged, open circles 0.15 μg/ml, open squares 0.75 μg/ml, open triangles 2.0 μg/ml. Panels B, D and F: cells damaged with UV and diluted 1/5: closed circles undamaged, open circles 2 J/m^2, open squares 6 J/m^2, open triangles 24 J/m^2. Panels A and B: cfu on sBHI. Panels C, D, E and F: ratio of cfu on sBHI with and without novobiocin. Panels C and D: cells incubated with MAP7 DNA at the indicated time. Shaded regions show typical transformation frequencies of fully competent cultures. Panels E and F: cells incubated without DNA. The data for 0.15 μg/ml mitomycin C and for 24 J/m^2 UV are from a separate experiment. The following data points are upper limits because no Nov^R colonies were detected from one ml of culture: Panel C, 0.75 μg/ml dose: 0, 30, 70 min; 2.0 μg/ml dose: 0, 30, 70, 135 min. Panel D, 2.0 J/m^2 dose: 0 min, 6.0 J/m^2 dose: 0, 70 min; 24 J/m^2 dose, 30 min, 70 min. Panel E, 0.75 μg/ml dose: 30, 70 min; 2.0 μg/ml dose: 0, 30, 70 min. Panel F, undamaged and 2.0 J/m^2 doses: 30 min; 6.0 J/m^2 and 24 J/m^2 doses: 30, 70 min.

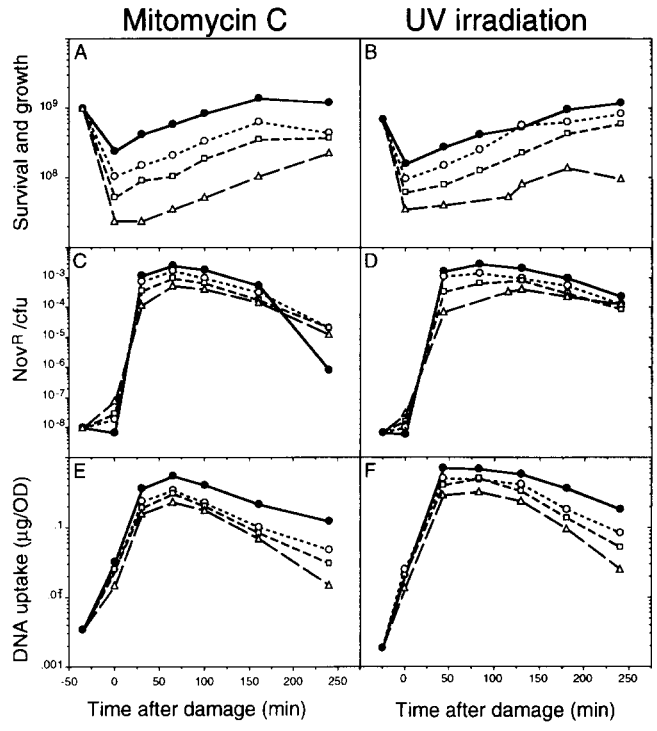

FIGURE 5.—Effect of DNA damaging agents on competent *H. influenzae* cultures. BC200 cells at OD_{600} = 0.25 in sBHI were damaged at room temperature, diluted in MIV, and returned to culture at time 0. Panels A, C and E, cells damaged with mitomycin C and diluted threefold: closed circles no damage, open circles 0.25 μg/ml, open squares 0.75 μg/ml, open triangles 2.0 μg/ml. Panels B, D and F, cells damaged with UV and diluted fivefold: closed circles no damage, open circles 2.0 J/m^2, open squares 6.0 J/m^2, open triangles 12 J/m^2. Panels A and B: cfu on sBHI. Panels C and D: ratio of cfu on sBHI with and without novobiocin. Panels E and F: uptake of ^{3}H-labeled KW26 DNA (as DNase I-resistant cpm), normalized for culture density measured as OD_{600}. The following data points for 0 min samples are upper limits because no Nov^R colonies were detected from 1 ml of culture. Panel C, undamaged, 0.25 μg/ml dose, 2.0 μg/ml dose; panel D, all doses.

and 10^{-7} per cfu, Figure 2, E and F). Both mitomycin C and UV caused substantial increases in the frequency of Met$^+$ colonies, but this was independent of exposure to transforming DNA (Figure 2, E and F are drawn to the same scale as Figure 2, C and D), indicating that the increases were due to damage-induced mutagenesis rather than to competence. In all cases the frequency of Met$^+$ colonies remained less than 1% of that produced by competent cultures induced by growth in GM1 and GM2 (shaded bars at the tops of Figure 2, C and D; for comparison see Figure 3, C and D).

Although damage did not induce detectable competence, the sensitivity of these experiments was limited by the density of surviving cells in the cultures: most of the data points for the highly damaged cultures in Figure 2, C and E, are based on three or fewer colonies from 1 ml of culture, and several are upper limits as no Met$^+$ colonies could be detected (such points are specified in the legend to each figure). In some cases sensitivity was also limited by the frequency of reversion mutations to Met$^+$, however selection for Trp$^+$ gave similar results (data not shown). Although the *trpC2* mutation reverts less frequently than does *metB5* (it is thought to be a small deletion), it also transforms less efficiently, and the sensitivity was again limited primarily by the survival.

Effects of DNA damage on competent *B. subtilis* cultures: The above experiments showed that DNA damage is not the primary inducer of competence in *B. subtilis*. However, the possibility remains that DNA repair is a secondary function of transformation, and that in rich medium induction of competence is actively prevented by other regulatory mechanisms. Accordingly I tested whether the presence of DNA damage might enhance the development of competence under standard inducing conditions (Figure 3).

Maximal competence in *B. subtilis* is customarily induced by growing cells into stationary phase

("T_0+90") in the minimal salts/glucose/amino acids/yeast extract medium GM1, and diluting them 10-fold into GM1 supplemented with Mg^{2+} and Ca^{2+} (GM2), where they reach maximal competence in 1 hr (BOYLAN *et al.* 1972). In the experiment shown in Figure 3, cells at T_0+90 in GM1 were exposed to mitomycin C or UV before dilution into GM2, and survival, transformation and mutation frequencies were followed as before. Undamaged cultures and induction of *din-22* were again followed under identical conditions.

Figure 3, A and B, shows the survival and growth of the YB886 cultures. The three doses of mitomycin C and of UV reduced survivals at $t = 0$ to 51%, 18% and 0.6%, and 77%, 27% and 0.6%, respectively. Figure 3, E and F, shows that these doses also induced high levels of β-galactosidase activity.

The upper sets of lines in Figure 3, C and D, show the frequency of Met^+ cfu from samples incubated with transforming DNA; the lower groups of lines are from the control samples incubated without DNA. As expected, undamaged cultures reached maximal competence at 1 hr, with transformation frequencies of 3–10%. Damaged cultures showed very similar kinetics and transformation frequencies, except that competence development was slightly delayed by heavy damage. Competence was decreasing in all cultures by the end of the experiment. Although the DNA damage did induce some mutations, these did not limit the sensitivity of the experiment; the mutation frequencies were always lower than the transformation frequencies, and the maximum transformation frequencies were always at least 1000-fold higher than the mutation frequencies. This experiment thus demonstrates that DNA damage does not enhance the development of competence in *B. subtilis* under permissive conditions.

H. influenzae

The natural transformation system of *H. influenzae* typifies those of gram negative bacteria, and appears to be unrelated to that of *B. subtilis* and other gram positive organisms (STEWART and CARLSON 1986). It is therefore suitable for testing the generality of the above results. The BC200 derivative of the standard *H. influenzae* Rd strain was used, because it does not induce a defective prophage in response to DNA damage and hence is less sensitive to damaging agents than its parent (SETLOW *et al.* 1973). Similar results were seen with the standard Rd strain KW20 (data not presented).

Effects of DNA damage on noncompetent *H. influenzae* cultures: The basic design was similar to that used for the *B. subtilis* experiments described above. *H. influenzae* growing exponentially in the rich medium sBHI exhibits transformation frequencies of less than 10^{-8}, but the frequency increases spontaneously to about 10^{-4} at the onset of stationary phase (REDFIELD 1991). Accordingly, BC200 cells were grown in sBHI to mid-log (OD_{600} = 0.2), damaged, and returned to sBHI at a fivefold dilution; growth and competence were followed for 4 hr (Figure 4).

The solid lines and circles show that after dilution the undamaged cells initially grew exponentially and then entered stationary phase (Figure 4, A and B). As expected, transformation frequencies during exponential growth were about 10^{-8} (Figure 4, C and D), only slightly higher than the mutation rate (Figure 4, E and F), and spontaneous competence peaked at the onset of stationary phase, giving transformation frequencies of about 10^{-4}.

The three doses of mitomycin C and of UV reduced survivals to 66%, 11% and 1.6%, and 53%, 20% and 9%, respectively. These doses did not detectably increase transformation frequencies in exponential growth, and reduced the spontaneous competence seen at the end of the experiment. Although the transformation and mutation frequencies appear to be initially increased by DNA damage, these data points are upper limits, because no Nov^R colonies were obtained from the heavily damaged cultures for the first 2 hr of the experiment. All of these values are far below the fully induced competence levels indicated by the shaded bars in Figure 4, C and D, (compare also to those in Figure 5, C and D), indicating that DNA damage did not induce or enhance competence development in *H. influenzae* in rich medium.

Effects of DNA damage on competent *H. influenzae* cultures: In *H. influenzae* maximum competence develops after rapidly growing cells are transferred to the starvation medium MIV (transformation frequencies about 10^{-2}) (HERRIOTT, MEYER and VOGT 1970). Accordingly cells at OD_{600} = 0.2 in sBHI were washed with MIV, exposed to mitomycin C or UV, and washed and resuspended in MIV at a final dilution of 1/3 or 1/5. Growth, transformation, and DNA uptake by these cultures were followed for 4 hr (Figure 5).

As expected, undamaged cells ceased dividing after transfer to MIV, and transformation frequencies peaked at 70 min. The peak competence of 3×10^{-3} was slightly lower than usually seen, presumably due to the delays incurred during the damaging and washing steps. The three doses of mitomycin C and of UV reduced survivals to 43%, 22% and 9.7%, and 62%, 40% and 23%, respectively. The transformation frequencies of the damaged cultures were all lower than those of the controls until the final time point, and as seen with *B. subtilis*, the most heavily damaged cultures showed some delay in reaching maximal competence. All cultures showed decreasing competence by the end of the experiment, confirming that suffi-

cient time had been allowed for any damage-induced changes in competence.

Measurement of DNA uptake provides an independent assay of the competence of the entire culture, not just of those cells capable of forming colonies. Figure 5, E and F, shows that DNA damage decreased uptake of ^{3}H-labeled DNA in parallel with the decreases in transformation frequencies. Thus all of the evidence shows that, in *H. influenzae* as in *B. subtilis*, DNA damaging agents neither induce nor enhance the development of competence.

DISCUSSION

Transformation has usually been assumed to serve the same function as sex, where an evolutionary advantage is thought to accrue from the production of recombinant progeny, enabling populations to better cope with deleterious mutations or changing environments [see collection of articles in MICHOD and LEVIN (1988)]. Because the advantages of sexual recombination remain controversial, the evolution of transformation may best be approached by investigating the other ways it might benefit the cell. Recombinational repair with transforming DNA is a plausible alternative function, because cells must cope with DNA damage much more often than with changing environments or deleterious mutations, and because the consequences of damage are more likely to be immediately lethal.

Previous experiments have attempted to demonstrate that DNA repair using transforming DNA increases the survival of *B. subtilis* cells after DNA damage (MICHOD, WOJCIECHOWSKI and HOELZER 1988; WOJCIECHOWSKI, HOELZER and MICHOD 1989; HOELZER and MICHOD 1991). However, the effect is expected to be small, because the frequency of successful repair will be limited by the amount of DNA the cells take up, usually equivalent to only a small fraction of the genome. The enhanced survival seen in these experiments was often much larger than could be attributed to transformational repair, unless a novel mechanism was postulated that enabled cells to take up *only* the DNA fragments that matched their damaged sequences.

The need for caution in interpreting these experiments is emphasized by recent attempts to reproduce the *B. subtilis* effects in *H. influenzae*, where cell survival can be measured directly rather than inferred from the transformation frequency. The presence of transforming DNA after damage with UV or mitomycin C was found to have no effect on the survival of cultures that had completed competence development (REDFIELD 1993; MONGOLD 1992).

An alternative approach is to infer the function of transformation systems from an understanding of their regulatory mechanisms. Competence is tightly regulated in both *H. influenzae* and *B. subtilis*, and because these regulatory mechanisms evolved to permit transformation only under conditions when it would benefit the cell, they provide direct windows into transformation's functions in the natural environments where the regulatory genes evolved. Thus, if the primary function of transformation is indeed DNA repair, we expect that competence, like other repair pathways, will be induced by DNA damage.

In investigating whether competence is a component of the SOS response, it was not possible to test all permutations of damaging agents and culture conditions, but a reasonable range has been covered. Two damaging agents were used; both are potent inducers of the SOS response, and both can generate lesions requiring recombinational repair [reviewed by KUSHNER (1987)]. UV causes mainly single-strand pyrimidine photodimers that can be repaired without an exogenous template, although the lethality associated with high UV doses may result from other photoproducts and strand breaks, and from replication and segregation of strands containing unrepaired lesions (BRASH 1988). The bifunctional alkylating agent mitomycin C reacts with guanine residues to cause double-strand crosslinks, which are lethal unless repaired by recombination. Cells were tested under two culture conditions, one where competence did not spontaneously develop (to find out whether damage could induce competence) and one where competence development had been initiated (to find out whether damage would enhance competence under conditions known to permit its development). The 4 hr of observation should be sufficient for any damage-induced effects to appear. *H. influenzae* can develop competence in as little as 30 min, although maximal induction by starvation medium usually takes 90–100 min. Comparable times are not known for *B. subtilis*, but high levels of β-galactosidase activity from the *din-22* fusion were induced in all controls. Under conditions where competence developed, it had usually peaked and begun to decrease by the end of each experiment.

Although the present experiments revealed no induction of competence by DNA damage, the converse does not hold: some DNA repair processes are induced by competence. In *H. influenzae* both prophage induction and recombination by the RecA-homolog Rec-1 are increased in competent cells (BOLLING and SETLOW 1969; SETLOW *et al.* 1973). RecA mediates generalized recombination as well as regulating DNA repair, so this induction may reflect its role in processing incoming DNA. In *B. subtilis* the connection between competence and the SOS response is much broader, and most damage-inducible processes and damage-inducible promoters are induced in undamaged competent cells, possibly by competence-specific activation of the *recA* and *dinR* promoters (YASBIN,

CHEO and BAYLES 1991; RAYMOND-DENISE and GUILLEN 1992). We do not know whether this evolved only to provide competent cells with enhanced recombination capabilities, or whether the associated induction of other repair processes is in itself adaptive.

Our present knowledge about the regulation of competence provides some support for an alternative function for competence, use of DNA as a nutrient. In *H. influenzae* competence is triggered by addition of cyclic AMP (WISE, ALEXANDER and POWERS 1973), and is completely blocked by mutations in adenylate cyclase and in the catabolite regulatory protein CRP (I. DOROCICZ and R. REDFIELD, unpublished results; CHANDLER 1992), suggesting participation in a global carbon-energy regulon (BOTSFORD and HARMAN 1992). In *B. subtilis* competence development is controlled by a complex regulatory cascade (DUBNAU 1991), several of whose components also regulate starvation-induced genes and other postexponential processes.

The lack of evidence for regulation of competence by DNA damage does not mean that cells do not use transforming DNA for recombinantional repair, but only that any such benefits have been too small to influence the evolution of the regulatory mechanisms. Similarly, the evidence for nutritional regulation does not preclude a benefit from DNA repair or from production of recombinant genomes. Because most deleterious mutations and environmental changes will have their main impact on the cell's nutritional state, nutritional regulation could reflect a recombinational function. Furthermore, because the DNA strand displaced by recombination with transforming DNA is subsequently degraded, cells can have their genetic cake and eat it, too, benefiting from both the new genetic information and all of the nutrients in the DNA they take up.

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