The Prophage Behaviour in Crosses between Lysogenic and Non-lysogenic derivatives of *Streptomyces coelicolor* A3(2)

By NATALIE D. LOMOVSKAYA, LIDA K. EMELJANOVA,
NORA M. MKRTUMIAN AND S. I. ALIKHANIAN

*Institute of Genetics and Selection of Industrial Micro-organisms,
Moscow, U.S.S.R.*

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SUMMARY

The prophage ϕ C31 was shown to be a genetic determinant of lysogeny in *Streptomyces coelicolor* A3(2). In mapping experiments with strains cured and relysogenized with mutant phages the prophage was located on the *Streptomyces* chromosome.

Allele ratios of non-selected markers for crosses between lysogenic and non-lysogenic derivatives of A3(2) differ strikingly from those of crosses between non-lysogenic parents. It is suggested that these differences are due to the loss of certain recombinant classes containing the complete chromosome of the non-lysogenic parent and a fragment from the lysogenic one, as a result of zygotic induction.

INTRODUCTION

Interest in lysogenic actinomycetes was developed because they were a principal source of lysis in the antibiotic industry. As a result actinophages and their host relationships were generally studied with a view to practical applications, in particular, to obtain actinophage-resistant cultures. Genetic control of lysogeny in actinomycetes remained uninvestigated until now.

Study of lysogeny in actinomycetes encounters more difficulties than in other bacterial systems because of the structural peculiarities of actinomycetes and their inadequate genetic characterization. There is no doubt that more detailed studies are needed to elucidate the genetic control of lysogeny in actinomycetes. The only representative for which a detailed genetic map has been constructed was chosen for investigation with the temperate actinophage ϕ C31 isolated from *Streptomyces coelicolor* A3(2) (Lomovskaya, Mkrtumian, Gostimskaya & Danilenko, 1972). This prophage has been located on the A3(2) chromosome (Lomovskaya, Emeljanova & Alikhanian, 1971).

We present in this communication more precise data on the prophage location and report the effects of the prophage on the results of crosses between non-lysogenic A3(2) strains and their lysogenized derivatives.

METHODS

Strains. Genetically marked strains used in this work are listed in Table 1. Genetic symbols are as given by Hopwood (1967). Strain 853 used in this work was taken from a group of polyauxotrophic derivatives of A3(2) kindly supplied by D. A. Hopwood. Non-lysogenic (ly)⁻ and lysogenized (ly)⁺ derivatives of A3(2), S134, S26 and S12, were obtained in our

Table 1. *Characteristics of genetically marked strains of Streptomyces coelicolor A3(2)*

Strain	Genetic markers
853	<i>hisA1 uraA1 adeA3 cysD18 tps33</i>
S134	<i>pheA1 mthB2 strA1</i>
S26	<i>hisA1 strA1</i>
S12	<i>proA1 uraA1 adeA3 cysD18 argA1</i>
S92	<i>proA1 adeA3 cysD18 argA1</i>
S146	<i>proA1 uraA1 cysD18 argA1</i>

Fig. 1. Plaques produced by a mixture of wild-type phage ϕ C31 and mutant phage ϕ C31*clo1*. Arrows indicate: (1) wild-type phage ϕ C31; (2) mutant phage ϕ C31*clo1*.

laboratory. Recombinant descendants of A3(2), S92 and S146, containing the mutant ϕ C31*clo1* prophage were constructed for use in crosses. Phage ϕ C31*clo1* differs from wild-type phage in plaque morphology producing turbid plaques completely and evenly overgrown by lysogenic cells while wild-type phage produces plaques with sharp rings of cell growth (Fig. 1). To obtain a strain lysogenic for mutant prophage ϕ C31*clo1* the lysogen carrying wild-type phage was treated with ultraviolet light (u.v.). Survivors were replicated on to plates seeded with indicator strain spores in a soft agar and a colony carrying the mutant prophage ϕ C31*clo1* was isolated.

Actinomyces coelicolor strain 66 was used as a sensitive culture for phage assays (Lomovskaya *et al.* 1972).

Media, phage assays and crossing procedure. Corn-steep liquor medium was used for phage assays, complete and minimal media used in crosses between actinomycetes strains, and the procedure for crosses followed by haploid recombinant analyses, are the same as described previously (Lomovskaya *et al.* 1971; Lomovskaya *et al.* 1972).

In crosses between non-lysogenic and defective-lysogenic A3(2) strains the presence or absence of the prophage in recombinants was detected by immunity and sensitivity, respectively, to ϕ C31. In crosses between non-defective lysogens and non-lysogenic cultures

Fig. 2. Location of prophage ϕ C31 on the A3(2) chromosome. Markers of the two parents are indicated on the two circles. Solid triangles indicate selected markers. Letters between circles show map intervals between markers. Numbers are the frequencies of each allele in the sample of recombinants analysed.

a mixture of parental strains was incubated in the presence of the phage-specific antiserum. Only those crosses were analysed where mixed culture of parental strains did not display free phage during the growth period. In addition, phage-resistant strains which did not adsorb the phage and as a result survive high concentrations of the phage were used as non-lysogenic parents in all crosses.

To test the prophage type in crosses between lysogens carrying different prophages the spores of recombinant colonies arising on the selective media were picked with a sterile pin and transferred to plates seeded with indicator strain spores. Halos of lysis formed by the phage, liberated during growth of lysogenic spore cultures, were characteristic of each prophage type.

RESULTS

Prophage location

We previously reported the prophage chromosomal location between *cysD18* and *argA1* (Lomovskaya *et al.* 1971). However, these markers are not close to each other on the genetic map of A3(2). To determine more precisely the map position of the prophage the polyauxotrophic strain 853 carrying *tpS33* marker located between *cysD18* and *argA1*, was used in crosses as a lysogenic parent. Like the wild-type A3(2), the strain was characterized by a very low spontaneous rate of phage liberation, due possibly to defective

Table 2. Analysis of *hisA1*⁺ and *mthB2*⁺ selected recombinants from the cross 853(*ly*)⁺*hisA1uraA1adeA3cysD18tps33* × S134(*ly*)[−]*pheA1mthB2strA1**

		Single crossover classes†			
Genotype,‡	r.h. arc . . .	<i>ly</i> [−]	<i>cysly</i> [−]	<i>cysly</i> ⁺	<i>cys ly</i> ⁺ <i>tps</i>
Genotype, l.h. arc	Crossover in interval§	f	g	h	i
<i>uraade</i>	a	38	11	6	50
<i>ade</i>	b	4	2	—	9
<i>pheade</i>	c	—	—	—	1
<i>phe</i>	d	11	2	2	10
<i>phestr</i>	e	10	5	1	3

* The cross is illustrated in Fig. 2.

† Amongst the 168 recombinants three multiple recombinants were obtained. Each multiple recombinant was produced in intervals *adei*, *adeg* and *adef* accordingly.

‡ Wild-type alleles omitted.

§ See intervals in Fig. 2.

lysogeny, and by an immunity that may be overcome only following infection with virulent mutants of ϕ C31.

Fig. 2 illustrates the results of the cross 853(*ly*)⁺*hisA1uraA1adeA3cysD18tps33* × S134(*ly*)[−]*pheA1mthB2strA1*, where *hisA1*⁺ and *mthB*⁺ were the selected alleles. All the other markers, including the prophage, which was to be mapped, were non-selected. Prophage location was deduced in the manner described by Hopwood (1967). The allele ratio of 85(*ly*)[−]:83(*ly*)⁺ served to locate the prophage either between *cysD18* and *tps33* or between *uraA1* and *hisA1*. Table 2 shows the correct location of the prophage between *cysD18* and *tps33*, since only three recombinants/cross need more than the minimum number of crossovers. If the prophage was located between *uraA1* and *hisA1*, 26 of the recombinants would have required multiple crossovers.

The isolation of A3(2) derivatives cured of prophage and the preparation of true lysogens following infection of cured strains with ϕ C31 (Lomovskaya *et al.* 1971; Lomovskaya *et al.* 1972) enabled us to determine the chromosomal location of the prophage after lysogenization of these cured variants. The lysogenic parents for use in such crosses were obtained as follows. Strain s26(*ly*)[−] was lysogenized with wild-type phage, the resulting strain s26(ϕ C31) was treated with u.v. and a lysogen carrying the mutant prophage ϕ C31*clor* was isolated from the survivors (see Methods). From the progeny from the cross s26(ϕ C31*clor*) × S12(ϕ C31) the recombinant strains S146 and S92 were selected, which instead of wild-type prophage contained the mutant prophage ϕ C31*clor*, which was expected to occupy the same place on the chromosome as the wild-type phage.

Fig. 3(a) and Table 3 summarize the results of the cross between strains s26(ϕ C31) and S92(ϕ C31*clor*), where *hisA1*⁺ and *strA1* were the selected alleles. Prophage location, deduced in the manner described above, indicates a position between *adeA3* and *proA1*. The correctness of this conclusion was confirmed in crosses between strains s26(ϕ C31) and S92(ϕ C31*clor*), where *proA1*⁺*adeA3*⁺*strA1hisA1*⁺ (Fig. 3b) or *hisA1*⁺*argA1*⁺*cysD18*⁺*strA1* and *adeA3*⁺ (Fig. 3c) were used alternatively as selected markers. Selected markers in a cross of first type were designed to obtain recombinants obligatorily including in their genomes that fragment of strain s26 situated between *proA1*⁺ and *strA1*. As expected, all these recombinants carried the wild-type prophage, while if the prophage were located above *cysD18* marker, then recombinants could have carried whether wild-type prophage or

mutant prophage. In a cross of second type the right fragment of the chromosome situated between *argA1*⁺ and *adeA3*⁺ was contributed to all recombinants. If the prophage was located above *cysD18* marker then all the recombinants would have carried the wild-type prophage. However, 18 out of the 188 recombinants liberated the mutant phage during spontaneous induction. Consequently the prophage location between *adeA3* and *proA1* is considered to be more likely. On the other hand, the results of the cross between strains s26 and s146 (Fig. 3*d*, Table 4) suggest that the prophage is located between *uraA1* and *strA1*. Thus, we conclude that the prophage ϕ C31 is located between *uraA1* and *adeA3* markers.

Table 3. *Analysis of hisA1⁺ and strA1 selected recombinants from the cross s26(ϕ C31)hisA1strA1 $\times$ s92(ϕ C31clo1)proA1argA1adeA3cysD18**

Genotype, r.h. arc . . . Genotype, l.h. arc Crossover in interval‡		Single crossover classes†		
		<i>cysarg</i>	<i>cys⁺arg</i>	<i>cys⁺arg⁺</i>
		e	f	g
+++	a	0	30	19
pro++	b	3	138	17
proclo1+	c	0	20	3
proclo1ade+	d	0	14	2

* The cross is illustrated in Fig. 3(a).

† No multiple crossover classes observed.

‡ See intervals in Fig. 3.

Table 4. *Analysis of hisA1⁺ and strA1 selected recombinants from the cross s26(ϕ C31) hisA1strA1 $\times$ SI46(ϕ C31clo1)proA1argA1uraA1cysD18**

Genotype, r.h. arc . . . Genotype, l.h. arc Crossover in interval‡		Single crossover classes†		
		<i>cysarg</i>	<i>cys⁺arg</i>	<i>cys⁺arg⁺</i>
		e	f	g
+++	a	7	26	8
pro++	b	23	58	18
proura+	c	4	3	0
prouraclor	d	25	13	1

* The cross is illustrated in Fig. 3(d).

† No multiple crossover classes observed.

‡ See intervals in Fig. 3(d).

Table 5. *Analysis of hisA1⁺ and strA1 selected recombinants from the cross SI2(ly)⁻ $\times$ S26(ly)⁻*

Genotype, r.h. arc . . . Genotype, l.h. arc Crossover in interval		Single crossover classes %*		
		<i>cysarg</i>	<i>cys⁺arg</i>	<i>cys⁺arg⁺</i>
		e	f	g
+++	a	5	10	8.5
pro++	b	15	30	6
proura+	c	5	5	0.5
prouraade	d	10	5	—

* No multiple crossover classes observed amongst the 201 recombinants.

Effect of the prophage presence on the results of crosses

To test the influence of the presence of non-defective ϕ C31 prophage on the results of crosses non-lysogenic A3(2) strains and their lysogenic derivatives were used as parents. In the control cross SI2(ly)⁻ $\times$ S26(ly)⁻ the frequencies of crossovers on either side of selected markers hisA1⁺ and strA1 are compatible (Table 5) with the lengths of the intervals estimated by Hopwood (1966*a, b*). Hence relatively equal numbers of recombinants arose which contained a complete chromosome contributed from one of the parents and fragments

Fig. 4. Allele frequencies of non-selected markers in selected recombinants inheriting the alleles *proA*⁺ and *hisA*⁺ in crosses: (a) *s12(ly)*⁻ × *s26(ly)*⁻; (b) *s12(ly)*⁻ × *s26(ly)*⁺; (c) *s12(ly)*⁺ × *s26(ly)*⁻.

from the other. The data indicate that both parents belong to the same fertility type, that is, NF type, since NF × NF cross is characterized by an absence of merozygote asymmetry and polarization (Vivian & Hopwood, 1970). A similar result was obtained in a cross between lysogenic parents (Tables 3, 4).

In reciprocal crosses between lysogenic and non-lysogenic strains where *hisA*⁺ and *strA*⁺ served as selected markers the selected marker was situated in the *de* region of the chromosome of the lysogenic strain *s26*, whereas when strain *s12* was used as a lysogenic parent the selected marker was located just across, in the *ag* region. This fact makes it more difficult to analyse the results of these crosses.

Fig. 4 and Table 6 present the results of reciprocal crosses where *proA*⁺ and *hisA*⁺, located in the same region of the genome, served as selected markers. Even in these crosses fragments belonging to different halves of the genome were included in recipient cells, depending on which of the parents was lysogenic. Analysis of these results revealed considerable differences in allele ratios of nonselected markers between crosses *s12(ly)*⁻ × *s26(ly)*⁺ or *s12(ly)*⁺ × *s26(ly)*⁻ and those obtained in the cross *s12(ly)*⁻ × *s26(ly)*⁻.

The *ly*⁺ chromosome very rarely contributes to recombinants a fragment carrying *pro*⁺ (cross *s12ly*⁻ × *s26ly*⁺). A fragment carrying *his*⁺ is contributed by the *ly*⁺ chromosome with a lower frequency (cross *s12ly*⁺ × *s26ly*⁻) than by the *ly*⁻ chromosome. As a result, recombinants derived from the complete parental *ly*⁺ chromosome and a fragment from the *ly*⁻

Table 6. Analysis of *hisA1*⁺ and *proA1*⁺ selected recombinants from crosses *s12(ly)*⁻ × *s26(ly)*⁻, *s12(ly)*⁻ × *s26(ly)*⁺ and *s12(ly)*⁺ × *s26(ly)*⁻*

Genotype	Crossover in interval	Recombinants in crosses (%)†		
		<i>s12(ly)</i> ⁻ × <i>s26(ly)</i> ⁻	<i>s12(ly)</i> ⁻ × <i>s26(ly)</i> ⁺	<i>s12(ly)</i> ⁺ × <i>s26(ly)</i> ⁻
<i>uraade</i> + <i>cysarg</i>	b	38	1	48
+ <i>ade</i> + <i>cysarg</i>	c	4	1	31
+ + + <i>cysarg</i>	d	6	0	1
+ + <i>str cysarg</i>	e	5	1	1
+ + <i>str</i> + <i>arg</i>	f	28	56	10
+ + <i>str</i> + +	g	16	38	6

* Each cross between lysogenic and non-lysogenic strains was conducted in the presence of phage-specific antiserum and by using phage-resistant variants as non-lysogenic parents (see Methods). Results obtained in both sets of experiments were identical. The data given are those which were obtained in crosses with phage-resistant strains.

† In this arc crossovers arise in interval a. In the cross *s12(ly)*⁻ × *s26(ly)*⁻, amongst the 141 recombinants four multiple recombinants were obtained, in the cross *s12(ly)*⁻ × *s26(ly)*⁺, amongst the 157 recombinants five multiple ones were obtained and in the cross *s12(ly)*⁺ × *s26(ly)*⁻, amongst the 145 recombinants four multiple ones were obtained.

chromosome are predominant. This effect may be explained by zygotic induction. The killing of zygotes occurs after prophage induction which takes place when the prophage-carrying chromosome enters a non-lysogenic cell which contains no repressor. Therefore recombinants consisting of the complete chromosome from the non-lysogenic strain and a fragment carrying the prophage from the lysogenic parent could not be detected.

DISCUSSION

The results of crosses between polyauxotrophic derivatives of the wild-type strain A3(2), which seems to carry a defective prophage, and variants cured of prophage demonstrate the location of the prophage on the chromosome between *cysD18* and *tps33*. After relysogenization of cured variants the prophage was mapped between *uraA1* and *adeA3*. A possible explanation for this observation is that curing of strain A3(2) following heavy u.v. treatment was due to a deletion of the chromosomal region carrying the prophage attachment site. Consequently, the phage is unable to occupy its former site.

It was observed that lysogenization of cured variants took place with high frequency. We take this to mean that the prophage is readily integrated into the site between *uraA1* and *adeA3*. Three independently lysogenized strains had the same site of the prophage location between *uraA1* and *adeA3*. Thus, following lysogenization of this cured strain the phage appears to integrate into this site of the chromosome. The number of sites on the chromosome available for prophage insertion must await further work.

The mapping experiments with strains cured and relysogenized with mutant phages permit us to conclude that prophage ϕ C31 is a genetic determinant of lysogeny and is located on the chromosome of the host strain.

Interpretation of the results obtained in crosses between lysogenic and non-lysogenic strains of NF type is rather difficult. It was shown by Hopwood (1967) that in NF × NF crosses each of the parental strains may serve alternatively as a donor and as a recipient. It is therefore not easy to evaluate each parental contribution to recombinants. To analyse

recombinant progeny of this cross we must consider it as though it consisted of two crosses, each parent behaving as a donor and as a recipient alternatively. The difference in allele ratios of nonselected markers in crosses between lysogenic and non-lysogenic strains is very likely due to the killing of merozygotes following zygotic induction. It is possible that even for selected markers distant from the prophage location site, some recombinants may nevertheless be absent because zygotic induction kills the merozygotes before recombinant formation. Consequently, we observed differences in the ratios of recombinant genotypes. The effect of zygotic induction seems to be manifested in reciprocal crosses where *proA*⁺ and *hisA*⁺ served as selected markers. In both crosses the analysed recombinants were mostly derived from the complete *ly*⁺ chromosome and a fragment of the *ly*⁻ chromosome.

We cannot decide at present whether the prophage is contributed shortly after commencement of transfer of the chromosome or not. We hope to solve this question in polarized crosses where lysogenic and nonlysogenic strains of various fertility types would serve as parents.

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