Proc. Nat. Acad. Sci. USA
Vol. 69, No. 2, pp. 366–370, February 1972

Establishment of Repression by Lambdoid Phage in Catabolite Activator Protein and Adenylate Cyclase Mutants of *Escherichia coli*

(cyclic AMP)

TERRI GRODZICKER, R. RITA ARDITTI, AND HARVEY EISEN*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115; and * Department of Biochemistry, University of California, San Francisco, Calif.

Communicated by Allan Campbell, November 10, 1971

ABSTRACT **Lambdoid phages form clear plaques and show reduced ability to establish immunity in strains of *Escherichia coli* that lack adenylate cyclase or catabolite activator protein. The absence of the activator protein or cyclic AMP reduces the frequency of lysogenization, but does not prevent steady-state repressor synthesis of a lysogen. Lambda phage mutants able to form turbid plaques on strains that lack catabolite activator protein or adenylate cyclase have been isolated and analyzed.**

Infection of sensitive bacteria by temperate lambdoid phage leads to one of two responses. The phage may infect by the lytic pathway, with eventual lysis of the host cell and release of a burst of progeny phage, or the phage genome may follow the lysogenic pathway and become inserted into the host chromosome as a prophage. In the lysogenic state, the only phage genes known to be expressed are the *rex* gene and the repressor gene cI, the expression of all other genes being directly or indirectly blocked by the repressor.

The decision whether to follow the lytic or lysogenic pathway is influenced by various phage functions. Unlike the repressor of the lactose operon, synthesis of the λ repressor is regulated (1, 2). Initiation of repressor synthesis upon infection requires the action of the products of genes cII and cIII (3–6). Subsequently, the product of the *cro* gene prevents repressor synthesis even though this gene is directly under repressor control (5, 6). Therefore, it seems that the final decision whether to lysogenize or to grow lytically might depend on the ratio of repressor to *cro* product, and factors influencing this ratio would influence frequency of lysogenization in a given population of infected cells.

It was of interest to see whether host factors could influence the frequency of lysogenization by lambdoid phages. One likely candidate is the system involved with catabolite repression. In *Escherichia coli*, RNA polymerase alone cannot efficiently initiate transcription at promoters of catabolite-sensitive operons. An additional factor, catabolite activator protein (CAP protein), in conjunction with a small molecule, cyclic AMP, is required for the maximal expression of such operons (7, 8). Thus, mutants that lack CAP protein or adenylate cyclase, (the enzyme that catalyzes synthesis of cAMP) have pleiotropic effects. For example, these mutants produce low levels of the enzymes of the lactose, arabinose, maltose, glycerol, or rhamnose operons (9–11).

Abbreviations: CAP protein, catabolite activator protein, *cya*$^-$, the genotype for inability to synthesize adenylate cyclase.

We shall show here that in mutants that lack adenylate cyclase (*cya*$^-$) or CAP protein (*CAP*$^-$), expression of phage functions is also altered. When λ or related phages infect mutants that lack CAP protein or cAMP, the frequency of lysogenization is reduced.

By analogy with certain bacterial promoter mutants (12, 13), we have isolated λ mutants that efficiently establish repression in the absence of CAP protein and cAMP. Moreover, upon infection of a wild-type host, these phage mutants are channeled almost exclusively into the lysogenic (or repressed) state. Thus, the concentration of host-cell factors plays a role in determining the decision made by the phage to enter the lytic or lysogenic pathway.

Similar observations have independently been made upon infection of *Salmonella typhimurium* with phage P22 (15).

MATERIALS AND METHODS

Strains. The genotypes and derivation of the strains used are listed on Table 1.

Media. Cells were grown on LB broth (14). Phage was plated on H top agar (14) containing the indicator on Tryptone–Yeast Extract (TYE) plates (14). When cAMP or AMP (Sigma) were to be used, 0.2 ml of a 0.1 M solution was added to the H top agar. The nucleotides were dissolved in buffer containing per liter: 14 g K_2HPO_4–6 g KH_2PO_4–2 g $(NH_4)_2SO_4$–1 ml of 1 M $MgSO_4$. TM buffer contains per liter: 5.8 g Tris–6 g maleic acid–1 g $(NH_4)_2SO_4$–0.1 g $MgSO_4 \cdot 7H_2O$–0.25 mg $FeSO_4 \cdot 7H_2O$–5 mg $Ca(NO_3)_2$ (pH 6). M63 (standard dilution medium) contains per liter: 7 g K_2HPO_4–3 g KH_2HPO_4–2 g $(NH_4)_2SO_4$–0.2 g $MgSO_4 \cdot 7H_2O$ (pH 7).

Frequency of Lysogenization of λh80. Cells were grown to 5×10^8/ml, centrifuged, and resuspended in 0.01 M $MgSO_4$. Phage was added at a multiplicity of infection of 10 and allowed to adsorb to bacteria at 37°C for 10 min. Unadsorbed phage was measured by dilution in M63 medium containing chloroform. Infected cells were centrifuged, resuspended in LB broth, and treated with antiserum to φ80 (antiserum courtesy of C. Yanofsky) for 10 min at a final $k = 1(5)$ at 37°C. Cells were then diluted and plated on TYE plates containing antiserum. Surviving colonies were counted and tested for immunity with λ *virh*80 and λ cI *h*80. Results are expressed as percent of infected cells forming lysogens.

Mutagenesis. CA8000 (λ *h*80) was grown to 2×10^8/ml, centrifuged, and resuspended in an equal volume of TM buffer.

A crystal of nitrosoguanidine (Aldrich) was added and the culture was incubated for 1 hr at 37°C. It was then centrifuged, resuspended in a 0.5 volume of 0.1 M $MgSO_4$, and induced with UV light. An equal volume of LB broth was added, and the induced cultures were shaken at 37°C until lysis occurred.

These lysates were titered and scored for clear-plaque mutants on CA8000 to check the effectiveness of the mutagenic treatment. They were then plated on CA7910 to look for turbid-plaque mutants.

Crosses of λ *h80 with* λ i^{434} *h80 and* λ $i^{21}h80$. CA7910 was grown to 5 × 10^8 cells/ml, centrifuged, and resuspended in an equal volume of 0.01 M $MgSO_4$. The cells were infected at a multiplicity of 5 of each phage, adsorbed for 20 min at room temperature, and diluted into 50 volumes of LB broth. They were then incubated for 2 hr at 37°C and treated with chloroform. Since the turbid-plaque mutants have a high frequency of reversion, each parent of the cross was grown separately in strain CA7910 as a control and the phage progeny were examined. Total phage produced were scored on strain CA7910, and phages having immunity to lambda phage were scored by plating the lysates on strain WD5037. Results are expressed as percent recombinants amongst the total yield.

RESULTS

Growth of phages in *CAP*⁻ and *cya*⁻ mutants

CAP⁻ and *cya*⁻ mutants have a greatly reduced extent of transcription of catabolite-sensitive operons. We have found that the expression of genes of various temperate bacteriophages infecting these strains is also altered.

These phages normally form turbid plaques due to the growth of lysogenic bacteria in the center of the plaques. All of the phages tested form clear plaques on the *CAP*⁻ and *cya*⁻ strains (Table 2). However, these phages form turbid plaques on the *cya*⁻ strain when cAMP is added to the plate, while when 5′-AMP is added, the phages still form clear plaques. cAMP, as expected, has no effect when added to the *CAP*⁻ or *CAP*⁻*cya*⁻ mutants.

Although the phages form clear plaques, it is possible to obtain lysogens from all of the mutant bacterial strains. These lysogens, on repeated isolation and purification, are stable, and the amount of free phage in these lysogenic cultures is the same as in the cultures of lysogens of the parent

TABLE 1. *Bacterial and phage strains used and their derivation*

Bacteria	Description	Origin
CA8000	HfrH prototroph Sm^s	J. G. Scaife (7)
CA7901	*CAP*⁻	Mutagenesis of CA8000 (9)
Other *CAP*⁻ (CA7900, CA7904, CA7905, CA7906)	*CAP*⁻	Mutagenesis of CA8000 (9)
CA7902	*cya*⁻ (ochre)	Mutagenesis of CA8000 (9)
Other cya⁻ (CA7903, CA7904)	*cya*⁻	Mutagenesis of CA8000 (9)
X7901	F⁻(Δ*prolac*) *trp*⁻*CAP*⁻Sm^R	J. R. Beckwith (7)
CA7910	*cya*⁻*CAP*⁻Sm^R	CA7902 transduced to *CAP*⁻Sm^R with P1 lysate derived from X7901 (T. Grodzicker, unpublished result)
WD5021	F⁻$gal_{1,2}$⁻Sm^R*su*⁻	From I. Herskowitz
WD5037	WD5021 (λi^{434}*Q*73)	From I. Herskowitz
WD5066	WD5021 (λi^{21}*Q*73*Q*501)	From I. Herskowitz
1100	HfrH Sm^s	R. Perlman (8)
PP78	HfrH cya⁻*CAP*⁻SM^R	From 1100 (R. Perlman)
QD5007	*su*III⁺ *pro*⁻ Sm^R	From E. Signer
*rif*ʳ*cro*ˢ	Sensitive to λcI857*h*80 *cro*⁻ at 30°C, rifamycin resistant	Mutagenesis of QD5007
Phage		
λ*h*80	—	From J. R. Beckwith
λcI857*ind*⁻*h*80	—	From J. R. Beckwith
λi^{434}*h*80	—	From E. Signer
λi^{21}*h*80	—	From E. Signer
φ80	—	From J. Beckwith
λcI857*cro*⁻27*h*80	—	From cross of λcI857*cro*⁻27 and λi^{434}*h*80
λcI857*cro*⁻27*h*80	—	N. Franklin

TABLE 2. *Properties of various phages on CAP⁻ and cya⁻ strains*

	Bacterial strains							
	CA8000 Plaque morphology		CA7901(*CAP*⁻)		CA7902(*cya*⁻)		CA7910(*CAP*⁻*cya*⁻)	
Phage	−cAMP	+cAMP	−cAMP	+cAMP	−cAMP	+cAMP	−cAMP	+cAMP
φ80	*t*	*t*	*c*	*c*	*c*	*t*	0*	0
λ*h*80	*t*	*t*	*c*	*c*	*c*	*t*	*c*	*c*
λcI857*h*80 32°C	*t*	*t*	*c*	*c*	*c*	*t*	*c*	*c*
37°C	*c*	*c*	*c*	*c*	*c*	*c*	*c*	*c*
λi^{434}*h*80	*t*	n.d.	*c*	n.d.	*c*	n.d.	*c*	n.d.
λi^{21}*h*80	*t*	n.d.	*c*	n.d.	*c*	n.d.	*c*	n.d.

t, forms turbid plaques; *c*, forms clear plaques; 0, does not plate; n.d., not done.

All phages are *h*80, since λ does not plate on *CAP*⁻ and *cya*⁻ strains, which are maltose negative and do not adsorb λ.

* It is not clear why φ80 fails to plate on CA7910, since it does plate on derivatives of this strain.

TABLE 3. *Frequency of lysogenization by λh80*

Bacterial strain	% Lysogens among infected cells −cAMP	+cAMP
CA8000	21	23
CA7901(*CAP*⁻)	5	6
CA7902(*cya*⁻)	6	19
CA7910(*CAP*⁻*cya*⁻)	3	3

wild-type bacteria (Arditti, unpublished). Thus, the maintenance of phage repression does not seem to be affected. As expected from their clear plaque morphology, the proportion of λ *h80* and λc1857*h80* phages lysogenizing is decreased in the *CAP*⁻*cya*⁻ mutant as compared to the wild-type parental strain (Table 3).

In summary, when the temperate phages tested infect *E. coli* mutants that lack CAP and/or cAMP, lysogenization is infrequent.

Behavior of λ_{cro}^- mutants on *CAP*⁻ and cyclase⁻ strains

λ *cro*⁻ mutants cannot form a plaque under conditions where active *c*I repressor is made. Thus λc1857 *cro*⁻ phage form plaques at 40°C, where the repressor is nonfunctional, but do not plate at 33°C where repressor is active (5). However, mutations that abolish the *c*II and *c*III functions also permit the *cro*⁻ phage to plate, even under conditions permissive for repressor (5). This is not surprising, as *c*II and *c*III are both required for initiation of repressor synthesis. To see whether or not the CAP protein–cAMP system is required for induction of repressor synthesis, we tested the plating ability of λc1857 *cro*⁻$_{27}$*h80* strains on *CAP*⁻, *cya*⁻, and *CAP*⁻*cya*⁻ hosts at 33°C (where they cannot form plaques on the parent strain). As seen in Table 4, the absence of the CAP protein–cAMP system allows the *cro*⁻ phage to plate. This finding strongly suggests that this system is important for induction of λ repressor.

Other host mutations affecting lysogenization by λ

The fact that the *cro*⁻ phenotype is suppressed on *CAP*⁻ or *cya*⁻ hosts suggests a selection for other host mutations that affect the synthesis of λ repressor. We have picked bacterial mutants that permit λc1857*cro*⁻$_{27}$*h80* to form plaques at 33°C. Mutagenized bacteria were plated on TYE plates and, after 24 hr, the colonies were replicated to plates seeded with 10^9 λc1857*cro*⁻$_{27}$*h80* phage. Those colonies showing lysis were picked and tested for the ability to plate the λc1857*cro*⁻$_{27}$*h80* phage. *Cro*-sensitive mutants were also picked from among the rifamycin-resistant population by plating of the mutagenized bacteria on TYE plates containing 65 μg/ml of rifamycin. The mutants were scored for *CAP* function by their inability to grow on glycerol, maltose, or lactose as carbon source, their insensitivity to added cAMP, and their linkage to the *str* (streptomycin) A gene. Mutants that were unable to grow on the sugars mentioned above except in the presence of cAMP were scored as *cya*⁻. In addition to *CAP*⁻ and *cya*⁻ mutants, we found a third class of mutants that map at the *rif* (rifamycin) locus (15). These mutants grow poorly on sugars other than glucose, and are partially resistant to phages with λ host specificity. However, phages with φ80 host specificity plate well and are as clear as they are on *CAP*⁻ or *cya*⁻ strains.

TABLE 4. *Growth of λc1857cro*⁻$_{27}$*h80*

Bacterial strain	Efficiency of plating 33°C	37°C
CA8000	3×10^{-5}	1
CA7901(*CAP*⁻)	0.42	0.96
CA7902(*cya*⁻)	0.23	1.02
CA7910(*CAP*⁻*cya*⁻)*	0.01	0.01
1100	1.2×10^{-5}	1
PP 78(*CAP*⁻*cya*⁻)	0.15	1.10
QD5007	4.6×10^{-5}	1
Rif^r*cro*^{s}	0.67	0.92

* λc1857*cro*⁻$_{27}$*h80* will not grow on CA7910 at any temperature; it is not clear why not, as the phage grows on PP 78, another *CAP*⁻ *cya*⁻ strain.

Properties of turbid-plaque phage mutants

Since the temperate phages studied form clear plaques on *CAP*⁻ and *cya*⁻ strains, by isolating turbid-plaque mutants on such strains, one should obtain phage that have lost the CAP protein and cAMP requirement for efficient lysogenization. In fact, we have obtained λ*h80* mutants that have the expected properties.

The λ*h80* mutants (*t* mutants) were isolated as phages able to form turbid plaques on the *CAP*⁻*cya*⁻ strain. We isolated 10 independent mutants by nitrosoguanidine treatment. These mutants fall into two classes (Table 5). Class I turbid-plaque mutants either fail to plate or form only minute plaques on wild-type strains, while the class II mutant forms extremely turbid plaques. Both class I and class II mutants give stable lysogens at extremely high efficiency (>70%) on these strains.

Although the *t* phage mutants do not enter the lytic cycle upon infection of a wild-type host, when the lysogens that they form are treated with UV radiation, they are efficiently induced and yield high titer phage lysates. Since upon induction, these phages grow normally, this suggests that their failure to form plaques upon infection is not due to a defect in any essential phage gene required for lytic growth.

Both class I and class II turbid-plaque mutants revert to wild-type λ*h80* at extremely high frequency (about 0.5%). The revertants form clear plaques on the *CAP*⁻*cya*⁻ strain and form normal turbid plaques on the wild-type parent. However, pure mutant *t*-phage lysates (<10^{-4} revertants) can be obtained by induction of a lysogen, so that the phage undergo only one cycle of growth.

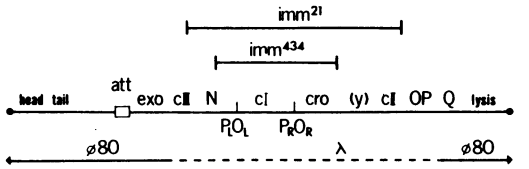

FIG. 1. Vegetative map of λ–*h80* hybrid. The *arrows* indicate the genetic origin of the portions of the λ *h80* genome. *Imm*^{434} indicates the segment of 434 present in λ *i*^{434}, while *imm*^{21} indicates the segment of 21 present in λ *i*^{21}. The *c*I gene is the structural gene for the λ repressor. *c*II and *c*III gene products are necessary for efficient lysogenization. The *cro* gene product prevents the synthesis of repressor during phage growth. For an explanation of the rest of the markers, see ref. 18.

TABLE 5. *Properties of turbid-plaque mutants*

λ *h*80 *t* Mutants	Number isolated	Host			Derivation
		CA7910(*CAP*$^-$*cya*$^-$)	CA8000 (wild type)	WD5037	
Class I (t3, *t*11, *t*12, *t*15, *t*20, *t*21, *t*23, *t*24, *t*25)	9	*t*	0	Tiny plaques	Nitrosoguanidine mutagenesis of CA8000 (λ *h*80)
Class II (*t*18)	1	Semi-turbid	Very turbid	Very turbid	
$i^{434}h80$ *t* recombinants					
Class I (*t*23)	9	*t*	0	0	Recombination between
(*t*24)	13	*t*	0	0	λ*t*23 *h*80 x $\lambda^{i434}h80$ λ*t*24 *h*80

λ *t* *h*80 mutants were obtained at a frequency of 0.1%. This is the same frequency at which clear mutants are found by screening on CA8000.

The $\lambda^{i434}t$ *h*80 recombinants were found among the progeny of the cross, at a frequency of 0.5%

In order to determine whether the mutations leading to turbid-plaque formation mapped in the region of nonhomology between λ and λi^{434} and λ and λi^{21}, the *t* mutants were crossed with $\lambda i^{434}h80$ and $\lambda i^{21}h80$. If the *t* mutation lies outside the nonhomology region, two types of recombinants are expected, namely wild-type λ*h*80 and λi^{434} *t* *h*80 or λi^{21} *t* *h*80. If the *t* mutation lies within the regions of nonhomology of these phages, only λ*h*80 revertants can be produced, with no recombinants.

Crosses were performed with two class I mutants and the class II mutant (*Methods*). As seen in Table 5, λi^{434} *t* *h*80 recombinants were obtained at a frequency of about 0.5% from crosses of the class I mutants with $\lambda i^{434}h80$. The λi^{434} *t* *h*80 recombinants formed smaller turbid plaques than did the λ *t* *h*80, just as λi^{434} *h*80 forms a smaller clear plaque on the *CAP*$^-$-*cya*$^-$ strain than does either λ *h*80 or λi^{21} *h*80 phage. In the case of the class II mutant, no such recombinants were observed although the scoring procedure would have detected 0.1% recombination. The proportion of λ *h*80 phages among the progeny of the cross was not significantly different from the reversion frequency. The λi^{434} *t* *h*80 recombinants have all the expected properties listed in Table 5. In addition, like the λ *t* *h*80 mutants, they lysogenize wild-type strains at high efficiency, produce pure, high-titer phage lysates upon UV induction, and revert to wild-type $\lambda i^{434}h80$ at the usual high frequency. Thus, the mutations of the two *t* phages of class I tested map outside the region of nonhomology between λ and λi^{434}, while the class II mutation maps either within or very close to this region.

Similar crosses with λi^{21} *h*80 failed to yield either λ*h*80 recombinants in excess of the reversion frequency or λi^{21} *t* *h*80 recombinants. Since the *t* phage mutants are screened on the *CAP*$^-$*cya*$^-$ strain, if the λi^{21} *t* *h*80 recombinant did not look appreciably different from the λ *t* *h*80 parent, it would go undetected.

DISCUSSION

We have shown that CAP protein and cAMP, factors necessary for the transcription of catabolite-sensitive bacterial operons, are also required for efficient establishment of lysogeny. In the absence of CAP protein and cAMP, infecting phages tend to enter the lytic cycle. λ *cro*$^-$ mutants, which produce excess repressor and thus fail to plate on wild-type strains, produce plaques on *CAP*$^-$ and *cya*$^-$ strains.

To identify the phage site sensitive to CAP protein and cAMP, we isolated turbid-plaque mutants of λ *h*80 that overcome this requirement. CAP protein and cAMP might act directly on this site or through a CAP protein–cAMP controlled bacterial gene product. If CAP protein and cAMP are directly involved, the simplest explanation of their effects is that they act directly as transcription initiation factors at the promoter of some phage operon, by analogy with their function in bacterial transcription. If, on the other hand, the CAP protein–cAMP effect is mediated through a CAP protein–cAMP controlled bacterial product, it could affect either the transcription or translation of mRNA from λ phage or the activity of a λ gene product. Evidence that suggests that CAP protein and cAMP act directly derives from the observation that when one selects bacterial mutants that can support the growth of λ *cro*$^-$ phage, the majority of the bacteria are mutant in either adenylate cyclase or CAP protein.

Furthermore, the other class of *cro*$^-$-suppressing host mutations, which are linked to *rif*^{r}, behave as though they have an altered response to cAMP. They grow extremely slowly on sugars other than glucose, and do not respond to added cAMP. It is possible that these are mutations in the host RNA polymerase that prevent the enzyme from interacting with CAP protein and cAMP.

What stage of phage development is sensitive to CAP protein and cAMP? Our results suggest that the initiation of repressor synthesis requires cAMP and CAP protein. Steady-state repressor synthesis can occur in the presence or absence of CAP protein or cAMP. Initiation of repressor synthesis upon infection requires the products of genes *c*II and *c*III (4–6), and it is probable that transcription begins in the *y* region of the phage genome (1, 4, 6, 16). Since the *t* mutation in two of the turbid-plaque mutants of class I maps close to, but outside of, the λ immunity region, these could be mutations affecting *c*II or *c*III activity. They clearly do not map in (*a*) the promoter of the operons containing *c*II or *c*III; (*b*) the *c*I promoter, which functions in the lysogenic state or; (*c*) the *cro* gene, because all of these sites lie within the immunity region (5, 6, 17). These mutations might represent the introduction of new promotors at the *c*II–*c*III-dependent site in the *y* region. Further mapping and analysis should precisely define

their location, as well as determine whether or not these mutations lie in the site directly controlled by the CAP protein/cAMP effect.

The results presented here demonstrate that the physiological state of the host cell influences the decision of the lambdoid phages to lysogenize or to grow lytically. This influence is at least partially mediated by CAP protein and its effector, cAMP.

We thank Ira Herskowitz for stimulating discussions and for providing some of the bacterial and phage strains used, and acknowledge helpful conversations with Drs. Bruce Konrad and Jon Beckwith. We are grateful to Ronnie MacGillivray for excellent and cheerful assistance. H. E. acknowledges conversations with J. Hong, G. Smith, and R. Ames, whose findings for P22 phage prompted his investigations of the effects of cAMP on λ phage. This work was supported by grants to J. Beckwith from the National Science Foundation and the American Cancer Society, and to H. Eisen from the National Institutes of Health. T. G. was a postdoctoral fellow of the National Institutes of Health.

1. Eisen, H. A., Pereira da Silva, L. & Jacob, F. (1968) *C. R. H. Acad. Sci.* **266**, 1176–1178.
2. Calef, E. & Z. Newbauer (1968) *Cold Spring Harbor Symp., Quant. Biol.* **33**, 765–767.
3. Kaiser, A. D. (1957) *Virology* **3**, 42–61.
4. Echols, H. and Green, L. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2190–2194.
5. Eisen, H. A., Brachet, P., Pereira da Silva, L. & Jacob, F. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 855–862.
6. Reichardt, L. & Kaiser, A. D. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2185–2189.
7. Zubay, G., Schwartz, D. & Beckwith, J. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 104–110.
8. Emmer, M., De Crombrugghe, B., Pastan, I. & Perlman, R. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 480–487 (1970).
9. Schwartz, D. & Beckwith, J. (1970) in *The Lactose Operon*, ed. Zipser, D. & Beckwith, J. (Cold Spring Harbor Laboratory), pp. 417–422.
10. Perlman, R. L. & Pastan, I. (1969) *Biochem. Biophys. Res. Commun.* **37**, 151–157.
11. Eron, L., Arditti, R., Zubay, G., Connaway, S. & Beckwith, J. R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 215–218.
12. Silverstone, A. E., Arditti, R. R. & Magasanik, B. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 773–779.
13. Silverstone, A. E., Magasanik, B., Reznikoff, W. S., Miller, J. H. & Beckwith, J. R. (1969) *Nature* **221**, 1012–1014.
14. Gottesman, S. & Beckwith, J. (1969) *J. Mol. Biol.* **44**, 117–127.
15. These mutants appear to be similar to *rif*^{r} mutants isolated by Hong, Smith, and Ames, (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2258–2262.
16. Brachet, P. & Thomas, R. (1969) *Mutat. Res.* **7**, 257–260.
17. Eisen, H. A., Fuerst, C. R., Siminovitch, L., Thomas, R., Lambert, L., Pereira da Silva, L. & Jacob, F. (1966) *Virology* **30**, 224–241.
18. Herskowitz, I. & Signer, E. (1970) *Cold Spring Harbor Symp., Quant. Biol.* **35**, 355–368.