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# Transformation and Transfection in Lysogenic Strains of *Bacillus subtilis*: Evidence for Selective Induction of Prophage in Competent Cells

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Lysogenic strains of *Bacillus subtilis* 168 were reduced in their level of transformation as compared to non-lysogenic strains. The level of transformation decreased even further if the competent lysogenic cells were allowed to incubate in growth media prior to selection on minimal agar. This reduction in the frequency of transformation was attributable to the selective elimination of transformed lysogenic cells from the competent population. Concurrent with the decrease in the number of transformants from a lysogenic competent population was the release of bacteriophage by these cells. The lysogenic bacteria demonstrated this dramatic release of bacteriophage only if the cells were grown to competence. Both the selective elimination of transformed lysogens and the induction of prophage was prevented by the inhibition of protein synthesis. Additionally, competent lysogenic cells released significantly higher amounts of exogenous donor transforming deoxyribonucleic acid than did competent non-lysogenic cells or competent lysogenic cells incubated with erythromycin. These data establish that the induction of the prophage from the competent lysogenic cells was responsible for the selective elimination of the lysogenic transformants. A model is presented that accounts for the induction of the prophage from competent lysogenic bacteria via the induction of a repair system. It is postulated that a repair system is induced or derepressed by the accumulation of gaps in the chromosomes of competent bacteria. This hypothetical enzyme(s) is ultimately responsible for the induction of the prophage and the selective elimination of transformants.

Lysogenic conversion can markedly influence the capacity of a wide range of bacterial species to undergo deoxyribonucleic acid (DNA) mediated transformation and transfection. For instance, *Staphylococcus aureus* can be made competent only in the presence of temperate bacteriophage $\phi$11 (29, 34). Similarly, *Bacillus stearothermophilus* requires the temperate bacteriophage TP-12 for the development of competence (N. E. Welker and M. E. Eager, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, V98, p. 201). Apparently this virus enables the cell to produce the competence factor(s) required for the binding of DNA to the cytoplasmic membrane (38). On the other hand, in *Bacillus subtilis* (27, 44, 45, 47) and streptococci (25), lysogeny inhibits DNA-mediated transformation. Although transformation is reduced in competent lysogenic cultures of *B. subtilis*, these same bacteria are capable of normal levels of DNA-mediated transfection (44, 45, 47).

Previously, we suggested five possible explanations for the decrease in transformation observed in lysogenic cultures of *B. subtilis* (45): (i) restriction of unmodified DNA, (ii) deficiencies in recombination enzymes or other enzymes needed in transformation, (iii) alterations of cell wall or membrane resulting in reduced uptake of bacterial DNA, (iv) preferential lysis of competent cells by their prophage, and (v) preferential degradation of bacterial genes by nucleases. Based upon our results, we concluded that the more plausible explanations were an alteration in the cell surface, a differential inactivation of bacterial DNA, or the preferential lysis of the competent population (45). Since the report of our studies, the following information has been obtained: in *Haemophilus influenzae* (33) and in *B. subtilis* (15, 16) the addition of transformation DNA is capable of inducing resident prophages from lysogenic

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competent populations. The selective elimination of recombinant bacteria can be accomplished by the infection of transformed cultures of *B. subtilis* with temperate bacteriophage SPO2 (37). In addition, as previously noted, strains of *B. subtilis* lysogenic for both bacteriophages $\phi$105 and SPO2 have a lower efficiency of transformation than either of the two single lysogens (45). Furthermore, certain defective mutants of bacteriophages $\phi$105 and SPO2 do not decrease the frequency of transformation (B. Reilly and A. Garro, personal communication). These results all strongly suggest that competent cells are more susceptible to lysis from temperate bacteriophages than are noncompetent cells.

To investigate the molecular mechanism by which lysogeny influences the frequency of transformation, we evaluated the induction of prophages during the onset of competence. The data to be presented demonstrate that preferential lysis of the competent lysogenic population is occurring. These studies have led to the formulation of a new model to explain changes in the chromosome of competent cells.

## MATERIALS AND METHODS

**Strains and methods of propagation.** Most of the strains of *B. subtilis* 168 used in these experiments have been described in detail previously (45). Strain BR151 carries *lys*-3, *trp*C2, and *met*B10; strain BR151($\phi$105) is lysogenic for the temperate bacteriophage $\phi$105 and strain RUB818 is wild type. Strains RUB814 and RUB813 were isolated as bacteriophage-resistant transformants of BR151 and BR151($\phi$105), respectively, using DNA obtained from strain CU548. S. Zahler isolated strain CU548 as a mutant that was resistant to bacteriophage $\phi$105cl-z (a clear plaque mutant of bacteriophage $\phi$105; 43). This strain is also resistant to bacteriophages $\phi$29 and SPO1 (R. E. Yasbin, V. C. Maino, and F. E. Young, manuscript in preparation). The propagation and maintenance of the bacterial cultures as well as the bacteriophage stocks have been previously described (43, 45).

**Media.** The minimal salts consisted of Spizizen minimal medium without glucose (36). The standard growth medium (GM1) was minimal salts supplemented with 22 mM glucose, 0.02% acid hydrolyzed casein (pH 7.0, Nutritional Biochemical Co., Cleveland, Ohio), 0.1% yeast extract (Difco), and 50 $\mu$g of the amino acid(s) required to supplement the auxotrophic requirements of the strains per ml. GM2 medium was similar to GM1 except that $CaCl_2$ and $MgCl_2$ were added to bring the final concentrations to 0.5 and 2.5 mM, respectively. The tryptose blood agar base (Difco), Penassay broth (PB, antibiotic medium no. 3, Difco), minimal agar (minimal salts, 22 mM glucose and 1.4% agar), and M agar were utilized as described previously (1, 43, 45).

**Procedures for genetic exchange.** Transformation and transfection were accomplished as described previously (1, 45). Essentially, cells were grown in GM1 for 90 min ($T_{90}$) after the cessation of logarithmic growth ($T_0$), diluted 10-fold into GM2, and incubated at 37 C on a New Brunswick gyratory shaker at 250 rpm. Unless otherwise specified, DNA (1 to 5 $\mu$g/ml) was added 1 h after the cells had been diluted into GM2, incubation continued for 25 min, and the reaction was terminated by the addition of 100 $\mu$g of deoxyribonuclease (DNase) per ml. After an additional 5 min at 37 C, the cells were assayed for transformation. Infectious centers were assayed on M agar plates overlayed with semisolid M agar (45). Bacterial DNA was isolated by a modification of the procedure of Wilson et al. (40). Bacteria, from a culture grown 18 h in PB (50 to 100 ml), were centrifuged, resuspended, in 10 ml of 0.15 M Tris [tris(hydroxymethyl)aminomethane hydrochloride], 0.1 M EDTA (ethylenediaminetetraacetic acid) buffer (pH 8.0), recentrifuged, and finally suspended in 5 ml of the Tris-EDTA buffer containing 1 mg of lysozyme per ml (Worthington Biochemicals, Freehold, N.J.). After incubation for 30 min at 37 C, pronase (Calbiochem, San Diego, Calif., 1 mg/ml) was added, and the culture was incubated at 50 C for 10 min and then for 50 min at 37 C before the addition of sufficient sodium lauryl sulfate and sarkosyl (NL-97, K and K Laboratories, Plain View, N.Y.) to produce a final concentration of 1%. Incubation was continued at 50 C until the cytoplasmic membranes were dissociated. The DNA was then extracted three times with buffer (0.1 M Tris, pH 8.0)-saturated redistilled phenol at 55 C (5 ml of DNA solution), precipitated in 0.1 M NaCl with 10 ml of cold 95% ethanol, wound on a glass rod, washed in three successive 70% ethanol solutions, and redissolved in 10 mM Tris buffer containing 1 mM EDTA (pH 7.5). Bacteriophage DNA was obtained after the purification of the bacteriophage by equilibrium centrifugation in CsCl (40). The bacteriophage were suspended in Tris-EDTA buffer (pH 8.0) and incubated with 1% sodium lauryl sulfate prior to extraction and precipitation as described for the isolation of bacterial DNA. The preparation of radioactive DNA and the binding of radioactive DNA to competent cells has been described previously (45).

**Stock solutions of antibiotics.** Rifampin, a gift of R. Doi, was dissolved in 0.2 ml of 95% ethanol and diluted in glass-distilled water to a final concentration of 500 $\mu$g/ml. 6-(*p*-Hydroxyphenylazo)-uracil (obtained from B. W. Langley) was dissolved in 0.05 N NaOH at a final concentration of 5 mg/ml. Erythromycin (Abbott Laboratories, North Chicago, Ill.) and chloramphenicol (Sigma Chemical Co., St. Louis, Mo.) were dissolved in glass-distilled water at final concentrations of 400 and 500 $\mu$g/ml, respectively. All stock solutions were filtered through a type HA filter (0.45 $\mu$m, Millipore Corp., Bedford, Mass.) and stored in the dark at 4 C.

## RESULTS

**Frequency of transformation in lysogenic and non-lysogenic competent cells.** Previously we demonstrated that the frequency of transformation varied considerably for lysogenic strains throughout the period of competence (44, 45).

This fluctuation in the frequency of transformation could be due to changes in the ability of competent lysogenic cells to take up transforming DNA or to the selective elimination of lysogenic cells from the competent population. To resolve this question, DNA (1 μg/ml) from strain RUB818 was added to competent cultures of strain RUB814 and lysogenic strain RUB813 at $T_{90}$ (the time of dilution into GM2). At half-hour intervals, samples were removed and diluted in minimal salts, and the number of $Trp^+$ transformants was determined. As shown in Fig. 1, there was a selective elimination of the transformants in competent lysogenic cultures. The number of transformants in the non-lysogenic strain (RUB814) reached a maximum and maintained that state over the 6-h period of competence. In contrast, after strain RUB813 reached a maximal level of transformation, the number of $Trp^+$ transformants began to decrease and then eventually increased. These data suggest that whereas the level of transformation is initially decreased in lysogenic cultures as compared to the non-lysogen, this reduction is even greater if the transformed cells are permitted to incubate in GM2 before being diluted and plated onto minimal agar.

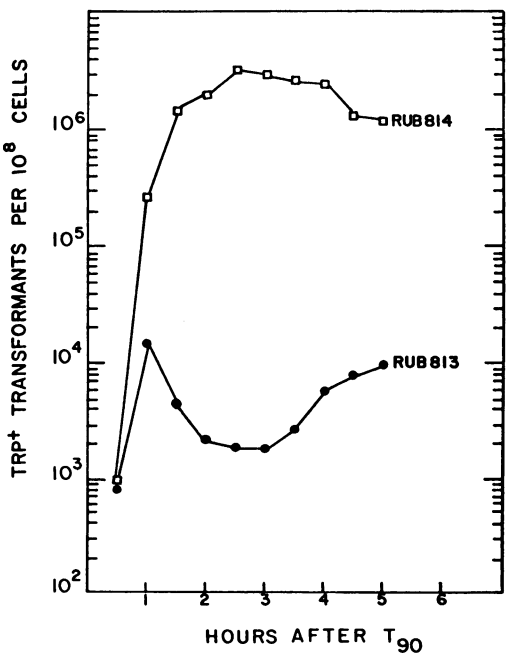


FIG. 1. *The effect of additional incubation on the level of transformation. DNA was added to competent cultures of strain RUB814 (□) and strain RUB813 (●) at $T_{90}$. At half-hour intervals, samples were taken and the number of $Trp^+$ transformants per $10^8$ cells was determined as described.*

This apparent reduction in the number of transformants which occurred upon additional incubation of competent lysogenic cells was investigated further. The non-lysogenic strain RUB814 and the lysogenic strain RUB813 were grown to competence, and transforming DNA was added at half-hour intervals after $T_{90}$. After a 20-min incubation with DNA and a 5-min incubation with DNase, samples were collected and an immediate determination of the number of transformants was made. Additionally, each sample was held until after the 210-min sample had been collected, and then a delayed determination of the number of transformants was made (Fig. 2). It is important to note that the time of the additional incubation for each delayed sample was not constant. For instance, the sample collected 60 min after $T_{90}$ was incubated for an additional 150 min, whereas

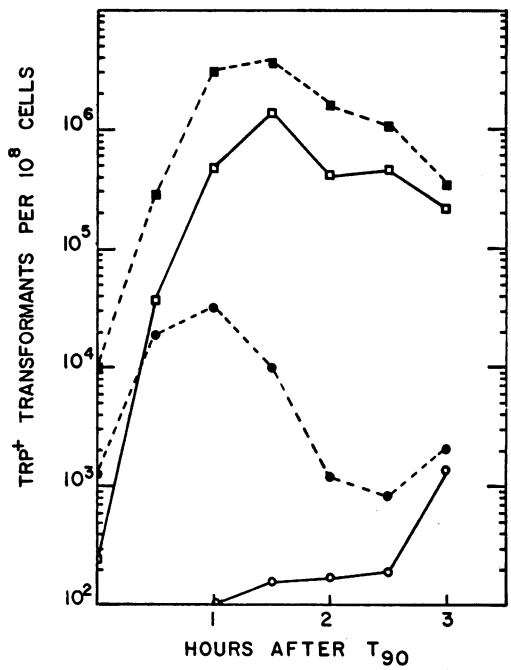


FIG. 2. *The selective elimination of transformants from a competent lysogenic culture. DNA was added to competent cultures of strain RUB814 and strain RUB813 at half-hour intervals after $T_{90}$. DNA incubation was for 20 min followed by a 5-min incubation with DNase as described. A determination of the number of $Trp^+$ transformants was done for each sample immediately after the DNase incubation and again after the 210-min sample had been taken. Symbols: the number of $Trp^+$ transformants for RUB814 immediately assayed ((■) and for the delayed assay (□); the number of transformants for RUB813 immediately assayed (●) and for the delayed assay (○).*

the sample collected 180 min after $T_{90}$ was incubated for an additional 30 min. These experiments (Fig. 2) show that at the maximal time of competence, a delay in the plating of transformed lysogenic cells onto minimal agar definitely results in a drastic loss in the number of transformants. Although there is some loss in the number of transformants in the non-lysogenic strain, this decrease is not of the same magnitude as that shown for the lysogenic cells. This slight loss of transformants in the non-lysogenic culture will be discussed later.

**Effects of growth on the elimination of transformed lysogenic cells from competent cultures.** The observation that transformed lysogenic cells were lost if the competent culture was allowed to incubate in GM2 prior to plating on minimal agar suggested that bacterial growth might be involved in the elimination of the transformants. To determine which aspect of growth was involved in the decrease in the level of transformants in competent lysogens, strains RUB814 and RUB813 were grown to competence, transformed, and incubated in GM2 for an additional 120 min in the presence of various inhibitors. The characteristic loss of transformants after incubation of strain RUB813 in GM2 was observed when DNA synthesis was inhibited with 6-(*p*-hydroxyphenylazo)-uracil (3, 17), as shown in Table 1. However, incubation in GM2 for the additional 120 min did not result in loss of any transformants if protein synthesis was inhibited with either erythromycin or chloramphenicol (26), and only a slight loss of transformants was obtained when ribonucleic acid synthesis was inhibited with rifampin (4). Therefore, the results in Table 1 demonstrate that protein synthesis is an absolute requirement for the selective elimination of transformed lysogens from a competent population.

To determine when protein synthesis must be inhibited to prevent the selective elimination of the transformed lysogens, strains RUB814 and RUB813 were transformed and incubated with erythromycin for various intervals after the addition of DNase. In these experiments, competent cultures were incubated with DNA and DNase for 10 and 5 min, respectively. The data (Fig. 3) indicate that protein synthesis must be inhibited within 30 min after the addition of DNA to prevent loss of transformed lysogens.

**Coincidence of prophage release and selective elimination of transformed lysogens.** The induction of the resident prophage could explain the observed reduction in the number of transformed lysogens. To determine if the resident prophages were being induced, DNA isolated from strain RUB818 was added to strain RUB813 at $T_{90}$. Samples were taken every half hour and the number of $Trp^+$ transformants was measured. Additionally, each sample was centrifuged (8,000 × *g*) and the number of plaque-forming units (PFU) was scored. Because strain RUB813 cannot adsorb bacteriophage $\phi$105, most of the bacteriophages released during the development of competence could be detected. As shown in Fig. 4, as the number of transformants begin to decrease, there was a concomitant rise in the number of PFU. Significantly, addition of erythromycin to the competent culture prevented the decrease in the number of transformants as well as the increase in the PFU. Surprisingly, the increase in the number of PFU was found to occur even in the absence of exogenous transforming DNA. Thus, it was important to ascertain whether the increase in PFU was directly related to the cells becoming competent or merely a result of the dilution of stationary cells into fresh media. To answer this question, strain RUB813 was grown in GM1 and in PB. Both cultures were treated as if they were being grown to competence. At

TABLE 1. *Effect of inhibitors on loss of transformants*

| Expt. | Additional incubation (min) | Incubation media[a] | $Trp^+$ transformants per $10^8$ cells | |
|---|---|---|---|---|
| | | | RUB814 | RUB813 |
| 1 | None | NA | 300,000 | 19,000 |
| | 120 | GM2 | 180,000 | 160 |
| | 120 | GM2 + HPUra (250 μg/ml) | 120,000 | 180 |
| | 120 | GM2 + chloramphenicol (50 μg/ml) | 320,000 | 28,000 |
| | 120 | GM2 + erythromycin (20 μg/ml) | 270,000 | 34,000 |
| 2 | None | NA | 4,900,000 | 57,000 |
| | 120 | GM2 | 2,400,000 | 4,300 |
| | 120 | GM2 + rifampin (25 μg/ml) | 1,400,000 | 41,000 |

[a] NA, Not applicable; HPUra, 6-(*p*-hydroxyphenylazo)-uracil.

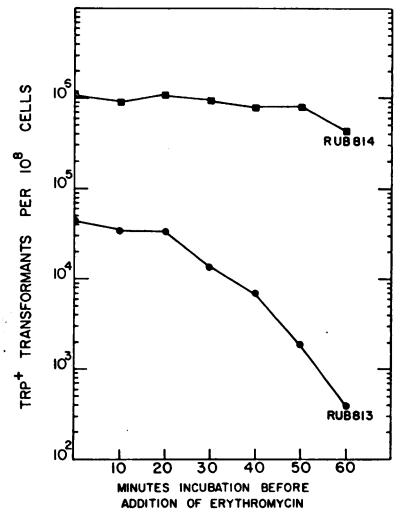


FIG. 3. *The loss of transformants from lysogenic populations as a function of time before the addition of erythromycin. Competent cultures of strain RUB814* (■) *and strain RUB813* (●) *were incubated with transforming DNA 1 h after* $T_{90}$. *DNA incubation was for 10 min followed by a 5-min incubation with DNase. At 10-min intervals after the addition of DNA, portions were added to erythromycin (20 μg/ml) and incubated with aeration at 37 C until 180 min after* $T_{90}$. *The number of Trp+ transformants was subsequently determined.*

$T_{90}$, the first culture was diluted into GM2 whereas the second culture was diluted into fresh PB. *B. subtilis* does not develop a significant level of competence in PB (48). The results (Fig. 5) indicate that induction of the prophage occurred only when the population developed competence. Prior to dilution at $T_{90}$, the PFU were approximately 1.0% of the number of colony-forming units in both the culture grown in GM1 and the culture grown in PB. However, in the culture grown to competence (diluted in GM2), the number of PFU after dilution was eventually 237% of the colony-forming units, whereas in the culture diluted in PB, the number of PFU was only 2.8% of the colony-forming units. Therefore, there was a significant release of bacteriophage from strain RUB813 only when it was grown to competence.

Preferential lysis of transformed lysogens should result in the release of transforming DNA from competent lysogens. To explore this possibility, strains RUB814 and RUB813 were grown to competence and incubated with [$^3$H]thymidine-labeled DNA (0.01 μg/ml) for 20 min and with DNase for 5 min. Samples were removed periodically to determine the amount of radioactive DNA that remained bound to the competent cells in a DNase-resistant form. Additionally, the competent cells were incubated in the absence and presence of erythromycin (20 μg/ml). Over a 2-h period there was only a 50% loss in the radioactivity in the competent populations of strains RUB814, RUB814 treated with erythromycin, and RUB813 treated with erythromycin (Fig. 6). This amount of solubilization of donor DNA would be expected from the degradation of one of the strands to nucleotides during the early events in DNA-mediated transformation (10). However, over the same 2-h period, there was a loss of 95% of the radioactivity in the competent population of RUB813. Therefore, we conclude that preferential lysis of competent lysogens is responsible for the selective elimination of transformed lysogens.

## DISCUSSION

**Relationship among genetic recombination, repair of DNA, and induction of prophage.** The biochemical genetic defects in re-

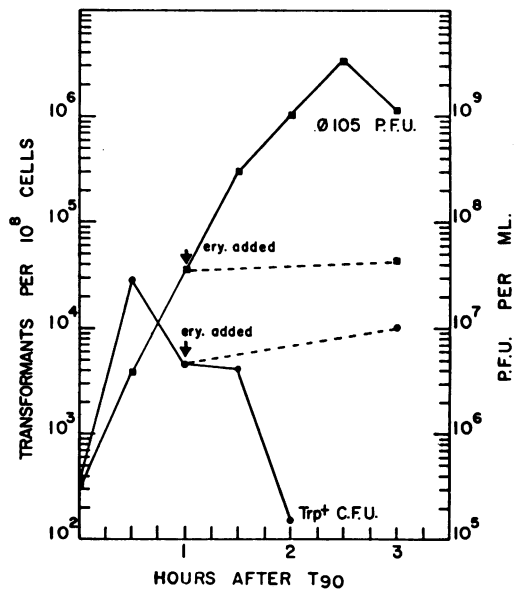


FIG. 4. *The loss of transformants from competent lysogenic cultures and the coincident release of bacteriophage. DNA was added to a competent culture of strain RUB813 at* $T_{90}$, *and DNase was added 60 min later. Samples were taken at half-hour intervals starting with 20 min after the addition of the transforming DNA. The number of Trp+ transformants* (●) *and the number of φ105 PFU* (■) *were determined. PFU determination was made after centrifugation of the samples (8,000 × g for 2 min). At 60 min after the addition of DNA, a sample was removed and placed in erythromycin (20 μg/ml), and incubation was continued for an additional 120 min. DNase was added to the 0- and 30-min sample at the time of assay.*

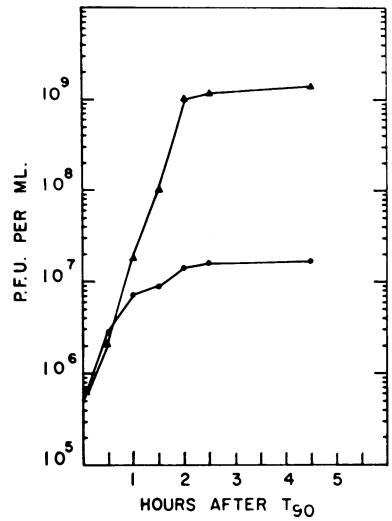


FIG. 5. *The release of bacteriophage from lysogenic cells grown to competence. Strain RUB813 was grown to competence as described. In addition, a second culture of RUB813 was grown in PB using the competence regime. The number of PFU was determined after the dilution of the competent culture into GM2 (▲) and dilution of the second culture into fresh PB at $T_{90}$ (●).*

combination deficient mutants and mutants deficient in the repair of damaged DNA have been recently reviewed (7). Interestingly, in *Escherichia coli*, some recombination and repair deficient mutants were also unable to induce resident prophages. Specifically, *recA*$^-$, *lex*$^-$, and *zab*$^-$ mutants were reduced to some degree in their ability to induce prophage lambda either spontaneously or after treatment with agents such as ultraviolet irradiation (UV) and mitomycin C (6, 7). Repair of damaged DNA was blocked by altering one of the dark repair pathways present in *E. coli* (2, 6, 28, 35). The three suggested dark repair systems consist of excision repair, postreplication (recombination) repair, and a SOS inducible repair (28, 35, 42). The proposed inducible or SOS repair is the system involved with the induction and release of prophage. This inducible repair system, as well as the induction of prophage lambda and mutagenesis after UV treatment, is believed to be under the control of the *recA*$^+$, *lex*$^+$, and *zab*$^+$ gene products (7, 24, 28, 42). Both direct and indirect UV reactivation of bacteriophage lambda are regulated by these genes (8, 18). Where genetic and biochemical analyses were possible, all of the functions that were under the control of the *recA*, *lex*, and *zab* genes were inhibited when protein synthesis was blocked (28, 42). A thermosensitive mutant, *tif*-1, whose expression mimics the effect of UV irradiation has been isolated (5, 6, 41). This mutation is suppressed by a mutation in the *recA*, *lex*, or *zab* genes. These results have led Witkin and George (42) and Radman et al. (8, 18, 28) to suggest that this inducible repair system does exist. Additionally, these workers have concluded that this inducible repair system is error prone since mutants that inhibit the induction of this system also prevent UV mutagenesis. It is proposed that after damage to chromosomal DNA, a signal is released which, upon reaching threshold levels, induces a repair system which is efficient but inaccurate (6, 28, 42). The inducible system will not be activated if most of the damage to the DNA is repaired via one of the other mechanisms (i.e., photoreactivation, excision repair, and recombination repair). Radman suggests (28) that the SOS repair is necessary for the passing of dimers (or other damage in the DNA) during replication and/or

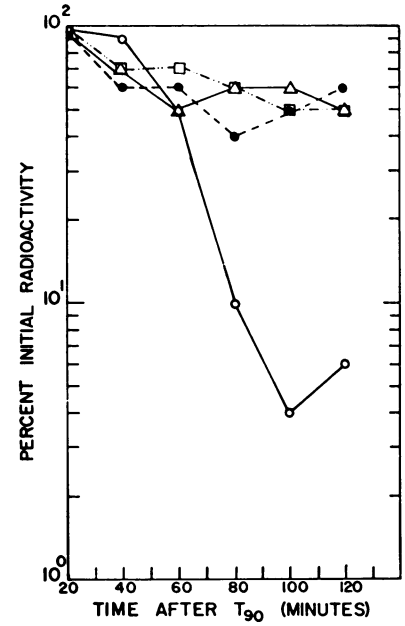


FIG. 6. *Release of transforming donor DNA from competent lysogenic and non-lysogenic cells. Competent cultures of strain RUB814 and strain RUB813 were incubated with [$^3$H]thymidine-labeled DNA at 1 h after $T_{90}$. DNA incubation was for 20 min followed by a 5-min incubation with DNase. The cultures were allowed to incubate at 37 C with aeration in the presence and absence of erythromycin (20 μg/ml). At 20-min intervals, samples were taken and the amount of labeled DNA bound to the cells in a DNase resistant form was determined as described. The percentage of the initial radioactivity is given for RUB814 (□), RUB814 + erythromycin (△), RUB813 (○), and RUB813 + erythromycin (●).*

the filling of gaps caused by the passing of dimers during replication. The signal that activates this error prone (SOS) repair system is probably also responsible for the induction of prophage, direct and indirect UV reactivation, and formation of long filamentous cells in UV-irradiated bacteria or in the shift of the *tif*-1 mutant to the nonpermissive temperature (5, 6, 28, 42).

The characterization of recombination and repair deficient mutants has not been as extensively investigated in *B. subtilis* as in *E. coli* (12, 13, 30, 31, 44). Nevertheless, some recent studies have resulted in the characterization of mutants that alter recombination and that are deficient in repair processes. In addition, some of these mutants are unable to induce and release prophage (30, 44). These early studies therefore suggest that the repair and recombination systems in *B. subtilis* may be analogous to repair and recombination in *E. coli*.

**Role of lysogeny in decreasing transformation frequency.** Harris and Barr observed that up to 5% of the chromosome of competent *B. subtilis* contained single-stranded regions (19–21). Their data suggested that this single strandedness is the result of gaps in the bacterial chromosome. Furthermore, they demonstrated that 4 to 10% of the host cell DNA was released as the cells grew to competence and that both this release of DNA and the further accumulation of gaps were blocked by the addition of puromycin (20). This inhibition in the accumulation of gaps was not found when actinomycin D was added to the competent cells. The DNA of competent cells accumulated gaps even in the absence of exogenous transforming DNA (21). Harris and Barr suggested that during transformation, single-stranded donor DNA lines up in the gaps and repair processes fill in the gaps to provide the recombinational event associated with transformation (21).

The suggestion of gap accumulation in competent cells of *B. subtilis* and the hypothesis of the SOS repair system in *E. coli* has led us to formulate the following model to explain the mechanism by which lysogeny decreases the level of transformation in competent *B. subtilis* (Fig. 7). In stage I, noncompetent cells begin to become competent. At this point, metabolic changes occur which enable the competent cells to irreversibly bind DNA and to allow this DNA to penetrate the cell wall and membrane. Additionally, the cells begin to produce an enzyme which we have designated "GAPase". GAPase could represent a multifunctional enzyme or

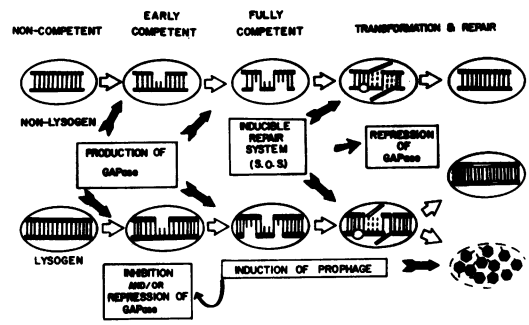


FIG. 7. *A model presenting the changes in the recipient chromosome during the process of transformation. As described, the model explains how lysogeny affects the levels of transformation.*

several enzymes whose functions are to nick the host DNA (endonucleic activity) and then to create a single-strand gap in the chromosome. In stage II, early competent cells become fully competent. The number of gaps in the chromosome has reached a maximum. As the gaps accumulate, a signal is released which eventually induces a SOS repair system. This repair system closes the gaps and represses and/or inhibits GAPase. Transforming DNA will be incorporated into the recipient chromosome as the repair system is functioning. In stage III, the gaps in the chromosome of the competent cells have been closed and recombination has occurred between the donor transforming DNA and the recipient chromosome. In addition, no further gaps are produced since GAPase has been repressed and the cells are now capable of growth and replication. Our model would suggest that, as gaps are beginning to accumulate (stage II), not only is the inducible repair system activated but prophage induction also occurs. Therefore, in lysogens, the number of transformants would be reduced since some of the transformed cells would lyse and produce bacteriophage.

The following key observations support the proposed model. Harris and Barr found that single-strand gaps exist in the chromosomes of competent cells and that the number of gaps can be decreased by the inhibition of protein synthesis in these cells (19–21). The decrease in the number of transformed lysogens after incubation in GM2 or PB can be explained by the further accumulation of gaps resulting in the induction of more prophage (Fig. 1, 2, and 4). The inhibition of protein synthesis not only prevents the production of gaps but it also increases the survival of transformed lysogens. In addition, the inhibition of protein synthesis prevents the dramatic induction of prophage

which normally accompanies the development of competence in lysogenic strains (Fig. 4 and 5). The data presented in Fig. 2 show that even in the non-lysogenic strain of *B. subtilis* (RUB814), there is some loss in the number of transformants upon additional incubation of the competent culture. A similar observation has been made by C. Hadden (personal communication) and an explanation for this phenomenon is suggested by our model. Strain RUB814 is a derivative of *B. subtilis* 168 and therefore carries the inducible defective prophage PBSX (32, 39). When strain RUB814 is incubated after the addition of transforming DNA, some induction of defective phage PBSX should occur resulting in a small decrease in the number of transformants.

Finally, the model presented in this paper is in agreement with the concept that only a minimal amount of DNA synthesis is required for the recombination of donor and recipient DNA in the process of transformation (9, 11, 23). Thus the conclusions of Laird et al. (22) and Ganesan (14) that integration of transforming DNA occurs randomly around the chromosome are compatible with our model.

## ACKNOWLEDGMENTS

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