Control of Lysogenization by Phage P22

I. The P22 cro Gene

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P22 cro− mutants were isolated as one class of phage P22 mutants (cly mutants) that have a very high frequency of lysogeny relative to wild-type P22. These mutants: (1) do not form plaques and over-lysogenize relative to wild-type P22 after infection of a wild-type Salmonella host; (2) are defective in anti-lysogeny; and (3) fail to turn off high-level synthesis of P22 c2 repressor after infection.

P22 cro− mutations are recessive and map between the P22 c2 and c1 genes. P22 cro− mutations are suppressed by clear plaque mutations in the c1 gene, one of which is simultaneously cγ−. They are also suppressed, but incompletely, by mutations in the c2 (repressor) gene, especially those that do not completely abolish c2 gene function.

Salmonella host mutants have been isolated that are permissive for the lytic growth of the P22 cro− mutants.

1. Introduction

The genomes of the temperate Salmonella phage P22 and the temperate coliphage λ share the same overall functional organization. They also share some common DNA sequences as evidenced by DNA–DNA hybridization (Skalka & Hanson, 1972) and by the ability of the two phages to recombine in vitro (Gems et al., 1972; Botstein & Herskowitz, 1974).

The immunity systems of the two phages differ in that P22 has two distinct regions that participate in immunity and repression (the immc and immu regions; Bezdék & Amati, 1968; Botstein et al., 1975; Levine et al., 1975), whereas λ has only one immunity region (Hopkins & Ptaszynski, 1971). If, however, the P22 immu region is deleted, the phage is still able to establish and maintain lysogeny normally. Furthermore, a P22 hybrid phage, which contains the immc region of P22 and the rest of λ, can be established and maintain lysogeny normally (Gems et al., 1972; Botstein & Herskowitz, 1974; Hilliker & Botstein, 1976).

The P22 immc region and the A immunity region are structurally and functionally similar (Fig. 1). Each codes for a repressor necessary to maintain lysogeny (Levine, 1971).

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Fig. 1. Genetic map of the P22 innu and the B immunity regions. P22 genes are above the line, A genes are below the line. Lines above the map denote the transcription patterns. Pm is the promoters for the establishment mode transcription of the repressor gene (Pm of P22) (of λ). Jones et al. (1979); Schmeisser et al. (1980). Pm is the promoter for maintenance mode transcription of the repressor genes. Gansin et al. (1973); L. E. Potente, unpublished data. Pp is the promoter for the genes to the left and right, respectively. Koida et al. (1980); Roberts, 1985; Heitmann and Spiegelman, 1979; Hilliker & Botstein, 1975, 1976.

1957; Kaiser, 1957) and the structure of the operator sites for repressor binding are similar for the two phages (Potente et al. 1980). In addition, both phages code for immunity-specific proteins necessary for high-level repressor synthesis during establishment of lysogeny. cII and cIII for λ (Kaiser, 1957; Reichardt & Kaiser, 1971), and cI and c3 for P22 (Levine, 1957; Gough & Tokuno, 1975).

The λ cro gene codes for an immunity-specific protein that is essential for lytic growth of λ after infection of a wild-type Escherichia coli host. The λ cro function has been studied in great detail. It has been shown to repress directly phage gene expression from the promoters Pm, Pp, and Pp (Reichardt & Kaiser, 1971; Reichardt, 1975; Johnson et al., 1978; Meyer et al., 1980). It has also been shown to repress indirectly expression of the cI repressor gene from the Pm promoter by repressing expression of the cII and cIII genes from Pp and Pp, respectively (Zehs et al., 1973; Reichardt, 1976).

λ cro- mutants have several characteristic phenotypes: (1) they do not grow lytically after infection of a wild-type E. coli host; (2) they do not allow a defective prophage to become "anti-immune" (Cald & Neubauer, 1968; Eisen et al., 1970; Cale et al., 1971); and (3) they fail to turn off high-level repressor synthesis after infection (Reichardt & Kaiser, 1971). Due to the inability of λ cro- mutants to grow lytically after infection, virtually all λ cro- strains also carry the cI57 mutation, which specifies a thermolabile repressor. The cI57 allele suppresses the cro- defect at 37°C, but not at 30°C or 42°C (Eisen et al., 1975).

Given the ability of the P22 innu region to substitute for the λ immunity region and the fact that the λ cro protein is essential for lytic growth, it seemed logical to anticipate that P22 would code for a cro function. Suggestive evidence for a P22 cro function has been reported (Hilliker et al., 1978) but a direct demonstration of such a function has been lacking.

In the course of isolation and analysis of P22 mutants that lysogenize at greatly increased frequency relative to wild-type P22 (P22 3lB mutants; Hong et al., 1971; Roberts et al., 1976), we found three different classes of mutants, which we have named clyA, clyB and clyC (Winston, 1980; Winston & Botstein, 1981, accompanying paper). The P22 cly mutants studied by Hong et al. (1971) appear to fall into what we call the clyA class. In addition, these mutants, like the previously identified P22 3lA mutants, lysogenize at very high frequency and do not form plaques on a wild-type Salmonella host. They can form plaques on the cly-permissive hosts identified by Hong et al. (1971) and on new hosts reported here. In this paper, we shall refer to the P22 clyB mutants as P22 cro- mutants. The Salmonella hosts permissive for lytic growth by these and other classes of P22 cly mutants will be referred to as cly permissive hosts.

In this paper we present evidence for a P22 cro function, the isolation and analysis of P22 cro- mutants, and the isolation and preliminary characterization of new hosts permissive for lytic growth of P22 cro- mutants. By this analysis we have found, first, that P22 cro- mutants do not grow lytically (and lysogenize at very high frequency) after infection of wild-type Salmonella hosts but can grow lytically after infection of certain mutant Salmonella hosts. By use of these cly-permissive Salmonella hosts, we have been able to study P22 cro- mutants without the presence of a conditional clear-plaque mutation, which otherwise would be required to suppress conditionally the cro- phenotype. Second, we found that different cly-permissive mutations affect different host functions based on their phenotypes. Third, we found that a defective P22 cro- lysogen can become anti-immune and that a cro- mutation blocks the ability of a defective P22 lysogen to become anti-immune. Fourth, we found that a P22 cro- mutant fails to turn off high-level synthesis of c2-repressor and at least one other early phage-coded protein after infection of a wild-type host. These last two phenotypes are similar to the phenotypes of λ cro- mutants that are cited above.

2. Materials and Methods

(a) Bacteria

Bacterial strains are listed in Table 1. All Salmonella typhimurium strains are derivatives of LT2. DB1000 was actually used as the standard en- host. Most often, the cly-permissive hosts used contained the cI10 allele. The strains carrying the pclA, pclB and pclC alleles were isolated as Tn10 insertion mutants permissive for P22 cly mutants (see below). DB147 contains a short (c2+ mar-) deletion of the prophage, which begins outside the left end of the P22 prophage and extends into the cI gene; DB329 contains a long prophage deletion (c2+ mar-), which deletes everything between a Tn10 insertion in the P22 region and the cI gene (Chou & Botstein, 1972). All lysogens (except DB107) were made by selecting for ampicillin resistance since all the phage strains used to construct lysogens carried the Ap31/31 deletion-substitution (see below).

(b) Phage

All bacteriophage strains used in this work are derivatives of wild-type P22. The alleles used are listed in Table 2. Phages constructed in this work contain various combinations of these alleles. The croH100 allele was initially called cly3, clyA3 and clyH1 are the same as cly3 and clyH1 (Hong et al., 1971). The c27 mutation is the same as the c22 mutation previously described (Tokuno & Gough, 1976). We call c27 because of its similarity to c2 mutations of phage λ.

(c) Construction of P22 cro- clear double mutants

A large number of phage constructions in this work involved 2 classes of markers affecting the plaque morphology: clear (cI1, c22 and cly+) and cro-. For construction of P22 cro- cI1
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source/Reference</th>
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<tbody>
<tr>
<td>Non lysogenic</td>
<td>L72, prototroph</td>
<td>Botstein (1968)</td>
</tr>
<tr>
<td></td>
<td>recA</td>
<td>Wing et al. (1968)</td>
</tr>
<tr>
<td></td>
<td>hsdR316 (bio auR)</td>
<td>B. Ames via G. Walker</td>
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<tr>
<td></td>
<td>cya-408</td>
<td>B. Ames</td>
</tr>
<tr>
<td></td>
<td>ara-las5414</td>
<td>D. Botstein</td>
</tr>
<tr>
<td></td>
<td>ara-las5414 rpsL</td>
<td>Hilliker &amp; Botstein (1973)</td>
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<td></td>
<td>ara-las5414 koc-6002767 exph10</td>
<td>Winston et al. (1979)</td>
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<td></td>
<td>ara-las5414 koc-6002767 exph290</td>
<td>Winston et al. (1979)</td>
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<td>ara-las5414 koc-6002767 exph390</td>
<td>Winston et al. (1979)</td>
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<td></td>
<td>ara-las5414 koc-6002767 exph90</td>
<td>Winston et al. (1979)</td>
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<td>ara-las5414 rpsL99</td>
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<td></td>
<td>pell13::Tnl0</td>
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<td>ara-las5414 rpsL99 cya-408</td>
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</tbody>
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| Lysozyme | ara-las5414 [P22 recA c2+299 sicA1 as3] | D. Botstein |
|——|——|——|
| P22 cro− mutants | ara-las5414 rpsL (P22 recA+32424 24 amn836 | This work |
| | c2+299 sicA1 as317 361 gpl1 | This work |
| | ara-las5414 rpsL (P22 recA+32424 24 amn836 | This work |
| | c2+299 sicA1 as317 361 gpl1 | This work |

| Prophage deletions | ara-las5414 rpsL99 (P22 recA c2+299 sicA1 as3) | This work |
|——|——|——|
| DB147 | Chan & Botstein (1972) |
| DB268 | Chan & Botstein (1972) |

double mutants, the effect of these markers on plaque morphology was useful in detecting the desired recombinant phage. All of the P22 cro− c2− double mutants were initially identified as plaques that were of intermediate turbidity relative to the c2− and cro− parents. The same principle was applied to separate the mutations in P22 cro− clear double mutants.

The construction of P22 cro− c2− double mutants was accomplished in 2 different ways. The P22 cro− c2− double mutant was constructed simply by crossing the 2 single mutants by each other, plating the progeny on a wide-type 0+ host, and then scoring the small clear plaques for plaque morphology on 2 amber suppressor hosts, DB7153 (expF) and DB7156 (expF). On DB7155, the P22 cro− c2− double mutant makes a plaque that is more turbid than that of the c2− amn836 parent and furthermore does not plate on DB7156, which is a better suppressor of the c2− amn836 mutation.

Four-factor crosses were used to construct P22 cro− c2− to double mutants. In every case one parent was 22− amn8342 c2− and the other was c2− cro− . Crosses were done in a rpsL99 host (permissive for cro− mutants) and the outside markers 22− and cro− were selected on a rpsL99 host (DB7590). All constructions were tested by recovering the individual component mutations after a backcross to wide-type P22. The P22 cro− c2− double mutants were found by scoring the inside markers and looking for the recombinant class that was different from either parent or from wide type. In one case (P22 cro− c2− to c2− c290) this double mutant was found as a class that grew more poorly on DB7700 at 40°C and for the other case (P22 cro− c2− to c2− c290) this double mutant was found as a class that made a more turbid spot on DB7700.

In Construction of plaque strains used for the anti-immunity experiments

Phage strains used for the anti-immunity experiments were constructed using the same general principles described above. In order to preserve the desired inside markers during construction, parent phages were constructed that contained all cro and clear alleles desired.
in the final recombinant and carrying either the 12' am H342 or the 24' am SS6 markers. Then these 12' and 24' derivatives were crossed with each other to produce the final recombinant.

(iii) Construction of P22 Ap31pcf1

In order to have a strong selection for lysogens, a P22 strain was constructed that contained the β-lactamase gene from Tn1, thereby allowing selection for lysogens by selecting for AmpR colonies. This strain also contains a deletion of non-essential P22 material so that the phage DNA in particles will be terminally redundant, a feature necessary for P22 to grow after single infection (Botstein & Matz, 1970).

The parent strain was P22 Ap31, a strain containing a Tn1 insertion in the non-polar orientation in the carboxy-terminal end of the P22 nst gene (Weinstock et al., 1970). The non-polar orientation allows expression of the tail gene (gene 9), which is downstream (with respect to transcription) of nst. The presence of the Tn1 element results in a genome too large to fit inside the phage head. Therefore, the packaged DNA is not terminally redundant and the Tn1-carrying phage cannot grow by single infection. Selection for plaque-forming revertants (pfrs) is therefore a selection for deletions.

pfrs were selected, purified and then scored for whether they still carried the β-lactamase gene by testing for formation of AmpR lysogens. One pfr strain that carries the intact β-lactamase gene has been designated P22 Ap31pcf1.

Genetic tests showed that this phage had become recA- (since the parents phage contained the recA44 mutation (Suskind et al., 1974), loss of the recA gene function could not be scored). By electron microscopy of heteroduplex molecules, this phage has a deletion of approximately 5000 base-pairs beginning within the Tn1 element and extending leftward (D. Koshland, unpublished data).

(c) Growth of phage stocks

Concentrated phage stocks were prepared either by infection or by ultraviolet induction of lysogens (Botstein & Matz, 1970). These lysates made by u.v. induction are tail deficient (Israel, 1967) they were treated with approx. 10^4 plqge equivalents/ml of P22 tails (provided by P. Berget) at 37°C for 1 h prior to concentration.

For growing high titer stocks of some of the P22 ero+ mutants by infection, a slightly different procedure was used. The phage host was grown to 2 x 10^8/ml at 37°C, infected at a multiplicity of approximatively 1 and grown for 90 min at 37°C. Several drops of chloroform were added to the cultures, which were then put back to shake slowly at 37°C for approx. 10 min. The lysate of phage stocks with chloroform seemed to be greatly aided by illumination of the lysate at 37°C rather than at room temperature. The lysates were then concentrated by the usual procedures.

(d) Phage crosses

(i) Standard phage crosses

The procedure used for standard P22 crosses was adapted from that of Gough & Levine (1968) and of Botstein & Matz (1970). Each parent was infected at a multiplicity of infection (m.o.i.) of 7.

For some crosses the m.o.i. was shifted (to 7 for one parent and 1 for the other parent) to help bias in favor of a particular marker. For example, to construct a 12' am etl' am double mutant, the etl parent, whose plaque morphology is easily identifiable, would be infected at a multiplicity of 1 to increase the probability of recombination with a genome carrying the 12' am allele.

(ii) Four factor crosses

Four-factor crosses were done by the standard cross procedure. The crosses were done in strain DB17333 (r698 supE) for mapping cly mutations and in strain DB17155 (supE) for mapping cly mutations. For each set of crosses performed, the parents alone were infected in the identical procedure at a multiplicity equal to the total multiplicity in the co infection.

All of the 4-factor crosses were done with outside markers in genes 12', 12' and am H342 and ero (ero' and ero2). The progeny from the crosses were assayed on a permissive host (DB51323 and DB7155) and the non-permissive host DB471 (recA +), which only allows growth of the 12' ero' recombinants. Once the titers of the 12' ero' recombinants was determined, the crosses were replated on DB47 to examine a larger number of 12' ero' recombinants for the frequency of clear and turbid (c') plaques.

The relative positions of the 2 inside markers in question were always determined by the relative frequencies of turbid progeny in reciprocal crosses where the 12 and ero markers were in combination with each inside marker.

(f) Media

Liquid media used were LB broth (Levine, 1967), M9 minimal medium and M9 supplemented with charcoal-clarified Casamino acids (MDCA: Smith & Levine, 1964).

Solid media used were LB plates and agar plates (Singer & Weil, 1968) and green indicator plates (Levine & Cortis, 1961; Suskind et al., 1971). Dilutions were made in dilution fluid (Botstein & Matz, 1970) or buffered saline (Botstein, 1968). Nutrient top agar (Levine, 1967) was used in all cases. Drugs were used at the following concentrations: tetracycline, 25 μg/ml and ampicillin, 25 to 100 μg/ml. When needed, amino acids were supplemented at 20 μg/ml.

(f) Chemicals and radioactive isotopes

The sources of chemicals were as follows: tetracycline, Calbiochem-Behring Corp. (La Jolla, CA) and Sigma Chemical Co. (St. Louis, MO); ampicillin, Bristol Laboratories (Syacaue, N.Y.); and Sigma: hydroxyamine, J. T. Baker Chemicals (Phillipsburg, N.J.); anaerobic, Eastern Kodak Co. (Rochester, N.Y.); biacrylamide, Eastman and Bio-Rad Laboratories (Richmond, CA); Tumor, Bio-Rad; amosinum perruthenate, Bio Rad and Mallinckrodt Inc. (St. Louis, MO); and sodium dodecyl sulfate, BDH Chemicals. [35S]methionine was purchased from Amersham (Arlington Heights, IL).

(g) Hydroxyamine mutagenesis

Hydroxyamine mutagenesis was adapted from the procedure of Hall & Teasman (1966). Phage were mutagenized in eireo for 20 to 24 h to a survival of approx. 1%. The frequency of clear plaques mutants was generally near 1%.

(h) Isolation of cly mutants by plaque morphology

This procedure, adapted from that of Hong et al. (1971), was to plate a hydroxyamine-mutagenized P22 lysate for 100 to 200 plaques per plate on a cly permissive host, either DB17160 (r698) or DB17321 (recA4). Plates were incubated at either 30 or 37°C until the majority of the plaques on the plate began to have a faint turbid or bull's eye center. At that time the plates were removed from the incubator and screened visually for fully turbid plaques. The turbid plaques were purified and tested for the cly phenotype of no plaque formation on a cly-non-permissive (wild-type) host by either streaking or plates out a single-plaque suspension. Those that grew on the cly-permissive host and did not grow on the cly-non-permissive host were grown into lysates for further study.

(i) Isolation of cly mutants by lysogen selection

This procedure selects for mutants that are able to lysogenize efficiently under conditions where lysogens are extremely rarely formed. Strain DB17198 (r698) was grown in LB to a
concentrate of 5 x 10⁷/ml. The cells were centrifuged and resuspended in buffered saline to the same concentration. The cells were then infected at a multiplicity of 0.1 with P22 Ag31ϕJ1. After adsorption for 20 min at room temperature, 0.1 ml of P22 anti-serum was added and the infected cells were incubated an additional 10 min at room temperature. The infected cells were then plated on green plates containing ampicillin; the plates were incubated at 37°C.

Colonies that grew on the plates after a day or two were purified on ampicillin plates and then on LB plates. After purification, these strains were tested initially for their immunity by cross-streaking (Susskind et al., 1971). Those that were sensitive to P22 ant° and immune to P22 ant¬ were repurified and tested for spontaneous phase release by staining single colonies of the lysogens into lawns of clg non-permissive and clg permissive hosts. For those lysogens that warranted further testing, u.v.-induced lysates were grown.

(i) Isolation of clg-permissive hosts by Tn10 insertion mutagenesis

After Tn10 mutagenesis of strain DB21 (adapted from Kleckner et al., 1975), the Tet° colonies were replica-plated onto green tetracycline plates and green tetracycline plates seeded with 10⁸ P22 clgN3 phage (Hong et al., 1971), which were then incubated at 37°C. The replica plates were then examined for those colonies that were nibbled by the clgN3 phage (Susskind et al., 1971). These candidates were purified and retested for clg-sensitivity by cross-streaking and then by streaking-out clg mutants on lawns of the candidates. For those insertions isolated that did render DB21 clg-sensitive, the Tn10 insertion mutation was moved by P22 generalized transduction (selecting Tet°) into the original parental strain, DB21 (Kleckner et al., 1975). One hundred per cent of the Tet° transductants were clg-sensitive, demonstrating that the Tn10 insertion mutation caused the clg-sensitivity.

(k) Selection for revertants of P22 cro° mutants

Individual plaques on a T7β9 clg-permissive host were picked and replated on a non-permissive host, either DB7000 (ex°) or DB7304 (ex°). This generally yielded between 5 and 100 revertant plaques/plate with an occasional jackpot (Luria & Delbrück, 1943).

(l) Spot tests for identification of clear mutants

In order to distinguish among clear mutants we used spot complementation tests similar to those developed by Levine (1957). These distinguish cl mutations from clg, cyg and K5 permissive mutants.

A procedure to test for K5 mutants was developed by Bronson & Levine (1971) and relies on the fact that neither a Yz (left-side virulent) nor a K5 (right-side virulent) mutant alone can overgrow an immunity-infecting P22 cro- lysogen; however, a lysogen that is co-infected by the 2 phages will allow plaque growth.

A spot test to distinguish between cl and cy mutants (both of which complement as cl°) was adapted from the colony-spacing test developed by Susskind et al. (1974). In this test, one infects a strain that is lysogenic for P22 cl⁰ cro° in the clgN3444 at 40°C, a temperature non-permissive for growth of this lysogen. If the super-infecting phage carries a wild-type cl template, then wild-type cl repressor can be made at high temperature and the lysogen will survive. The exact mechanism by which the lysogen is able to survive is not clear (presumably repressor is being made from the good template in the maintenance mode). If the super-infecting phage carries a defective cl allele, no functional repressor can be made and the temperature-sensitive lysogen will die.

For this test phage candidates that complement as cl° are spotted on a lawn of DB607 (a P22 cl° 6289 614.44 lysogen) and the plates are incubated at 40°C. If a patch of survivors grows where the phage were spotted then the candidate is a cy mutant; if not, then the superinfecting phage carries a defective cl gene.

(m) Non-permissive marker rescue from prophage deletions

Non-permissive rescue was done as described by Chan & Botstein (1972). For rescue from DB147 an additional overlay of DB7000 was added to the plates to improve the plaque morphology.

(n) Frequency of lysogenic measurements

The frequency of lysogeny by wild-type P22 and by P22 cro° mutants was measured in the clg non-permissive (wild-type) host DB7000. Cells were grown in M9CAA to 2 x 10⁶/ml at 37°C, infected with the phage at the desired multiplicity and incubated for 15 min at 37°C. The infected cells were then diffused and spread on plates seeded with 5 x 10⁶ P22 clg and incubated at 37°C. Under these conditions only lysogens form colonies. The frequency of lysogeny is calculated from the number of infected cells and the number of lysogens formed.

(c) Dominance tests

The basic procedure for dominance tests was the same as for the standard cross with respect to growing the cells and the infection, growth and titering of the phage. Two different dominance tests were done. First, dominance tests for phage growth were done under conditions where each parent infected, at low multiplicity (<10), the clg non-permissive (and ex°) host DB7000; each parent carried a complementing amber mutation in a P22 late gene. Thus one parent carried a 5 am mutation and the other parent carried an 8 am mutation. In this way, only cells which were co-infected by each parent would produce a burst of phage and these co-infected cells would virtually always be infected by no more than one of each parent.

Second, dominance tests for frequency of lysogeny were done at a high multiplicity of infection (5) in strain DB7128 (ex°). The cells were infected, incubated at 37°C and 20 min later plated for lysogens as described above.

(p) Anti-immunity tests

If one constructs a lysogen that is defective in early functions and carries a clg-permissive mutation in the cl represor, one can test for the ability of this lysogen to become anti-immune by inducing the lysogen at a temperature non-permissive for the cl° mutation and then returning the temperature to the permissive temperature. If the Cro function of the lysogen can be expressed, it will inhibit the restoration of immunity upon return to the permissive temperature, allowing indefinitely the continued expression of the Cro function instead of immunity (cl represor function). Anti-immunity can be observed conveniently by superinfecting with a phage that ordinarily would fail to grow because of the presence of repressor. Anti-immunity is a phenomenon unrelated to the ability of superinfecting phages that can synthesize anti-repressor to grow: anti-repressor directly inactivates repressor while Cro function interferes with its synthesis. In the case of P22, anti-immunity tests could be carried out in such a way as to allow plating of anti-repressor producing phages to serve as a control, since all lysogens were deleted for the immB region. In such lysogens, all ant° P22 phages can grow but ant° phages cannot grow unless the lysogen has lost its immunity (Botstein et al., 1975).

Lysogens were tested for whether or not they could become anti-immune by growing them at 40°C (a temperature non-permissive for the cl° mutation in the prophage) to a concentration of 2 x 5 x 10⁶ Test phage phages were plated using these cultures and the plates incubated at 35°C. The tester phages used were wild type P22 and P22 Ag31ϕJ1; wild type P22 serves as a control since it is ant° and, therefore, grows on all the lysogens tested regardless of their anti-immunity state. P22 Ag31ϕJ1 grows only on anti-immune hosts since its ant gene is partially deleted. Lysogens used in these tests carry the rpmA allele to make the host more non-permissive for the P22 34° am366 allele.
3. Results

(a) Isolation of P22 cro" mutants by plaque morphology

We initially set out to isolate P22 mutants that over-lysogenize after infection of wild-type Salmonella. Such mutants had already been isolated by Hong et al. (1971). Those mutants, which they named cly mutants (for control of lysogeny), have the properties that: (1) they do not make plaques and virtually always lysogenize after infection of wild-type Salmonella; and (2) they can grow lytically after infection of Salmonella cya" , ccr" or rfi99 hosts and make turbid plaques on these hosts (on which wild-type P22 makes clear plaques). Hong et al. mapped these mutations between the P22 genes c1 and 18.

The initial method we used to isolate more mutants of this type was the same as that used by Hong et al. (1971): to screen for P22 mutants that make turbid plaques on a host permissive for cly mutants.

In this procedure, P22 stocks were mutagenized with hydroxylation and plated on a cly-permissive host (rfi99) to look for meningococcal that form turbid plaques. Among the candidates for cly mutants, the final criterion used was whether or not the cly was able to grow on the cly non-permissive (wild-type) host. Salmonella strain DB7000. The procedure yielded seven mutants in the 15,000 mutagenized plaques screened. The seven cly mutants isolated represent approximately 10% of the turbid plaque-formers tested. Most of the plaque that formed more turbid plaques than P22" on the cly-permissive host also appeared to plate normally on DB7000 and were not analyzed further.

(b) Isolation of cly mutants by lysogenization frequency

This procedure selects for mutants that can lysogenize more often than P22" in 4 host in which P22" lysogenizes at an extremely low frequency. The host carries the rfi99 allele; the phage for this selection is the strain P22 Ap31/pr1, which carries a gene coding for β-lactamase (ampβ) and whose construction is described in Materials and Methods. P22 Ap31/pr1 lysogenizes at very low frequency after low multiplicity infection of a rfi99 host (approximately 10$^{-3}$ lysogens per infected cell), as expected since P22" makes clear plaques on this host.

cly mutants were selected using both unmutagenized and hydroxylation-mutagenized phage stocks. Among 99 Amp⁸ colonies tested, 16 had a cly mutant as a prophage. The strength of the selection is indicated by the isolation of a spontaneous cly mutant. All 16 cly mutants isolated by this procedure were in the clyB or clyC class (see below).

(c) Preliminary mapping of cly mutations and designation of cly mutants

Preliminary mapping of the cly mutations by non-permissive rescue from two prophage deletion strains allows the cly mutants to be grouped into two sets.

The two prophage strains used, DB147 and DB5201, are deleted in from the left end and the right end of the prophage map, respectively, ending in the c1 region. Chan & Botstein (1972) showed that the prophage deletion in DB147 includes the c2 gene but not the 18' am100 allele and that the deletion in DB5201 does include 18' am100 but not the c2 gene. We have shown further that the deletion in DB147 includes almost all markers in the c1 gene (Winston & Botstein, unpublished results).

The results (Fig. 2) show that the cly mutants fall into two categories: those that can form cly" recombinants with the deletion prophage strain DB147 and those that cannot. All of the mutants can form cly" recombinants with the deletion prophage in strain DB5201.

These preliminary mapping results, in conjunction with the plating phenotypes of the cly mutants that show clyH125 and clyH122 to be in a different class from the other cly mutants based upon their host range (Winston & Botstein, 1981, accompanying paper), define three classes of P22 cly mutants: clyA, clyB and clyC.

The one class of mutants that map under deletion DB147 and have the same host

\[
\begin{align*}
\text{clyA}: & \quad \text{clyA} \\
\text{clyB}: & \quad \text{clyB} \\
\text{clyC}: & \quad \text{clyC}
\end{align*}
\]

Fig. 2. Fine structure map of the P22 cya-cry-c1 region. Map positions were determined by a factor crosses (Table 3) and non-permissive rescue from the prophage deletion strains DB147 and DB5201. The clyA mutants map between the endpoints of the 2 deletions and the clyB mutants map under the DB147 deletion. The clyC mutants also map under DB147 (not shown). The cly alleles mapped by non-permissive rescue are listed in Table 2.
range as the H100 mutant (clyB mutants) will be discussed further here. The set of clyB mutants will hereafter be referred to as P22 cro- mutants.

(d) Mapping P22 cro- mutations by four-factor crosses

In order to determine more precisely the map positions of the P22 cro- mutations, four-factor crossovers were used to order cro mutations relative to known P22 markers. The results of mapping the cro mutations by four-factor crosses (Table 3) order the markers croH100 and croH105 relative to the P22 markers K5, cgl7, c1'7 and c1'tes101 and to each other (Fig. 2). The four-factor crossovers place croH100 and croH105 to the right of K5, which defines P22 Dk (Bronson & Levine, 1971; Potente et al., 1980), and to the left of the cy region. These crossovers also definitively order croH100 to the left of croH105 thus demonstrating that not all the cro mutations are identical.

(e) Frequency of lysogeny by P22 cro mutants

Measurements of the frequency of lysogeny by different P22 cro- mutants after infection of a cro-non-permissive host (Table 4) show that they greatly over-

TABLE 3
Four-factor crossovers for mapping cro- mutations

<table>
<thead>
<tr>
<th>Cross</th>
<th>a</th>
<th>b</th>
<th>12' am</th>
<th>a\ig</th>
<th>ab</th>
<th>\ig</th>
<th>a\ig</th>
<th>ab</th>
<th>\ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>cgl7</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>a</td>
<td>ab</td>
<td>+</td>
<td>a</td>
<td>ab</td>
<td>+</td>
</tr>
<tr>
<td>c1'tes101</td>
<td>+</td>
<td>b</td>
<td>0</td>
<td>Single</td>
<td>Single</td>
<td>Single</td>
<td>Single</td>
<td>Triple</td>
<td></td>
</tr>
</tbody>
</table>

Reciprocal cross

| a | b | 12' am | Single | Triple | Single | Single |

Depending upon the relative position of the inside unselected markers, either the cross or the reciprocal cross will require a triple crossover to generate wild-type progeny. For each pair of crosses listed below, the cross is listed first, the reciprocal cross second. The \ig refers to the frequency of wild-type plaques among progeny recombinant for the outside markers. For cross 5, frequencies were determined by directly plating for cro- recombinants, p.e., plaque-forming units.

<table>
<thead>
<tr>
<th>Unselected markers</th>
<th>p.f.u.</th>
<th>12'</th>
<th>Deduced map order</th>
</tr>
</thead>
<tbody>
<tr>
<td>croH100</td>
<td>342805</td>
<td>115</td>
<td>12'c-roH100-croH105-c1'7-c1'tes101</td>
</tr>
<tr>
<td>cgl7</td>
<td>362780</td>
<td>451</td>
<td>c1'tes101-roH100-c1'7-croH100</td>
</tr>
<tr>
<td>c1'tes101</td>
<td>172940</td>
<td>181</td>
<td>croH100-c1'7-c1'tes101</td>
</tr>
<tr>
<td>K5</td>
<td>852210</td>
<td>203</td>
<td>croH100-K5-croH100</td>
</tr>
<tr>
<td>cgl7</td>
<td>252175</td>
<td>961</td>
<td>c1'tes101-c1'7</td>
</tr>
<tr>
<td>c1'tes101</td>
<td>169253</td>
<td>180</td>
<td>croH105-croH100-croH105</td>
</tr>
<tr>
<td>c1'tes101</td>
<td>242462</td>
<td>241</td>
<td>c1'tes101-c1'tes101-c1'7</td>
</tr>
</tbody>
</table>

(f) Dominance tests

Dominance tests show that the P22 cro- mutants are recessive to wild-type P22 for both growth and high frequency of lysogeny.

(f) Low multiplicity dominance tests for phage growth

In order to determine the dominance or recessiveness of P22 cro- mutants under conditions where the number of infecting phage is guaranteed to be one of each parent per cell, low multiplicity dominance tests for phage growth were done as described in Materials and Methods.

These results (Table 5) show that the P22 mutants croH100 and croH105 are recessive to wild-type P22 for growth under these conditions. The burst size in the cro- + P22+ infections, while reduced a few fold from the P22+ single infection, is over fiftyfold larger than the burst of cro- infections alone. Also, in the mixed infection, approximately equal numbers of P22+ and P22 cro- phage are produced. We conclude that the cro- mutants are missing a diffusible product; lack of this product results in over-lysogenization after infection.
Co-infection by P22 croH100 and P22 croH105 yields a burst size typical of either mutant parent alone, demonstrating lack of complementation between these two cro' mutations.

(ii) High multiplicity dominance tests for frequency of lyoseny

Since the P22 cro' mutants lyoseny at a much greater frequency than wild-type P22, a second type of dominance test is possible, i.e. one that measures dominance of the ability to lyoseny at high frequency in a cro-permissive host. These experiments were done in a rif29 host where the frequencies of lyoseny of wild-type P22 and P22 cro' mutants are significantly different from each other after a high multiplicity of infection.

These dominance test results (Table 6) confirm the results of the low multiplicity dominance tests: recessiveness of P22 croH100 to wild-type P22.

Table 6

High multiplicity dominance tests in DB7180 (rif29)

<table>
<thead>
<tr>
<th>Phage</th>
<th>% Lyoseny</th>
</tr>
</thead>
<tbody>
<tr>
<td>P22' + P22 croH100</td>
<td>36</td>
</tr>
<tr>
<td>P22'</td>
<td>27</td>
</tr>
<tr>
<td>P22 croH100</td>
<td>106</td>
</tr>
</tbody>
</table>

The procedure is described in Materials and Methods. Single parent infections were done at a multiplicity equal to the total multiplicity in the co-infections.

(g) Demonstration of an anti-immunity function of P22

Demonstration of an anti-immunity function for P22 was done in a manner similar to that used for phage λ by Calef & Neubauer (1968) and Eisen et al. (1970). We constructed the P22 lysogen DB7621 and DB7630 (see Materials and Methods), which are isogenic except for the cro alleles: the prophage in DB7621 is cro' and the prophage in DB7630 carries the croH100 allele. Both prophages contain a c2' to mutation and amber mutations in genes 24 and 72 so that the lysogen will not die after thermal induction. The prophages are also int' to prevent excision of the prophage and carry the Ap31pf1 marker, which makes the prophages int'A' (which allows superinfecting P22 to inject their DNA; Sauskind et al., 1974) and provides a convenient marker (amp') to score for their presence. Calef & Neubauer (1968) and Eisen et al. (1970) have shown for the analogous λ cro' lysogen that after growth at high temperature and a shift to low temperature the lysogen did not regain immunity and furthermore directed all superinfecting phage into the lytic pathway of growth. The λ cro' lysogen was able to regain immunity.

The results of plating P22 on DB7621 and DB7630 grown under the same conditions (Table 7) demonstrate that P22 does have an anti-immunity function.

Table 7

<table>
<thead>
<tr>
<th>Phage</th>
<th>Host</th>
<th>Relevant prophage marker</th>
<th>Temp at which host grown (°C)</th>
<th>Plating efficiency (relative to DB7621)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P22 Ap31pf1</td>
<td>DB7621</td>
<td>cro'</td>
<td>30</td>
<td>&lt;1.5 x 10^-5</td>
</tr>
<tr>
<td>P22 Ap31pf1</td>
<td>DB7630</td>
<td>croH100</td>
<td>30</td>
<td>&lt;1.5 x 10^-5</td>
</tr>
<tr>
<td>P22 Ap31pf1</td>
<td>DB7639</td>
<td>croH100</td>
<td>30</td>
<td>&lt;1.5 x 10^-5</td>
</tr>
<tr>
<td>P22 croH100 Ap31pf1</td>
<td>DB7621</td>
<td>cro'</td>
<td>30</td>
<td>&lt;8.5 x 10^-5</td>
</tr>
<tr>
<td>P22 croH100 Ap31pf1</td>
<td>DB7630</td>
<td>croH100</td>
<td>30</td>
<td>&lt;8.5 x 10^-5</td>
</tr>
<tr>
<td>P22 croH100 Ap31pf1</td>
<td>DB7639</td>
<td>croH100</td>
<td>30</td>
<td>&lt;8.5 x 10^-5</td>
</tr>
</tbody>
</table>

Plating efficiencies are calculated by titrating the tester phage (first column) on the desired host (second column) at either 35 or 40°C and dividing this titer by the titer measured on DB7621 at 40°C (permissive conditions). The prophages in the host all contain the 16° sizeH342, 24° sizeS30, 62° sizeC77, cro' sizeL37 and Ap31pf1 markers. They differ only by their cro alleles.

and that this function is defective in the P22 croH100 mutant. After being grown at 40°C (a temperature non-permissive for the c2' to30 allele), and plated at 35°C (permissive for the c2' to allele) the cro' lysogen (DB7621) fails to regain immunity as shown by the plating of the P22 Ap31pf1 (int') strain. The lysogen remains immune when grown at 30°C and plated at 35°C. The plaques that form on this strain after it has been grown at the high temperature are clear, indicating that no lysogens are formed and that the strain has become anti-immune. Furthermore, the anti-immune host is also permissive for P22 cro' mutants probably because it expresses cro function constitutively.

The cro' lysogen (DB7630) is deficient in expressing the anti-immunity function; after being grown at 40-5°C and plated at 35°C, this strain is immune, as evidenced by the failure of P22 Ap31pf1 to form plaques. These results further confirm that P22 cro' mutations define a gene similar to the cro gene of phage λ.
(h) The pattern of P22 protein synthesis in P22 wild-type and P22 cro'-infected cells

The rates of synthesis of P22 proteins in wild-type P22 and P22 croH100 infections were analyzed by pulse-labeling infected cells at various times after infection and then examining the labeled products on sodium dodecyl sulfate/polyacrylamide gels. Results of these experiments (Figs. 3 and 4) demonstrate: first, that wild-type P22 synthesizes c2-repressor at a high rate for a short time after infection and then reduces the rate of synthesis to a low level; second, that P22 croH100 has the same initial kinetics of c2 synthesis as wild-type P22 but continues to synthesize repressor at a high rate until a much later time after infection; third, that at least one other P22 early protein, the product of the cef gene, is also synthesized at a higher rate later in infection in the P22 croH100 infection than in the P22 wild-type infection; and fourth, that the P22 croH100 infection results in a lower rate of synthesis of the major capsid protein (gene 5) product than the wild type P22 infection, indicating that expression of the P22 late genes is defective in the P22 cro' infection.

These results are all consistent with the idea that the P22 cro function normally serves to turn down expression of P22 early gene expression. This deficiency in turn-off of early gene expression in the P22 cro' infection is similar to the Tof' phenotype observed in a λ cro' infection (Radding, 1964; Eisen et al., 1966; Peru, 1971). Harvey et al. (1970) also showed that this same P22 mutant overproduces c2-repressor.

![Graph](image1)

Fig. 3. Rates of protein synthesis after infection by wild-type P22 and P22 croH100. Samples of infected cells were pulse-labeled with 35S-methionine and run on sodium dodecyl sulfate/polyacrylamide gels as described in Materials and Methods. Lanes are labeled with the time the 1-min pulses were begun. Lanes labeled u are uninfected samples.

![Graph](image2)

Fig. 4. Rates of synthesis of cef protein and c2-repressor after infection by wild-type P22 and P22 croH100. Infections and pulse-labelings were performed as described in Materials and Methods. The curves indicate the relative rates of synthesis of c2-repressor and cef protein. Autoradiograms were traced and the areas were normalized to the amount of trichloroacetic acid-precipitable radioactivity in each gel slot.

(i) Partial suppression of a 24-am mutation by the croH100 mutation

Franklin (1971) and Court & Campbell (1972) noted that λ cro mutations can often suppress λ N' mutations (Fed phenotype). We tested for this phenotype with P22 cro' mutants and found that the croH100 mutation weakly suppresses a P22 24' am mutation. The relative efficiency of plating (data not shown) of a P22 croH100 24' am836 double mutant is approximately 0.2 on an su' host with respect to plating on an su' host. For the P22 24' am836 single mutant, the relative efficiency of plating is less than 0.1. Since both phage strains used here carry the c2 ly30 mutation, which suppresses the growth defect of the croH100 mutation, these strains grow on cly non-permissive hosts.

(j) Isolation and characterization of pseudo-revertants

Among revertants of P22 cro' phages, there is a high frequency of clear-plaque pseudo-revertants. Clear pseudo-revertants of the croH100 mutant were selected, purified and tested by spot complementation tests (as described in Materials and
Method) for the type of clear mutation they carried. Clear pseudo-revertants were also tested for temperature-sensitive or nonsense phenotypes. The results of this analysis show: first, that both e1 and e2 mutations suppress the CrO' phenotype; second, that e1 mutations comprise the predominant class of clear pseudo-revertants (54 of 67 tested were e1 mutants); and third, that one pseudo-revertant defines a new class of clear mutation, which confers both a cy' and CrO' phenotype on the phage.

The cy pseudo-revertants of P22 croH100 grow normally on cyC- non-permissive hosts, indicating total suppression of the CrO' phenotype. The e2 pseudo-revertants of P22 croH100 do not grow as well on cyC- non-permissive hosts, making smaller plaques.

In addition to e1 and e2 pseudo-revertants of the croH100 mutant, one cy pseudo-revertant, P22 croH100e12, was also found. However, the cy12 mutation, the cy mutation in P22 croH100e12, is different from any previously identified in P22 or in 8R as it is also cy'. A second cy' e1' mutation of this type has also been isolated as a pseudo-revertant of a cyA mutant (Winston & Botstein, 1981, accompanying paper). For 8R, sequence analysis suggests that some 8H1 cy mutations are within the structural gene for the 8H1 protein although all 8H1 cy mutations are still e1' (Schwarz et al., 1978; Rosenberg et al., 1978; Wulf et al., 1980).

For croH100e12, the two mutations were separated and then the double mutant was reconstructed, demonstrating suppression of croH100. The P22 croH100e12 double mutant makes a smaller plaque than cro' cy12. Fine structure mapping of the P22 cy e1 region places cy12 at the far left end of the e1 gene (Winston, 1980).

(k) Construction and analysis of cro-clear double mutants

Construction and analysis of cro-clear double mutants confirmed and extended what was inferred from the isolation and analysis of the cro' pseudo-revertants. First, e1 mutations suppress P22 cro' mutations; second, a cy1 amber mutation suppresses croH100 unless the e1 amber mutation is well suppressed, in which case the cro' phenotype returns; third, e2 missense (including temperature-sensitive) mutations suppress cro' mutations; and fourth, a e1 amber mutation suppresses croH100, but the suppression is temperature-sensitive.

(i) Construction and analysis of cro' e1' double mutants

To construct a P22 cro' e1' ts double mutant, croH100 was crossed with e1'ts2 and recombinants that would make a more turbid plaque than the e1'ts2 parent at 37°C were found at low frequency. These recombinants made turbid plaques at 37°C and clear plaques at 40°C. The e1'ts2 parent makes clear plaques at both temperatures. When the presumed P22 croH100 e1'ts2 double mutant was crossed by wild-type P22, both single mutations could be recovered, thereby confirming the genotype. These results confirm that a e1 mutation will suppress a P22 cro' mutation.

The suppression of croH100 by e1 mutations is not allele specific for the particular e1 mutation. P22 cro' e1'ts double mutants were constructed using two different P22 cro' mutations (croH100 and croH105) and two other e1'ts mutations, e1'ts101 and e1'ts97. In every case, the double-mutant genotype was confirmed by crossing the strains back to P22' and re-isolating the single parental mutations. The fact that every one of these double mutants is able to grow on a cyC- non-permissive host even at low temperature demonstrates that a wild-type level of e1 activity is crucial for the cro' phenotype of non-growth on a wild-type host.

For two P22 croH100 e1'amber double mutants, the cro' phenotype is restored on a particular amber suppressor host; that is, on one su' host (supF), the e1' am mutation is suppressed well enough that the e1-dependent cro' phenotype is manifested. For two other P22 croH100 e1'am double mutants, this is not the case: rather, the double mutants grow on all of the su' hosts, plating clearly on some and turbid on others. The four e1'am mutations used in these experiments were all isolated as pseudo-revertants of either croH100 or a cyA mutant, cyG100 (Winston & Botstein, 1981, accompanying paper). As for e1'ts mutations, then, a e1'am mutation is able to suppress a P22 cro' mutation.

(ii) Construction and analysis of P22 cro' e2' double mutants

Construction and analysis of P22 cro' e2' double mutants demonstrates that cro' mutations can be suppressed by e2 mutations; however, the suppression is not so efficient as suppression by e1 mutations.

P22 cro' e2' ts double mutants were constructed as described in Materials and Methods using the croH100 mutation and the e2'ts30 and e2'ts29 alleles. The croH100 mutation affects the e2' ts plaque morphologies in a manner resembling its effect on the e1' ts plaque morphologies; the presence of a cro' mutation makes the plaque more turbid than it is in a cro' background at all temperatures tested.

The P22 croH100 e2'amO8 double mutant has a temperature-sensitive phenotype, which is dependent upon the suppressor strain on which it is grown. As can be seen from the results in Table 8, on supE, this plaque is quite temperature-sensitive. On these two hosts the e2'amO8 mutation, judging by the plaque morphology, is unsuppressed and well-suppressed, respectively. On the supE host, where the e2'amO8 mutation is suppressed to an intermediate level, this plaque shows only slight temperature sensitivity. These results suggest that the quantity or quality of the e2 made is important in the temperature-sensitive mutant.

<table>
<thead>
<tr>
<th>Table 8</th>
<th>Growth of P22 croH100 e2'amO8 on different hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Relevant genotype</td>
</tr>
<tr>
<td>DB7158</td>
<td>supF</td>
</tr>
<tr>
<td>DB7000</td>
<td>su</td>
</tr>
<tr>
<td>DB7155</td>
<td>supF</td>
</tr>
<tr>
<td>DB7156</td>
<td>supF</td>
</tr>
</tbody>
</table>

Phage dilutions were plated on the above hosts at 30 and 40°C. All strains were grown at 37°C. Under the same conditions the P22 croH100 e2' ts mutants grew at both temperatures.
phenotype. The non-growth at high temperature has not been investigated further but is reminiscent of the \( \lambda \) Tro phenotype (Eisen et al., 1975; Georgiou et al., 1976).

(1) Isolation and characterization of new cly-permissive hosts

After insertion mutagenesis of the wild-type Salmonella strain DB21 with the translocatable tetracycline resistance element, Tn10, approximately 30,000 colonies were tested for sensitivity to clyN3 by replica-plateing onto plates seeded with this cly mutant and looking for "nibbled" colonies (Susskind et al., 1971). Three clyN3-sensitive mutants were found and are the strains pclA, pclB and pclC (pcl for permissive for cly). These three hosts are permissive for lytic growth of all of the P22 cro^- mutants tested.

These three strains have been tested for several characteristics and the following is known about them: they are cpr^- and cpr^+, they are prototrophic and they are not u.v.-sensitive. Preliminary Hfr crosses (not shown) indicate that the pclA, pclB and pclC mutations map in different locations. The efficiency of plating of four P22 cro^- mutants on the set of Salmonella cly-permissive hosts is shown in Table 9.

### Table 9

<table>
<thead>
<tr>
<th>P22 cro^- mutant</th>
<th>pclA</th>
<th>pclB</th>
<th>pclC</th>
<th>Non-permissive</th>
</tr>
</thead>
<tbody>
<tr>
<td>P22 croH100</td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>0.2</td>
</tr>
<tr>
<td>P22 croH105</td>
<td>10</td>
<td>10</td>
<td>07</td>
<td>0.4</td>
</tr>
<tr>
<td>P22 croH116</td>
<td>10</td>
<td>09</td>
<td>12</td>
<td>0.4</td>
</tr>
<tr>
<td>P22 croH124</td>
<td>10</td>
<td>15</td>
<td>17</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The efficiencies of plating were normalized to the titer on the rif^R host DB138. Platings were all done at 37°C.

These hosts are all significantly more permissive for the P22 cro^- mutants than the parent strain, DB21. Additionally, wild-type P22 makes a clearer plaque on all of the cly-permissive hosts so far identified, although the degree of clarity differs among the different hosts. Grodzicker et al. (1972) showed that an E. coli cpr^- host is more permissive for a \( \lambda \) cro^- mutant than a cpr^+ host.

We also tested the plating of P22 croH100 on the set of permissive hosts at a range of temperatures and found this mutant to be quite temperature-sensitive for growth on one host (rif^R 40.5°C), to have an intermediate temperature-sensitive phenotype on two hosts (pclA and pclB) and to not be temperature-sensitive on two other hosts ( pclC and cpr^R 408). These results suggest that different host loci are affected among the different cro-permissive hosts and that they may affect plaque growth in different ways. However, they seem to affect all plaque with the Cyl phenotype in the same general way, i.e. by allowing lytic growth.

### 4. Discussion

The experiments presented here define the P22 cro^- gene and also demonstrate that certain mutant Salmonella hosts are able to suppress the Cro^- phenotype in that they allow P22 cro^- mutants to grow lytically.

(a) Identification of the P22 cro^- gene

The P22 cro^- gene has been identified by an ensemble of several results: (1) demonstration of an anti-immunity state in P22 c2^-ts cro^- lysogen; (2) demonstration that the P22 cro^- mutants are defective in the anti-immunity function as well as in turning off at least some P22 early gene expression; (3) demonstrating that these mutations map in a region between the P22 O1 and cy regions; (4) demonstration that the P22 cro^- mutations are recessive; and (5) demonstration that a P22 cro^- mutation can weakly suppress a P22 c2'-ts mutation, a phenotype that is analogous to the Fed phenotype of \( \lambda \) cro^- mutants (Franklin, 1971: Court & Campbell, 1972).

The simplest interpretation of these results is that the P22 cro^- gene codes for a protein that acts as the \( \lambda \) cro protein does, i.e. to repress early gene expression at the transcriptional level by binding to the operators, O1 and O2. In a P22 cro^- infection, then, early gene expression is not turned off and c1 protein continues to be made, resulting in greater c2-repressor synthesis and a greater frequency of lysogenization. This interpretation is based on the postulate that the P22 Cro^- phenotype is mediated by overproduction of the P22 c1 protein. More direct evidence that the P22 cro^- protein binds to O1 and O2 to repress transcription must await purification of the P22 cro^- protein and demonstration of its function in vitro.

The virtual total suppression of P22 cro^- mutations by c1 mutations both supports the interpretation that the overproduction of c1 protein causes the Cro^- phenotypes and strengthens the analogy to \( \lambda \) cro^- mutations that are suppressed by mutations in the analogous \( \lambda \) cII gene (Reichardt, 1975c).

The suppression of P22 cro^- mutations by c2^- mutations is not straightforward. Both c2^-ts alleles used suppress the croH100 mutation. However, suppression of croH100 by the c2^- amO8 mutation is temperature-sensitive. For phage \( \lambda \), a c2 croT7 d'6857 double mutant does not grow at 42°C (Tro phenotype: Eisen et al., 1975), presumably because of overproduction of some \( \lambda \) gene products in the absence of any repression by either c2 or cro. For the P22 c2^- amO8 croH100 double mutant a similar argument can be made if the mutant c1 protein made in a P22 c2^- amO8 croH100 infection does not lose all activity until 40.5°C. This predicts that this infection should also have the other Tro phenotypes (Eisen et al., 1975; Georgiou et al., 1979).

Unlike \( \lambda \) cro^- mutations, which are suppressed by \( \lambda \) cII mutations, indirect evidence suggests that P22 c3^- mutations do not suppress P22 cro^- mutations. Although a P22 cro^- c3^- double mutant has not been constructed, no c3^- mutations were obtained as pseudo-revertants of cro^- mutations. In conjunction with the result that on the growth media generally used for P22 a P22 c3^- mutant makes a turbid plaque (Levine, 1975) and lysogenizes only two to threefold less well than wild type P22 (Winston & Botstein, unpublished), this result probably reflects the
less than critical role of the P22 c3 protein in establishment of lysogeny under these conditions.

(b) The role of host functions in establishment of lysogeny

The role of bacterial host functions in regulating establishment of lysogeny after P22 or A infection is still largely obscure. Results of others (Hong et al., 1971) and those presented here have indicated that at least six different Salmonella loci (cya, cya, fip, pelA, pelH, and pelC) can be altered to lower the frequency of lysogenization by wild-type P22 and by P22 cya mutants (which include P22 cya' mutants). The steps at which these host mutations act to affect establishment are unknown. The fact that some of the host mutations could have multiple effects also makes any interpretation of their effect more difficult. For example, the cya-permissive Salmonella rifA9 mutation could be affecting a step as direct as initiation of transcription at the P22 pyr promoter or a factor as indirect as altering the host physiology in a general way resulting in lower establishment of lysogeny.

E. coli hfl mutants, which allow efficient establishment of lysogeny by a λ cII mutant (Beloff & Wulf, 1971, 1974), are the best-characterized host mutants that affect establishment of lysogeny. Epp (1978) has shown that the λ cII protein is more stable after infection of an E. coli hfl host. Furthermore, Beloff & Wulf (1974) have shown that a λ cIII mutant can establish lysogeny at normal frequencies in an E. coli cya hfl double mutant. From these results and in conjunction with the results that the cya1 mutation (which stabilizes the λ cII protein) suppresses a λ cII' mutation (Jones & Herskovits, 1978; Epp, 1978), and that the λ cII1 protein appears to stabilize the λ cII protein, Jones & Herskovits (1978) and Epp (1978) have put forth the model that the E. coli hfl product is at least partially responsible for the instability of the cII protein and that cyclic AMP and the cya protein positively regulate a protein, "Z", which is a negative regulator of hfl. By this model, a mutation that lowers or abolishes Z function or expression would be expected to over-produce hfl protein, lower λ cII stability, and therefore lower the frequency of establishment of lysogeny.

Assuming that there is an hfl-like function in Salmonella and knowing that Salmonella cya and cpa mutations affect P22 lysogeny as E. coli cya and cpa mutations affect λ lysogeny, it is logical to hypothesize that the Salmonella pel mutations, isolated as permissive hosts for P22 cya mutants, may affect a Salmonella Z function. One could postulate that there are several Z functions to account for the different loci that affect establishment of lysogeny by λ and P22.

While it is attractive to think that all host mutations affecting establishment of lysogeny mediate their effect by affecting activity of the P22 cII protein or the λ cII protein, there is little evidence to support this belief. Host mutations that affect expression of the cya gene or stability of the cya protein for either λ or P22 would also affect establishment of lysogeny. Possible examples of such host mutants are E. coli ER437 (Oppenheim et al., 1974) and Salmonella typhimurium px01 (Tokuno & Gough, 1975).

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Control of Lysogenization by Phage P22

II. Mutations (clyA) in the cI Gene that Cause Increased Lysogenization

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P22 clyA mutants lysogenize at very high frequency after infection of wild-type Salmonella. One class of P22 clyA mutations, clyA mutations, are shown to map within the P22 cI gene and to increase the activity of the cI gene product during establishment of lysogeny. The P22 clyA mutations also appear to affect cI repressor gene expression in cis and to cause a Cro - phenotype, despite the fact that the clyA mutations map far downstream (with respect to transcription) of the end of the P22 cro gene.

1. Introduction

The decision between lysis and lysogeny after infection by the temperate phages P22 and λ is subject to influence by several phage and host components. These components serve to regulate the level of repressors (the cI and mII gene products of P22 and the cl gene product of λ) that are necessary to repress most phage gene expression, thereby allowing establishment of lysogeny.

The immc region of P22 is functionally and structurally analogous to the immunity region of coliphage λ (Gough & Tokumo, 1975; Winston & Botstein, 1981, accompanying paper) and, although repression and immunity of P22 are mediated by two immunity systems, immc and immI (Bezdèk & Amati, 1968; Botstein et al., 1975; Levine et al., 1975), a P22 strain with immI deleted is able to establish and maintain lysogeny normally (Winston, 1980). Also, λ P22 hybrid phages, which contain the immc region of P22 substituted for the λ immunity region, are able to establish and maintain lysogeny normally (Gemski et al., 1972; Botstein & Herskowitz, 1974).

Levine (1957) and Kaiser (1957) demonstrated for P22 and λ, respectively, that clear-plaque mutations in genes other than those coding for the repressor (the cl and cI genes of P22 and the cI and cII genes of λ) greatly lower the frequency of establishment of lysogeny but do not affect the maintenance of lysogeny.

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