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DNA-Mediated Prophage Induction in *Bacillus subtilis* Lysogenic for ϕ105c4

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Prophage was induced when strains of *Bacillus subtilis* 168 lysogenic for ϕ105c4 were grown to competence and exposed to specific bacterial DNAs. The time course of phage production was similar to that observed for mitomycin C induction of wild-type prophage. Induction was directly dependent upon DNA concentration up to levels which were saturating for the transformation of bacterial auxotrophic markers. The extent of induction varied with the source of DNA. The burst of phage induced by DNA isolated from a W23 strain of *B. subtilis* was fivefold less than that induced by DNA from *B. subtilis* 168 strains, while *B. licheniformis* DNA was completely inactive. This order of inducing activity was correlated with the ability of the respective DNAs to transform auxotrophic markers carried by one of the ϕ105c4 lysogens. Differences in inducing activity also were observed for different forms of ϕ105 DNA. The DNAs isolated from ϕ105 phage particles and ϕ105c4 lysogens were inactive, whereas DNA from cells lysogenized by wild-type ϕ105 induced a burst of phage. When tested for transforming activity, however, both ϕ105c4 and ϕ105 lysogen DNAs were equally effective. An induction mechanism which involves recombination at the prophage insertion site is proposed to explain these differences.

Prophage induction involves a release from repression of phage genes required for lytic development. The molecular basis of this repression has been resolved for coliphage λ, and a similar mechanism appears to function for a number of other inducible temperate phages (6). In the prophage state transcription of most λ genes is prevented by a phage-coded protein which binds reversibly to operator loci on the prophage genome (21). Release from repression may ensue either from inactivation of the repressor or an alteration in an operator locus which renders it insensitive to the repressor (10). Within this context, thermal induction is readily understood as the inactivation of a mutationally altered thermolabile repressor.

A variety of physical and chemical treatments such as UV irradiation, mitomycin C (MC), X rays, and thymine deprivation also cause prophage induction. These agents, all of which inhibit DNA replication, do not act directly on the repressor and are unable to induce prophage from a class of radiation-sensitive bacterial mutants which are also defective in genetic recombination (5, 22). However, the relationship between these mutations and their effects on genetic recombination and prophage induction is not understood.

In the preceding paper I reported that the prophage formed by the c4 clear plaque mutant of the temperate *Bacillus* phage ϕ105 was induced when transforming DNA was added to competent lysogenic cultures (12). This observation suggested a possible relationship between this pathway of genetic recombination and prophage induction and led to the present study in which DNAs from different sources were examined for their capacity to act as inducers. It was hoped that any specificities observed among these DNA samples might provide some insight into the mechanism behind this novel method of induction.

MATERIALS AND METHODS

Bacteria and phage. A listing of the bacterial strains used is included in Table 1. The isolation of the ϕ105c4 lysogens and the general procedures used for growing and assaying bacteria and phage are described in the preceding paper (12).

DNA preparations. Bacterial DNA was isolated by the Marmur method (19). Bacteriophage ϕ105 DNA was isolated by phenol extraction from CsCl-purified phage induced from BR95(ϕ105). The lysogen

TABLE 1. *Inducing activities of different DNAs*

DNA source	Levels of phage (PFU/ml)[a] induced from			
	BR95(φ105c4)	R[b]	SR135(φ105c4)	R[b]
None	1.8 × 10³	1.0	6.0 × 10²	1.0
B. subtilis 168 strains				
BR95 *phe, ilv, try-2*	5.6 × 10⁴	31.3	4.1 × 10⁴	68.5
SR135 *spoA-9, try-7, su*⁺3	5.3 × 10⁴	31.0	4.8 × 10⁴	80.0
MB54 *metC*	4.5 × 10⁴	25.0	2.7 × 10⁴	46.1
SB-1 *his-2, try-2*	4.8 × 10⁴	26.7	3.8 × 10⁴	61.5
B. subtilis W23	1.2 × 10⁴	6.7	6.7 × 10³	10.6
B. licheniformis ATCC *8480*	1.5 × 10³	0.9	6.2 × 10²	1.0
φ105 lysogens				
BR95(φ105)	5.0 × 10⁴	27.6	5.1 × 10⁴	85.0
SR135(φ105)	5.7 × 10⁴	31.6	5.3 × 10⁴	88.1
BR95(φ105c4)	2.4 × 10³	1.3	6.0 × 10²	1.0
SR135(φ105c4)	2.0 × 10³	1.1	5.8 × 10²	1.0
Purified φ105	1.8 × 10³	1.0	5.5 × 10²	0.9

[a] PFU/ml due to free phage measured 120 min after DNA addition.
[b] Ratio of PFU, induced:noninduced cultures.

was grown in VY broth, and the phage was induced by the addition of 0.4 μg of MC (Kyowa Hakko Kogyo Co., Ltd.) per ml. Phage was concentrated from the lysate by the polyethylene glycol precipitation procedure described by Yamamoto et al. (26), and the concentrated phage was banded in a CsCl step gradient consisting of 15 ml of phage concentrate, 13 ml of aqueous CsCl with a refractive index (η) of 1.3738, and 10 ml of aqueous CsCl (η = 1.3953). The gradient was centrifuged in an SW27 rotor at 25,000 rpm for 16 h, and the visible phage band was collected and dialyzed against 1,000 volumes of standard saline citrate (SSC; 0.15 M NaCl, 0.015 M Na citrate, pH 7), and the DNA was extracted by gentle mixing with an equal volume of phenol which had been equilibrated with buffer (0.006 M Na_2HPO_4, 0.002 M NaH_2PO_4, 0.001 M EDTA, pH 7.4). Phenol was removed from the DNA preparation by dialysis against 0.1 × SSC.

Isopycnic banding of phage. Phages φ105 and φ105c4 were mixed with a solution of CsCl (η = 1.3800) in 0.01 M Tris (pH 7.4), 10^{-3} M $MgCl_2$, and 0.01% gelatin and centrifuged for 40 h in a type 50 Ti rotor at 30,000 rpm. Fractions of 0.15 ml were collected dropwise and then diluted with the Tris buffer described above and assayed for PFU.

RESULTS

Time course of induction. Incubation of competent cultures of *B. subtilis lysogenic for* φ105c4 with DNA isolated from *B. subtilis* 168 results in a burst of phage (Fig. 1). With both lysogenic strains, BR95 (φ105c4) and SR136 (φ105c4), there was a 60-min lag between exposure of the cells to DNA and the first detectable rise in the levels of free phage. No burst was detected if the DNA was added to noncompetent broth cultures, if the inducing DNA was pretreated for 15 min with pancreatic DNase (10 μg/ml), or if MC (0.4 μg/ml) was used as the inducing agent.

Induction as a function of DNA concentration. Since the conditions for φ105c4

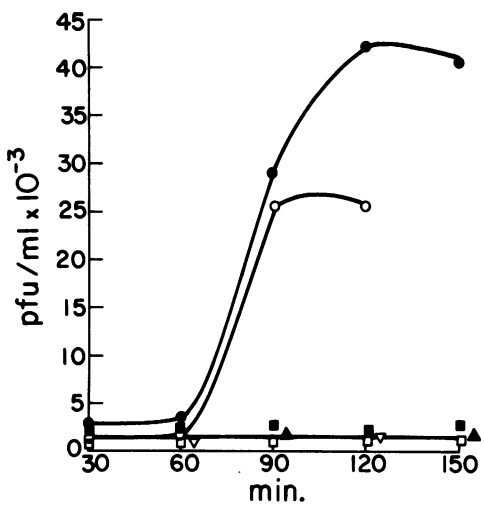

FIG. 1. *Time course of phage production. Levels of free phage in competent cultures of BR95(φ105c4) (closed symbols) and SR135(φ105c4) (open symbols) which were exposed at 0 min to: MB54 DNA, 4 μg/ml (●,○); MC, 0.4 μg/ml (■,□); MB54 DNA, 4 μg/ml, pretreated for 15 min with 10 μg/ml of pancreatic DNase (▲,△). Free phage in noncompetent cultures grown in VY broth and exposed to MB54 DNA, 4 μg/ml (▼,△).*

induction were the same as those required for DNA-mediated transformation, a process which is directly dependent upon DNA concentration, the relationship between induction and DNA concentration was examined. The lysogen BR95(ϕ105c4) *phe ilv try* was chosen for these experiments as the presence of auxotrophic markers in this strain made it possible to monitor induction and transformation simultaneously. The markers *phe* and *ilv* were of particular interest since they bracket the prophage insertion site (20) and are linkable by cotransformation.

BR95(ϕ105c4) *phe ilv* try was grown to competence, and 1-ml samples were exposed to varying concentrations of MB54 DNA for 30 min, followed by 10 μg of pancreatic DNase per ml. Samples were withdrawn and streaked on minimal plates to select ilv^+ and phe^+ ilv^+ transformants. The remaining portions of the cultures were incubated for an additional 90 min and then assayed for free phage. As seen in Fig. 2 the dose response curves for induction and single- or double-marker transformation are linear up to DNA concentrations of approximately 10^{-1} μg/ml, above which the level of DNA approaches saturation. It is also evident from this experiment that the efficiency of induction is lower than the efficiency of single marker transformation especially if an approximate burst of 10 to 50 is assumed for each induced cell (2).

Relationship between induction and the source of DNA. Competent cells can take up DNA from a variety of sources including those which are genetically unrelated to the recipient cell and thus incapable of producing genetic recombinants. To determine whether induction showed any dependence on genetic homology between the inducing DNA and the ϕ105c4 lysogens, DNA samples isolated from different strains and species of *Bacilli* and from ϕ105 lysogens and purified ϕ105 phage were tested for their effectiveness as inducers. The induction response of BR95(ϕ105c4) to DNA isolated from strains of *B. subtilis* 168, *B. subtilis* W23, and *B. licheniformis* is shown in Fig. 3A. The burst of phage induced by W23 DNA was approximately fivefold less than that induced by 168 DNA, whereas *B. licheniformis* DNA was completely ineffective. Similar experiments in which DNAs from BR95(ϕ105), BR95(ϕ105c4), and purified ϕ105 were compared revealed that the only effective inducer among this group was BR95(ϕ105) DNA (Fig. 3B). The burst produced by this DNA, which contains the genome of the wild-type turbid plaquing phage, was made up of greater than 90% clear plaques.

Additional experiments in which the induction of phage from SR135(ϕ105c4) also was examined are summarized in Table 1. The response of both ϕ105c4 lysogens to the various DNA samples was qualitatively the same. The order of inducing activity of the bacterial DNAs from nonlysogenized strains was found to be *B. subtilis* 168 > *B. subtilis* W23 > *B. licheniformis*. The DNAs from *B. subtilis* 168 strains lysogenized by wild-type ϕ105 induced prophage while those from ϕ105c4 lysogens or purified phage did not.

Comparison of inducing activity with recombinant production. If one assumes that the ineffectiveness of *B. licheniformis* DNA as an inducer is indicative of a requirement for genetic homology between the inducing DNA and the lysogen, then the relatively poor activity of

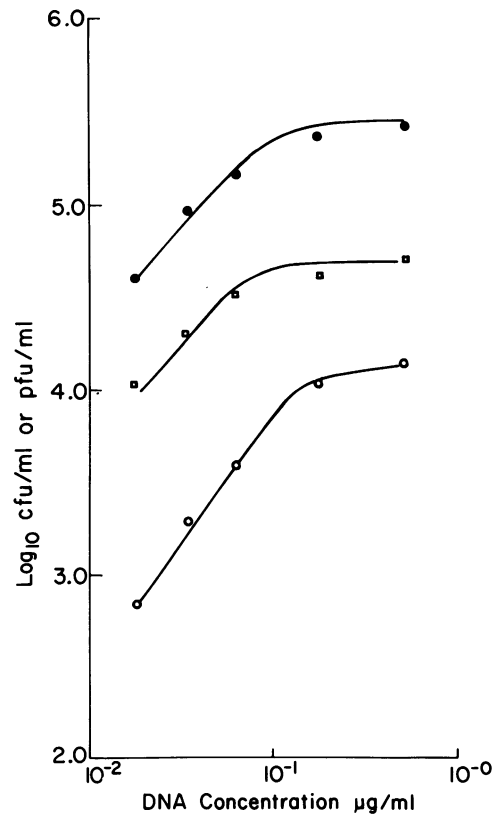

FIG. 2. *Relationship of induction and transformation to DNA concentration. Varying concentrations of MB54 DNA were added to 1-ml samples of a competent culture of BR95(ϕ105c4) and incubated with shaking at 37 C for 30 min. Pancreatic DNase was added to a final concentration of 10 μg/ml, and after 15 min samples were plated for phe^+ (●), and phe^+ ilv^+ (○) transformants. Incubation was continued for a total of 120 min, and the cultures were assayed for ϕ105c4 PFU (□).*

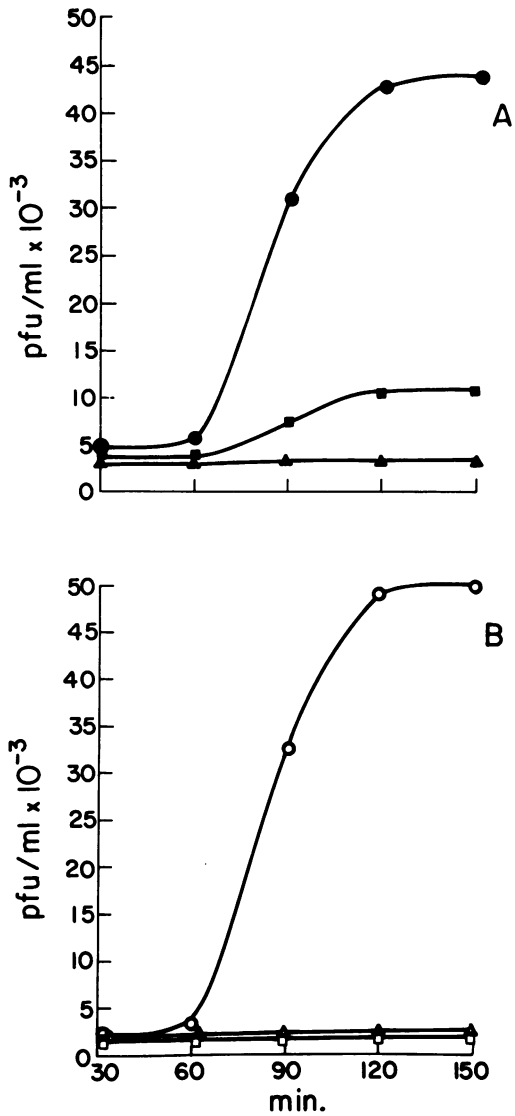

FIG. 3. *Relationship of induction to DNA source. Samples of a competent culture of BR95(φ105c4) were exposed at 0 min to 4 μg of DNA per ml, and the levels of free phage in the cultures were followed. Sources of DNA: B. subtilis 168 strain MB54 (●), B. subtilis W23 (■), B. licheniformis (▲), BR95(φ105) (○), BR95(φ105c4) (△), φ105 phage (□).*

B. subtilis W23 DNA is surprising. Although W23 and 168 strains of *B. subtilis* are readily distinguishable by a number of criteria, nevertheless the DNAs from these strains exhibit extensive homology by hybridization, and W23 DNA is generally as efficient as 168 DNA in transforming most *B. subtilis* 168 strains (8). However, since some strain differences have been noted with respect to this latter property, the efficiency with which DNAs from *B. subtilis* W23 and 168 transformed BR95 and its lysogenic derivatives was examined. The results (Table 2) show that W23 DNA transformed all the BR95 strains less efficiently than the 168 DNA. However, this reduced efficiency was most evident with the φ105 lysogens, in which the relative frequency of transformation with W23 DNA was reduced by approximately the same factor as the frequency of induction (cf. Fig. 3). No transformants for the markers listed in Table 2 were ever detected using *B. licheniformis* DNA.

In contrast to these results, the poor inducing activity of DNA from φ105c4 lysogens was not correlated with a reduced capacity to produce transformants. When competent cultures of BR95(φ105c4) were exposed to saturating concentrations (4 μg/ml) of SR135(φ105c4) and SR135(φ105) DNAs no differences were observed in the numbers of *phe*$^+$ or *ilv*$^+$ transformants produced (Table 3).

Buoyant densities of φ105c4 phage. Because of the marked differences in inducing activity of DNAs from φ105c4 and φ105 lysogens, the possibility that the c4 mutation was a macrodeletion was examined. If the clear plaque phenotype of φ105c4 phage were due to a substantial deletion of the φ105 genome then the buoyant density of c4 particles would be less than that of the wild-type phage (17). However, when a mixture of φ105c4 and φ105 phage articles were

TABLE 2. *Relative frequencies of transformation*[a]

Recipient strain	Marker selected		
	ilv-1	*phe-1*	*try-2*
BR95	0.8	0.9	0.7
BR95(φ105c4)	0.2	0.1	0.1
BR95(φ105)	0.3	0.2	0.2

[a] Competent cultures were transformed with saturating concentrations of DNA (4 μg/ml). The values presented represent the ratio of transformants obtained using DNA from *B. subtilis* W23 to the number obtained using DNA from *B. subtilis* 168 strain MB54.

TABLE 3. *Transformation of BR95(φ105c4) by DNA from φ105 and φ105c4 lysogens*[a]

DNA from:	Transformants/10^8 cells	
	phe$^+$	*ilv*$^+$
SR135(φ105)	1.0×10^5	0.9×10^5
SR135(φ105c4)	0.8×10^5	1.3×10^5

[a] DNA was added to a final concentration of 4 μg/ml.

centrifuged to equilibrium in a CsCl gradient, the phages banded at the same density (Fig. 4).

DISCUSSION

The experiments presented demonstrate that the induction of ϕ105c4 prophage produced by addition of DNA to competent cultures is a function of both the concentration and source of the DNA.

A number of mechanisms have been considered to explain this mode of induction. The possibility that the inducing DNA directly inactivates the ϕ105 repressor is unlikely on the basis of the observed kinetics of induction (Fig. 1). The 60-min lag which elapses between DNA addition and the burst of phage production is similar to that seen with MC induction (2) and significantly longer than the 30- to 40-min lag observed during heat induction of ϕ105ts23 which makes a thermolabile repressor (1). Similarly, a mechanism involving the titration of repressor, as is observed when λ lysogens are infected at high multiplicities by c1 mutants (16, 25), can be rejected because of the direct dependence of induction on DNA concentration (Fig. 2). Furthermore, the lack of inducing activity of mature ϕ105 DNA in contrast to that of bacterial DNAs which do not carry the ϕ105 genome also argues against such a mechanism.

One possible explanation, which is consistent with the inducing activity of *B. subtilis* 168 DNAs, is that induction results from either the formation of processing of recombinant DNA molecules produced during transformation. This mechanism would presuppose an ability to recombine with host genes as a necessary condition for inducing activity. The inactivity of mature ϕ105 DNA may reflect either a requirement for recombination with some bacterial genes or may be due to the destruction, during DNA uptake, of some critical phage genes situated near the end of the ϕ105 genome (23). Similarly, the absence of inducing activity of *B. licheniformis* DNA would be related to its limited genetic homology with *B. subtilis* (9) while the relatively low levels of induction produced by *B. subtilis* W23 DNA may be correlated with its poor transforming activity for BR95(ϕ105c4) (Table 2). The basis for this reduced activity is not known but is probably due to slight differences in base sequence homology between W23 and 168 strains (8). The fact that this effect is accentuated in ϕ105 lysogens is interesting since this phage does not replicate on W23 strains, and the possibility that ϕ105 may restrict W23 DNA should be considered.

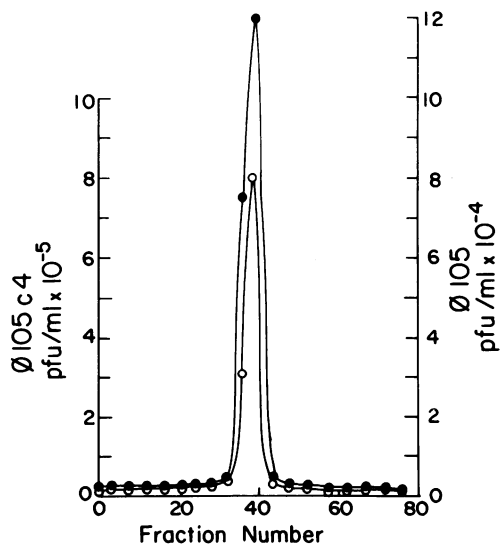

FIG. 4. *Equilibrium density gradient centrifugation of ϕ105 (●) and ϕ105c4 (○). The phages were mixed in a CsCl solution and centrifuged to equilibrium as described in Materials and Methods. Fractions were collected and assayed for PFU on lawns of SR135.*

An ability to recombine with the host chromosome appears to be a necessary but not sufficient condition for inducing activity. This conclusion derives from the differences in inducing activities of DNAs isolated from lysogens of wild-type and c4 mutant ϕ105. Although both of these DNAs transform BR95(ϕ105c4), only the wild-type lysogen DNA is active as an inducer (Tables 1, 3, and Fig. 3). Nevertheless, a recombination-based induction mechanism can account for these differences if one postulates that induction results only from recombination initiated in the region of prophage insertion by DNAs which are inhomologous for some prophage genes. The DNAs from the *B. subtilis* 168 strains which were not lysogenized by ϕ105 clearly fulfill this requirement and would be inhomologous for all prophage genes. The extent of inhomology which exists between wild-type prophage and c4 prophage, however, is not known. Although it was not possible to detect any indication that ϕ105c4 is a deletion mutant of ϕ105, the phages may differ appreciably on the prophage level. In the preceding paper, the possibility that ϕ105c4 formed an aberrant prophage either in terms of its exact integration site or gene order was suggested to explain the unusual properties of these lysogens (12).

What would be the effect of such inhomologies on the type of recombinant molecules produced? Since transformation produces recombinants by the integration of a single-

stranded segment of donor DNA which replaces a corresponding single-strand on the recipient genome (3, 7, 11, 13), inhomologous regions which could not completely base pair would result in DNA heteroduplexes (3, 4, 14, 24). If binding of the ϕ105 repressor to the prophage operator site(s) were interfered with by these structures, then heteroduplex formation might be the primary inducing event. Alternatively, induction might result from inactivation of the ϕ105 repressor during the repair process which converts heteroduplexes to homoduplexes. Evidence for such a correction mechanism in *B. subtilis* has been obtained by clonal analysis of transformed cells (3) and by the use of artificially prepared heteroduplex phage DNA which was used to transfect competent cultures (24). Since heteroduplex conversion may be analogous to the repair of UV- and MC-induced damage (4), it is interesting to note that MC-treated *Escherichia coli* appears to contain some product which inactivates the λ repressor in vitro (Johnston and Echols, Abstr. 1972 Cold Spring Harbor Bacteriophage Meeting).

The proposed induction mechanism also provides an explanation for the relatively small increases in free phage produced by DNA induction and concomitantly why this mode of induction is not detectable with wild-type ϕ105 lysogens (12, 20). First of all, only a minority of the cells, less than 20%, in competent cultures of *B. subtilis* are actually in a state of competence (15, 18) and thus potentially inducible. Secondly, the process by which heteroduplexes are formed requires that the donor or inducing DNA be base paired with recipient DNA on both sides of the inhomologous region. If this regions amounts to any substantial length of the inserted prophage, then the formation of heteroduplexes which lead to induction would occur with a frequency similar to that observed for simultaneous transformation of poorly linked genes. Taking the frequency of *phe*$^+$*ilv*$^+$ transformants as an example of this type of transformation, it can be seen in Fig. 2 that the number of double transformants is less than 10% that of single marker transformants. In the case of wild-type ϕ105 lysogens the relatively high levels of free phage present in the cultures as a result of spontaneous induction (11) would mask any increases in free phage due to such a low-efficiency process.

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