NOTES

Growth of Bacteriophage ϕ 105 and Its Deoxyribonucleic Acid in Radiation-Sensitive Mutants of *Bacillus subtilis*

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Growth of phage ϕ 105 and its deoxyribonucleic acid (DNA) was studied in radiation-sensitive mutants of *Bacillus subtilis*. The *recA* gene is required for optimal prophage induction with mitomycin C and for infectivity of prophage DNA. *recB* gene is required for marker rescue from mature DNA. The importance of bacterial genes for phage DNA activity seems to depend on phage DNA structure.

Mutants with increased sensitivity to irradiation have been isolated in many bacterial species. Besides their radiation sensitivity, such mutants are often defective in genetic recombination (6, 13). In *Bacillus subtilis*, two distinct loci, *recA* and *recB*, of importance for genetic recombination in transformation and transduction have been identified (8, 9). Another such mutation, *rec-4*, has been recently identified by C. Anagnostopoulos; we are not aware of any published report on its position on the *B. subtilis* map.

Transfection, e.g., infection of sensitive cells with naked viral nucleic acid, has been demonstrated with deoxyribonucleic acid (DNA) from several *Bacillus* phages (12). Transfection is often used as a system for studying uptake of biologically active DNA by competent cells.

We have studied infectivity and marker rescue of various forms of DNA from the temperate *Bacillus* phage ϕ 105 and have demonstrated that infectivity is strongly dependent on phage DNA structure (2, 3, 10, 11). The present experiments were undertaken to study growth of bacteriophage ϕ 105 and its DNA in radiation-sensitive mutants of *Bacillus subtilis* to investigate whether phage growth or genetic recombination in the phage is dependent on any of the host functions defined by the radiation-sensitive mutations *recA1*, *recB2*, *rec-4*, and *uvr-1*. The strains employed are listed in Table 1. Before use, the mutant character of the strains was verified by testing their sensitivity to mitomycin C as described by Hoch et al. (9).

All strains employed are readily lysogenized by ϕ 105. The frequency of lysogenization of

rec⁺ bacteria by wild-type phage is 10 to 30% at multiplicities of infection of 1 to 10 (5), as measured by the technique of Bertani and Nice (4). The frequency of lysogenization of *rec⁻* strains is generally slightly lower. None of the bacterial mutations employed depresses phage growth, nor is phage genetic recombination in mixedly infected bacteria dependent on any one of the *recA*, *recB*, *rec-4*, or *uvr-1* functions (*data not shown*). Prophage ϕ 105 is inducible with mitomycin C (5) to the same degree in *rec⁺*, *recB2*, *rec-4*, and *uvr-1* strains measured as yield of infectious centers immediately after induction and as final burst size (Table 2). Lysogenic *recA1* bacteria, however, reproducibly give lower yields of infectious centers and of phage. *RecA1* is leaky, allowing 1 to 10% of the wild-type level of recombination in transformation (9). It resembles *recA*-type mutants in *Escherichia coli*, however, in giving a decreased phage yield after induction with mitomycin C but not after heat induction of a temperature-inducible prophage (7).

The biological activity of prophage DNA extracted from W168 (ϕ 105) was assayed in the various strains, grown to competence as described by Anagnostopoulos and Spizizen (1). Transformation of bacterial markers *metB4* or *hisA4* was measured. Transformation of *recA1* bacteria was about 1% of that of *rec⁺* bacteria in these experiments (Table 3), whereas in *recB2* bacteria transformants were four- to fivefold reduced and in *uvr-1* not at all. In these, as well as all other experiments, we failed to detect any significant

biological activity of any DNA added to *rec-4* bacteria grown to competence as described. In *recA1* bacteria, prophage infectivity is reduced to the same level as transformation, whereas the level of marker rescue is similar to that observed in *rec+* bacteria. In *recB2* bacteria, prophage DNA is as active as in *rec+* bacteria; this is also true for *uvr-1* bacteria. These experiments confirm our previous observation of decreased infectivity of prophage DNA in *recA1* bacteria (10).

TABLE 1. Properties of bacterial strains used

Strain	Properties ^a	Source
W168	Prototrophic, <i>su</i> ⁻	J. Spizizen
W168 (φ105)	W168 lysogenized by us	Our collection
SR135	<i>spoA9</i> , <i>trp-7</i> , <i>su</i> ⁺	J. A. Hoch
GSY 1025	<i>trpC2</i> , <i>metB4</i> , <i>recA1</i>	J. A. Hoch
GSY 1026	<i>trpC2</i> , <i>metB4</i> , <i>rec+</i>	J. A. Hoch
GSY 1027	<i>trpC2</i> , <i>metB4</i> , <i>uvr-1</i>	J. A. Hoch
GSY 1028	<i>trpC2</i> , <i>metB4</i> , <i>recB2</i>	J. A. Hoch
GSY 908	<i>argC4</i> , <i>hisA4</i> , <i>rec-4</i>	C. Anagnostopoulos

^a Properties: *su*⁻, nonsuppressing; *su*⁺, carries the *su3* suppressor gene; *spo*, asporogenous; *trp*, tryptophan; *met*, methionine; *arg*, arginine; *his*, histidine.

TABLE 2. Induction of prophage φ105 in radiation-sensitive strains of *Bacillus subtilis*

Strain	Induction ^a	Yielders ^b	Burst size
<i>rec+</i>	MC	1.4	71
<i>recA1</i>	MC	0.41	6
<i>recB2</i>	MC	1.2	115
<i>rec-4</i>	MC	1.4	185
<i>uvr-1</i>	MC	1.0	47
<i>rec+</i>	Heat	1.3	94
<i>recA1</i>	Heat	1.3	81
<i>recB2</i>	Heat	1.2	42
<i>rec-4</i>	Heat	1.4	106
<i>uvr-1</i>	Heat	1.2	39

^a Mitomycin C (MC) induction: the bacteria were lysogenized with wild-type φ105 and induced as previously described (5, 12). Lysis occurred within 120 min after induction except with the *recA1* strain which gave a slower and incomplete lysis. Heat induction: the bacteria were lysogenized with the heat-inducible φ105 *cts23* (to be published). The cells were grown at 30°C, induced at 45°C for 5 min, and then shifted to 37°C. All cultures lysed within 60 min.

^b Yielders, ratio of infectious centers after induction but before burst to viable cells before induction.

Infectivity of mature φ105 DNA does not depend on any of the radiation-sensitive genes studied (except *rec-4*). Marker rescue frequency, however, is reduced in *recB2* bacteria to a level comparable to that of transformation, whereas marker rescue in *recA1* and *uvr-1* is as efficient as in *rec+* bacteria (Table 4).

Vegetative DNA from φ105 is defined by us as DNA of increased infectivity compared to mature

TABLE 3. Transformation, transfection, and marker rescue with DNA from W168 (φ105) in radiation-sensitive strains of *Bacillus subtilis*^a

Strain	Total colony formers ^b	Transformants ^c	Infectious centers ^c	Marker rescue ^c
<i>rec+</i>	77	392	148	322
<i>recA1</i>	52	3	4	110
<i>recB2</i>	48	60	95	173
<i>rec-4</i>	42	<0.01	<0.01	<0.01
<i>uvr-1</i>	60	231	80	153

^a The methods for DNA extraction and assays of viable cells, transformants, infectivity, and marker rescue have recently been described (2, 11, 12). A 1-μg amount of DNA was added per ml of competent bacteria. φ105 *sus19* (2) was used as superinfecting phage in marker rescue experiments. *Rec-4* was assayed for *his+* transformants; all other strains were assayed for *met+* transformants.

^b Values to be multiplied by 10⁶.

^c Values to be multiplied by 10³.

TABLE 4. Infectivity and marker rescue of mature φ105 DNA in radiation-sensitive strains of *Bacillus subtilis*^a

Strain	Total colony formers ^b	Transformants ^c	Infectious centers ^d	Marker rescue ^c
<i>rec+</i>	84	300	363	488
<i>recA1</i>	78	10	295	346
<i>recB2</i>	54	40	243	49
<i>rec-4</i>	42	<0.01	<0.01	<0.01
<i>uvr-1</i>	82	381	312	238

^a Preparation of mature φ105 DNA, as well as methods used in transformation, transfection, and marker rescue have recently been described (2, 3, 11). A 1-μg amount of W168 DNA per ml was used for transformation and 1 μg of mature φ105 DNA per ml was used for transfection and marker rescue. φ105 *sus19* (2) was used as superinfecting phage in marker rescue experiments. *Rec-4* was assayed for *his+* transformants; all other strains were assayed for *mer+* transformants.

^b Values to be multiplied by 10⁶.

^c Values to be multiplied by 10³.

^d Values to be multiplied by 10¹.

DNA, which appears at the onset of phage DNA synthesis in infected or induced bacteria (10). In contrast to prophage and mature DNA which are molecules of relatively well defined structure (3, 5, 11), the structure of vegetative DNA is varied and may also depend on the type of infection (*unpublished data*). A DNA preparation from infected cells is a mixture of mature and replicating phage DNA. This makes it less meaningful to study marker rescue in such preparations, whereas infectivity is our means of defining this DNA species. DNA from induced bacteria (or infected bacteria) in which phage DNA synthesis is occurring is equally infectious in all strains tested, except in the sterile *rec-4* strain (Table 5).

Our experiments thus show that the *recA* gene is needed for prophage DNA infectivity and the *recB* gene function for marker rescue from mature DNA. An explanation in molecular terms of these observations must await an understanding of the exact functions of the *recA* and *recB* genes. The possibility that the normal frequency of rescue of markers from prophage DNA in *recB2* bacteria is spurious, due to simultaneous entry of prophage DNA and the wild-type allele of *recB*, seems unlikely considering that *recB* is unlinked to any prophage genes (8; *unpublished data*). It has recently been proposed that recombinant molecules, unable to replicate, are formed in the absence of *recB* gene product (R. Davidoff-Abelson and D. Dubnau, Proc. Nat. Acad. Sci.

U.S.A., *in press*). If this proposal is correct, it indicates that alternative mechanisms of recombinant formation may operate in marker rescue from $\phi 105$ DNA. Which mechanism is employed may then (at least in part) be determined by the structure of the DNA molecule taken up by the competent cells. A similar suggestion has been made to explain the diverse effects of the *recA1* mutation on transduction by phages SP10 and PBS1, respectively (8, 9). Finally, our results indicate that some caution must be exercised when assuming that transfection of *B. subtilis* occurs independently of any mechanism related to integration of bacterial DNA in transformation.

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TABLE 5. Transforming activity and infectivity of DNA from prophage-induced W168 ($\phi 105$) in radiation-sensitive strains of *Bacillus subtilis*^a

Strain	Total colony formers ^b	Transformants ^c	Infectious centers ^c
<i>rec</i> ⁺	55	230	150
<i>recA1</i>	73	19	121
<i>recB2</i>	94	59	180
<i>rec-4</i>	40	<0.01	<0.01
<i>uvr-1</i>	63	142	145

^a DNA was extracted from mitomycin C-induced W168 ($\phi 105$) 70 min after induction (5, 12). All techniques employed were as recently described (2, 11, 12). About 1 μ g of DNA was added per ml of competent cells. *Rec-4* was assayed for *his*⁺ transformants; all other strains were assayed for *met*⁺ transformants.

^b Values to be multiplied by 10⁶.

^c Values to be multiplied by 10³.