THE GENETIC LOCATION OF PROPHAGE ON THE CHROMOSOME OF *STREPTOMYCES COELICOLOR*

N. D. LOMOVSKAYA, L. K. EMELJANOVA AND S. I. ALIKHANIAN

Institute of Genetics and Selection of Industrial Microorganisms, Moscow, USSR

Received December 18, 1970

THE genetic structure of *Streptomyces coelicolor* A3(2), the only strain among the actinomycetes for which a genetic map has been constructed, was studied in detail by HOPWOOD (1967) and SERMONTI (1969). For genetic analysis, HOPWOOD and SERMONTI utilized the capacity of mutant strains of A3(2) to undergo genetic recombination. To enlarge the possibilities of genetic analysis of the actinomycetes by using a temperate actinophage and to genetically analyze the actinophage in a suitable host, an actinophage from *S. coelicolor* strain A3(2) was isolated (LOMOVSKAYA, MKRTUMIAN and GOSTIMSKAYA 1970).

The actinophage was designated ϕC31; it was isolated from a culture of *S. coelicolor* A3(2) on the indicator strain, *Actinomyces coelicolor* 66. The spontaneous rate of actinophage induction was very low. It was possible to increase it by a factor of 10 or 100 when germinated spores were treated with ultraviolet (UV) light and then with N-methyl-N′-nitro-N-nitrosoguanidine. LOMOVSKAYA (1970) suggested that such a low spontaneous rate of actinophage ϕC31 induction could result from a defective lysogenic nature of A3(2). If so, actinophage ϕC31 must be a mutant of an induced defective prophage that had acquired the ability to grow on the indicator strain. The actinophage ϕC31 appeared to be a temperate phage which produces large turbid plaques and causes formation of true lysogenic cultures in indicator cultures and in cultures cured of prophage. The actinophage ϕC31 could be adsorbed on strain A3(2) but failed to reproduce.

The present communication solves the question of whether the prophage is located on the A3(2) chromosome or not. Lysogeny segregates among the recombinants in crosses between lysogenic and nonlysogenic strains in precisely the same way as other genetic markers do. Hence, it was possible to locate the prophage on the A3(2) linkage map.

MATERIALS AND METHODS

Strains: Wild-type A3(2) and its derivatives:

749, *proA1 argA1 cysD18 uraA1;*
916, *hisA1 mthB2 phe A1 strA1;*
s85, *hisA1;*
and s86, *metA1 pheA1 strA1*
were kindly supplied by D. A. HOPWOOD and G. SERMONTI.

Recombinant derivates:

137 (ϕC31)+ *proA1 pheA1 strA1 mthB2 cysD18 argA1;*
407 (ϕC31)- *uraA1;*
282 (ϕC31)+ *proA1 cysD18 argA1;*

and also s85 and s86 cured of prophage were obtained in our laboratory.

Markers: requirement for histidine (*hisA1*), requirement for proline (*proA1*), requirement for uracil (*uraA1*), requirement for phenylalanine (*pheA1*), requirement for homoserine (*mthB2*), requirement for methionine (*metA1*), requirement for cysteine (*cysD18*), requirement for arginine (*argA1*), and resistance to streptomycin (*strA1*). Strains with prophage are designated, for example, as s85 (ϕC31)+ or *ly*+ (lysogenic and strains cured of prophage as s85 (ϕC31)- or *ly*- (nonlysogenic).

Crosses: Crosses were performed in the way described by HOPWOOD (1967). The strains were crossed on complete medium. Complete medium contained (per liter): glucose, 10 g; NaCl, 5 g; $CaCO_3$, 0.5 g; peptone, 5 g; corn steep liquor, 5 g; agar, 20 g; medium adjusted to pH 7.0–7.2.

Spores obtained from the cross were plated on the medium selective for one allele from each parent (selected alleles at distant loci), and haploid recombinants were scored. Minimal medium contained (per liter): asparagine, 0.5 g; K_2HPO_4, 0.5 g; KOH, 0.3 g; $MgSO_4 \times 7\ H_2O$, 0.2 g; $FeSO_4 \times 7\ H_2O$, 0.01 g; agar, 15 g; and glucose, 10 g. Selective media were supplemented with (per liter): amino acids, 50 mg; uracil, 10 mg; dihydrostreptomycin sulfate, 50 mg.

Recombinants sampled from the selective plates were characterized according to the non-selected markers, including the prophage to be mapped. The presence or absence of prophage was evaluated by the immunity or sensitivity, respectively, of recombinants to ϕC31.

RESULTS AND DISCUSSION

Isolation of strains cured of prophage: The immunity of strain A3(2) to actinophage ϕC31 superinfection and a low frequency of spontaneous induction of actinophage in A3(2) have been the principal indications of the presence of prophage in this strain. The most convincing evidence for the presence of prophage in A3(2) was the isolation of A3(2) variants cured of prophage.

We tried to obtain variants of wild-type strain A3(2) which had been cured of prophage following treatment of A3(2) with high doses of ultraviolet light (surviving faction 10^{-3}–10^{-4}). Among the survivors we managed to obtain variants sensitive to ϕC31 actinophage. From mutant derivatives of A3(2), s85 and s86, variants sensitive to ϕC31 actinophage were also obtained. The frequency of sensitive variants in these mutant strains was comparable to that in wild type, and it was about 1–2%. To demonstrate that the phage-sensitive variants obtained had been cured of prophage and did not result from a possible mutation from phage resistance to phage sensitivity, lysogenization of the sensitive variants of A3(2), s85, and s86 was performed.

The immune survivors from infection with ϕC31 were studied for lysogenicity following repeated single-colony isolations and treatment with specific antiserum. After a 6-hr incubation, a germinated spore suspension of survivors (10^8 germinated spores / ml) contained approximately $2–5 \times 10^6$ free phage particles per ml. Chloroform was added to the samples to kill the cells, and the free phage was titrated. Before incubation this suspension contained 1×10^2 free phage per ml and 1×10^6 spores per ml producing phage. Thus, during nearly 14 hours of growth on the lawn of a sensitive culture, one out of 100 germinated spores yielded phage progeny as a result of spontaneous induction.

In addition, by using the sensitive indicator as lawn, it was observed that all the colonies of survivors were surrounded by a turbid halo of lysis formed by phage. Phage ϕC31-sensitive variants obtained following UV treatment for A3(2)

strains did not display this capacity and did not produce free phage when incubated. Hence the survivors from infection with ϕC31 actinophage proved to be truly lysogenic. Re-lysogenization of sensitive A3(2) strains revealed that they were variants which had lost the prophage following UV treatment.

Cured strain s85 retained the histidine requirement, and cured strain s86 retained both the methionine requirement and streptomycin resistance.

Plating efficiency and morphology of actinophage ϕC31 plaques was the same on strains A3(2) $(\phi C31)^-$, s85 $(\phi C31)^-$, s86 $(\phi C31)^-$, and on the indicator strain *A. coelicolor* 66.

Prophage location: Obtaining A3(2) variants cured of prophage made it possible to follow the prophage behavior (inheritance of lysogeny) in crosses between lysogenic (ly^+) and nonlysogenic (ly^-) variants of A3(2).

Variants with a very low spontaneous frequency of actinophage induction were employed in the crosses as lysogenic parents. Thus we managed to avoid lysogenization of the nonlysogenic parent in crosses and to evaluate the presence or absence of prophage only by immunity and sensitivity of recombinants to ϕC31.

Figure 1 (A,B,C) illustrates the results of three typical crosses between A3(2) ly^+ and A3(2) ly^- strains. From the allelic ratios it is apparent that lysogeny segregates among recombinants, i.e., prophage behaves like any other marker. Therefore we can map prophage just as well as any mutant gene the location of which is not known.

Figure 1A summarizes the results of the first cross [749 $(\phi C31)^+$ *proA1 uraA1 cysD18 argA1* × s85 $(\phi C31)^-$ *hisA1*] where *his*$^+$ and *ura*$^+$ were the

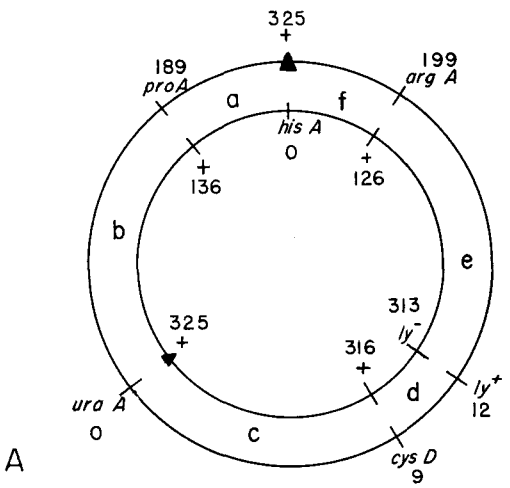


FIGURE 1A.—Location of the prophage state of actinophage ϕC31 on the A3(2) chromosome of *Streptomyces coelicolor*. Markers of the two parents in each cross (Figures 1A, 1B, & 1C) are indicated on the two circles. Filled triangles indicate the selected alleles. Letters between circles show map intervals between markers corresponding to intervals a, b, . . . h in Tables 1, 2, and 3. Th number beside each allele indicates its frequency among the sample of recombinants analyzed. *ly* indicates the position of prophage inferred from scoring progeny of the cross.

TABLE 1

*Location of prophage state of actinophage φC31 in the cross**
749 (φC31)+ proA1 uraA1 cysD18 argA1 × s85 (φC31) hisA1

Genotype Left-hand arc↓	Right-hand arc→ Crossovers†	SINGLE CROSSOVER CLASSES *cys, ly+, arg* c	*cys+, ly+, arg* d	*cys+, ly−, arg* e	*cys+, ly−, arg* f	Totals
pro+	a	3	. .	65	68	136
pro	b	5	3	123	57	188
Totals		8	3	188	125	324

Genotype	MULTIPLE CROSSOVER CLASSES Crossovers†	Number observed
pro, ly+, cys, arg+	c e f b	1

* The cross is illustrated in Figure 1A. Recombinants containing *his+ ura+* were selected and classified for nonselected markers.
† See intervals in Figure 1A.

selected alleles (indicated by closed triangles). All the other markers including the prophage which was to be mapped were not selected. Prophage location was deduced in the manner described by HOPWOOD (1967). It is evident from the allele ratios that prophage lies either between *pro* and *ura* or between *cys* and *arg*. The position of prophage has been chosen in such a way as to minimize the total number of crossovers required. Table 1 shows the correctness of prophage location between *cys* and *arg*, since in this case only one recombinant per cross needs more than the minimum number of crossovers for its production. If prophage had been located between *ura* and *pro*, four of the recombinants would have required multiple crossovers.

To obtain a more complete gradient of allele ratios between selected markers, we used as one parent strain 407 $(\phi C31)^-$ *uraA1*, isolated as one of the recombinants from the first cross, and as the other parent strain 137 $(\phi C31)^+$ *proA1*

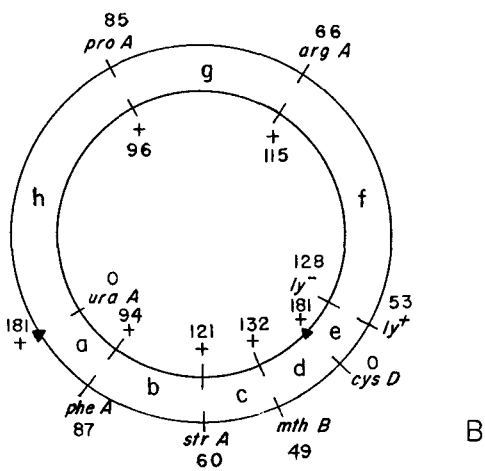


FIGURE 1B.—Location of the prophage state of φC31 on the *S. coelicolor* chromosome.

TABLE 2

*Location of prophage in the cross**
137 (ϕC31)+ *proA1 pheA1 strA1 mthB2 cysD18 argA1* × 407 (ϕC31)− *uraA1*

Genotype Lower arc↓	Upper arc→ Crossovers†	SINGLE CROSSOVER CLASSES *ly*+, *arg*, *pro* e	*ly*−, *arg*, *pro* f	*ly*−, *arg*+, *pro* g	*ly*−, *arg*+, *pro* h	Totals
phe+, *str*^{s}, *mth*+	a	2	6	17	66	91
phe, *str*^{s}, *mth*+	b	..	3	5	66	28
phe, *str*^{r}, *mth*+	c	1	2	..	7	10
phe, *str*^{r}, *mth*	d	49	..	..	..	49
Totals		52	11	22	93	178

Genotype	MULTIPLE CROSSOVER CLASSES Crossovers†	Number observed
phe+, *str*^{r}, *ly*+, *mth*+, *arg*, *pro*+	a b c e g h	1
phe+, *str*^{s}, *ly*−, *mth*+, *arg*, *pro*+	a f g h	2

* The cross is illustrated in Figure 1B. Recombinants containing *ura*+ *cys*+ were selected.
† See intervals in Figure 1B.

pheA1 stA1 mthB2 cysD18, a recombinant from the cross [916 (ϕC31+ *hisA1 mthB2 pheA1 straA1* × 282 (ϕC31)+ *proA1 cysD18 argA1*]. According to the allele ratio gradient of nonselected markers (Table 2) in this cross (Figure 1B), the prophage could be located either between *cys* and *arg* or between *str* and *mth*.

Prophage location between *cys* and *arg* appears to be more likely for the following reasons. When prophage is located between *cys* and *arg*, multiple crossing over is necessary in three cases, but not in five, as would be the case if prophage had been located between *str* and *mth*. Besides, in the former location, all the recombinant classes with the *ly*+ genotype which were obtained as a result of a single crossover in each arc must have the *arg* genotype, while in the latter location, the *ly*+ recombinants could be *arg* or *arg*+. Table 2 shows that all recombinants have an *arg* genotype, which again indicates the prophage is located between *cys* and *arg*.

Moreover, this prophage location was confirmed a third time in the cross [137 (ϕC31)+ *proA1 pheA1 straA1 mthB2 cysD18* × 407 (ϕC31)− *uraA1*], where the selected markers *str* and *arg*+ were used (Figure 1C). Allele ratio gradient analysis (Table 3) indicates that the minimum number of recombinant classes which require multiple crossing over is two, not six; this again indicates that the prophage is located in the right-hand arc between *cys* and *arg*.

Further analysis of segregation of progeny from the cross shown in Figure 1C allows locating the prophage with respect to *str* (selected marker) and *mth* (unselected marker). Presence of recombinants with the genotype *mth ly*− but not *mth*+ *ly*+ in a sample of recombinants provides evidence for the order indicated in Table 4. Hence, prophage location between *straA1* and *mthB2* is excluded.

Conclusions: The results of these crosses clearly indicate that the prophage behaves just like any other marker on the A3(2) genetic map. The fact that the prophage state of ϕC31 is located in a region of the chromosome which is almost devoid of known genes is of particular interest. The discovery of actinophage in

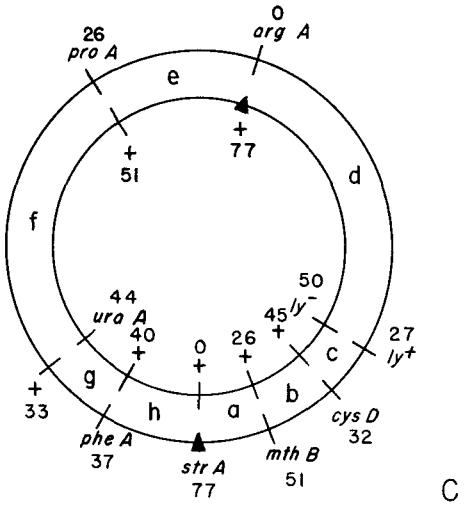


FIGURE 1C.—Location of the prophage state of ϕC31 on the *S. coelicolor* chromosome.

the prophage state on the chromosome reveals new approaches to genetic study. Obtaining variants cured of prophage and mapping the prophage on the chromosome provide the prerequisites for carrying out transduction experiments. Crosses between truly lysogenic and nonlysogenic strains would clarify the genetics of immunity of actinomycetes to actinophages. Furthermore, by employing a host with a known genetic map, one can investigate the genetic structure of the actinophage itself and also the genetic control of lysogeny, a phenomenon widespread among actinomycetes.

We acknowledge the help of Mr. ROBERT GILLEN in preparing the figures.

TABLE 3

*Location of prophage in the cross**
137 (ϕC31)+ *proA1 pheA1 strA1 mthB2 cysD18 argA1* × 407 (ϕC31)− *uraA1*

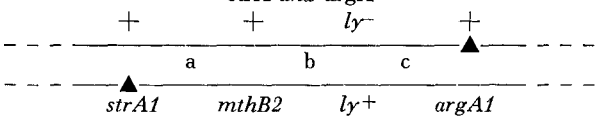
Genotype Left-hand arc↓	Right-hand arc→ Crossovers†	SINGLE CROSSOVER CLASSES *pro, ura*+, *phe* e	*pro*+, *ura*+, *phe* f	*pro*+, *ura, phe* g	*pro*+, *ura, phe*+ h	Totals
mth+, *cys*+, *ly*−	a	1	..	..	24	25
mth, cys+, *ly*−	b	..	5	..	15	20
mth, cys, ly−	c	2	..	2	..	4
mth, cys, ly+	d	22	3	1	..	26
Totals		25	8	3	39	75

Genotype	MULTIPLE CROSSOVER CLASSES Crossovers†	Number observed
mth, cys, ly+, *pro, ura, phen*	d e f g	1
mth+, *cys, ly*−, *pro*+, *ura, phe*+	a b c h	1

* The cross is illustrated in Figure 1C. Recombinants containing *strA1 argA1*+ were selected.
† See intervals in Figure 1C.

TABLE 4

Mapping two loci, lysogeny* (ly) *and* mthB, *with respect to outside selected markers* strA *and* argA



Genotype	Number observed	Crossovers
mth+, *ly*⁻	26	a
mth, *ly*⁻	24	b
mth, *ly*+	27	c
mth+, *ly*+	0	a b c

* A sample of recombinants from the cross shown in Figure 1C was analyzed with respect to the unselected markers *ly* and *mthB2*. The presence in the sample of recombinants with genotype *mth*, *ly*⁻ but not *mth*+, *ly*+ indicates the order of markers shown above.

SUMMARY

The data obtained in the present study clearly indicate that in crosses between lysogenic and nonlysogenic strains of *S. coelicolor* A3(2), the prophage behaves just like any other marker on the genetic map of A3(2). The data indicate the chromosomal location of the prophage state of actinophage φC31 is between the *cysD* and *argA* loci.

LITERATURE CITED

HOPWOOD, D. A., 1967 Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. **31**: 373–403.

LOMOVSKAYA, N. D., 1970 Studies of the lysogenic system of *Streptomyces coelicolor* A3(2) and actinophage φC31. Proc. 1st Intern. Symp. Genet. Indust. Microorganisms **40**:

LOMOVSKAYA, N. D., N. M. MKRTUMIAN and N. L. GOSTIMSKAYA, 1970 Isolation and characteristics of *Streptomyces coelicolor* actinophage. (in Russian with English summary) Genetika **6**(12): 135–136.

SERMONTI, G., 1969 *Genetics of Antibiotic-Producing Microorganisms*. Wiley–Interscience, New York.