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Effect of 6-(*p*-Hydroxyphenylazo)-Uracil on the Homologous and Heterologous Transduction Processes in *Bacillus subtilis*

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We have studied the effect of 6-(*p*-hydroxyphenylazo)-uracil on the recombination processes that operate in the homologous and heterologous transduction mediated by PBS1 and SP10 phages of *Bacillus subtilis*. The results obtained demonstrate that the process of heterologous genetic exchange is sensitive to this compound, whereas the homologous process is not. The present data, along with those of our previous work (U. Canosi, A. G. Siccardi, A. Falaschi, and G. Mazza, J. Bacteriol. **126**:108-121, 1976), suggest that the DNA polymerase III is involved in the recombination process that operates in transformation and heterologous transduction, whereas homologous transduction follows a partially independent pathway not involving this protein.

In bacteria the genetic recombination mechanism consists of sets of reactions that lead to the integration of a fragment of exogenous DNA into a recipient genome. The isolation and characterization of mutants of *Bacillus subtilis* defective in functions involved in recombination (*rec*) have been described by several authors (2, 4, 6, 7, 10, 12, 13, 16, 17). These studies have led to the identification of several *rec* genes and to a detailed phenotypic characterization of the properties altered in these mutants. Enzymic studies have at present identified only one enzyme of DNA metabolism that plays a role in genetic recombination, i.e., the ATP-dependent deoxyribonuclease (2, 4).

We have previously described the specific inhibitory effect of 6-(*p*-hydroxyphenylazo)-uracil (HPUra) on transformation and on the recombinatory step required for transfection, and we have observed that DNA polymerase III (PolIII) is consequently involved in the recombination events that occur in these processes (1).

We have extended these studies on a different recombination system, i.e., PBS1 transduction, whether homologous (*B. subtilis* 168 to *B. subtilis* 168) or heterologous (*B. subtilis* W23 to *B. subtilis* 168). These two recombination processes follow two partially different recombination pathways, as suggested by genetic studies with *rec* mutants (8, 16). We have also considered an SP10 transduction system which allows homologous exchange between *B. subtilis* W23 and *B. subtilis* W23 and heterologous exchange between *B. subtilis* W23 and *B. subtilis* 168 (11). The heterologous SP10 transduction probably follows the same pathway of recombination as heterologous PBS1 transduction, DNA transformation, and transfection (8).

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The origin and the description of the strains used in this work are reported in Table 1. The phages PBS1 (20) and SP10 (21) were used for transduction experiments.

Culture media. Spizizen minimal medium MT (18) was used to prepare competent cells. Medium Y (23), Penassay broth (antibiotic medium no. 3, Difco), and tryptose blood agar base (Difco) were used in PBS1-mediated transduction experiments. PA and NBYA media (21) were used to produce W23 spores lysogenized with SP10, to determine SP10 plaque-forming units and for SP10 propagation. The minimal medium of Davis and Mingioli (3) was used for selection of transformants and transductants. Nutrient broth (Oxoid), solidified with agar, was used to determine cell titer.

Reagents. HPUra was obtained from B. W. Langley Imperial Chemical Industries, London.

DNA preparation. DNA was prepared according to the methods described by Marmur (15).

Effect of HPUra on transformation. Competent cells were prepared as described by Stewart (19).

The effect of HPUra was assayed by following the procedure previously described (1).

Transformation to HPUra resistance was performed by plating on nutrient agar plates containing 20 μg of the inhibitor per ml.

Effect of HPUra on transduction. PBS1-mediated transduction was performed following the procedure of Hoch et al. (13). The phage grown on SB 19 strain was used for homologous transduction, whereas the same one grown on W23 strain was used for the heterologous process.

To assay the effect of HPUra on transduction, 1 ml of cells, about 2 h after the end of the log phase, was

diluted in a volume of PY containing the drug to obtain a concentration of 10^8 cells per ml. After 10 min of incubation at 37°C, PBS1 was added at a multiplicity of infection of 1, and incubation was carried out for 30 min. Before plating, the culture was concentrated 10-fold by centrifugation, and the cells were suspended in minimal medium.

SP10-mediated transduction was performed as described for PBS1. Since SP10 phage is able to infect and to reproduce only in W23 strains but can also transduce 168 derivatives, SP10 grown on W23 was used as the donor phage, and W23 or 168 was used as the recipient strain in homologous and heterologous transduction, respectively.

RESULTS

Effect of HPUra on PBS1 transduction. The effect of HPUra on PBS1 transduction has been explored by the procedure described above. The results reported in Table 2 show that at doses of 2 to 10 μg of HPUra per ml the homologous transduction process was not affected, whereas a reduction to about 5% of the control was observed in the heterologous process. The specific reduction of heterologous recombination is reproducible and suggests a common HPUra-sensitive step which operates in transformation and heterologous PBS1 transduction but not in homologous transduction. As has already been demonstrated (1), the transformation process is significantly inhibited by this drug at these same doses (Table 3).

The sensitivity of heterologous transduction to HPUra suggests that PolIII, the specific target of this drug, could be involved in this process as well as in transformation/transfection. If this is true, in a mutant resistant to this drug because of an alteration of the structure of this enzyme we would expect the heterologous process also to be insensitive to the drug. For this purpose we have used the *azp-12* mutant, which has a drug-resistant PolIII (14).

Since the heterologous transduction for the biochemical markers present in the original strain carrying the PolIII resistance mutation *azp-12* shows a very low frequency, according to the data of Dubnau et al. (8), we transferred the *azp-12* mutation to another strain by congression (see Table 1). We confirmed the identity of the *azp-12* mutation in this new strain (PB 1770) by determining the map linkage with *pyrA*.

The results regarding the effect of HPUra using as recipient a strain with a resistant PolIII are reported in Table 4. In these conditions, a much lower effect was observed at doses that strongly affect the heterologous process in normal strains, confirming that PolIII is the molecule responsible for the reduction of heterologous transduction by HPUra.

Effect of HPUra on SP10-mediated transduction. The effect of HPUra on transduction was also assessed on a different bacteriophage (SP10) having properties quite different from those of PBS1. This phage cannot grow on *B. subtilis* 168 but is able to transduce cells of the nonpermissive host. It is thus possible to perform homologous (W23 to W23) and heterologous (W23 to 168) transduction. These experiments (Table 5) demonstrate that in this case too HPUra is able to affect heterologous ex-

TABLE 1. *List of B. subtilis strains used*

Strain	Genotype[a]	Origin
SB 19	Prototroph	J. Lederberg
SB 202	*tyrA1 hisB2 trpC2 aroB2*	J. Lederberg
BR 151	*lys-3 trpC2 metB10*	F. Young
BD 54	*metB5 ile-1 leu-8 spcB azp-12*	N. Brown
PB 1728	*tyrA1 hisB2 trpC2 aroB2 azp-80*	U. Canosi
PB 1770	*trpC2 aroB2 azp-12*	From SB 202+ DNA BD 54
W23	Prototroph	J. A. Hoch
W23 Trp^-	*trp*	J. A. Hoch
W23 Thy^- His^-	*thyX1 thyY1 his*	A. Adams

[a] Symbols: *tyr*, *his*, *trp*, *aro*, *lys*, *met*, *ile*, *leu*, and *thy*, requirement for tyrosine, hystidine, tryptophan, shikimic acid, lysine, methionine, isoleucine, leucine, and thymine, respectively; *spc* and *azp*, resistance to spectinomycin and HPUra, respectively.

TABLE 2. *Effect of HPUra on homologous and heterologous PBS1-mediated transduction*[a]

Transduction	Conditions	Viable cells per ml	Survival	Trp^+ transductants per ml	Frequency of transduction	HPUra/control ratio
Homologous	Control (−HPUra)	3.21×10^9	1.00	3.95×10^3	1.23×10^{-6}	1.00
	+HPUra, 2 μg/ml	3.15×10^9	0.98	3.8×10^3	1.2×10^{-6}	0.97
	+HPUra, 5 μg/ml	2.82×10^9	0.87	3.82×10^3	1.35×10^{-6}	1.1
	+HPUra, 10 μg/ml	2.65×10^9	0.82	3.4×10^3	1.28×10^{-6}	1.04
Heterologous	Control (−HPUra)	3×10^9	1.00	5.7×10^2	1.9×10^{-7}	1.00
	+HPUra, 2μg/ml	2.64×10^9	0.88	1.2×10^2	4.54×10^{-8}	0.23
	+HPUra, 5 μg/ml	2.26×10^9	0.75	3×10^1	1.33×10^{-8}	0.07
	+HPUra, 10 μg/ml	2.11×10^9	0.70	2×10^1	9.5×10^{-9}	0.05

[a] Recipient strain: SB 202.

TABLE 3. *Effect of HPUra on transformation*[a]

Conditions	Viable cells per ml	Survival	Transformation to Met+			Transformation to Azr		
			Transformants per ml	Frequency of transformation	+HPUra/control ratio	Transformants per ml	Frequency of transformation	+HPUra/control ratio
Control (−HPUra)	2.14×10^8	1.00	5.35×10^5	2.5×10^{-3}	1.00	7.26×10^3	3.4×10^{-5}	1.00
+HPUra, 2 μg/ml	1.91×10^8	0.89	3.68×10^5	1.92×10^{-3}	0.77	5.24×10^3	2.74×10^{-5}	0.80
+HPUra, 5 μg/ml	1.94×10^8	0.91	3.31×10^5	1.7×10^{-3}	0.68	4.73×10^3	2.43×10^{-5}	0.71
+HPUra, 10 μg/ml	2.04×10^8	0.95	3.27×10^5	1.6×10^{-3}	0.64	2.99×10^3	1.46×10^{-5}	0.42
+HPUra, 20 μg/ml	1.93×10^8	0.90	2.13×10^5	1.1×10^{-3}	0.44	1.75×10^3	9×10^{-6}	0.26

[a] Competent cells of strain BR 151 were treated with 1 μg of strain PB 1728 DNA per ml.

TABLE 4. *Effect of HPUra on homologous and heterologous PBS1-mediated transduction in a PolIII-resistant strain (PB 1770)*

Transduction	Conditions	Viable cells per ml	Survival	Trp+ transductants per ml	Frequency of transduction	+HPUra/control ratio
Homologous	Control (−HPUra)	3.54×10^9	1.00	4.53×10^3	1.29×10^{-6}	1.00
	+HPUra, 2 μ/ml	3.23×10^9	0.91	4.21×10^3	1.3×10^{-6}	1.01
	+HPUra, 5 μg/ml	3.15×10^9	0.89	4.18×10^3	1.32×10^{-6}	1.03
	+HPUra, 10 μg/ml	3.04×10^9	0.86	3.69×10^3	1.21×10^{-6}	0.94
Heterologous	Control (−HPUra)	3.13×10^9	1.00	4.6×10^2	1.47×10^{-7}	1.00
	+HPUra, 2 μg/ml	2.98×10^9	0.95	3.5×10^2	1.17×10^{-7}	0.80
	+HPUra, 5 μg/ml	2.8×10^9	0.89	3×10^2	1.07×10^{-7}	0.73
	+HPUra, 10 μg/ml	2.34×10^9	0.75	2.3×10^2	9.83×10^{-8}	0.67

TABLE 5. *Effect of HPUra on SP10-mediated transduction*

Transduction	Recipient strain	Conditions	Viable cells per ml	Survival	Trp+ transductants per ml	Frequency of transduction	+HPUra/control ratio
Homologous	W23 *trp*−	Control (−HPUra)	7.5×10^8	1.00	3.7×10^2	4.9×10^{-7}	1.00
		+HPUra, 5 μg/ml	7.4×10^8	0.98	3.3×10^2	4.4×10^{-7}	0.90
		+HPUra, 10 μg/ml	7.5×10^8	1.00	3.2×10^2	4.2×10^{-7}	0.86
		+HPUra, 20 μg/ml	6.8×10^8	0.91	3.1×10^2	4.5×10^{-7}	0.92
Heterologous	SB 202	Control (−HPUra)	1.27×10^9	1.00	1.7×10^2	1.34×10^{-7}	1.00
		+HPUra, 5 μg/ml	1.16×10^9	0.91	2×10^1	1.72×10^{-8}	0.12
		+HPUra, 10 μg/ml	1.17×10^9	0.92	1×10^1	8.5×10^{-9}	0.06
		+HPUra, 20 μg/ml	1.19×10^9	0.94	0.5×10^1	4.2×10^{-9}	0.03

change preferentially, whereas homologous exchange is not affected.

DISCUSSION

The dissection of the bacterial recombination pathway by genetic and biochemical studies is still far from satisfactory; a recent summary of the studies on transformation has allowed Dubnau (5) to sketch a plausible sequence of basic events, but the recognition of the role of particular gene products in any of these steps is still not possible. Even less information is available on the transduction process. Thus, the use of drugs that inhibit the recombination process may offer another set of tools which, when coupled to the evidence gathered by genetic and biochemical studies, might help in furthering the understanding of the interlocking pathways of bacterial recombination.

The properties of the *rec* mutants of *B. subtilis* have indicated two pathways of recombination, which probably merge into a common one at certain stages. In particular, some *rec* mutants (*recA* and *recG* notably) are altered in

heterologous transduction and in transformation/transfection (the latter two processes being always coupled as far as the *rec* mutants go), but normal in homologous transduction; several other mutants are instead altered in all three types of recombination. One could thus surmise that the homologous transduction process enters the *rec* pathway at some stage after that catalyzed by the products of genes A and G. No mutant with the opposite behavior (altered in homologous transduction and not in heterologous) has ever been observed. Also the hypothesis that a restriction-like phenomenon is at the basis of the phenotype of *recA* mutants has been suggested (9).

The data reported in this paper support the existence of a pathway for homologous transduction which is partially different from the heterologous pathway as well as from that for transformation/transfection. HPUra (which specifically inhibits the polymerizing portion of PolIII) does not harm homologous transduction, whereas it affects significantly the other three processes. What the basis of the different sensitivity is, or, in other words, why some gene products (including PolIII and the products of *recA* and *recG* genes) are necessary only in some types of recombination, is not clear. The basis of the differential inhibition of the heterologous process by HPUra may lie in the length of the DNA piece handled by the cell before or during integration, which could be of the same size in heterologous transduction and transformation and larger in the homologous process. The heterologous DNA may be highly susceptible to nucleolytic attack either because of restriction-like processes or because of perturbations in base pairing leading to a PolIII-dependent repair and/or recombination. The longer the heterologous DNA molecule being processed, the more susceptible it could be to the phenomenon.

PBS1 and SP10 in heterologous exchanges give recombination values of the order of those of transformation, indicating extensive fragmentation of their DNA (respectively, of 1.9×10^8 and 5.9×10^7 molecular weight [11]) upon entering on strain 168 background. The finding of Tyeryar et al. (22) in *Bacillus licheniformis*, that the linkage of markers was greater with SP10 transduction than in transformation, supports this hypothesis. Alternatively, it is possible that double-stranded DNA integration is involved in homologous transduction (in contrast with the single-stranded integration), clearly demonstrated in transformation, and conceivable in heterologous transduction.

The observations reported here may orient research on the recombination mechanisms in *B. subtilis* towards a study of the reasons for the difference between the two main pathways, which could reside in some essential aspects of the process itself.

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