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Genetics and Molecular Biology of *Streptomyces* Bacteriophages

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INTRODUCTION

The streptomycetes are genetically among the most tractable of bacteria. In the best-studied strain, *Streptomyces coelicolor* A3(2), chromosome mapping is straightforward, and a single circular linkage map of more than 100 markers has been established (36). Extremely efficient protoplast fusion can be induced by polyethylene glycol (40, 41). Plasmids have been demonstrated both physically and genetically, and some of them are self-transmissible sex factors having a variety of interactions with the host chromosome (reviewed in reference 16). Reintroduction (transformation) of isolated plasmid deoxyribonucleic acid (DNA) into protoplasts

occurs at high frequency in the presence of polyethylene glycol (8). These features are important in the study of several special characteristics of streptomycetes, in particular, their remarkable status as antibiotic producers (6) and their morphological complexity (20, 47). Much greater progress will be possible in these investigations when the techniques already available are supplemented by gene cloning (9) and transposon genetics and by systems for fine-structure mapping. Among the important requirements for the development and optimum exploitation of such techniques is an understanding of *Streptomyces* phages, particularly of their genetics and DNA.

About a decade ago it was possible for major reviews of *S. coelicolor* genetics (35) and *Streptomyces* phages (89) to be completely independent of each other; the potential benefits of using a genetically studied host in phage studies, and vice versa, were unfulfilled. More recently, considerable advances have been made in the genetic and physical analysis of these phages and their interactions with their hosts. Thus, when temperate phages acting on *S. coelicolor* A3(2) were isolated (30, 55), it became possible to initiate studies of such problems as the relationship between phages and differentiated bacteria, comparison with model eubacterial phage-host systems, the genetic basis of lysogenization in streptomycetes, the structure and function of the genomes of *Streptomyces* phages, and the development of *Streptomyces* phages as DNA-cloning vectors. Both temperate and virulent phages have been instrumental in understanding *Streptomyces* host-controlled restriction and modification (RM) systems, and they have also helped in various aspects of the genetic analysis of streptomycetes.

The most extensive studies have been of the temperate phage ϕC31, and we will focus on this phage as a main theme, with occasional variations provided by observations on some other phages. We shall then consider these and other results in the context of the use of phages as vectors for the introduction of particular DNA segments into streptomycetes, and finally review interactions between the phages and host-controlled RM systems.

GENERAL BIOLOGY OF *STREPTOMYCES* PHAGES

Isolation and Plating Conditions

Streptomyces phages can easily be isolated from most soils of pH higher than 5.0 (S. T. Williams, personal communication) by plating of soil extracts. This can be done either directly or after a short period of enrichment by incubation with freshly germinated *Streptomyces* spores (29) in a conventional soft-agar overlay containing spores of an indicator strain. Plaques are usually seen after overnight incubation at 28 to 30°C (the temperature range used in most of the experiments described in this review). There are also several reports of the isolation of temperate phages from natural lysogens (e.g., 42, 71), the difficulty being to predict suitable indicator strains.

The media used for *Streptomyces* phage work have usually been based on corn steep liquor agar medium and peptone broth (56) or nutrient agar (Difco Laboratories, Detroit, Mich.) and nutrient broth (Difco) (29). To satisfy requirements for divalent cations during adsorption, Ca^{2+} is usually added to base plates at about 4 mM, though optimal concentrations vary. The further addition of Mg^{2+} may also increase efficiency of plating (EOP), plaque size, and yield. High-titer lysates have usually been obtained from just confluently lysed top layers, though shaken liquid culture lysates have sometimes been used (e.g., 29).

Stability and Storage

The phages which have been studied in detail are all rather stable in simple storage solutions such as nutrient broth (Difco), peptone broth, SM buffer (22), and even water. It is advantageous to store stocks under oil. Many of the phages are sensitive to chloroform (though there is no evidence that any contain lipids). Most can be apparently permanently preserved by lyophilization of suspensions in nutrient broth or in 1.5% gelatin plus 5% sucrose (sometimes with the addition of an equal volume of bovine serum).

Morphology

Nearly all the *Streptomyces* phages examined belong to Bradley's group 2 (11), having polyhedral heads and long tails with little, if any, more complex structure. Three phages with elongated heads (MSP2 [12], MSP8 [44], R2, [27]) and one with a very short tail (ϕ17 [59]) have been found, but none have been described which lack tails, have contractile tails, or are filamentous. As with other bacteriophages of similar morphology, all the *Streptomyces* phages examined contain double-stranded DNA.

Host Range

Host range may be narrow or wide, both for virulent and for temperate phages. It seldom extends outside the genus *Streptomyces* (but see reference 70). Although adsorption specificity is the commonest factor limiting host range (e.g., 57, 65), there may also be important intracellular barriers; for example, in at least one phage (R4 [19]), host-specific restriction and modification

appears to be of great significance. Limited searches of soil extracts have not revealed any phages whose ability to form plaques on *S. coelicolor* A3(2) depends on the presence or absence of either of the two sex plasmids SCP1 (86) and SCP2 (7).

Adsorption

The most efficient adsorption is usually to the mycelium growing out from freshly germinated spores (usually 6 to 8 h after inoculation). Typically, adsorption constants (1) are around 10^{-9} ml min^{-1}. In practice, with a spore density of 5 $\times 10^7$ ml^{-1}, this means that 50% adsorption takes place after about 15 min. Meaningful interpretation of adsorption coefficients is difficult because of the complex morphology of the host (29).

Some workers have successfully used sheared mycelial fragments in phage studies (72; R. H. Baltz, personal communication), and in the latter case a rather high adsorption coefficient (>10^{-8} ml min^{-1}) was observed for adsorption of FP4 to ultrasonically fragmented late-exponential-phase *S. fradiae* mycelium.

One-Step Growth Experiments

Table 1 summarizes data on the one-step growth parameters of a number of *Streptomyces* phages. Although typical values of 40 to 60 min for the latent period, 40 to 60 min for the rise period, and 10 to 50 for the burst size have been obtained, some exceptional cases of longer latent periods (up to 360 min) and burst sizes of several hundreds have been reported for a variety of phages, especially of *S. griseus*. For one virulent phage (VP12), the growth cycle became resistant to rifampin at 25 min after infection, suggesting that a significant change in the transcription apparatus took place at this stage (K. F. Chater, unpublished data). Conceivably, VP12 (other *Streptomyces* phages have not been tested) may specify a new ribonucleic acid polymerase for late transcription, as was observed in coliphage T7 (13).

GENETICS OF TEMPERATE PHAGE φC31

The temperate phage φC31 is by far the best-studied *Streptomyces* phage. In this section we review these studies in detail, adding observations on other temperate phages where appropriate.

Origin and Morphology of φC31

φC31 was discovered as a result of testing liquid cultures of *S. coelicolor* A3(2) on lawns of *S. anthocyanicus* strain 31 and *S. lividans*

TABLE 1. *One-step growth parameters for Streptomyces phage-host systems*[a]

Phage	Host for one-step growth expt	Latent period (min)	Rise period (min)	Burst size (PFU)	Reference
Temperate					
φC31	*S. coelicolor* A3(2) (φC31[s])	40	50	20–30	64
VP5*c*1[b]	*S. coelicolor* A3(2)	45	15	30–40	30
ACP13*c*1[b]	*S. coelicolor* A3(2)	50	20	10–50	—[c]
R4*c*1[b]	*S. parvulus* ATCC 12434	45–50	20–30	10–50	19
Pg2	*S. griseus* 806	150	60	200	92
RP2	*S. rimosus* ATCC 10970 (R7)[d]	360	240	100–120	42
Virulent					
VP11	*S. coelicolor* A3(2)	40	30	30–35	29
VP12	*S. coelicolor* A3(2)	40–50	15–35	20–100	—[c]
FP4	*S. fradiae* ATCC 19609	40	30	75	—[e]
Pg81	*S. griseus* Kr15	60	45	300	92
W-2a	*S. griseus*	90	?	226	2
MSP8	*S. venezuelae*	95	80	60	50
S1	*S. virginiae* ATCC 13161	115	175	164	—[f]
B, W-1a, C-131	*S. griseus*	120	?	118–340	2
Pg100	*S. griseus* Kr15	120	90	100	92
514-3	*S. griseus*	125	30	15–20	34
W-1, W-5	*S. griseus*	160	?	125–168	2
W3	*S. griseus*	180	?	108	2

[a] We have excluded some published results either because of difficulties in interpreting extreme variation between reports (φ17; 90; C. M. Calberg-Bacq, Ph.D. thesis, University of Liege, Liege, Belgium, 1974) or because the published times of sampling were too far apart to permit detailed interpretation (MSP2; 45).

[b] Clear-plaque mutants were used for these experiments.

[c] Chater and Carter, unpublished data.

[d] R7 is a derivative cured of the naturally present RP2 prophage.

[e] Baltz, personal communication.

[f] Konvalinkova, Ph.D. thesis, 1977.

strains 66 and 130. The phage did not form plaques on strain A3(2). Phage-sensitive variants were obtained after ultraviolet light (UV) treatment of A3(2) and its multiauxotrophic derivatives (53, 55). These variants and *S. lividans* strain 66 were then used as indicator strains. In most work strain 66 was used because the large plaques obtained with it allowed the detection of plaque morphology mutants; on the phage-sensitive variants of A3(2), the phage produces small turbid plaques.

Electron microscopy showed that φC31 phage particles have a polyhedral head with a hexagonal outline (57 by 54 nm) and a noncontractile tail (123 by 10 nm) with a basal plate (80).

Adsorption

To determine at which growth stage adsorption and infection were most efficient, spores of phage-sensitive A3(2) were infected at various times during germination (64). Adsorption was determined in three ways: (i) the phage in the supernatant was titered after centrifugation of the adsorption mixture; (ii) a streptomycin-susceptible host was used in the adsorption mixture, which was then plated on a streptomycin-resistant strain in the presence of streptomycin to estimate unadsorbed phage; and (iii) the portion of adsorbed phage which infected the cells and gave progeny was assayed after treatment of the adsorption mixture with phage-specific antiserum. Methods (i) and (ii) estimated total adsorption, and method (iii) measured only adsorption events in which penetration of DNA followed immediately.

Significant adsorption to dormant spores was found by the centrifugation method, but the adsorbed phage was not killed in the streptomycin assay (i.e., was presumably reversibly adsorbed) and was inactivated by antiserum. As germination proceeded, phage adsorption increased, as determined by centrifugation and by the streptomycin assay. However, infected spores were able to initiate plaque formation in the antiserum assay only when at least 5 h of germination in peptone broth had elapsed before adding phage, indicating that penetration of DNA was possible only into spores that had reached a certain stage of germination. Subsequently, 5- to 6-h-old germinating spores having a short germ tube with two to three nucleoids have been used for phage infection. In a typical experiment, 65 and 51% of phage particles were associated with cells after 15 min of adsorption as determined by the centrifugation and streptomycin methods, respectively. However, only 20% of the adsorbed particles produced plaques after treatment with antiserum. The low rate of adsorption (adsorption coefficient, 8.0×10^{-10} ml min^{-1}) and small proportion of adsorption events giving productive infections appear to be connected with the asynchrony of germination and consequent variation in competence for phage infection.

One-Step Growth Experiments

In one-step growth experiments (24, 56, 60), a short period of adsorption (no more than 5 min) was followed by treatment with antiserum to inactivate free phage. Infected germinating spores (initial spore concentration of not less than 10^8 ml^{-1}) after dilution to about 10^4 ml^{-1} were assayed for plaque-forming units (PFU) at intervals to determine various parameters of infection (e.g., Fig. 3). At 28°C the latent period of phage growth in A3(2) sensitive to φC31 was 40 min. The long rise period (60 min) indicated that phage release from single infected cells was asynchronous. This may reflect variation in cell volume in the germ tubes. Burst size ranged from 10 PFU per infected cell in some experiments to as many as 100 in others.

At 37°C the yield of φC31 was less than 1 PFU per infected cell. In one-step growth experiments in which the infected cells were shifted from 28 to 37°C at intervals, it was found that the gene product responsible for temperature sensitivity had accumulated in sufficient quantities and functioned by 25 min of the latent period (56). Temperate phage R4 also failed to produce plaques at more than 34°C, and temperature shift experiments indicated that, just as with φC31, the temperature sensitivity was expressed only in the first 25 to 30 min of infection (19).

Mutants that could grow at both 28 and 37°C could only be isolated from clear-plaque (*c*) mutants of φC31 (56). It proved possible to construct a heat resistant c^+ derivative by recombination, and this was used in all further work because it allowed the isolation of temperature-sensitive mutants. (This lineage is illustrated in part of Fig. 8.) The frequency of lysogenization was the same for the original φC31 and for its heat-resistant c^+ variant.

R4 mutants capable of growth at 37°C could not be obtained, either from the wild-type phage or from a clear-plaque mutant (19).

Characterization of φC31 as a Temperate Phage

Subculturing from zones of growth within φC31 plaques gave rise mainly to lysogens, which were immune to φC31 infection, i.e., could adsorb the phage, but yielded no progeny upon infection with a low multiplicity of infection. Infection of A3(2) (φC31) with a multiplicity of infection of about 20 using a *c* mutant unable to lysogenize resulted in phage multiplication, pre-

sumably by overcoming repression in the lysogen (54, 56, 61).

The immune cultures, after being freed from exogenous phage by treatment with antiserum, continued to produce phage spontaneously. The titer of phage remaining in the supernatant after centrifugation of suspensions of 3×10^8 lysogenic spores per ml varied from 1×10^2 to 5×10^3 PFU ml^{-1}. The ratio of the number of plaques produced when a lysogenic spore suspension was treated with anti-ϕC31 serum and plated on a sensitive lawn to the total number of lysogenic spores plated was 0.01 for *S. coelicolor* A3(2) (ϕC31) and 0.1 for *S. lividans* 66 (ϕC31). In experiments on various lysogens carrying phage R4, there was also variation in this ratio from one wild-type species to another (19).

UV induction of prophages ϕC31 (93), VP5 (30), and R4 (19) was not detected, but a low level of UV induction of SH10 has been observed by H. Krügel (personal communication).

To estimate the frequency of lysogenization of indicator strains by ϕC31, a sample taken at the end of the latent period of a one-step growth experiment was plated in the presence of 10^7 PFU of a *c* mutant of ϕC31. Typically 30 to 40% of infection events resulted in lysogenization (60).

A3(2) (ϕC31) lysogens were stable, in that immunity and the ability to produce phage were retained after successive single-colony subculturing. Among 2×10^3 lysogenic colonies, no spontaneous cured variants were detected.

Genetic Mapping of the ϕC31 Chromosomal Attachment Site and Zygotic Induction of ϕC31 Prophage

To map ϕC31 prophage in a variant of *S. coelicolor* A3(2) which had been made sensitive to ϕC31 (by UV treatment) and then relysogenized, a prophage mutant (ϕC31*clo*1), which gave rise to "cloudy" plaques, was used. By recombination, the *clo*1 prophage was placed in a host genetic background complementary to that of the original (*clo*$^+$) lysogen from which the mutant had been derived. Conventional genetic analysis (54) utilizing the *clo*1/*clo*$^+$ plaque morphology difference identified a map location between *adeA* and *uraA* for the prophage (in the single lysogen examined). More recent mapping (Fig. 1 and 2) has placed it between *pheA* and *uraA* (in independent lysogenization events, prophage was also mapped between *pheA* and *uraA* by C. Stuttard [personal communication]). This was a surprising result because earlier studies (30, 53) had shown that ϕC31 resistance in the original A3(2) strain mapped elsewhere (i.e., between *cysD18* and *tps-33*; Fig. 2). The original suggestion that this ϕC31 resistance was due to a resident defective ϕC31 prophage in A3(2) therefore needs reexamining. It was based on the apparent origin of infectious ϕC31 from strain A3(2), an event of extreme rarity, and on the high frequency (about 1%) at which sensitive variants of A3(2) occurred after UV treatment. More recently, it has been observed during one-step growth experiments that A3(2) can in fact support a low level of ϕC31 multiplication and produce infectious centers and that it is lysogenized at the same high frequency as sensitive variants. It therefore appears more likely that strain A3(2) contains some unstable or curable genetic determinant other than ϕC31 prophage, located between *cysD* and *tps-33*, which interferes with ϕC31 vegetative reproduction.

An attachment site on the *S. coelicolor* A3(2) chromosome for VP5, which is heteroimmune to ϕC31, has also been mapped for two independent lysogens (Fig. 2; 30; Chater, unpublished data). Neither the ϕC31 nor the VP5 attachment site is close enough to known genetic markers to permit a meaningful search for specialized transduction.

Genetically marked ϕC31 lysogenic and nonlysogenic *S. coelicolor* A3(2) strains were used to study zygotic induction (32; Tables 2 and 3). In ultrafertile reciprocal crosses between lysogenic (ly$^+$) and nonlysogenic (ly$^-$) donor (NF)

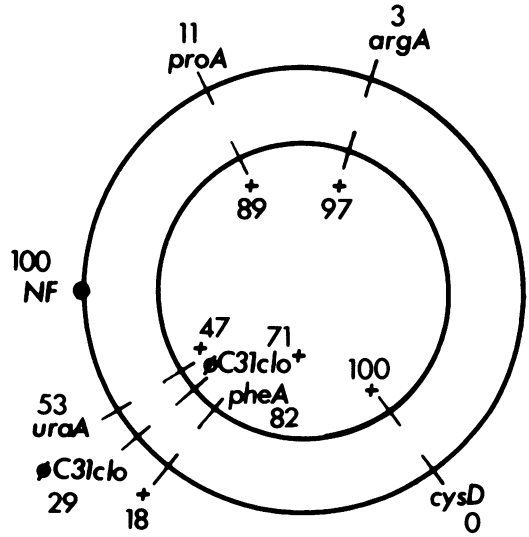


FIG. 1. *Mapping of prophage ϕC31 on the S. coelicolor A3(2) linkage map. S146 (ϕC31clo1, NF) (outer circle) was crossed with S32 (ϕC31clo$^+$, SCP1$^-$) (inner circle). Nonselective analysis of recombinants was as in reference 36. Numbers indicate allele frequencies (percentage) among recombinants. The allele gradient indicated a location either between NF and proA or between uraA and pheA. The latter location was chosen because clo segregation was independent of proA, but not of pheA.*

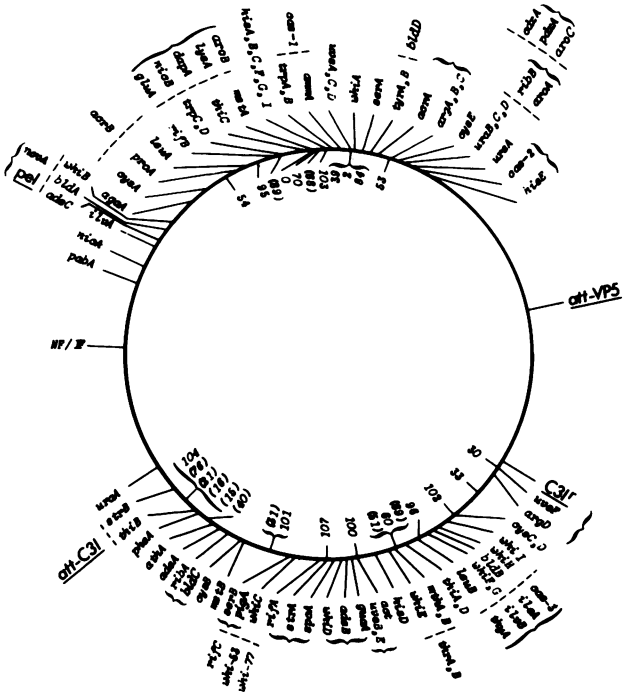


FIG. 2. *Locations of chromosomal sites affecting interactions between phages and S. coelicolor A3(2). The linkage map is based on that in reference 38. The loci of interest are shown in heavier typeface. The pel mutation (at 10 o'clock) gives rise to resistance not only to VP11 (29), but also to other phages including VP5. It does not affect VP11 adsorption (29). The locus for φC31 resistance, φC31r (4 o'clock; 53), was formerly thought to be a φC31 attachment site (but see text). It does not affect φC31 adsorption. The true φC31 attachment site (att-C31) is located at 8 o'clock (see Fig. 1), and the VP5 attachment site (att-VP5) is located at about 3 o'clock (30).*

TABLE 2. *Effect of zygotic induction in crosses between S. coelicolor A3(2) derivatives S12 (proA1 argA1 cysD18 adeCv10 uraA1 NF) and S32 (pheA1 SCP1⁻) in different states of lysogeny for prophage φC31 (selected markers, pheA⁺ and cysD⁺)*

Expt	Frequency of recombinant formation in crosses	
	S12ly⁺ × S32ly⁻	S12ly⁻ × S32ly⁺
I	1.4×10^{-3}	3.0×10^{-1}
II	3.0×10^{-3}	6.2×10^{-1}
III	1.6×10^{-3}	1.9×10^{-1}
IV	2.8×10^{-3}	5.4×10^{-1}
V	3.4×10^{-3}	6.8×10^{-1}

and recipient (SCP1⁻) strains (see reference 38 for a discussion of these fertility types), the high frequency of recombination made it possible to conduct nonselective analysis of recombinants. (Mutant strains unable to adsorb φC31 were used in these experiments to avoid complications arising from the presence of extracellular phage.)

The frequency of recombination in ly⁺ NF × ly⁻ SCP1⁻ crosses was about 100-fold lower than in reciprocal crosses (Table 2), and during growth of the mixed parental strains the titer of free phage became 10^3 times higher for ly⁺ NF × ly⁻ SCP1⁻ than for ly⁻ NF × ly⁺ SCP1⁻ strains (Table 3). We conclude that zygotic induction takes place when φC31 prophage is introduced into the cytoplasm of a nonlysogen, as would be expected if a repressor is necessary to maintain lysogeny. This result also suggests that extensive cytoplasmic mixing does not occur in these crosses, unless twofold dilution of prophage repressor is sufficient to cause induction.

TABLE 3. *Extracellular phage in mixed cultures of S. coelicolor A3(2) strains S12 (proA1 argA1 cysD18 adeCv10 uraA1 NF) and S32 (pheA1 SCP1⁻) in different states of lysogeny for prophage φC31*

Length of mixed culture (h)	Mean phage titer for five independent expt	
	S12ly⁺ × S32ly⁻	S12ly⁻ × S32ly⁺
0	2.1×10^3	1.6×10^3
6	6.5×10^3	1.7×10^3
12	1.2×10^6	4.6×10^3
27	4.3×10^6	4.0×10^3

Genetic Control of Lysogeny

Clear-plaque mutants. The ability of temperate phages to initiate the reactions leading to

lysis or lysogeny is genetically determined. The outcome of infection may therefore be affected by mutations in phage genes concerned with different stages of the lysogenization process (31, 46, 52). For example, mutations in genes responsible for the establishment or maintenance of lysogeny would increase the ratio of lysed to lysogenized cells (46, 52). Such mutants normally have clear-plaque phenotype, which facilitates their recognition and scoring. The frequency of *c* mutants was 1×10^{-3} to 5×10^{-3} in different stocks of ϕC31c^+. One hundred and thirty-two independent *c* mutants were isolated and placed into phenotypic groups according to the degree of plaque turbidity. Phenotypic differences proved to be related to different allelic states of a gene designated *y*, which appears to regulate the ratio of lysis to lysogenization (61). Evidence for this was provided by comparing burst sizes and frequencies of lysogenization in a c^+ *y* recombinant (obtained in a cross of c^+ y^+ phage with *cy* mutant) with those of a ϕC31 c^+ y^+ mutant. The presence of the *y* mutation caused a 2.5- to 3-fold increase in phage yield and a 1.5- to 2-fold decrease in frequency of lysogenization, thus changing the ratio of lysed to lysogenized cells. This suggests that the y^+ gene product is responsible for inhibition of lytic growth. Thus, in *y* mutants the lytic response takes place in a portion of cells which would normally be destined for lysogenization.

Among sensitive cells infected by *c* mutants of each phenotypic group, lysogenization could not be detected (frequency less than 0.01%). Stable lysogens for *c* mutants were not produced during mixed infections with c^+ phage (though c^+ lysogens were obtained), indicating that the c^+ gene product was required for maintenance of lysogeny in addition to any possible role in its establishment.

No lysogens were found when sensitive cells were infected by pairs of *c* mutants, either in spot tests on plates or in one-step growth experiments (61). Although this suggested that they all belonged to a single complementation group, it should be borne in mind that if a pair of mutants were defective in different cistrons involved in maintaining lysogeny, mixed infection could produce stable lysogens only if the prophage were a c^+ recombinant or if double lysogens were formed.

These experiments failed to reveal clear-plaque mutants which (like λc_{II} or λc_{III} mutants; 10) were defective solely in the establishment of lysogeny. Either such mutants are rare, for unspecified reasons, or there is no ϕC31 gene product which functions like the c_{II} and c_{III} gene products of λ.

When *c* mutants were isolated at 37°C, some gave turbid plaques at 28°C (*cts* mutants; spontaneous frequency of about 10^{-4}; 63). These mutants gave no detectable lysogens at 37°C and a reduced frequency of lysogenization at 28°C (2 to 15%, compared with 30 to 40% for the wild type). Mixed-infection experiments with *c* and *cts* mutants carried out at 37°C did not result in lysogenization. For reasons discussed above, this result did not allow the conclusion that *c* and *cts* mutations belonged to the same complementation group, but this possibility is not excluded. However, the main implication of this result, that in *cts* mutants a repressor protein for maintenance of lysogeny was temperature sensitive, was confirmed by the finding that the mutant *cts* prophage failed to maintain the stable lysogenic state at a high temperature, leading to induction in the majority of cells. The number of PFU in a germinated population of A3(2) (ϕC31*cts*) spores was 2% in the absence of a heat shock. After a brief heat treatment, the number of PFU immediately increased to 50 to 60% of the initial number of colony-forming units (CFU). As seen in Fig. 3, this was followed after a 40-min lag by the release of free phage. The rise period was shorter in heat-induced lysogens

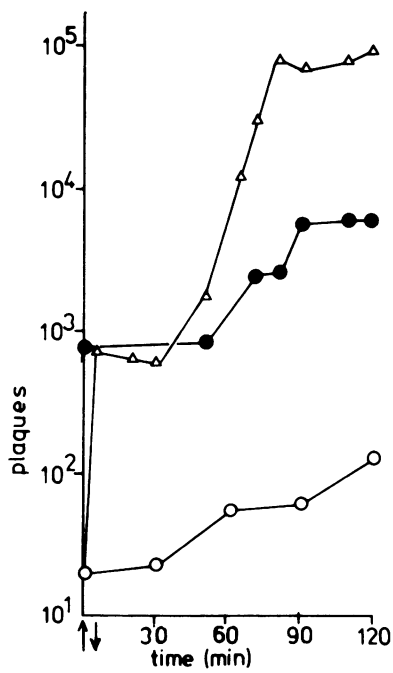


FIG. 3. *Development of ϕC31cts1 (a heat-inducible mutant) in S. coelicolor A3(2). Host spores were germinated for 6 h before the experiments. Symbols: ●, one-step growth curve in an infected sensitive culture; ○, spontaneous plaque production by an uninduced ϕC31cts1 lysogen; △, phage production after a heat shock (arrows; 40°C for 5 min) of a ϕC31cts1 lysogen. (After reference 24.)*

than in lytic infection, suggesting that phage release took place more synchronously in heat-induced spores.

Lysogens for *cts* phage were used to study intracellular phage development, avoiding adsorption. A heat shock did not cause killing of dormant spores or release of infectious phage. Heat induction of 3- to 4-h-old spores resulted in spore death, but not phage release. Heat induction leading to phage production took place only in spores with germ tubes, which appeared at 5 to 6 h (the number of PFU coinciding with the number of spores which possessed germ tubes).

Very similar results were obtained with a heat-inducible mutant (*hin*63) of VP5 (21). This study also showed that aerial mycelium of a VP5 *hin*63 lysogen could not give rise to productive induction, although heat killing (specific to the lysogen) was observed. If it is assumed that phage gene expression is necessary for killing of the host, then it follows that the specificity of the host's transcription/translation apparatus, if it is changed at all, is not completely changed in aerial hyphae from that found in the vegetative cells.

Although the wild-type (c^+) allele of φC31 was dominant to most *c* mutations, some *c* and *cts* mutations were dominant to c^+ (25). Not only did such mutations make the percentage of lysogenized cells lower than that for wild-type infection alone, in mixed infections with the wild type, but the mutant phages were also able to grow in wild-type lysogens when the multiplicity of infection was about 5 or more. The addition of a recessive *c* mutation to the genome of dominant *c* mutants eliminated both the dominance and the partial virulence. As with phenotypically similar λ clear-plaque mutants (67, 68), the transdominance and weak virulence of these φC31 mutants may reflect negative complementation, caused by interaction of mutant repressor subunits with normal ones. This would require that active repressor be an oligomeric protein.

Virulent mutants. Virulent mutants are important both in an industrial context, as one of the factors causing lysis in antibiotic fermentation using lysogenic actinomycetes, and in their potential for elucidating the mechanisms of maintenance of lysogeny.

Virulent (*v*) mutants of φC31 occurred only with a low frequency and only provided that a *c* or *cts* mutant was used; no virulent mutants were found in a c^+ stock (25, 91). Virulent mutants arose in a population of dominant *c* or *cts* mutants at about 10^{-8}. Superinfection of A3(2) (φC31) with *v1* (a representative *v* mutant) resulted not only in reproduction of the *v* mutant but also in prophage induction (Fig. 4). Mutant

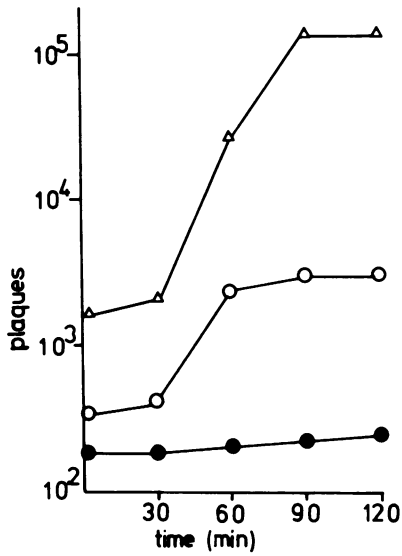


FIG. 4. *Growth of φC31v1 in S. coelicolor (φC31). Host spores were germinated for 6 h before the experiments. Symbols: ●, spontaneous plaque production by the uninfected lysogenic host; ○, φC31v1 PFU (assayed on a lysogen); △, prophage induction (assay values on a nonlysogen, minus values on a lysogen). (After reference 91.)*

v1 also gave productive infections in a nonlysogenic strain, and was thus nondefective. A possible explanation of the complete virulence might be that a change has taken place in an operator-promoter region, reducing its interaction with negatively acting repressor specified by the *c* gene sufficiently to allow transcription to take place in the conditions of low effective repressor concentration found in c^+ lysogens infected with dominant *c* or *cts* mutants. This would be analogous to the finding that adding the c_{17} mutation (occurrence of a new promoter) to a partially virulent clear-plaque mutant (CP) of λ generated completely virulent double λCPc_{17} mutants (68).

The frequency of virulent mutants in recessive φC31*c* mutants was very low (about 10^{-10}). Single infections of wild-type lysogens by such mutants caused prophage induction. It was found, however, that the mutants could not grow in nonlysogenic hosts, which implies that they were defective for lytic growth. These mutants were called *vd* (virulent defective). Thus, growth of *vd* mutants in a lysogenic host required functions specified by the induced prophage. Evidence that only a part of the *vd* genome was needed to express virulence was obtained by comparing survival curves after UV irradiation of *vd*, using different indicator strains. With a lysogenic indicator strain, measuring *vd* survival, the inactivation curve was less steep than that

obtained for the induced resident phage particles on a nonlysogenic indicator strain. Thus, inactivation of the virulence function of *vd* particles involved a target considerably smaller than the normal φC31 genome (91) (although subsequent physical analysis [see below] has revealed that the *vd* genome is not extensively deleted).

To find out which prophage gene products are needed for production of viable progeny of *vd* mutants, lysogens carrying prophages with *ts* mutations from various complementation groups (see below) were infected at the nonpermissive temperature by a *vd* mutant (25). Neither phage production nor prophage induction was seen in any of these experiments, suggesting that all the prophage genes tested by means of *ts* mutations were needed for *vd* reproduction, and that the *vd* genome did not complement *ts* mutations in the prophage genome. These results were confirmed by experiments in which *vd* was propagated in lysogens carrying wild-type prophage or a prophage with a host range (*h*) mutation. Infection of an h^+-resistant, *h*-sensitive strain by h^+ *vd* previously propagated in lysogens for *h* phage resulted in reproduction of *vd*, whereas no yield was observed when the *vd* phage used in infection had been grown in a strain lysogenic for h^+. Therefore *vd* possesses the adsorption organ specified by the prophage present in the lysogen in which *vd* was last propagated.

The properties of *vd* mutants of φC31 somewhat resemble those of bacteriophage P4. P4 grows in the presence of prophage P2 helper (E. W. Six, Bacteriol. Proc., p. 138, 1963). P4 DNA is 30% of the molecular weight of P2 DNA (44), and all known P2 genes are needed for reproduction of P4 (73) (but note that these two phages are heteroimmune and have different sites of prophage integration; 74). It is likely that P4 is a deletion mutant of a phage related to P2 and that this mutant is capable of using the products of P2 phage genes to provide for P4 reproduction. If the analogy were correct, φC31*vd* might be a deletion mutant of φC31, requiring products of φC31 prophage. Consistent with this, a *vd* preparation consisting of *vd* and resident phage particles was visualized in a CsCl gradient as two closely situated bands of different densities (75). The size of DNA molecules from the denser particles corresponded to that of φC31 DNA, whereas only slightly smaller DNA molecules were obtained from the less dense *vd* particles. Heteroduplex analysis of φC31/φC31*vd* (discussed in more detail below) confirmed that the mutant DNA possessed a small deletion, whereas a nondefective φC31*v* mutant appeared to have no deletions. Presumably, therefore, the defective phenotype is not due to the absence of structural genes, but rather to some aberration in their control.

It was of interest to examine whether virulent mutants of φC31 could cause induction of other prophages. A *v* mutant of φC31 caused induction of prophages φC43 and φC62, both of which belong to the same immunity group as φC31, but not of the heteroimmune phages VP5 and φ448 (58). Thus, antirepression provided by superinfection with the *v* mutant was relatively specific. This is in contrast with the lack of specificity in the action of P22 antirepressor (83).

Turbid-plaque mutants defective in establishing lysogeny. Recent studies (76, 78) have revealed a class of viable deletion mutants (see below) which, despite retaining apparently wild-type plaque morphology (i.e., turbid plaques), do not establish lysogeny (*lyg* mutants). Further genetic studies will be necessary to determine the extent to which φC31*lyg* mutants are analogous to λ*att* and λ*int* mutants, which have specific defects preventing prophage λ integration and which give turbid plaques (31).

Isolation, Complemenation Analysis, and Phenotypic Characterization of Temperature-Sensitive Mutants of φC31

Use of conditional lethal mutants (temperature sensitive [*ts*] and suppressor sensitive [*sus*]) is essential in studying phage genetics. φC31*ts* mutants unable to produce plaques at 37°C were isolated from φC31c1 phage after UV or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (24). Both mutagens induced mutants at a frequency 0.5 to 2%. Burst sizes of *ts* mutants at 37°C were not more than 1% of those at 28°C. Complementation tests were made by spotting together about 10^7 PFU of two mutants on a sensitive lawn at 37°C. Using 50 mutants with sufficiently low reversion rates ($\leq 10^{-4}$), 21 complementation groups were recognized. Since 12 of these complementation groups contained only one representative, there were presumably still many genes left to be discovered by using *ts* mutants.

To confirm the spot test data, quantitative complementation and recombination tests were carried out. It is not possible to obtain accurate estimates of complementation or recombination by normal methods, because the number of mixedly infected germinated spores is rather low, and therefore the progeny from mixed infections may be masked by yields from singly infected cells. A modified method was therefore used in these measurements (24). One-step growth experiments were conducted at the nonpermissive temperature after infection by a pair of mutants. The number of infectious centers was then estimated at the nonpermissive tem-

perature, as shown by plating samples during the latent period. These could only have arisen as a result of complementation and recombination. A complementation index was obtained as the ratio of this value to a theoretical value for doubly infected cells (obtained as the product of the fraction of cells infected in parallel experiments, at the permissive temperature, with each phage separately). If the index was more than 0.1, complementation took place in more than 10% of the total mixed infected spores. If the index was less than 0.05, the two mutants tested were assigned to the same complementation group. Nineteen complementation groups were established in this way.

Although recombination was likely to contribute to what was being measured as complementation, it was evident that this was not a major effect. For example, the extent of complementation obtained for pairs B2:L24, F14:I21, and C4:E8 (0.66, 0.36, and 0.80), situated close together (Fig. 5), significantly exceeded that of a number of other pairs, such as B2:E8, F14:R31, G16:C4, and J22:K23 (0.27, 0.19, 0.12, and 0.19), located further from each other (Fig. 5). The number of recombinants obtained in crosses of mutants from different complementation groups was two to three orders of magnitude more than that obtained in crosses of mutants belonging to one complementation group, indicating that complementation itself facilitated recombination; such a great difference could not be explained solely by differences between recombination frequencies related simply to distances between mutations, and probably reflects the greater possibility for recombination brought about by the replication of DNA in complementing infections.

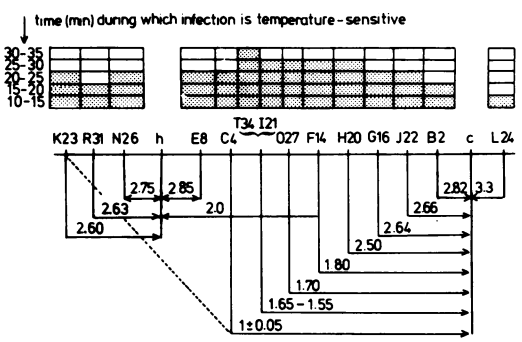


FIG. 5. *Genetic map of φC31 and times of gene expression. Map locations were determined by two- and three-factor crosses (see text for details). Symbols: h, host range mutation (presumably specifying part of tail); c, gene controlling lysogenic response (presumably specifying repressor); B2, C4, E8,..... T34, temperature-sensitive mutations representative of complementation groups. The relative order of T34 and I21 (brace) is undetermined. Numbers below the map indicate linkage to c or h determined by three-factor crosses. Times given above the map indicate times of gene expression determined by temperature shift experiments done with ts mutants (data from reference 24).*

In temperature shift-up (28 to 37°C) experiments, the time needed for synthesis of the gene product altered in the *ts* mutants was determined (24). After 5 min of adsorption (to give approximately simultaneous infection), infected germinated spores were incubated at 28°C, and during the latent period samples were removed at intervals to 37°C. Noncomplementing mutants behaved identically to each other in these experiments (Fig. 5). The shortest time (10 to 15 min) before infection became heat resistant was obtained with mutant L24.

Genetic Mapping of *ts* Mutations

Crosses were done by infecting germinated spores with a pair of mutants under permissive conditions (24). Preliminary $ts\ c \times ts^+\ c^+$ crosses were used to generate $ts\ c^+$ phages, which were then used in reciprocal three-factor crosses of the type $ts_x\ c \times ts_y\ c^+$ and $ts_x\ c^+ \times ts_y\ c$. The relative order of *ts* mutants with respect to the *c* marker was established by measuring the ratio of *c* to c^+ phages among ts^+ recombinants (Fig. 5). This ratio also gave the linkage between *c* and the closest *ts* marker in each cross. The nearer the ratio to 1, the farther the nearest *ts* mutation is situated from the *c* region. The only anomaly was the behavior of the L24 mutation. The c/c^+ ratio in a cross of L24 with B2 (the nearest mutation to *c*) was about 1. However, in crosses with other *ts* mutants more distant from *c*, linkage of L24 to *c* was demonstrated. These findings suggested that L24 is situated close to *c* and that *c* is between L24 and the other mutations. This was supported by low-frequency recombination in two-factor crosses of L24 with B2 and J22.

The three-factor crosses indicated that representatives of nine complementation groups (L24 to T34) were linked to the *c* region (Fig. 5). Those unlinked to *c* were mapped both by two-factor crosses and by three-factor crosses involving a host range marker (*h*) (25; Fig. 5). Recombination frequencies in the two-factor crosses were determined in one-step growth experiments under nonpermissive conditions. This allowed the estimation of recombination frequencies with respect to the yield from mixedly infected spores only. (Control experiments with spores infected at the nonpermissive temperature with each mutant separately were also needed to allow calculation of the recombination percentage.) Since considerable variations were

obtained in replicate experiments, each cross was repeated. Mutants for functions expressed relatively late (25 to 35 min) were clustered in a single region in the middle of the genetic map, with earlier functions at either end of the map.

The high frequency of recombination between the terminal *ts* markers (24%) suggested that the φC31 genetic map is linear, although still far from saturation. This is consistent with the nonpermuted structure of φC31 DNA isolated from virions, as determined by denaturation, heteroduplexing, and restriction mapping (see below).

Suppressor-Sensitive Mutants of φC31

S. coelicolor A3(2) derivatives (e.g., S39 Rv1) carrying a suppressor were discovered among revertants of strain S39 *proA argA mthB* which had lost requirements for arginine and homoserine (*mth* = requirement for homoserine) (3, 28). Suppressor-sensitive mutants (*sus*) of UV-irradiated φC31 (i.e., growing in S39 Rv1 but not in S39) were found only at 37°C. At 28°C they developed normally in both hosts and in *S. lividans* 66. Possibly S39 and *S. lividans* naturally carry suppressors which cause insertion at the mutant codon of an amino acid which is acceptable only at low temperature. Further studies with the *sus* mutations were hampered by their high reversion frequencies, although at least three complementation groups were discernible. However, the mutants proved useful in further studies of *S. coelicolor* A3(2) and *S. lividans* 66 suppressor genes (3).

Temperate Phages Homoimmune to φC31

φC31 did not form plaques on *S. lividans* 803 or *S. caesius* 55. This proved to be due to the presence in these strains of prophages which were designated φC43 and φC62, respectively. φC43, φC62, and φC31 were serologically related as well as homoimmune. Virulent mutants of φC31 were able to overcome the immunity of lysogens carrying φC43 and φC62 prophages and caused prophage induction (75), suggesting similar mechanisms for repression. Prophage induction caused by φC31*vd* mutants indicated considerable genetic homology between these three phages, since it had previously been shown that many φC31 prophage genes were needed to ensure *vd* growth. A high degree of homology (93 to 96%) was also demonstrated in electron microscope analysis of φC31, φC43, and φC62 DNA heteroduplexes (see below). The three phages were able to recombine vegetatively. Some differences in host ranges of φC31 and φC43 were found. Extracellular phages φC31, φC43, and φC62 were similarly sensitive to high temperature, but differed in their stability to the chelating agents ethylenediaminetetraacetic acid (EDTA) and sodium citrate (Fig. 6). These nonsystematic differences show that DNA molecular weight is not the only factor in determining sensitivity to heat and chelating agents (see below) and emphasize the potential importance of testing a variety of chelating agents when seeking a treatment that enriches for deletion mutants.

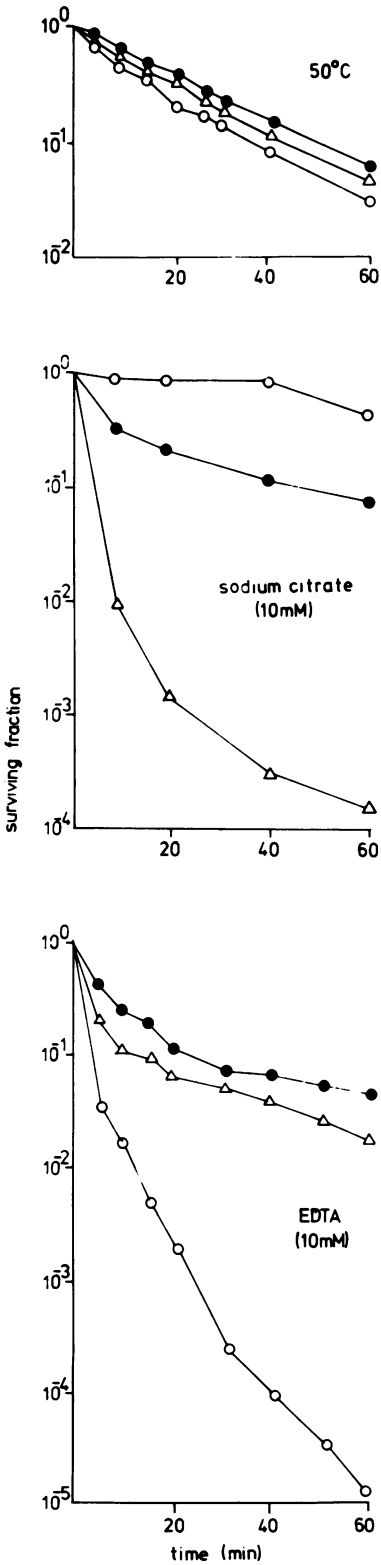
All three phage DNAs had very similar restriction profiles when treated with a number of restriction endonucleases, except for *Bam*HI. No *Bam*HI sites were found in φC31 and φC62 DNAs, whereas one site was discovered in φC43 DNA. The restriction analysis, combined with heteroduplex and electron microscope denaturation sites (see below), suggests that φC62 and φC31 (Norwich stock; see below and Fig. 7 and 8) may be essentially identical.

Electron Microscopy of Deoxyribonucleic Acid of φC31, φC62, and φC43

Physical studies of φC31 carried out independently in our two laboratories have revealed a difference between our wild-type φC31 stocks (see below and Fig. 7 and 8). For clarity, in what follows we therefore specify which of these (i.e., Moscow or Norwich stock) was used in a given experiment.

The physical properties of φC31 (Moscow stock) DNA have been studied by electron microscopy (77). When heated, phage particles were disrupted and ejected DNA in a linear form. After incubation of molecules in 0.2 M NaCl at 60°C for 30 min, they were converted into a circular form as the temperature was gradually lowered implying that the linear molecules have cohesive ends. Linear and circular molecules were 37.7 ± 0.5 kilobases (kb) in length.

A map of the distribution of regions of the DNA which melted after alkali treatment was constructed (Fig. 7d). The existence of peaks and the coincidence of the locations on histograms for two levels of denaturation points to a nonpermuted structure for the φC31 DNA partial denaturation map. Compared with λ DNA (43), φC31 DNA has a relatively random base distribution, since melting takes place over only 2.5°C in 0.2 × SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate) compared with a much wider interval for λ DNA; thus φC31 DNA lacks extended regions that denature more easily. The most pronounced peaks were located at positions 0.25, 0.33, 0.73, and 0.99 from the left end of the denaturation map. Similar results were obtained with φC62 DNA (I. A. Sladkova, Abstr. 11th Conf. Electron. Microsc. Jallin, U. S. S. R., 1979).



DNA heteroduplexes involving φC31, φC43, and φC62 (76), and other φC31 derivatives (75, 78) are presented schematically in Fig. 7. Heteroduplexes of φC31 (Moscow stock) and φC62 DNA had a single-strand loop close to one end. This end was arbitrarily defined as the right end of the molecule and was chosen as the starting point for calculating distances along heteroduplexes. The loop represented additional DNA present in φC62, since molecules of φC62 DNA were longer than those of φC31 (39.4 ± 0.6 and 37.7 ± 0.5 kb, respectively). DNA of φC31*vd* carried a further deletion near to that already seen in φC31 (Moscow stock). Although the novel deleted region in φC31*vd* DNA corresponds to no more than two genes, a great number of prophage genes are needed to overcome the defect in vegetative growth of *vd*, as shown by the genetic evidence described earlier. This suggests that the deleted region may contain a *cis*-acting regulatory region.

Two single-stranded loops observed in φC31*c*5/φC31 (Moscow stock) heteroduplexes were assigned to different strands, since the two phage DNAs were equal in length. The central loop was interpreted as a deletion in the φC31*c*5 molecule, because a similar deletion was seen in φC62/φC31*c*5 heteroduplexes. The second loop indicated that φC31*c*5 did not carry the same deletion as was seen in φC31 wild-type Moscow stock (in comparison with φC62).

The origin of this deletion in wild-type (Moscow stock) DNA is obscure. A similar, presumably the same, deletion was also observed in DNA of φC31*cts*, whereas DNA of φC31 wild-type (Norwich stock) did not contain this deletion (17). The identical nature of the deletion in the Moscow stock and in φC31*cts* is also confirmed by restriction enzyme analysis (see below) and heteroduplex analysis. Figure 8 illustrates the pedigree of the various stocks and shows that all the differences can be explained by a single spontaneous deletion event during routine subculture in Moscow some time between 1970 (when the Norwich stock was originally sent from Moscow) and 1972 (when φC31*cts*1 was isolated in Moscow).

In heteroduplexes between φC62 and φC43, a small single-stranded loop (not shown in Fig. 7) and a "bubble" of nonhomology were seen in the center of the molecule, and two single-stranded loops were seen near one end (76: Fig. 7). The loop nearest the end of the heteroduplexes had a transposon-like structure flanked by very short

FIG. 6. *Survival of φC31, φC43, and φC62 during treatment with heat or chelating agents. Symbols:* ○, *φC31 (Moscow stock; see Fig. 8);* ●, *φC43;* △, *φC62.*

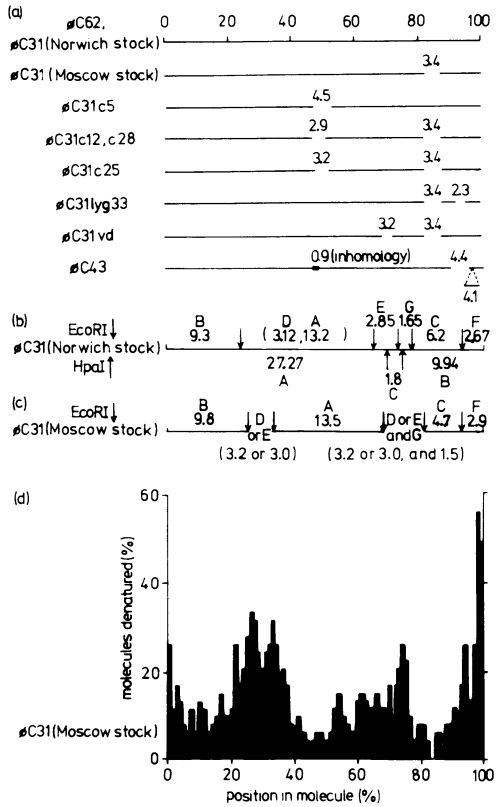


FIG. 7. *Physical mapping of φC31 DNA. The terms "Moscow stock" and "Norwich stock" are explained in the text and Fig. 8. Note that the scale in (a) and (b) is slightly smaller than that in (c) and (d). (a) Summary of heteroduplex results (58, 76, 78, 79). Gaps indicate the positions of deletions. Units are in percentage of φC62 genome length (39.4 ± 0.6 kb; see reference 58) (φC62 DNA and φC31 DNA [Norwich stock] are essentially identical.) (b) EcoRI and HpaI restriction map of φC31 (Norwich stock) and (c) EcoRI restriction map of φC31 (Moscow stock; 78). Letters are fragment designations, and numbers are fragment sizes in kilobases, obtained by comparison with standards provided by λ DNA digested with EcoRI, HindIII, or SalPI (procedures given in references 14, 22, and 78; see text for further details). (d) Distribution of denatured regions in 65 molecules of φC31 DNA (Moscow stock) at 11% denaturation, as determined by electron microscopy (75, 77).*

inverted repeats. Studies of φC43 homoduplexes, and heteroduplexes involving φC43 and its deletion mutants, showed that this structural modification was an insertion in φC43 DNA. Interestingly, the single *Bam*HI site of φC43 is 2.4 kb from the right end of the molecule, i.e., in the region flanked by inverted repeats. The second major single-stranded loop in φC62/φC43 heteroduplexes was presumably due to a deletion in φC43 DNA, since φC62 and φC43 DNA molecules were closely similar in length (39.4 ± 0.6 and 39.6 ± 0.8 kb, respectively).

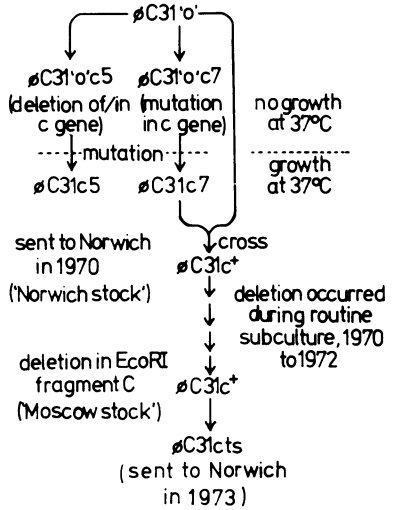


FIG. 8. *Pedigree of various φC31 stocks, including origin of deletions found in φC31 (Moscow stock) and φC31cts. The original φC31 isolate (φC31'o') was unable to grow at 37°C.*

Isolation of Viable Deletion Mutants

Packaging of DNA into temperate phage heads in vivo is usually by one of two means. In one, concatameric DNA is cut into lengths determined by the size of the phage head ("headfuls") in such a way as normally to give a short region of terminal redundancy (e.g., P22; 84). Deletion of a segment of such a phage DNA does not reduce the amount of DNA packaged, but merely increases its terminal redundancy. In the other mechanism (e.g., coliphage λ; 85), concatameric DNA is cut during packaging by a site-specific endonuclease which recognizes a base sequence present only once per genome length. Thus deletion of part of the genome results in the packaging of smaller DNA segments. The resulting reduction in the tightness of DNA packaging increases the stability of the mature phage particle to heat and chelating agents. By selecting mutants resistant to these treatments, viable λ deletion mutants lacking up to 23% of their genome have been obtained (69).

Since φC31 DNA was known to have cohesive ends, its DNA packaging seemed likely to resemble that of λ, so isolation of chelating-agent-resistant deletion mutants was attempted (79; K. F. Chater and E. A. Page, unpublished data). Survivors of repeated treatments with EDTA, citrate, or pyrophosphate were greatly enriched for *c* mutants (30% compared with 0.1% in the starting population). Among nine independently isolated *c* mutants found in the starting popu-

lation, one was resistant to chelating agents, whereas *c* mutants obtained after enrichment were invariably resistant (79). Two independent resistant mutants (*c*25 and *c*28) were retained for further study.

To reveal the presence of deletions in EDTA-resistant *c* mutants, DNA lengths were measured and heteroduplex analysis was done. DNA molecules of *c*25 and *c*28 were 36.5 ± 0.5 kb and 36.6 ± 1.1 kb in length, respectively, i.e., were shorter than those of φC31 (Moscow stock) DNA (37.7 ± 0.5 kb). Analysis of heteroduplexes between φC62 DNA and φC31*c*28 DNA showed that *c*28 DNA contained a deletion in a position similar to that of the φC31*c*5 deletion (Fig. 7). The relative locations of the deletions in *c*5, *c*25, and *c*28 were determined by analysis of *c*25/*c*28, *c*25/*c*5, and *c*28/*c*5 DNA heteroduplexes. The three deletions were situated in a region of 1.7 kb near the center of the molecule (at 46.2 to 50.6%) and overlapped, lacking a common region of 1.0 kb length (Fig. 7). *Eco*RI analysis of *c*28 and *c*5 DNA suggested that the deletions were from the largest *Eco*RI fragment. It appears that deletion of this region does not affect the lytic growth of phage but prevents lysogenization. In DNAs of EDTA-resistant mutants that retained the c^+ phenotype, deletions were also observed which were located outside the region of *c* deletions. In addition, φC31-*cts* (which contains a 1.7-kb deletion) was more resistant to chelating agents than was the φC31 wild-type Norwich stock (which did not have the deletion) (17).

Spontaneous chelating-agent-resistant deletion mutants have also been obtained for phages SH10 (M. Hartmann, F. Walter, and S. Klaus, personal communication) and VP5 and R4 DNA (17). One of the R4 deletion mutants lacked the single *Eco*RI cleavage site usually found in R4 DNA.

Restriction Enzyme Analysis of φC31 Deoxyribonucleic Acid

The fragments detected by agarose gel electrophoresis of φC31 DNA after restriction enzyme digestion are given in Fig. 7 and Table 4. Partial restriction maps have been independently obtained for the Norwich and Moscow stocks.

Norwich stock. *Eco*RI fragments B (9.3 kb) and F (2.67 kb) were shown to be termini carrying the cohesive ends of φC31 DNA by the inverse relationship between their intensity on gels and that of fragment A′ (12 kb), whose molecular weight equals the sum of the B + F molecular weights (17). In addition, DNA ligase treatment resulted in intensification of A′ and almost total loss of fragments B and F (J. E. Suarez, unpublished data). An *Eco*RI partial digestion fragment of 7.9 kb found with wild-type Norwich stock DNA was shown to comprise fragments C (6.2 kb) and G (1.65) kb because it was replaced by one 1.7 kb smaller in the *cts* mutant, which was known to possess a 1.7-kb deletion in fragment C (Chater, unpublished data). Thus C and G are adjacent fragments.

Further information came from considering *Hpa*I digestion which gave fragments of about 27, 10, and 1.8 kb (Chater, unpublished data). The smallest fragment (C) was not terminal, since *Hpa*I did not cut either terminal *Eco*RI fragment; therefore the two *Hpa*I sites are situated 10 and 11.8 kb from one end of the molecule. Since they occurred in the *Eco*RI fragments E and G, these fragments were adjacent. Furthermore, since the *Hpa*I fragment B was reduced by 1.7 kb in the *cts* mutant known to lack 1.7 kb from its *Eco*RI fragment C, it was possible to deduce that *Eco*RI fragment C was the only fragment between *Eco*RI fragments G and F. Thus the single ambiguity remaining in the map (given in Fig. 7b) was the relative orientation of fragments A and D.

Moscow stock. Sladkova and Rebentish (79), using Moscow stock DNA, obtained an *Eco*RI digestion pattern apparently identical to that given above for the Norwich stock DNA, except that fragment C was 1.7 kb smaller in the Moscow stock. A partial map of these fragments (Fig. 7c) was obtained by combining heteroduplex and restriction enzyme analysis. An important feature of this work was the use of a deletion mutant (*lyg-33*) in which fragments F and C had become fused by a short deletion (0.7 kb; Fig. 7a). The larger fragment so formed was shown to possess a cohesive end which combined with the other cohesive end (fragment B) to give an 18-kb band on gels. Thus fragment C was adjacent to the terminal fragment F.

The position of fragment A was defined by electron microscope analysis of *Eco*RI digests of φC31/φC43 heteroduplex molecules. Fragment A contained two regions of nonhomology, the positions of which could also be defined in the undigested heteroduplex. The resulting map thus consisted of four accurately located fragments, with ambiguities about the locations of the remaining three (Fig. 7c).

Combined restriction map. Although the two *Eco*RI restriction maps in Fig. 7b and 7c separately contain ambiguities, combining them gives an unambiguous map, since the Moscow map (Fig. 7c) requires that either fragment D or E be located between fragments B and A. According to the Norwich map, the correct fragment can only be D, and the result is the single possible fragment sequence B-D-A-E-G-C-F.

TABLE 4. *Physical properties of Streptomyces phage DNA*[a]

Phage	Immunity shared with:	G+C in DNA (mol%)[a]	Size of DNA		Restriction enzyme target sites						Reference
			Mol wt ($\times 10^6$)[b]	kb	*Eco*RI	*Hin*dIII	*Bam*HI	*Pst*I	*Sal*GI	Others	
Temperate											
φC31	φC43, φC62 (group I)	63 (ρ_b)	26.01 (R)	39.02	6	13	0	0	~31	*Hpa*I, 2; *Kpn*I, ~6; *Bcl*I, ~14; *Xho*I, 0	77, 78, —[c]
VP5	VP7, VP14, ACP13 (group II)	59 (ρ_b)	27 (R)	40.5	6	8	0	?	8	*Kpn*I, ~9	17, —[c]
S14	φ448 (group III)	59 (ρ_b)	35.9 (R)	53.85	4	?	0	0	7	*Kpn*I, ~13; *Bcl*I, ~17; *Xho*I, 0	17, —[c]
φ448	S14 (group III)	59 (ρ_b)	34 (R)	51	7	~10	0	1	5		17, —[c]
R4	SH10?[c] (group VI)	67 (ρ_b)	30 (R)	45	1	0	0	1	>30	*Kpn*I, ~8; *Bcl*I, 17; *Xho*I, 0; *Sma*I, 0	19, —[c]
SH3	?	73 (T_m, ρ_b)	34 (S, L)	51	2	?	8	?	?		48
SH10	R4?[c]	69 (T_m, ρ_b)	26.7 (S, L)	40.05	1	0	0	0	>20	*Hpa*I, 0; *Kpn*I, 9; *Bgl*II, 12; *Sma*I, 0; *Shy*I, 0; *Bgl*I, >20; *Hin*dII, >20; *Hpa*II, >50; *Bsp*I, >50	48, —[d]
SH11	?	71 (T_m, ρ_b)	26.1 (S, L)	39.15	0	?	0	?	?		48
SH12	?	69 (T_m, ρ_b)	28.7 (S, L)	43.15	2	?	0	?	?		48
Virulent											
VP12	—	67 (ρ_b)	26.3 (R)	39.45	0	0	0	0	~30		19, 23
Pal6	—	55 (ρ_b)	66 (R)	99	>40	>40	0	0	1	*Hpa*I, >16; *Kpn*I, >27; *Xho*I, 0	18, 19, —[c]
S1	—	64 (T_m, ρ_b)	45 (R)	67.5	4	?	?	?	?		—[e]
FP4	—	65 (T_m, ρ_b)	39 (S)	58.5	0	0	0	?	>20		—[f]

[a] Modes of determination; ρ_b, buoyant density in CsCl; T_m, melting temperature.
[b] Electron microscope length determination; R, restriction enzyme analysis; S, sedimentation analysis.
[c] K. F. Chater, J. E. Suarez, and C. J. Ellis, unpublished data.
[d] Hartmann et al., personal communication.
[e] Konvalinkova, Ph.D. thesis, 1977.
[f] Baltz, personal communication.

Correlation of the Physical and Genetic Maps of φC31

Sladkova (Abstr. 11th Conf. Electron Microsc., 1979) recently reported the alignment of the denaturation and heteroduplex maps of φC62, using electron microscope heteroduplexes (Fig. 7). Alignment of the heteroduplex map with the restriction map has also been obtained, using the deletion mutant *lyg-33*. As described above, heteroduplex studies placed this deletion close to the right end of the heteroduplex map, and *Eco*RI analysis showed that fragments C and F were fused, with the loss of about 0.7 kb of DNA (78). Thus a convention was adopted in which these fragments were placed at the right end of the restriction map (Fig. 7). It is evident that the right half of the map contains most of the physical variations seen in the different phages, as well as the *Eco*RI fragment C in which spontaneous deletions are commonly detected. This leads us to a preliminary orientation of the genetic map, assuming that the absence of markers to the right side of the *c* gene reflects the nonessential nature of a significant part of this region (Fig. 5). Confirmation of this orientation awaits genetic mapping of some of the deletions.

TEMPERATE PHAGES HETEROIMMUNE TO φC31

Several other immunity groups have been described for *Streptomyces* phages (Table 4). Essentially no attempts have been made to determine genetic relatedness either between or within these groups, though DNA molecular weight, base composition, and restriction patterns (Table 4) are rather similar for homoimmune phages where these have been examined.

Evidence that site-specific cleavage of concatameric DNA is required for VP5, R4, and SH10 packaging is provided by the occurrence of chelating-agent-resistant deletion mutants of these phages (17; Hartmann et al., personal communication). DNA restriction fragments of all three phages have been identified which apparently contain cohesive ends, in that they are usually present in lower quantities than other fragments, and the sum of their molecular weights corresponds to that of a fragment of higher molecular weight which is present in proportions inversely related to those of the cohesive end fragments (19, 48; Suarez, unpublished data).

Clear-plaque mutants have been obtained with great ease for VP5 (30; A. Sabatier and K. F. Chater, unpublished data), φ488, and S14 (Stuttard and Chater, unpublished data), but they are exceedingly rare in R4, where only a single occurrence of a clear-plaque mutant has been observed (19). Sabatier (unpublished data) carried out a limited analysis of VP5 clear-plaque mutants, among which he observed two different plaque morphologies. No complementation could be detected between or within the two groups of *c* mutants. Other VP5 mutants have also been isolated, including a heat-inducible mutant (see above reference 21) and numerous temperature-sensitive mutants (Sabatier, unpublished data). The latter mutants, which were induced by hydroxylamine, were usually extremely unstable, reverting at frequencies too high to allow genetic analysis. However, three relatively stable mutants were obtained and used in spot complementation tests. Two complementation groups were thus defined. An interesting avenue of research, yet to be explored, would be to extend these studies of VP5 to allow comparison with φC31 and to analyze functional and genetic interactions between these phages.

Genetic studies of R4 have not been attempted because it has not been possible to obtain plaques at temperatures higher than 34°C, but there is no obvious obstacle to such studies of S14, φ448, or the SH phages.

ANALYSIS OF OTHER PHAGE DEOXYRIBONUCLEIC ACID

Estimates of molecular weight, base composition, and numbers of restriction enzyme target sites for four virulent and nine temperate *Streptomyces* phages are given in Table 4. Base compositions apparently often depart markedly from that of the host's DNA (which contains more than 70% guanine plus cytosine [G+C]). Chater (17) discussed some aspects of the distribution of restriction enzyme target sites. Broadly, enzymes recognizing sites rich in adenosine plus thymine give very few cuts in high-G+C phage DNAs, but rather more than expected in low-G+C DNAs. On the other hand, enzymes recognizing sites rich in G+C give very few cuts in any of the DNAs, with the remarkable exception of *Sal*GI. Since this enzyme is specified by a streptomycete (4), the phages might be expected, if anything, to have evolved to resist restriction by this enzyme; yet the frequency of the site is invariably several times higher than expected in those DNA species having more than 60% G+C. One may deduce that this particular hexanucleotide sequence has some important role in the DNA of these phages, and its precise location in relation to structural genes and their controlling sites will surely prove interesting. We further discuss the occurrence of restriction enzyme target sites in the section on phage cloning vectors (below).

TRANSFECTION

A system allowing the introduction of purified phage DNA into cells, and its subsequent biological expression, has potential uses both in studies of phage genetics and in the use of phage DNA as a vector for the introduction of particular DNA segments into cells. Only one transfection system using physiologically competent, normal mycelial *Streptomyces* cells as recipient has been described (51). Here DNA of a narrow-host-range virulent phage (S1) was introduced into late-exponential/early-stationary-phase cultures of *S. virginiae*, leading to phage production after 24 h (no earlier assays were made). Two other systems have been described that use protoplasts. In the pioneering studies of Okanishi et al. (65, 66), DNA of phage PK66 was added to protoplasts of *S. kanamyceticus*. Free phage could be detected only after incubation of the mixture for at least 14 h. Estimates of the efficiency of this system, and that of *S. virginiae*, were difficult to make because there was no means of directly detecting transfected cells. However, strains normally resistant to the phages but sensitive to transfection were found for both systems (65; V. Konvalinkova, Ph.D. thesis, University of Liege, Liege, Belgium, 1977), and the data obtained with these recipients can be used to obtain approximate transfection frequencies of about 10^{-6} per CFU (mycelium) or per protoplast, and about 10^{-9} per DNA molecule, for each system. Recently, the difficulty of quantitation has been overcome by a simple modification of Okanishi's procedure (82), in which the transfection mixture is plated in a soft-agar overlay containing excess protoplasts on a base plate of protoplast regeneration medium. Each transfection event is detected as a plaque which is visible after 1 to 2 days of incubation. A second important modification of Okanishi's procedure has been the use of 20% polyethylene glycol to stimulate the interaction of the protoplasts and phage DNA (as in the plasmid transformation system of reference 8). In this way, transfection of *S. coelicolor* A3(2) and *S. lividans* 66 by a variety of temperate phages, giving between 10^3 and 10^5 plaques per 10^8 protoplasts, was obtained (Table 5). Ligation of the cohesive ends of ϕC31 DNA did not alter the frequency of transfection.

In several cases, the major factor limiting transfection was found to be protoplasts rather than DNA. Evidence that this resulted from the occurrence of a subpopulation of "competent" protoplasts was obtained in mixed transfection experiments, in which it appeared that ϕC31 DNA and R4 DNA infected the same cells, with the outcome that only ϕC31 was produced in most plaques (82).

TABLE 5. *Polyethylene glycol-assisted transfection of Streptomyces protoplasts*[a]

Host protoplasts	Phage DNA	Highest transfection frequency ($\times 10^{-6}$)	
		Per DNA molecule	Per viable protoplast
S. coelicolor A3(2)	ϕC31	7.5	2,000[b]
	VP5	2.8	15[c]
S. lividans 66	ϕC31	3.26	416[b]
	VP5	0.6	40[b]
	S14	3	330[d]
	ϕ448	1.23	51[d]
	R4	3.06	11.5[c]

[a] Data are summarized from reference 82 and Suarez (personal communication).
[b] No saturation by DNA.
[c] Values at DNA saturation.
[d] Minimum values (obtained in single experiments).

Transfection could be obtained with protoplasts of *S. parvulus* only when they had been prepared from very young (18 h) mycelia (Suarez, personal communication) even though protoplasts from older *S. parvulus* mycelia are normally efficient recipients of plasmid DNA in transformation (8). Since the phages tested usually give high EOP on the strain, this result was unexpected.

STREPTOMYCES PHAGES AS DEOXYRIBONUCLEIC ACID-CLONING VECTORS

DNA cloning into *Streptomyces* plasmids has recently been successfully achieved (M. J. Bibb, J. Schottel, and S. N. Cohen, personal communication; C. J. Thompson, J. M. Ward, and D. A. Hopwood, personal communication). Possible uses of cloning systems in applied (37) and basic research in *Streptomyces* are numerous, and some of those uses may be most easily achieved by the availability of phage cloning vectors. Potential advantages of such vectors (17) include their wide host range and the ease with which it can be determined; the introduction of DNA into prophages, where it should be stable and potentially inducible to a multicopy form by using heat-inducible prophage; the possibility of taking advantage of DNA packaging constraints in selection of cloned DNA (as in some coliphage λ vectors; reference 62); the ease with which hybridization to radioactive probe DNA can be used to detect plaques containing desired DNA sequences (5); and the use of efficient phage promoters to obtain high levels of transcription of cloned DNA.

The best candidates as cloning vectors would be temperate and possess wide host range and small numbers of appropriate restriction enzyme target sites in their DNA. Deletion mutants

lacking segments of DNA should be obtainable to allow packaging into virions of phage DNA containing inserted DNA. Efficient transfection is also a prerequisite. From the preceding sections, three phages appear to meet most of these criteria: φC31, R4, and SH10. In current attempts to clone DNA, the use of φC31 is favored mainly because of the availability of relatively large deletions, but also because of its extensive genetic characterization; this is despite the inconvenient distribution of restriction enzyme target sites in φC31 DNA (Table 4), which has so far necessitated the use of partially digested DNA as receptor. By this means, the small plasmid pBR322 of *Escherichia coli*, cut at its single *Eco*RI site, has already been inserted into φC31. Initial recognition of the clone was by in situ hybridization of plaques (5) with radioactive pBR322 DNA (Suarez and Chater, unpublished data). The φC31/pBR322 hybrid DNA has acquired a new *Eco*RI fragment which comigrates with pBR322 and possesses single target sites for *Bam*HI and *Pst*I, neither of which usually occurs in φC31 DNA but both of which are present in pBR322. The presence of the *Pst*I site in the hybrid DNA has also been demonstrated in vivo by taking advantage of the presence in a streptomycete, *S. albus* P, of a restriction-modification system operating on the same DNA sequence as that recognized by *Pst*I (14, 18; see below). The hybrid phage, unlike the parental φC31, was restricted and modified by *S. albus* P. Apart from demonstrating the feasibility of using φC31 as a useful vector in *Streptomyces*, the hybrid clone can now be used to explore the expression of pBR322 drug resistance genes in *Streptomyces*; might conceivably be introduced into *E. coli*, using the pBR322 replication functions; and provides us with a φC31 vector possessing single *Bam*HI and *Pst*I sites in a dispensable region.

This first success should not mask the fact that φC31 is rather inconvenient as a vector for *Eco*RI-generated fragments. This is not a problem with R4 and SH10 DNA, both of which contain single *Eco*RI target sites. In the case of R4, the *Eco*RI site can be deleted, and is therefore not in an essential gene; so it should be a suitable site for the introduction of foreign DNA (17). R4 also possesses a single *Pst*I site. Use of R4 and SH10 as vectors is being delayed by the absence of extensive deletions. Their further use as vectors will probably involve the insertion of target sites for other enzymes (e.g., *Bam*HI and *Hin*dIII), probably into the *Eco*RI or *Pst*I sites.

Additionally, segments of phage DNA may be useful in developing plasmid/phage hybrid vectors. For example, the plasmid SCP2* (about 27 kb; 7) might be used as a vector for the *Eco*RI fragments containing the cohesive ends of φC31 DNA (combined size, 12 kb; Fig. 7) to make a plasmid of about 39 kb potentially packageable into φC31 phage heads either in vivo or, as in cosmids (26), in vitro.

TRANSDUCTION

We are aware of only one well-established example of transduction in streptomycetes (83). In this system, UV-irradiated virulent phage SV1 was shown to give generalized transduction of strain 3022a (a chloramphenicol producer resembling *S. venezuelae*) at frequencies of about 10^{-6} per PFU (10^{-5} per CFU). Unfortunately, this phage has a very narrow host range. Preliminary transduction data have also been obtained with the temperate phage SH10, which has been used to transduce several *S. hygroscopicus* markers at very low frequencies (F. Suss and S. Klaus, personal communication). Attempts to transduce *S. coelicolor* A3(2) with temperate phages VP5, φC31, and R4 all failed (Stuttard, Chater, and Carter, unpublished data).

HOST-CONTROLLED RESTRICTION AND MODIFICATION OF PHAGES

General

Streptomycetes are a rich source of site-specific endodeoxyribonucleases (restriction enzymes): at the time of writing we know of 14 *Streptomyces* enzymes with characterized recognition sites and 14 less well studied enzymes. Only two (*Sal*GI and *Sal*PI) have so far been shown to be involved in in vivo restriction and modification of phages, though a number of other streptomycetes with RM systems acting on phages have been reported (Table 6).

Restriction by *S. albus* G

The best-characterized RM system is that involving the *Sal*GI (≡*Sal*GI) enzyme of *S. albus* G, which recognizes the sequence 5′-GTCGAC-3′ in double-stranded DNA (4). The involvement of *Sal*GI in restriction of the virulent phage Pal6 was proved by several criteria (22, 23). First, Pal6 (unmodified) had a 50-fold-lower EOP on *S. albus* G than did Pal6 modified by growth on *S. albus* G, and this correlated with almost complete (80 to 90%) protection of the single *Sal*GI target site in its DNA against in vitro *Sal*GI digestion after growth of the phage in *S. albus* G. Second, host mutants with increased sensitivity to Pal6 (unmodified) could be isolated which proved to be restriction deficient and usually to lack *Sal*GI in cell-free extracts. Third, a Pal6 mutant resistant to *Sal*GI restriction had lost the *Sal*GI target site from its DNA.

TABLE 6. *Interactions of phages with Streptomyces RM systems*

Phage	Host[a]	Restriction coefficient	Reference
Pal6sag	*S. albus* G	10^{-2}	22
VP12, A2, R4, φC31	*S. albus* G	$<10^{-8}$	23
R4G, φC31G	*S. albus* G	$\sim10^{-5}$	19, —[b]
R4G	*S. albus* P	10^{-2}	18
R4G	*S. rimosus* 1459	10^{-2}	19
	S. rimosus 2251		
R4[c]	*S. fradiae* 1172	10^{-4}	—[b]
Pg81	*S. griseus* Kr15	10^{-6}	58
Pg81	*S. griseus* Kr20, Rcg2[d]	10^{-6}	58
Pg81rs	*S. griseus* Kr20, Rcg2[d]	10^{-2}	58
VP5	*S. lividans* 67	10^{-4}	58
FF4	*S. olivaceus* ATCC 31126, *S. lipmanii* NRRL 3584, *S. cattleya* NRRL 8057	10^{-6}	33

[a] Strains in brace shared restriction-modification specificity.

[b] Chater and Ellis, unpublished data.

[c] Only an R4 deletion mutant has been tested in this system.

[d] Rcg2 is a hybrid between *S. coelicolor* A3(2) and *S. griseus* Kr15.

Role of Restriction in Resistance of *S. albus* G to Phages

The *Sal*GI restriction system is a major factor in determining resistance of *S. albus* G to phages: the restriction-proficient (R^+) parent is totally resistant, and the R^- mutants are fully sensitive, to many wide host range phages. The availability of R^- mutants which were modification proficient (M^+) allowed the growth of these normally totally restricted phages in conditions which should have led to their modification. However, in tests of this kind with virulent phages VP12 and A2, neither phage was enabled to form plaques on *S. albus* G R^+ M^+. It was proposed (15) that this might have resulted from the combination in these phages of inefficient modification with a large number of *Sal*GI target sites (note that Pal6 DNA was 10 to 20% unmodified at its single *Sal*GI site after growth of the phage on *S. albus* G R^+ M^+); indeed, VP12 proved to have about 30 such sites (23).

Interactions Between Phage R4 and Various Restriction-Modification Systems

A more complex interaction was seen between phage R4 and *S. albus* G. R4 cannot usually form plaques on *S. albus* G, but can do so on R^- mutants. After growth on an R^- M^+ host, an EOP of about 10^{-6} on *S. albus* G R^+ M^+ was obtained (19). All the plaques examined were of a mutant type termed R4G. R4G mutants showed a more conventional response to the *S. albus* G RM system, giving EOP values of 10^{-5} when unmodified and 10^0 after modification. Comparison of R4 and R4G DNA after ostensible modification by growth on an R^- M^+ mutant of *S. albus* G showed that R4 DNA was completely susceptible to *Sal*GI digestion, whereas R4G DNA was largely undigested (Chater, unpublished data). Thus, the G mutation increased the susceptibility of R4 DNA to *Sal*GI modification. This effect was not specific to the *Sal*GI system, since R4G mutants also seemed to have an increased ability to be modified at their single *Sal*PI target site during growth on *S. albus* P (18). (*Sal*PI endonuclease is produced by *S. albus* P; 14.) A further effect of the R4G mutation was manifested when *S. rimosus* strain 1459 or 2251 was used as host. Here it was found that, whereas R4 was not restricted by either strain, R4G was subject to 100-fold restriction (19). Thus, one and the same mutation had removed barriers to two different *S. albus* modification enzymes and to two probably identical *S. rimosus* restriction enzymes. The simplest explanation of these results, but one which awaits experimental proof, is to suppose that in the DNA of wild-type R4, but not in that of R4G, one of the four normal bases is substituted by an unusual base, and that the unusual base prevents target site recognition by the *S. albus* modification and *S. rimosus* restriction enzymes (19). We have recently found (K. F. Chater and A. Jefferies, unpublished data) that φC31 responds to the *S. albus* G system in a similar way to R4, and have isolated φC31G mutants. Further tests with these mutants will be needed to establish the extent of their similarity to R4G mutants.

Restriction of Phage Pg81

The genetically well studied strain *S. coelicolor* A3(2) appears to specify a restriction system acting on plasmids SCP1 and SCP2 (39; Bibb and Ward, personal communication), but attempts to detect restriction of phages by this strain have been unsuccessful (15, 87, 88). However, a hybrid strain, Rcg2, which was isolated in recombination experiments between *S. coelicolor* A3(2) and *S. griseus* Kr15, displayed restriction of the narrow-host-range virulent phage Pg81 previously grown on Kr15 (57). It appeared that Rcg2 had inherited this restriction system from A3(2), along with streptomycin resistance and the ability to adsorb another phage (VP5), whereas from Kr15 it had inherited the ability

to adsorb Pg81, together with several chromosomal markers. Moreover, Rcg2 lacked a restriction-modification system present in Kr15, so that the return of Pg81 from Rcg2 to Kr15 also resulted in its restriction. Another Pg81 host, *S. griseus* Kr20, was found to possess an RM system sharing specificity with the Rcg2 system. Pg81 mutants that had partially lost sensitivity to either the Kr15 or the Rcg2 system (but not to both) were easily isolated (87).

Pg81 also had a low EOP (10^{-6}) on *S. griseofovillus* 43, giving rise to mutant phages which subsequently had a high EOP on strain 43 regardless of the last host. Some of these mutants had become partially or totally resistant to restriction by Kr15 and Rcg2. This result, for which we do not have any explanation, provides further evidence of unusual interactions between *Streptomyces* phages and the RM systems of their hosts.

SUMMARY AND CONCLUSIONS

Much of this review has been concerned with the temperate phage φC31, and it is appropriate here to survey the present state of our knowledge of this phage. The extracellular phage is assumed to adsorb by its tail to the surface of susceptible cells, particularly to recently emerged germ tubes. The surface component to which it adsorbs is unknown (φC31 non-adsorbing host mutants can easily be isolated, but they have not been analyzed). The DNA (about 39 kb), which is in a nonpermuted linear form, enters the host by an unknown mechanism, and there it is assumed that the cohesive ends join and ligation follows to give a covalently closed circular molecule. In the case of the lysogenic response, this molecule is inserted into the host chromosome at a site located between *uraA1* and *pheA1*. This recombination event is presumed to be mediated by a phage gene product(s) recognizing particular base sequences in the phage and host DNA. This process may involve the gene(s) deleted by *lyg* mutants. There is no evidence of any other gene products involved solely in establishing lysogeny. Both establishment and maintenance of lysogeny involve the action of a repressor which is probably a homo-oligomeric protein (according to genetic evidence). The sites of repressor action are unknown. Reduction in the effective concentration of repressor causes induction (though only in nondormant cells). This may conveniently be achieved in the laboratory by the use of mutants that have temperature-sensitive repressors, but not by the use of UV. Of the events after induction we have little information, and this area represents an important subject for future research. This research will be greatly helped by the availability of mutations in at least 19 genes, 16 of which have been mapped (Fig. 5). The method devised for the complementation analysis of these mutations may be useful in other phage-host systems where adsorption is inefficient. No mutations have been discovered in genes expressed earlier than 10 to 15 min after infection, and the biochemical nature of the blocks has not been determined for any of the genes defined by temperature sensitivity for growth. However, we may assume that the gene *h*, which affects adsorption, specifies a component of the end of the tail, so that the genes most closely linked to it may also be tail components (by analogy with eubacterial phages). Thus the time of expression of the *N* and *E* genes (i.e., 20 to 25 min; Fig. 5) may reflect the time of expression of genes for tail components.

It seems probable that the late functions may all be specified by the left arm of the phage DNA, with the genes for some early functions (*B*, *L*) more centrally located, closely linked to the *c* gene. Structural modifications of the DNA have, on the other hand, been observed exclusively in the right arm.

Based on the evidence from cohesive ends, DNA replication probably involves the "rolling circle" mechanism (by analogy with λ; 85), generating concatameric molecules. Packaging of these into the mature phage would then involve site-specific staggered cutting of the concatamers into monomers with cohesive ends (and would thus require a specific endonuclease). The timing of DNA synthesis is unknown. The release of mature phage particles takes place 40 to 50 min from the initiation of the lytic cycle.

The information now available, combined with the physical studies of φC31 DNA, should act as a springboard for further studies of the degree of similarity of φC31 both with other temperate phages of streptomycetes and with λ. This information will no doubt be invaluable if the best use is to be made of *Streptomyces* phages as DNA-cloning vectors, a recent advance made possible by the development of a useful transfection system, coupled with the availability of deletion mutants and information about restriction enzyme target sites. We have discussed some of the uses of phages as cloning vehicles in streptomycetes, and we anticipate that the availability of the φC31/pBR322 hybrid potentially able to replicate in either *Streptomyces* or *E. coli* will present numerous additional benefits, for example, in facilitating the introduction of *E. coli* transposons into *Streptomyces*.

It may be useful here to point out two related uses for the phages developed for cloning purposes. The first is in a search for naturally oc-

curring transposons in streptomycetes, using transduction of, for example, drug resistance by phage deletion mutants as the basis of primary screening. Such transposons will be important if the maximum benefit is to be obtained from cloning experiments. The second use is the possibility of introducing very large segments of foreign DNA into new hosts by in vitro ligation into a temperate phage followed by transformation into a lysogen, so that natural recombination between the incoming and resident phage DNA should allow the stable incorporation of the foreign DNA into the prophage (17). In addition, phages may be constructed with artificially introduced target sites for *Streptomyces* restriction systems [especially that in *S. coelicolor* A3(2)], thus facilitating the isolation of restrictionless host mutants (as has already been done in *S. albus* G and *S. griseus* Kr15 with the help of naturally occurring restriction-sensitive phages).

Although it is evident from this review that important advances have been made in our understanding of *Streptomyces* phages in the last decade, numerous deficiencies are apparent, such as the absence of studies on the physiology of phage infection, the paucity of transduction systems, and the absence of plasmid-specific or RNA phages. It should, however, be obvious that an air of optimism surrounds the biology of *Streptomyces* phages at present, in terms of their potential applications both as tools in new genetical techniques and as significant subjects for research in their own right.

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