

Interspecies Transformation in *Bacillus*: Mechanism of Heterologous Intergenote Transformation

RONALD M. HARRIS-WARRICK†* AND JOSHUA LEDERBERG

Department of Genetics, Kennedy Laboratory of Molecular Medicine, Stanford University Medical School, Stanford, California 94305

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Bacillus subtilis-*Bacillus globigii* hybrids were made by integration of the *B. globigii* aromatic region (*aroB* to *aroE*) as an intergenote in the *B. subtilis* chromosome. Transformation of the heterologous intergenote by *B. subtilis* DNA (or vice versa) occurred at about 10% of the frequency of homologous transformations by hybrid donors into the same region. Heterologous intergenote crosses were unusually sensitive to shear fragmentations of donor DNA to sizes less than 30×10^6 to 40×10^6 daltons. In all cases, the entire intergenote was transferred en bloc. Homologous transformation of intergenote markers by *B. globigii* DNA was not unusually shear sensitive, and linkage was normal for markers in the intergenote. A model is proposed in which efficient heterologous intergenote transformation occurs by recognition and base pairing of homologous DNA sequences on both flanks of the intergenote.

In our previous paper (7), we constructed hybrid strains of *Bacillus subtilis* carrying an extended sequence of heterologous DNA (called an intergenote [4]) from *B. globigii*. From an analysis of transformations using these hybrids, we concluded that enzymatic restriction is not a barrier to interspecies transformation in this system. Rather, sequence nonhomology is the major barrier to the efficient incorporation and expression of *B. globigii* DNA in *B. subtilis* recipients.

In this paper, we report the results of more detailed studies on the mechanisms of transformation by foreign *B. globigii* DNA sequences carried on an intergenote in *B. subtilis* and of incorporation of *B. subtilis* DNA into the *B. globigii* intergenote in hybrid recipients. In both situations, the nonhomologous sequences to be transformed are surrounded on both flanks by DNA that is homologous between donor and recipient. We have tested the importance of these homologous flanks in the transformation of the intergenote by a study of the DNA concentration dependence of the transformation as well as its dependence on the size of the donor molecule. Measurements of the cotransfer linked markers in the intergenote transformation were also done. These results demonstrate that high-efficiency integration of the intergenote requires cotransfer of DNA sequences that are homologous to the recipient chromosome on both flanks of the intergenote sequence.

† Present address: Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

MATERIALS AND METHODS

Materials and methods were the same as in the previous paper (7).

RESULTS

Effect of DNA concentration on transformations with hybrids as recipients. In the accompanying paper (7), we isolated *B. globigii*-*B. subtilis* hybrids (called globimar hybrids) in which the *B. globigii* aromatic region from *aroB*⁺ to *aroE*⁺ had been integrated as an intergenote in the *B. subtilis* chromosome. *B. globigii* DNA could transform this region with the same efficiency as *B. subtilis* DNA, representing a 10^5 -fold increase in the interspecies efficiency of transformation (ET). If the basis for this increase is the presence of sequence homology for the *B. globigii* donor, one would expect a concomitant decrease in *B. subtilis* donor efficiency for the same region. This was not seen; however, the *B. subtilis* donor carried sequences that were homologous to the recipient chromosome adjacent to the heterologous intergenote region. It is possible that a subset of *B. subtilis* donor molecules is capable of transforming the heterologous intergenote due to integration via these adjacent sequences.

The aforementioned experiments were performed at saturating DNA concentrations (4 to 8 $\mu\text{g}/\text{ml}$). A further study was made of the effect of varying DNA concentration on intergenote crosses. Hybrid recipients 76-2 and 76-3 were transformed by *B. globigii* (SB512) and *B. subtilis* (SB19) donor DNA. Intergenote markers

(*trp_g* or *tyr_g*) and the *B. subtilis* marker (*lys_s*) were selected. The relationship between DNA concentration and ET is shown in Fig. 1. With decreasing DNA concentrations, the ET values rose for intergenote markers and fell for the *B. subtilis* marker. For example, at 0.01 $\mu\text{g}/\text{ml}$, *B. globigii* DNA transformed the homologous intergenote *trp_g* and *tyr_g* markers 8 and 12 times more efficiently than did *B. subtilis* DNA, while being unable to transform the heterologous *B. subtilis* marker, *lys_s*, at a measurable frequency.

In Fig. 2, the intergenote/*B. subtilis* marker transformation ratio is calculated as a function of DNA concentration. In the *B. globigii* \times hybrid crosses, SB512 \times 76-2 and SB512 \times 76-3, these ratios represent the ratio of homologous to heterologous transformation efficiency; they rose with decreasing DNA concentration until no *lys_s⁺* transformants could be obtained. By using *B. subtilis* DNA in the crosses SB19 \times 76-2 and SB19 \times 76-3, the intergenote/homogenote ratio represents the ratio of heterologous to homologous transformation efficiency; as expected, these values fell with decreasing DNA concentration. As a control, also shown in Fig. 2, the *trpC*/*lys* and *tryA*/*lys* ratios were calculated for the entirely homologous cross, SB19 \times SB1023; these did not change with DNA concentration.

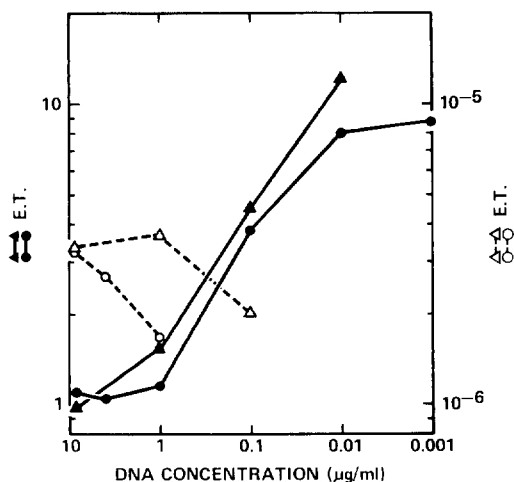


FIG. 1. DNA concentration dependence of efficiency of transformation of intergenote and *B. subtilis* homogenote markers by *B. globigii*. Hybrid recipients 76-2 and 76-3 were transformed by *B. globigii* (SB512) and *B. subtilis* (SB19) DNA at different concentrations. For each marker, the ET (ratio of *B. globigii* to *B. subtilis* transformants per milliliter) was calculated. The subscript for each marker refers to the origin of the marker in the recipient. Scales for the markers are as indicated. Recipients and markers: (●) 76-2, *trp_g⁺* selected; (○) 76-2, *lys_s⁺* selected; (▲) 76-3, *tyr_g⁺* selected; (Δ) 76-3, *lys_s⁺* selected.

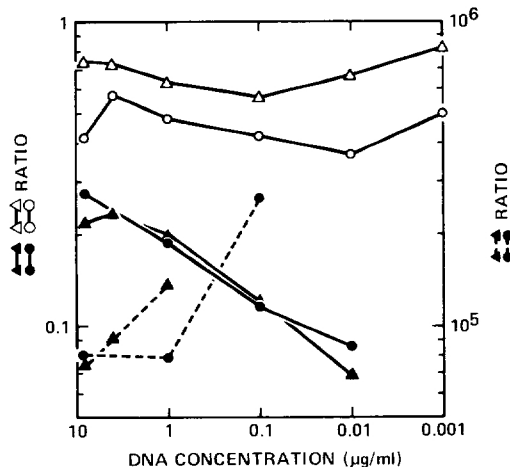


FIG. 2. Intergenote/homogenote transformation ratio: dependence on DNA concentration. Hybrid recipients 76-2 and 76-3 were transformed for intergenote and homogenote markers by SB512 and SB19 DNA at different concentrations. The frequency of transformation was calculated, and the ratio of intergenote to homogenote marker transformants was derived. As a control, the homologous ratios were calculated in the cross SB19 \times 1023. Crosses: (▲-▲) SB19 \times 76-2, *trp_g/lys_g*; (▲---▲) SB512 \times 76-2, *trp_g/lys_s*; (●-●) SB19 \times 76-3, *tyr_g/lys_s*; (●---●) SB512 \times 76-3, *tyr_g/lys_g*; (Δ-Δ) SB19 \times SB1023, *trpC_s/lys_s*; (○-○) SB19 \times SB1023, *tryA_s/lys_s*. The subscript for each marker refers to the origin of the marker in the recipient. Scales for the markers are as indicated.

For both donors, then, the ability to transform a heterologous DNA sequence was more dependent upon high DNA concentration than was the ability to transform a homologous sequence. This could be explained by the need for DNA molecules of a particular size or genetic composition to effect the heterologous cross; the likelihood of finding this subset of molecules would decrease with decreasing DNA concentration.

Transformation with globimar hybrid as donor. In the above experiments, *B. subtilis* DNA continued to retain a significant transformation efficiency for the heterologous *B. globigii* intergenote, even at very low DNA concentrations. As stated above, this can be explained by the presence of homologous flanks adjacent to the heterologous intergenote, which are present on a subset of the *B. subtilis* donor molecules. An analogous mechanism could also operate in transformation from a hybrid donor into a homogenetic *B. subtilis* recipient. Thus, intergenote transformation efficiencies should be comparable in *B. subtilis* \times globimar hybrid and globimar hybrid \times *B. subtilis* crosses.

To test this hypothesis, ^3H -labeled DNA was

extracted from the globimar hybrid strain 135-1, a thymine-requiring derivative of SB1112, and the *B. subtilis* strain SB1070, a *thyA thyB* derivative of SB19. As recipients, closely related strains were used: SB863, a *B. subtilis* homogenote carrying (among other markers) *trpC* and the unlinked *hisA* marker, and 99-12, a hybrid constructed by transforming the *trp_s* *B. globigii* intergenote from 76-2 into SB863. The entire intergenote was transferred in this cross (see below). The advantage of these transformations is that both donors are isogenic at the *hisA_s*⁺ locus, while both recipients are isogenic at *hisA_s*. Thus, any differences in competence of recipients or the physical state of the donor DNA, except the intergenote, will be seen in transformations to *hisA*⁺. Standardizing intergenote transformation frequencies relative to *hisA*⁺ transformation makes it possible to compare intergenote transformation efficiencies between different crosses. The results of these crosses are shown in Table 1. From the transformation frequencies, it is clear that globimar hybrid DNA can transform a *B. globigii* intergenote with higher efficiency than the corresponding *B. subtilis* sequence; when *hisA*⁺ ET values were normalized to 1.0, hybrid 135-1 DNA transformed the *trp_s* intergenote marker in 99-12 with an ET of nearly 13, but transformed the *B. subtilis* aromatic marker, *trpC*, in SB863 with an ET of less than 0.1. Transformation frequencies for the *trp* markers were normalized by dividing by the frequencies obtained for the isogenic *hisA_s* marker; when the ratio for the homologous *B. subtilis* × *B. subtilis* cross was arbitrarily set at 100%, both globimar hybrid × *B. subtilis* and *B. subtilis* × globimar hybrid crosses yielded *trp*⁺ transformants at about 8.5% efficiency, whereas

the homologous hybrid × hybrid cross efficiency was about 110%. Thus, both heterologous intergenote crosses are achieved at the same efficiency, while both homologous crosses occur about ten times more frequently, in keeping with the hypothesis. These experiments have been repeated using different donors (SB19, 76-2, 76-3) and recipients (SB1023, 76-3, 76-2) with identical results (data not shown); heterologous transformations can be achieved at fairly high frequency (about 10% of homologous frequencies) if the heterologous sequences are located on an intergenote surrounded by homologous sequences.

Effect of hydrodynamic shear on intergenote transformation. If effective heterologous intergenote transformation requires concurrent cotransformation of adjacent homologous sequences, this type of cross should require longer donor molecules than homologous crosses. To test size as a variable, DNA from SB1070 (*B. subtilis* homogenote), SB512 (*B. globigii* homogenote), and 135-1 (globimar hybrid) was hydrodynamically sheared to increasingly smaller sizes. The weight-average molecular weight of each sheared sample was determined from neutral sucrose gradient centrifugation, using P22 DNA as a size marker (data not shown). The peak of biological activity (transformants per microgram of DNA) in each gradient lay on the heavy shoulder of the peak of physical activity (counts per minute), so the weight-average molecular weight was calculated from bioassays of the gradients, using homologous recipient markers. It was not possible to test DNAs from these gradients for their ability to transform a heterologous marker; especially at higher shear, the DNA activity was too low

TABLE 1. *Biological activity of hybrid DNA compared to homogenote DNA*

Donor	Recipient	Recipient marker selected	Transformation frequency ^a	ET	Normalized ET ^b	Transformation frequency ratio <i>trp</i> ⁺ / <i>hisA</i> ⁺	Relative activity ^c (%)
SB1070 (<i>B. subtilis</i>)	SB863 (<i>B. subtilis</i>)	<i>trpC_s</i>	1.1			0.714	100
		<i>hisA_s</i>	1.6				(standard)
135-1 (hybrid)	SB863 (<i>B. subtilis</i>)	<i>trpC_s</i>	0.51	0.45	0.085	0.061	8.5
		<i>hisA_s</i>	8.4	5.35	1.0		
SB1070 (<i>B. subtilis</i>)	99-12 (hybrid)	<i>trp_s</i>	0.075			0.062	8.7
		<i>hisA_s</i>	1.2				
135-1 (hybrid)	99-12 (hybrid)	<i>trp_s</i>	3.8	50.0	12.9	0.797	112
		<i>hisA_s</i>	4.7	3.88	1.0		

^a Recipient strains were transformed by the donor strains at saturating DNA concentrations (4 μg/ml). The datum is the proportion of recipient cells transformed per 10⁴ exposed.

^b ET was normalized to *hisA_s*⁺ ET of 1.0, and other values were adjusted accordingly.

^c Relative activity is calculated from *trp*⁺/*hisA*⁺ ratios and standardized to the homologous cross SB1070 × SB863 as 100%.

for reliable transformation assays. Instead, samples of sheared and unsheared DNA were tested for their ability to transfer homologous and heterologous markers at saturating DNA concentrations (4 $\mu\text{g}/\text{ml}$). To standardize for variations in the physical states of the three DNA species, transformation values for the sheared DNA were calculated as a percentage of the unsheared values. These values were plotted as a function of molecular weight (Fig. 3). Two globimar hybrid strains, 99-12 (Fig. 3A) and 96-3-1 (Fig. 3B), and two *B. subtilis* strains, SB1023 (Fig. 3C) and SB863 (Fig. 3D), were used as recipients. For all homologous marker crosses, transformation frequencies showed a slow inactivation with shear, worsening at sizes below 10×10^6 daltons. In marked contrast, heterologous markers were much more sensitive to shear; at average sizes above 30×10^6 to 40×10^6 daltons, the heterologous crosses were still efficient, but at shear below 10^7 daltons they were selectively inactivated, retaining less than 0.1% of normal transforming activity after shear to less than 5×10^6 daltons. In Fig 3C and D, the effect of shear on cotransfer of the aromatic region from *aroB* to *tyrA* in the homologous crosses, SB1070 \times SB1023 and SB1070 \times SB863, is also shown: cotransfer of this long homologous sequence (12×10^6 daltons; L. Okun, Ph.D. thesis, Stanford University, Stanford, Calif., 1968) is also very sensitive to shear, though 10-fold less so than the heterologous intergenote cross. Thus, heterologous intergenote crosses are unusually sensitive to shearing of donor molecules below a critical size, as predicted by the hypothesis; this critical size is less than 30×10^6 daltons, since heterologous crosses are still fairly efficient at this size.

Cotransfer of linked markers in heterologous intergenote transformation. To test the genetic composition of DNA molecules capable of transforming heterologous intergenotes, the cotransfer frequency for linked markers in heterologous intergenote crosses was determined. For these experiments, globimar hybrid \times *B. subtilis* crosses was performed using 135-1 as donor and SB1023, carrying the linked aromatic *B. subtilis* markers *aroB*, *trpC*, *hisB*, and *tyrA*, which are present in the *B. globigii* intergenote of 135-1, as recipient. Transformations were performed at saturating DNA concentrations at the four levels of shear described previously, and each marker was selected separately. As a control, cotransfer was also measured with unsheared SB1070 (*B. subtilis* homogenote) DNA. The results are shown in Table 2. When the heterologous intergenote cross was attempted, all intergenote markers were cotransferred with essentially 100% efficiency; the few

colonies isolated that remained auxotrophic for one of the central markers were probably the results of rare multiple crossover events during recombination. The 100% cotransfer of intergenote markers occurred at all levels of shear, even when the weight-average molecular weight was less than 5×10^6 ; thus, with increasing shear, the transformation frequency of heterologous intergenote markers was drastically reduced, but the entire intergenote continued to be cotransferred in the few remaining transformants. The residual biological activity correlated with the residual level of molecules of size greater than 30×10^6 daltons at each shear level (data not shown). In the homologous control cross, cotransfer of all four markers did not exceed 50% of the transformants tested; normal linkage was seen, with significant numbers of colonies transformed for only one or two markers.

It is possible that the intergenote may possess unusual genetic properties that encourage high cotransfer in any cross. This possibility was eliminated by measuring the cotransfer of the linked markers *trp_k* and *tyr_k* in the hybrid \times hybrid cross 76-2 (*trp_k tyr_k⁺*) \times 76-3 (*trp_k⁺ tyr_k*); *tyr⁺* transformants were tested by replica plating for cotransfer of *trp*. Less than 50% of *tyr_k⁺* transformants were cotransformed to auxotrophy for tryptophan (Table 3).

Table 3 also shows cotransfer values for intergenote markers in homologous intergenote crosses of the type *B. globigii* \times globimar hybrid. New mutations were introduced into the intergenote by transformation or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis and penicillin selection; mutants corresponding in phenotype to the *hisB* and *aroE* loci were shown to be located in the intergenote by high transformation efficiencies using SB512 (*B. globigii*) DNA. Cotransfer of *trp-tyrA*, *hisB-tyrA*, and *tyrA-aroE* markers in homologous intergenote crosses (Table 3) was similar to that seen in the corresponding *B. subtilis* homogenote crosses (Table 2 and [8]), demonstrating a similar mapping order of the aromatic regions of *B. globigii* and *B. subtilis*.

DISCUSSION

The results shown in Table 1 demonstrate that foreign DNA sequences can be transformed at about 10% of homologous frequencies if they are located on an intergenote surrounded by DNA sequences homologous to the recipient chromosome. This is true for both globimar hybrid \times *B. subtilis* and *B. subtilis* \times globimar hybrid crosses. Figure 3 demonstrates that the efficiency of these crosses is highly dependent on DNA size; heterologous intergenote transformation was selectively inactivated at sizes below

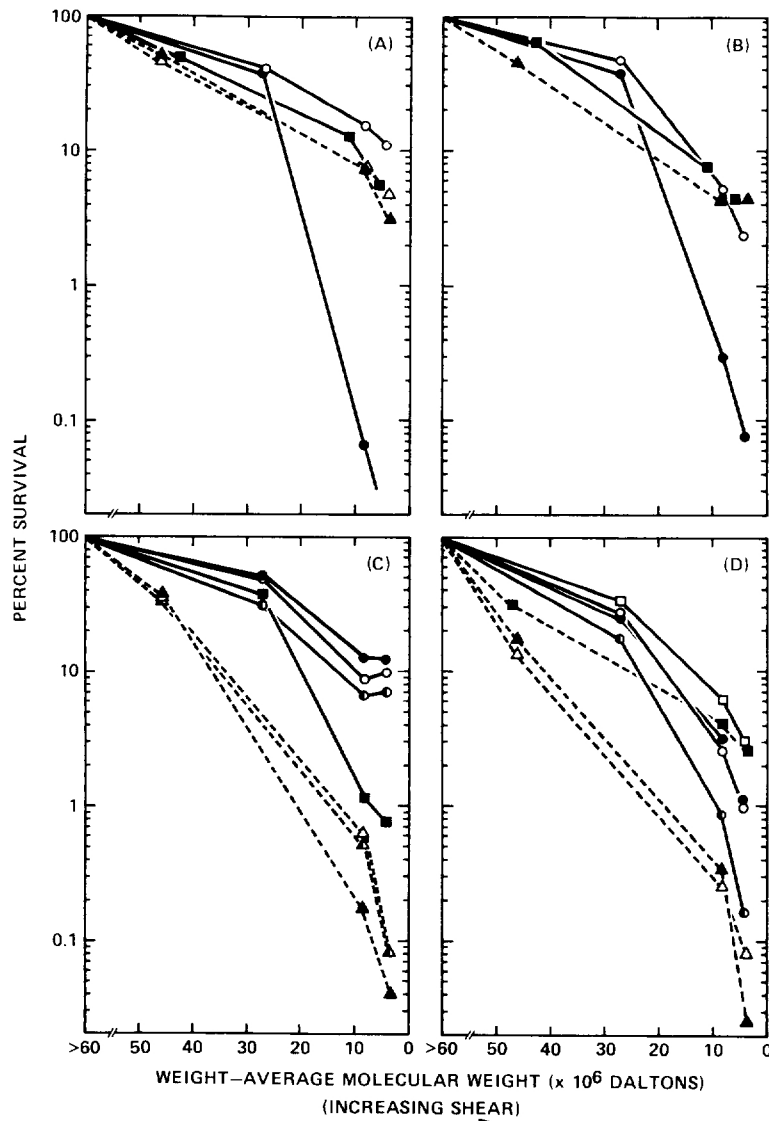


FIG. 3. Effect of shear on homologous and heterologous intergenote transformation. Hybrid and *B. subtilis* recipients were transformed with *B. subtilis* (SB1070), *B. globigii* (SB512), and hybrid (135-1) DNA sheared to various molecular weights. The frequency of transformation was calculated and expressed as a percentage of the unsheared transformation frequency for each DNA and each marker transformed. The first subscript for selected markers refers to the origin of the marker in the recipient; the arrow points to the origin of the marker in the donor, and hence the marker in the transformed recipient. (A) 99-12 as recipient: (●—●) SB1070 × 99-12, *trp_{R→S}* selected; (○—○) SB1070 × 99-12, *hisA_{S→S}* selected; (▲---▲) 135-1 × 99-12, *trp_{R→R}* selected; (△---△) 135-1 × 99-12, *hisA_{S→S}* selected; (■—■) SB512 × 99-12, *trp_{R→R}* selected. (B) 96-3-1 as recipient: (●—●) SB1070 × 96-3-1, *tyr_{R→S}*; (●—●) SB1070 × 96-3-1, *lys_{S→S}*; (▲---▲) 135-1 × 96-3-1, *tyr_{R→R}*; (■—■) SB512 × 96-3-1, *tyr_{R→R}*; (●—●) SB1070 × SB1023, *hisB_{S→S}*; (○—○) SB1070 × SB1023, *trpC_{S→S}*; (●—●) SB1070 × SB1023, *tyrA_{S→S}*; (■—■) SB1070 × SB1023 (*aroB_S-tyrA_{S→S}* coselected); (▲---▲) 135-1 × SB1023, *hisB_{S→R}*; (△---△) 135-1 × SB1023, *trpC_{S→R}*; (▲---▲) 135-1 × SB1023, *tyrA_{S→R}*. (D) SB863 as recipient: (▲---▲) 135-1 × SB863, *trpC_{S→S}*; (△---△) 135-1 × SB863, *tyrA_{S→R}*; (■---■) 135-1 × SB863, *hisA_{S→S}*; (●—●) SG1070 × SB863, *trpC_{S→S}*; (○—○) SB1070 × SB863, *tyrA_{S→S}*; (□—□) SB1070 × SB863, *hisA_{S→S}*; (●—●) SB1070 × SB863, *aroB_S-tyrA_{S→S}* coselected.

10×10^6 to 20×10^6 daltons. Table 3 shows that all the measurable markers on the intergenote are cotransferred during any heterologous inter-

genote transformation. A model of heterologous intergenote transformation is presented in Fig. 4. The recipient is assumed to be a globimar

TABLE 2. Cotransfer of linked markers in homologous and heterologous intergenote transformation into SB1023

Donor	Shear ^b	Primary marker	No. of colonies tested	Phenotypes found (%) ^a													
				1111	1110	0111	1011	1101	1100	0110	0011	1001	0101	1000	0100	0010	0001
135-1	0	<i>aroB</i> ⁺	160	158 (99)			2 (1)										
		<i>trpC</i> ⁺	389	388 (99.7)			1 (0.3)										
		<i>hisB</i> ⁺	300	300 (100)													
		<i>tyrA</i> ⁺	404	404 (100)													
135-1	18	<i>trpC</i> ⁺	258	258 (100)													
		<i>hisB</i> ⁺	343	343 (100)													
		<i>tyrA</i> ⁺	212	211 (99.5)			1 (0.5)										
135-1	23	<i>trpC</i> ⁺	122	122 (100)													
		<i>hisB</i> ⁺	119	119 (100)													
		<i>tyrA</i> ⁺	139	138 (99.3)			1 (0.7)										
135-1	27	<i>trpC</i> ⁺	69	67 (97.1)				2 (2.9)									
		<i>hisB</i> ⁺	33	33 (100)													
		<i>tyrA</i> ⁺	133	133 (100)													
1070	0	<i>aroB</i> ⁺	65	29 (44.6)	2 (3.1)				14 (21.5)				20 (30.8)				
		<i>trpC</i> ⁺	70	33 (47.1)	2 (2.9)	18 (25.7)	1 (1.4)	7 (10)	2 (2.9)					8 (11.4)			
		<i>hisB</i> ⁺	80	40 (50)	4 (5)	16 (25)	2 (2.5)				15 (18.8)					3 (3.8)	
		<i>tyrA</i> ⁺	69	21 (30.4)		15 (21.7)	8 (11.6)	1 (1.4)			18 (26.1)	1 (1.4)	1 (1.5)				4 (5.8)

^a Order of genes in 1111 is *aroB-trpC-hisB-tyrA*. In the phenotype number, 1 stands for the donor marker, i.e., transformants to prototrophy, and 0 stands for the recipient auxotrophy. Figures refer to number of absolute colonies found of each phenotype; parentheses indicate the percentage of total colonies tested that are of that phenotype.

^b Numbers indicate size of hypodermic needle used for hydrodynamic shearing.

TABLE 3. Linkage of markers in hybrid × hybrid and *B. globigii* × hybrid crosses

Donor	Recipient	Primary marker	Secondary marker	No. of colonies tested	Phenotypes			Cotransfer
					11 ^a	10	01	
76-2	76-3	<i>tyr_R</i> ⁺	<i>trp_R</i>	920	429	491		0.47
SB512	38-1	<i>tyr_R</i> ⁺	<i>trp_R</i> ⁺	20	13		7	0.66
		<i>trp_R</i> ⁺	<i>tyr_R</i> ⁺	60	40	20		
SB512	6-5	<i>tyr_R</i> ⁺	<i>his_R</i> ⁺	193	148	45		0.74
		<i>his_R</i> ⁺	<i>tyr_R</i> ⁺	178	126		52	
SB512	PheII	<i>tyr_R</i> ⁺	<i>aroE_R</i> ⁺	200	200	0		0.98
		<i>aroE_R</i> ⁺	<i>tyr_R</i> ⁺	309	299		10	

^a 1 and 0 are defined as in Table 2.

hybrid. The essential feature of this model is that efficient integration of the heterologous intergenote is accomplished only through se-

quence recognition and base pairing of homologous sequences on both flanks of the intergenote (Fig. 4A). These homologous sequences function

to locate the corresponding region of the recipient chromosome, align the heterologous sequence properly, and "splice" the intergenote into the recipient chromosome, holding it in place until the transformed region is rendered homogeneous at a later stage. An expected consequence of the requirement for integration of the entire intergenote is 100% linkage of all intergenote markers. The mechanism of conversion of the integrated heterologous intergenote to homogeneity is unknown but probably involves DNA replication or some form of repair. However, the excision repair mechanism does not seem to be active in gene conversion in *Haemophilus* (2). Transforming DNA that lacks the entire intergenote and homologous sequences on both sides is not efficiently integrated (Fig. 4B) due to difficulty in base pairing and covalent joining of the nonhomologous

end(s) of the donor molecule to the recipient chromosome; even if one end of the molecule is covalently bound to the chromosome, cellular exonucleases will digest it from the free end. Treatment of the DNA to reduce the number of molecules carrying the entire intergenote (such as shear, reduction in DNA concentration, or *in vitro* cleavage by the *B. globigii* restriction system [7]) will thus selectively inactivate heterologous intergenote integration; the surviving molecules should continue to transform recipients with 100% linkage of intergenote markers.

A similar mechanism to ours has been demonstrated for the transformation of deletion mutations in *B. subtilis* (1; R. Harris-Warrick, unpublished data). Deletions represent the extreme model for integration of foreign DNA sequences where no sequence homology exists. Integration of the sequence that is deleted in the

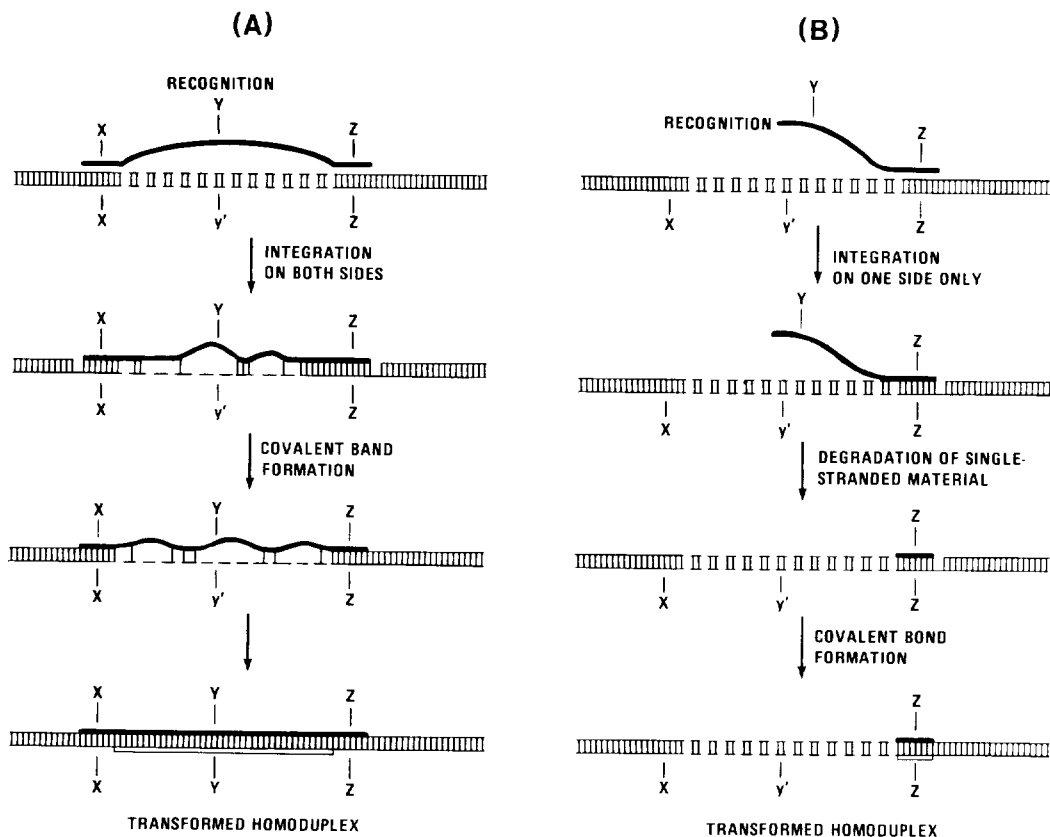


FIG. 4. Proposed mechanism of heterologous intergenote transformation. (A) Successful transformation of the intergenote *B. subtilis* × *globigii* hybrid cross. The homogeneous *B. subtilis* donor (—, represented by marker *y*) transforms the entire *B. globigii* intergenote (---, represented by marker *y'*) as well as homologous *B. subtilis* sequences (—) on both flanks of the intergenote, represented by markers *X* and *Z*. The integrated sequence is rendered homogeneous at a later stage either by gene conversion or semiconservative replication. (B) Unsuccessful intergenote transformation. Here the donor molecule lacks homologous sequences, represented by *X*, on one side of the intergenote. As a result, the heterologous sequence is not integrated and is digested by single-strand-specific nucleases.

recipient chromosome also occurs with 100% cotransfer of the deleted markers, again suggesting a requirement for homologous sequences on both flanks of the deletion.

Bernheimer and co-authors have studied a model whereby the capsular genome is transformed heterologously in its entirety by two possible mechanisms. (i) If the transforming DNA molecule contains the entire heterologous capsular genome and sequences homologous with the recipient chromosome on both flanks of it, the heterologous capsular genome is integrated at the site of the recipient capsular genome by a classical exchange mechanism. (ii) If the transforming molecule contains sequences homologous with the recipient chromosome on only one flank of the heterologous capsular genome, it is integrated ectopically by insertion at a site different from the resident capsular genome; the result of this addition is a binary strain carrying both capsular genomes, although only one may be expressed phenotypically. Our results involving interspecies transformation in *Bacillus* suggest that only the first kind of transformation can occur and that DNA molecules lacking sequences homologous to the recipient on one or both flanks of the heterologous intergenote are unable to transform the recipient. The reasons for this discrepancy are unknown, although it is likely that the increased genetic distance between *B. globigii* and *B. subtilis* compared to that between the different capsular types in *Pneumococcus* is a contributing factor.

Our model and data conflict with the results of Biswas and Ravin (5, 9); working with streptococcal-pneumococcal transformations, they did not find increased linkage between evolutionarily conserved antibiotic resistance markers in heterologous intergenote crosses. The discrepancy could be due to differences in heterologous transformation in conserved and nonconserved regions of the chromosome. In conserved regions, interspecies homology is high enough that recognition and integration may occur with short lengths of foreign donor DNA, and homologous nonforeign sequences may not be needed to facilitate recognition. The "linked" markers in the reported pneumococcal-streptococcal system may not even be located on a single continuous intergenote; identical linkage is seen when both intergenote markers are introduced simultaneously and when each marker is introduced independently in separate transformations (5). In nonconserved regions, DNA homology between two species is so low that effective integration is very rare; the presence of homologous nonforeign sequences adjacent to the continuous intergenote will have a significant effect on recognition capability. Alternatively, the discrepancy in our results could be due to species-spe-

cific differences in the mechanism of heterologous DNA integration.

We do not know the size of the *B. globigii* intergenote in our globimar hybrid strains. It extends across the entire aromatic region from *aroB* to *aroE*; the order of mapping of our markers in the intergenote (Table 3) suggests that the *B. globigii* sequence is organized similarly and should be the same length as the aromatic region of *B. subtilis*. Using *EcoRI*-cleaved *B. subtilis* DNA segments, the *trpE-aroE* linkage group (lacking only *aroB*, which is closely linked to *trpE* [8]) has been shown to lie on a segment of 12.5×10^6 daltons (6). Estimates of the *aroB-tyrA* linkage group based on shear sensitivity and cotransfer data yield similar values (L. Okun, Ph.D. thesis, Stanford University, Stanford, Calif., 1968). A minimal estimate of the intergenote size, then, would be slightly greater than these values, or 13×10^6 to 15×10^6 daltons.

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