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# Restriction-Like Phenomena in Transformation of *Bacillus subtilis recA*

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Genetic transformation in *recA1* strains of *Bacillus subtilis* was studied to test the hypothesis that, in these strains, a major pathway of recombination is missing, leaving only residual transformation via a pathway specific for transduction. The two putative recombinational pathways have been hypothesized to differ in either length of synapsed regions or specificity for nucleotide sequence homology. It was found that the efficiency of transformation of *recA1* cells by deoxyribonucleic acid (DNA) from the heterologous strain W23 was much lower than when a homologous donor DNA was used, the relative efficiency being different for different genetic markers. Because the frequency of recombination between linked markers is only slightly changed in *recA1* recipients, and because markers of heterologous origin in DNA from intergenotic strains are not discriminated against strongly by *recA1* recipients, it is concluded that neither a difference in length of synapsed DNA nor a difference in specificity for nucleotide sequence homology accounts for reduced transformation in *recA1* cells. It is proposed that at some time between uptake and integration, heterologous DNA is inactivated by restriction, and that aberrant restriction of repaired regions may account for reduced transformation by homologous DNA.

The efficiency of genetic exchange in *Bacillus subtilis* depends on both the source of the donor deoxyribonucleic acid (DNA) and the route of DNA transfer. Two varieties of *B. subtilis*, strain 168 and strain W23, differ to some extent in DNA sequence homology (20), cell wall composition (15), regulation of DNA replication (25), and some other parameters. In addition, derivatives of strain 168 are transformable, whereas derivatives of strain W23 are not (25). Derivatives of strain 168 can readily be transformed with DNA from other 168 strains or by DNA from strain W23. (For the purpose of this paper, these will be referred to as homologous and heterologous transformation, respectively.) However, the transduction efficiency of either of these strains with a lysate of the other (heterologous transduction) is reduced compared with transduction with a homologous lysate (13). Furthermore, the extent of reduction in efficiency varies with the marker selected (13). A mutation in a 168 strain, *recA1* (18), was shown to reduce both transformation and heterologous transduction to a small percentage of the wild-type level, while only moderately reducing homologous transduction (13, 19). These facts led Dubnau et al. (13) to conclude that there are two modes of recombinant formation in *B. subtilis*: one, controlled by the *recA1* gene, used for transformation and heterologous transduction (pathway I), and one used for homologous transduction (pathway II).

The residual transformation observed in *recA1* mutants could be interpreted in this scheme as resulting from leakiness of the mutation, allowing a small amount of transformation through pathway I or, alternatively, from the use of pathway II to process a small amount of transforming DNA. Reasoning that pathway II might be marker specific in discrimination against heterologous DNA in transformation as well as in transduction, I determined the ratios of transformation at several loci in *recA1* strains by use of heterologous and homologous DNA. Heterologous transformation in *recA1* strains is reduced for all markers, and some markers are discriminated against more than others. However, further investigation has led to the conclusion that there are probably not two pathways of recombinant formation. Instead, I hypothesize that after incorporation into the recipient cell, donor DNA in heterologous transduction of Rec$^+$ and *recA1* strains and in heterologous transformation of *recA1* strains is destroyed by host-specific restriction or a similar process.

## MATERIALS AND METHODS

**Bacterial strains.** All strains used in this study except strain W23 were derived from strain 168 and are therefore homologous. A series of *recA1* strains

was constructed by transformation, starting with strain GSY1025 as recipient (except for ORB74, for which GSY1025 was the donor). The *trpC2* or *metB4* mutation was maintained as a reference marker, and other mutations were introduced coincident with transformation to prototrophy at an expendable site. The *recA* character was determined by testing for sensitivity to 2 mM methyl methane sulfonate in nutrient agar. When the desired *recA1* strains were obtained, $Rec^+$ derivatives were prepared by transformation to resistance to 2 mM methyl methane sulfonate with DNA from strain GSY1026. Intergenotes were constructed by transformation with DNA from strain WB746, isolation of singly transformed derivatives, and transformation of those derivatives to prototrophy with DNA from strain W23. The heterologous origin of markers in intergenotes is indicated as follows: ORB127 is described as *hisA1* ($Trp^+$ · W23), meaning that the $Trp^+$ locus came from strain W23. The strains used in this study are listed in Table 1.

**DNA extraction.** For most experiments, DNA was extracted from stationary-phase cells grown in Difco antibiotic medium no. 3. The method of Marmur (22) was used, omitting treatment with ribonuclease and precipitation with isopropanol. When DNA was reextracted from transformed cultures, the samples were diluted with an equal volume of buffer containing 40 mM tris(hydroxymethyl)aminomethane (Tris) and 60 mM disodium ethylenediaminetetraacetate (EDTA) at pH 7.4. The cells were centrifuged and resuspended in 2 ml of 20 mM Tris–30 mM EDTA (pH 7.4). They were incubated for 20 min at 37°C with 0.4 mg of lysozyme per ml (Sigma Chemical Co., St. Louis, Mo.) and then lysed by the addition of sodium lauryl sulfate at a final concentration of about 0.075%. The lysates were shaken for 40 min at room temperature with an equal volume of phenol saturated with Tris buffer (20 mM, pH 7.2) and centrifuged, and the aqueous layers were dialyzed in the cold against four 1-liter changes of 15 mM NaCl–1.5 mM sodium citrate, pH 7.0.

**Transformation.** Cells were made competent by a nutritional step-down procedure (9), concentrated, and stored at −80°C in modified (5) Spizizen salts (1) plus 10% glycerol. Transformation was carried out at 37°C after diluting thawed competent cultures with Spizizen salts containing 0.5% glucose and 5 mM $MgSO_4$ and adding a saturating amount (about 10 μg/ml) of transforming DNA. Exposure to DNA was terminated by treatment with deoxyribonuclease (Calbiochem, San Diego, Calif.) at 20 μg/ml for 5 min. Cells were plated on the synthetic amino acid medium as described previously (16).

**Transduction.** Bacteriophage PBS2 (generously supplied by W. L. Carrier) was used to prepare a transducing lysate of strain W23. This lysate was then used to transduce cells of strain SB1. The methods used were described by Lovett and Young (21).

## RESULTS

**Effect of the *recA* mutation on heterologous transformation.** A number of closely re-

TABLE 1. *Bacterial strains used*

| Strain | Genotype | Origin |
|---|---|---|
| WB746 | Prototroph (homologous) | E. W. Nester |
| W23 | Prototroph (heterologous) | S. Greer |
| SB1 | *hisA1 trpC2* | E. W. Nester |
| SB5 | *hisA1 trpC2 pyrA1* | E. W. Nester |
| WB2676 | *purB6 metD3* | E. W. Nester |
| FB14 | *purA16 metB5 uvr-1* | C. T. Hadden |
| HA101 | *hisB leu metB* | B. Strauss |
| BR58 | *pyrA1 ilvA2 trpC2* | F. Young |
| GSY1025 | *trpC2 metB4 recA1* | J. Hoch |
| GSY1026 | *trpC2 metB4* | J. Hoch |
| ORB113 | *hisB leu metB4 recA1* | HA101-× GSY1025[a] |
| ORB114 | *trpC2 leu metB4 recA1* | GSY1025-× ORB113 |
| ORB116 | *leu ilvA2 trpC2 recA1* | BR58-× ORB114 |
| ORB117 | *hisA1 trpC2 metB4 recA1* | SB5 -× ORB114 |
| ORB118 | *pyrA1 trpC2 metB4 recA1* | SB5 -× ORB114 |
| ORB120 | *purA16 ilvA2 trpC2 recA1* | FB14 -× ORB116 |
| ORB128 | *hisB leu metB4* | GSY1026 -× ORB114 |
| ORB129 | *hisA1 trpC2 metB4* | GSY1026 -× ORB117 |
| ORB130 | *pyrA1 trpC2 metB4* | GSY1026 -× ORB118 |
| ORB131 | *purA16 ilvA2 trpC2* | GSY1026 -× ORB120 |
| ORB138 | *purB6 hisA1 trpC2 recA1* | WB2676 -× ORB117 |
| ORB139 | *purB6 metD3 trpC2 recA1* | WB2676 -× ORB138 |
| ORB140 | *purB6 metD3 trpC2* | GSY1026 -× ORB139 |
| ORB77 | *trpC2* | WB746 -× SB1 |
| ORB78 | *hisA1* | WB746 -× ORB77 |
| ORB79 | Prototroph ($Trp^+$ · W23) | W23 -× ORB77 |
| ORB80 | Prototroph ($His^+$ · W23) | W23 -× ORB78 |
| ORB127A-L | *hisA1* ($Trp^+$ · W23) | W23 $\xrightarrow{PBS2}$ SB1 |

[a] -× = transformation cross.

lated strains bearing various nutritional markers were constructed and used as recipients for transformation by DNA from strains WB746 (homologous) and W23 (heterologous). The cells were exposed to saturating concentrations of transforming DNA, and the relative levels of transformation of several markers with heterologous and homologous donor DNA were determined. The results, expressed as ratios (*R*) of heterologous to homologous transformation at a saturating concentration of DNA, are presented in Table 2. The values of *R* for transformation were significantly lower for all markers when assayed in *recA1* derivatives than in Rec$^+$ strains. The marker-specific variations in *R* for transformation of *recA1* strains were similar, with some exceptions, to those for transduction of Rec$^+$ strains (13). Since leakiness in pathway I should not result in marker-specific discrimination against heterologous DNA (as in transduction), and since pathway II should exclude heterologous DNA more effectively than was observed, neither of the proposed pathways seemed to be adequate to explain the results of transforming *recA1* recipients.

**Effect of *recA1* on linkage.** Dubnau et al. (13) suggested that the basis of the difference in marker-specific discrimination between transformation and transduction might be the size of the donor DNA molecules. They proposed that pathway II could act only on long regions of sequence homology between donor and recipient and suggested that heterologous transducing fragments from PBS1, being much larger than transforming fragments, would have more opportunity for sequence nonhomology, resulting in rejection by pathway II in the recipient cell. Pathway I was proposed to act only on short regions of homology, including both the small transforming fragments and the short regions of homology in heterologous transducing fragments. If only large fragments can be integrated by pathway II, and if only pathway II is used in *recA1* recipients, fragments in *recA1* recipients would be expected to be as large as transducing fragments. This was not observed in physical studies by Dubnau et al. (12), but a minority of large molecules might have been missed.

If the donor DNA fragments in transformation of *recA1* strains are large, an increase in linkage of markers should be observed. There was a decrease in recombination (increase in linkage) between the closely linked markers *hisB* and *trpC2* when strain GSY1025 was transformed with DNA from the His$^-$ strain HA101 (Table 3), confirming the results of Hoch et al. (19), but the increase in the linkage was only very slight. Furthermore, the loosely linked markers *metB* and *ilvA*, which are cotransduced by PBS1 at a frequency of around 90% (14), appeared no more closely linked (about 24%) in transformation of strain GSY1025 than in transformation of strain GSY1026 by DNA from strain BR58 (Table 3). Thus the inserted transforming DNA fragments appear to be slightly longer in *recA1*

TABLE 2. *Number of colonies scored and values of R (± standard deviation) for transformation of Rec$^+$ and recA1 strains*[a]

| Marker | Rec$^+$ recipients | | | *recA1* recipients | | |
|---|---|---|---|---|---|---|
| | Donor | | *R* | Donor | | *R* |
| | W23 | WB746 | | W23 | WB746 | |
| *hisA1*[b] | 298 | 521 | 0.566 ± 0.075 | 1,197 | 5,117 | 0.027 ± 0.013 |
| *hisB*[c] | 3,228 | 2,175 | 1.486 ± 0.181 | 871 | 2,185 | 0.354 ± 0.088 |
| *ilvA2*[d] | 271 | 429 | 0.637 ± 0.073 | 3,203 | 2,222 | 0.119 ± 0.023 |
| *leu*[e] | 1,335 | 1,558 | 0.884 ± 0.358 | 219 | 673 | 0.324 ± 0.018 |
| *metB4*[b,e] | 971 | 2,389 | 0.392 ± 0.088 | 1,903 | 4,382 | 0.048 ± 0.016 |
| *metB4*[c] | 3,737 | 4,793 | 0.778 ± 0.056 | 387 | 2,226 | 0.173 ± 0.015 |
| *metD3*[f] | 1,946 | 2,068 | 0.918 ± 0.072 | 527 | 865 | 0.054 ± 0.001 |
| *purA16*[d] | 1,245 | 3,493 | 0.309 ± 0.068 | 1,203 | 4,785 | 0.023 ± 0.006 |
| *purB6*[f] | 2,043 | 1,820 | 1.101 ± 0.138 | 392 | 1,032 | 0.282 ± 0.060 |
| *pyrA1*[e] | 584 | 1,288 | 0.460 ± 0.065 | 1,999 | 7,110 | 0.034 ± 0.020 |
| *trpC2*[b,d,e,f] | 3,736 | 2,980 | 2.158 ± 0.758 | 13,504 | 10,090 | 0.279 ± 0.136 |

[a] Competent cells were transformed with saturating concentrations of DNA from strains W23 (heterologous) and WB746 (homologous) as described in Materials and Methods. *R* is the ratio of transformant titer with W23 DNA to transformant titer with WB746 DNA. The ratios of total colonies scored are not the same as *R* because of differences in dilutions and because *R* is an average of ratios from several experiments.

[b] Measured in strains ORB117 and ORB129.

[c] Measured in strains ORB113 and ORB128.

[d] Measured in strains ORB120 and ORB131.

[e] Measured in strains ORB118 and ORB130.

[f] Measured in strains ORB139 and ORB140.

recipients, but not as long as in PBS1 transduction. This means that the proposed pathway II of Dubnau et al. (13) could not account for transformation in *recA1* recipients and also be absolutely specific for large DNA fragments, although its postulated requirement for close sequence homology might not depend on size of the paired regions when pathway I is missing.

**Transformation with DNA from hybrid strains.** Hybrid strains (intergenotes) prepared by transformation of markers from W23 into derivatives of strain 168 have short segments of heterologous DNA in a background generally homologous with other 168 derivatives. These heterologous markers behaved like homologous DNA when the intergenotes were used as either donor or recipient in the transduction experiments of Dubnau et al. (13). Similarly, the intergenotes ORB79 ($Trp^+ \cdot W23$) and ORB80 ($HisA^+ \cdot W23$) were prepared by transformation of strain SB1. The transforming activity for markers of W23 origin was the same when DNA from these strains was used to transform $Rec^+$ and *recA1* recipients (Table 4). Because the intergenotes were prepared by transformation, the length of the donor's heterologous region should be about the same as the regions of pairing in the test transformation of the *recA1* strain. This implies that sequence homology probably does not determine whether a DNA fragment will be rejected in transformation of *recA1* strains.

TABLE 3. *Effect of recA1 on linkage of closely and loosely linked markers in transformation*

| Transformant phenotype | Colonies from transformation of: | |
|---|---|---|
| | Strain GSY1026 ($Rec^+$) | Strain GSY1025 (*recA1*) |
| $Trp^+$ ($His^\pm$)[a] | 396 | 349 |
| $Trp^+$ $His^+$ | 105 | 58 |
| $\frac{Trp^+ His^+}{Total\ Trp^+}$[b] | 0.265 | 0.166 |
| $Met^+$ ($Ile^\pm$)[c] | 400 | 400 |
| $Met^+$ $Ile^+$ | 307 | 304 |
| $\frac{Met^+ Ile^+}{Total\ Met^+}$[b] | 0.768 | 0.760 |

[a] Competent cells of GSY1026 or GSY1025 were transformed with DNA from strain HA101 and plated on agar containing histidine and methionine. Colonies were restreaked and replica plated to determine the $His^+$ phenotype.

[b] Fraction with recombination between the markers tested.

[c] Competent cells of GSY1026 or GSY1025 were transformed with DNA from strain BR58 and plated on agar containing tryptophan and isoleucine. The recombinant phenotype was determined by restreaking and replica plating.

The experiment described above suffers because of uncertainty about the amount of heterologous DNA integrated in the test crosses. If the hybrid strains are prepared by PBS2 transduction, the heterologous region has to be much longer than the transforming fragment (4). $Trp^+ \cdot W23$ transductants of strain SB1 were prepared, using a PBS2 lysate of strain W23 (in this case no $His^+$ transductants were obtained, as predicted by Dubnau et al. [13]). To reduce the probability that the heterologous region of the intergenotes by chance began or ended very near the selected marker, 12 randomly chosen transductants were used as hybrid donors in a test transformation of the *recA1* strain, ORB118, and its $Rec^+$ derivative, ORB130. The ratios of transformants for the heterologous $Trp^+$ marker to the (homologous) resident $Ura^+$ marker with these DNA preparations, normalized to the ratio for DNA from strain WB746, were 0.77 for the *recA* recipient and 0.81 or the $Rec^+$ recipient.

TABLE 4. *Transformation of heterologous markers by transformation-generated intergenotes*[a]

| Donor | Marker | Recipient strain ORB129 ($Rec^+$) | | Recipient strain ORB117 (*recA1*) | |
|---|---|---|---|---|---|
| | | CFU[b]/ml ($\times 10^5$) | *R* | CFU/ml ($\times 10^5$) | *R* |
| WB746 | $HisA^+$ | 7.89 | 1.00 | 7.88 | 1.00 |
| | $TrpC^+$ | 4.34 | 1.00 | 5.82 | 1.00 |
| | $MetB^+$ | 10.96 | 1.00 | 10.46 | 1.00 |
| ORB79 | $HisA^+$ | 6.80 | 1.52 | 5.60 | 1.16 |
| | $TrpC^+$[c] | 3.10 | 1.26 | 3.32 | 0.93 |
| | $MetB^+$ | 6.23 | 1.00 | 6.39 | 1.00 |
| ORB80 | $HisA^+$[c] | 4.23 | 0.67 | 3.94 | 0.61 |
| | $TrpC^+$ | 3.54 | 1.01 | 4.69 | 0.99 |
| | $MetB^+$ | 8.82 | 1.00 | 8.53 | 1.00 |

[a] Competent cells of ORB117 and ORB129 were transformed with excess DNA from the indicated donor strains. Values of ratios (*R*) were calculated after normalizing the numbers of $His^+$ and $Trp^+$ transformants from each strain to the number of (homologous) $Met^+$ transformants to correct for differences in transforming activity among the DNA preparations.

[b] CFU, Colony-forming units.

[c] Marker of heterologous origin.

In this case the heterologous marker was equally discriminated against in both recipients, making a mechanism of discrimination based primarily on sequence homology very unlikely.

**Site of marker discrimination.** The results presented above are similar to those obtained when a bacterial strain is infected with bacteriophage previously grown on a host with a different restriction/modification specificity, leading to the hypothesis that the discrimination by *recA1* cells against heterologous DNA results from restriction. Restriction nucleases generally are specific for double-stranded DNA and usually do not restrict strongly if one strand has the modification pattern of the host (2, 6). This is true for *B. subtilis* R (7, 8) and presumably also for *B. subtilis* 168. Since there appears to be no intracellular double-stranded donor DNA during transformation until synapse with the recipient genome (10, 11), it seemed that restriction might occur on extracellular DNA. This possibility was ruled out by incubating a *recA1* strain with W23 DNA, removing the cells, and using the cell-free filtrate to transform other *recA1* and Rec$^+$ assay strains. This incubation neither decreased the relative activity of the highly restricted *pyrA1* marker in the Rec$^+$ recipient nor affected discrimination against *pyrA1* in the *recA1* recipient (Table 5).

Restriction of donor DNA after uptake by the recipient cells was tested in the following way. If all restrictable sites are attacked before, during, or immediately after integration of heterologous DNA in *recA1* recipients, then recombinant DNA molecules extracted after exposing competent *recA1* cells to W23 DNA should be inactivated at those sites. Therefore the yield of transformants for markers with restrictable sites should be the same in Rec$^+$ and *recA* assay strains and should reflect the low yield of transformants for those markers in the strain from which the recombinant DNA was extracted. If, on the other hand, restriction is associated with outgrowth of the transformants, recombinant DNA extracted from heterologously transformed *recA* cells should show restriction in *recA* assay cells but not in Rec$^+$ assay cells, even though the transformation frequencies of the culture from which the recombinant DNA was extracted reflect restriction. Furthermore, the extent of restriction in *recA1* assay cells of heterologous markers in recombinant DNA isolated from Rec$^+$ transformants should indicate whether modification takes place immediately after integration. Experimental results (Table 6) suggested that not all restrictable sites are attacked before integration in *recA1* recipients, but that restriction occurs very early in the

TABLE 5. *Test for extracellular restriction by recA1 cells*[a]

| Recipient strain | DNA source | Transformants/ml (×10$^4$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ade$^+$ | *R*[b] | Ile$^+$ | *R* | Trp$^+$ | *R* |
| ORB120 | WB746 | 8.50 | | 1.95 | | 4.85 | |
| | W23 | 0.125 | | 0.235 | | 0.85 | |
| | | | 0.015 | | 0.120 | | 0.175 |
| | | Transformants/ml (×10$^5$) | | | | | |
| | | PyrA$^+$ | *R* | Met$^+$ | *R* | Trp$^+$ | *R* |
| ORB130 | WB746 | 20.9 | | 22.25 | | 7.95 | |
| | W23 | 8.0 | | 9.65 | | 8.75 | |
| | | | 0.383 | | 0.434 | | 1.10 |
| | WB746 Sup | 25.45 | | 19.5 | | 8.95 | |
| | W23 Sup | 5.75 | | 5.55 | | 4.50 | |
| | | | 0.226 | | 0.285 | | 0.503 |
| ORB118 | WB746 | 4.36 | | 4.34 | | 1.12 | |
| | W23 | 0.066 | | 0.053 | | 0.126 | |
| | | | 0.015 | | 0.012 | | 0.112 |
| | WB746 Sup | 5.82 | | 4.03 | | 1.34 | |
| | W23 Sup | 0.310 | | 0.104 | | 0.097 | |
| | | | 0.053 | | 0.026 | | 0.072 |

[a] A competent culture of strain ORB120 was exposed for 30 min to a saturating concentration of DNA from either strain WB746 or strain W23. The cultures were sampled for determination of transformant titer and then were centrifuged. The supernatant liquids (Sup) were sterilized by filtration through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). Competent cultures of strains ORB130 and ORB118 were diluted into either these filtrates or fresh medium to which transforming DNA was subsequently added. After a 30-min incubation, the cultures were treated with deoxyribonuclease and then plated to determine transformant titers.

[b] *R* = ratio.

TABLE 6. *Restriction of recombinant DNA*[a]

| DNA source | Recipient strain | Met$^+$/ml | Trp$^+$/ml | His$^+$ or total/ml | Met$^+$/total ($\times 10^4$) | Trp$^+$/total ($\times 10^4$) |
|---|---|---|---|---|---|---|
| First transformation | | | | | | |
| WB746 (homologous) | GSY1026 | $5.27 \times 10^6$ | $4.38 \times 10^6$ | $1.42 \times 10^8$ | 371 | 308 |
| W23 (heterologous) | GSY1026 | $2.06 \times 10^6$ | $1.85 \times 10^6$ | $1.96 \times 10^8$ | 105 | 94.0 |
| WB746 | GSY1025 | $4.05 \times 10^5$ | $5.36 \times 10^5$ | $3.36 \times 10^8$ | 12.0 | 15.9 |
| W23 | GSY1025 | $1.90 \times 10^4$ | $1.04 \times 10^5$ | $2.99 \times 10^8$ | 0.64 | 3.5 |
| Assay of reextracted DNA | | | | | | |
| WB746 -× GSY1026[b] | ORB129 | $2.97 \times 10^4$ | $1.13 \times 10^4$ | $3.18 \times 10^6$ | 93.5 | 35.6 |
| W23 -× GSY1026 | ORB129 | $1.5 \times 10^3$ | $1.8 \times 10^3$ | $2.71 \times 10^6$ | 5.54 | 6.64 |
| WB746 -× GSY1026 | ORB117 | $4.70 \times 10^2$ | $4.15 \times 10^2$ | $8.45 \times 10^4$ | 55.7 | 49.1 |
| W23 -× GSY1026 | ORB117 | $1.2 \times 10^1$ | $2.0 \times 10^1$ | $7.35 \times 10^4$ | 1.63 | 2.72 |
| WB746 -× GSY1025 | ORB129 | $3.37 \times 10^3$ | $2.50 \times 10^3$ | $5.42 \times 10^6$ | 6.22 | 4.62 |
| W23 -× GSY1025 | ORB129 | $5.25 \times 10^1$ | $1.25 \times 10^2$ | $4.86 \times 10^6$ | 0.11 | 0.26 |
| WB746 -× GSY1025 | ORB117 | $1.34 \times 10^2$ | $1.20 \times 10^2$ | $2.40 \times 10^5$ | 5.59 | 5.0 |
| W23 -× GSY1025 | ORB117 | 0 | 2 | $2.15 \times 10^5$ | 0 | 0.09 |

| | Recipient | $R$[c] | |
|---|---|---|---|
| | | Met$^+$ | Trp$^+$ |
| First transformation | GSY1026 (Rec$^+$) | 0.284 | 0.304 |
| | GSY1025 (*recA*) | 0.053 | 0.219 |
| GSY1026 transformants | ORB129 (Rec$^+$) | 0.059 | 0.187 |
| | ORB117 (*recA*) | 0.029 | 0.055 |
| GSY1025 transformants | ORB129 (Rec$^+$) | 0.017 | 0.056 |
| | ORB117 (*recA*) | 0 | 0.019 |

[a] Experiment was carried out as described in the text.
[b] -× = transformation cross.
[c] $R$ = ratio.

transformation process. There was no indication of rapid modification of heterologous DNA in Rec$^+$ recipients.

The experiment performed was the following. Competent cells of GSY1026 and GSY1025 were exposed for 30 min to DNA from strain WB746 or strain W23. After deoxyribonuclease treatment, samples were plated to determine the transformation frequencies for Met$^+$ and Trp$^+$ (Table 6, upper portion), and DNA was extracted from the remainder of each transformed culture. Each of these DNA preparations was used to transform cells of ORB117 and ORB129, and the numbers of Met$^+$ and Trp$^+$ (donor-type) and His$^+$ (resident-type) transformants were determined (Table 6, middle portion). The ratios of heterologous to homologous donor-type transformants were then compared with the ratios calculated from transformation frequencies in the first transformation (Table 6, lower portion).

When recombinant DNA extracted from Rec$^+$ cells was assayed in the Rec$^+$ strain ORB129, the values of $R$ were reduced compared with the values observed in the first transformation, which would be expected if the discrimination step occurred at each transformation. Recombinant DNA from *recA1* transformants gave values of $R$ approximately equal to the product of $R$ for Rec$^+$ and *recA1* cells in the first transformation (0.066 for Trp$^+$ and 0.015 for Met$^+$), indicating that discrimination occurred at the level of integration rather than outgrowth, because in the latter case the values of $R$ should have been similar to those for Rec$^+$ donor cells. Therefore it appears that discrimination occurred within 30 min of integration, at least in the *recA1* cells, and that the Rec$^+$ cells did not modify the heterologous DNA significantly in that time. Apparently either not all restrictable sites are attacked immediately, or there is in Rec$^+$ cells a component of discrimination based on sequence homology.

When recombinant DNA was tested in the *recA1* strain ORB117, the yields of donor-type transformants were low, so quantitative conclusions cannot be drawn reliably. The values of $R$ were, however, similar when the two successive recipient strains were different in *recA1* character, regardless of which type was the initial recipient. When both resident and assay strains were *recA1*, the values of $R$ were at least 10 times lower than when both strains were Rec$^+$, demonstrating that restrictable DNA is integrated by both Rec$^+$ and *recA1* recipients and that not all restrictable sites had been attacked or modified at the time of DNA extraction.

**Evidence that discrimination is not lethal.** If the apparent restriction were a result of double-stranded scission of DNA molecules, the recipient cells would presumably be killed by such events. Low concentrations of DNA allow transformation at fewer sites in any given

cell than higher concentrations. On a graph of transformation of Rec$^+$ cells as a function of DNA concentration, the slope is 1 for transformation at a single site in the range of limiting concentrations of DNA, and the proportion of multiple transformants at unlinked sites increases as DNA concentration increases. If the restriction-like phenomenon described above is lethal to the cells, as DNA concentration is increased the probability of lethal transformation should increase faster than the probability of transformation at a given selected site, and the slope of the DNA concentration response in *recA1* cells should be less than 1. Experimentally the slope for transformation of *recA1* cells was about 0.85 with homologous DNA and about 1.3 with heterologous DNA for both a poorly restricted marker (*trpC2*) and a highly restricted marker (*pyrA1*) (Fig. 1). The same result with homologous DNA was previously shown by Hoch et al. (19). Further confirmation was provided by an experiment in which *recA1* cells were transformed with a mixture of limiting concentrations of homologous and heterologous DNA (Table 7). The mixture gave a number of transformants essentially equal to the sum of the numbers for heterologous and homologous DNA separately, whereas lethal transformation by heterologous DNA should have reduced the yield of transformants when the mixture was used instead of homologous DNA alone.

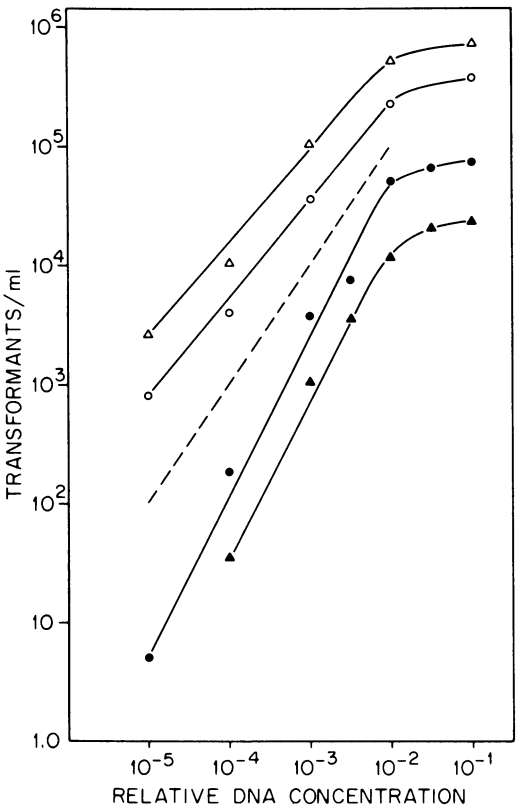


FIG. 1. *Concentration response of transformation of strain ORB74 (pyrA1 trpC2 recA1) with homologous and heterologous DNA. Competent cells of strain ORB74 were exposed for 40 min to various dilutions of stock preparations (about 100 μg/ml) of DNA from strain WB746 or strain W23. After treatment with deoxyribonuclease, appropriate dilutions were plated to select for Trp$^+$ or His$^+$ transformants. Open symbols, WB746 DNA; closed symbols, W23 DNA. ○, ●, Trp$^+$ transformants. △, ▲, Ura$^+$ transformants. The dashed line indicates a slope of 1.*

## DISCUSSION

Hoch et al. proposed (18, 19) that there might be two pathways for recombination in *B. subtilis*: one controlled by the *recA1* gene and responsible for transformation and for transduction by small DNA fragments, and the other responsible for transduction by long DNA fragments. Similarly, Dubnau et al. (13) proposed that the two pathways control transformation and heterologous transduction, in which there are short regions of sequence homology (pathway I), and homologous transduction, in which there are long regions of sequence homology (pathway II). However, it was never clear how the cell might discriminate between short and long regions of homology to determine which pathway was used to carry out recombination.

The evidence presented above does not support the hypothesis of discriminatory pathways based either on the size of integrated DNA fragments or on sequence homology. If the defect in *recA1* cells is in recombinant formation and if there are indeed two pathways of recombination, it is difficult to explain the results presented above. In particular, the hypothesis that the lower level of transformation in *recA1* cells results from a more stringent requirement for base sequence homology predicts that Rec$^+$ intergenotes should not be good donors for transformation of *recA1* cells, since the heterologous se-

TABLE 7. *Additivity of transformation of recA1 cells with homologous and heterologous DNA*[a]

| Donor DNA | CFU[b]/ml (×10$^4$) | |
|---|---|---|
| | Trp$^+$ | His$^+$ |
| WB746 (homologous) | 2.95 | 5.03 |
| W23 (heterologous) | 0.96 | 0.12 |
| Sum | 3.91 | 5.15 |
| Mixture | 3.34 | 5.32 |

[a] Competent cells of strain ORB117 were transformed with a limiting concentration of DNA from WB746 or W23, or a mixture equivalent to the sum of the two separately.

[b] CFU, Colony-forming units.

quences in the intergenotic DNA would not have been required to match the homologous sequences as closely as required for *recA1* transformation. In fact, the observed discrimination against heterologous sequences in intergenotic DNA occurs in both Rec$^+$ and *recA1* recipients.

It should be noted that Dubnau et al. (13) considered restriction as the most likely explanation for discrimination against heterologous donors in transduction. They found no restriction of plaque-forming ability of three *B. subtilis* phages and concluded that restriction did not occur. However, more recent publications (23, 26) have shown that some *B. subtilis* phages are restrictable by *B. subtilis* 168 but that others, including those studied by Dubnau et al. (13), are not. I suggest that discrimination against heterologous DNA in transduction and in transformation of *recA1* cells is a result of restriction, and that the *recA1* mutation actually causes a defect in the restriction/modification system. The nature of the defect is not clear, but several possibilities might be considered.

Because restriction is not observed during transformation of Rec$^+$ cells (7, 8, 26), recombinational intermediates must somehow be protected from restriction by the host restriction system, perhaps because they are either single stranded or paired with modified sequences. The *recA* mutation may alter the specificity of the restriction enzyme so that these intermediates are no longer protected. This would explain the absence of donor-recipient complexes in *recA* cells after uptake of transforming DNA (12), but it would not explain the fragmentation of repaired DNA in ultraviolet light (UV)-irradiated cells (17).

A more attractive hypothesis involves the apparently prophage-directed restriction/modification system (3, 27), which is induced by UV (27) or by the attainment of competence (28, 29). Prophage-directed modification is not seen in competent *recA* cells (28), so it may be that, in *recA* cells, irradiation or competence induces a faulty restriction/modification complex that not only cannot modify DNA but also allows restriction if only one DNA strand is modified. In this case, even homologous transforming DNA would be foreign to some extent, because the sequence recognized by the prophage-directed restriction system is different from that of the host (3, 27), resulting in a reduction in transformation even with homologous DNA. Restriction during heterologous transduction of Rec$^+$ cells might, but need not, involve the inducible system, because the double-stranded transducing DNA should be susceptible to any restriction enzymes in the recipient cell.

Studies of DNA repair to UV-irradiated *recA* cells have shown that damaged regions can be repaired, but that the DNA is subsequently fragmented at repaired sites (17). These repaired regions, including regions of DNA repaired during recombination, may be preferentially susceptible to the induced restriction system.

The markers most susceptible to restriction are also those which are selectively bound to the cell wall and selectively excreted from competent Rec$^+$ cells (24). Thus the structure of the recipient DNA and/or the donor-recipient complex may also determine whether a site will be available to be restricted. It seems clear that a number of factors interact to cause the peculiar phenotype of the *recA1* mutant. Some of them are probably not related to the *recA* mutation at all, but have little effect on the cell unless the *recA* mutation is present. Most of the effects of the mutation, however, can be explained in terms of induction of a faulty restriction/modification system that is defective in modification but not in restriction.

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