Proc. Nat. Acad. Sci. USA
Vol. 68, No. 9, pp. 2258–2262, September 1971

Adenosine 3′:5′-Cyclic Monophosphate Concentration in the Bacterial Host Regulates the Viral Decision between Lysogeny and Lysis

(*Salmonella typhimurium*/P22 phage/adenylate cyclase/RNA polymerase)

JEN-SHIANG HONG, GERALD R. SMITH, AND BRUCE N. AMES

Department of Biochemistry, University of California, Berkeley, Calif. 94720

Communicated by Paul Berg, June 25, 1971

ABSTRACT **Mutants of *Salmonella typhimurium* defective in adenylate cyclase (*cya* gene) or in cAMP receptor protein (*crp* gene) are lysogenized at reduced frequency by phage P22. One class of the bacterial mutants with an altered RNA polymerase (*rif* gene) is also lysogenized at reduced frequency. In the three types of mutant bacteria, the phage's decision between lysogeny and lysis is shifted to lysis and the phage form clear plaques. We propose that in wild-type bacteria the cAMP-receptor protein, in combination with cAMP, activates bacterial RNA polymerase to transcribe certain phage genes that are required for efficient lysogenization. Under conditions of strong catabolite repression, when the supply of energy and biosynthetic components is abundant and the concentration of cAMP is low, the phage would multiply and lyse the cell. When the supply of energy is deficient and the concentration of cAMP is high, the phage would lysogenize the cell.**

Phage mutants have been isolated that form turbid plaques on the three classes of bacterial mutants due to a higher frequency of lysogeny. These phage mutants have been shown by complementation to be defective in the same gene, which we have called the *cly* gene. These *cly* mutants lysogenize the wild-type bacteria with a 99% frequency and, thus, do not form plaques on them.

Other kinds of bacterial mutants are also lysogenized at reduced frequency by phage P22. They may be altered in other physiological control systems that influence the frequency of lysogenization.

When a temperate bacteriophage such as P22 or λ infects a bacterial cell, a decision is made during the first few minutes that results in either lysogeny or lysis. In lysogeny by these phages, the phage chromosome is incorporated into the bacterial chromosome as a dormant prophage. In lysis, the phage multiplies and produces a burst of progeny. For several bacterial viruses, the decision process is influenced by the physiological state of the bacteria (1–7).

Here we investigate the influence of the physiological state of the host bacteria on the lysogenization of *Salmonella typhimurium* by phage P22.

MATERIALS AND METHODS

Bacterial strains

Adenylate Cylase: cya Mutants. Mutants unable to grow on many carbon sources were isolated by T. Klopotowski in this laboratory, and were reported as being cotransducible with *hisR* and *metE* on the *S. typhimurium* chromosome (8). The same class of mutants was later isolated by Berkowitz (9) and by Yokota and Gots (10) in the same organism. These latter workers have reported that their mutants, called *cya*, respond to added cAMP, lack adenylate cyclase, and map next to *metE*. Adenylate cyclase mutants have also been isolated in *Escherichia coli* (11, 12).

We have isolated *cya* mutants in a similar way. Diethylsulfate-treated *thi-523* cells were plated on MacConkey indicator agar containing 1% D-mannitol–1% glycerol, and the plates were incubated overnight at 37°C. White colonies were isolated and a number of these were unable to grow on D-maltose, glycerol, D-xylose, or D-mannitol as carbon source, even though they grow on glucose. cAMP restores their ability to utilize these sugars. One of these mutants, TA2300 (*thi-523 cya-408*), was transduced to Thi^+ on minimal glucose medium with phage grown on wild-type strain LT-2 to obtain the resulting strain TA2301 (*cya-408*). The mutation *cya-408* is 47% linked to *hisR1223*. Phage P22 forms clear plaques on all of the *cya* mutants we have tested. Phage P22 forms turbid plaques on all the Cya^+ transductants tested (25/25). The mutation *cya-409* (TA2314 = *thi-523 cya-409*) causes a temperature-sensitive phenotype. P22 forms clear plaques on TA2314 at 42°C and turbid plaques at 25°C.

cAMP-Receptor Protein: crp Mutants.* A class of mutants, similar to *cya* in phenotype but not curable by cAMP, was isolated in *S. typhimurium* (9) and in *E. coli* (13, 14). Berkowitz (ref. 9, and personal communication) has shown that the *crp* gene of *S. typhimurium* is cotransducible with both *strA* (40%) and *cysG* (16%). In *E. coli* the gene has been named *crp* (15) or *cat* (12) and is in the homologous position, cotransducible with *str* (15). *E. coli* mutants have been shown to lack the cAMP-receptor protein (13, 14). The *crp* mutants that we have used were derived from wild-type *S. typhimurium* LT-2 by the same selection procedure described for *cya* mutants and have the same phenotype, except that they do not respond to cAMP. The mutation *crp-403* is 16% linked (14/86) to *cysG439*, in agreement with the mapping data of Berkowitz. P22 forms clear plaques on all 14 Cys^+Crp^- transductants tested and turbid plaques on all 27 Cys^+Crp^+ transductants tested.

Isolation of RNA Polymerase Mutants: the rif Gene. Rifampicin-resistant mutants (16–19) have an RNA polymerase with an altered β subunit (20), and are located on the *E. coli* chromosome in the *arg–thi* region (18, 19).

Our rifampicin-resistant mutants of mutant *thi-502* were isolated on minimal-glucose plates containing 50 μg/ml of

* This is probably the same protein that has been called catabolite gene activator protein (37).

rifampicin and 10 μg/ml of thiamine. Phage P22 formed clear plaques on one class of rifampicin-resistant mutants (about 3%) (e.g., *thi-502 rif-39*), which was distinguished by a wrinkled colony morphology and slow growth (2.5-times slower than wild type) on nutrient broth and minimal glucose–thiamine medium. These properties are present in strain *rif-39*, made by transducing the *rif-39* mutation into *argF88*. Moreover, when *thi-502* was transduced out with phage grown on *rif-39*, the rifampicin-resistant transductants 15/15 showed clear plaques, while the rifampicin-sensitive transductants (25/25) showed turbid plaques. Phage P22 forms turbid plaques on smooth rifampicin-resistant mutants that were tested (both slow- and fast-growing).

We have evidence for the gene order *argF-rif-thi*. Mutant *rif-13* (a smooth rifampicin-resistant mutant) is 4% linked (6/150) to *argF88* and 23% linked (12/53) to *thi-502*. The *argF88* mutation is less than 0.7% linked (0/165) to *thi-502*. Five other rifampicin-resistant mutants (including *rif-39*) have similar mapping properties.

The *rif-39* mutant has an altered RNA polymerase that is more resistant to rifampicin than is the wild type, as shown in Fig. 1.

Phage strains

Wild-type phage P22 and its derivative m_3*sie*-1 (made by M. Gough) (22) were obtained from D. Botstein, and amber mutants H200 and H201 (23) from John Roth. Mutant P22 *int-4* (24) was used for transductions.

Isolation of cly Mutants of P22. An exponential culture of LT-2 in nutrient broth was infected with P22 m_3*sie*-1 (10^6 phage per 10^8 cells per ml); 5 min after infection, 2 μg/ml of *N*-methyl-*N'*-nitro-N-nitrosoguanidine was added. After lysis, chloroform was added and cell debris was removed by centrifugation. The phage was diluted and mixed with *rif-39*, or *cya-408* or *cya-408 rif-39*, on a nutrient-broth plate. After incubation, 12 hr at 37°C, turbid plaques (0.02%) were picked and purified by streaking on the same hosts. Mutants *cly-2*, and *cly-3* were isolated on the *rif-39* strain. Mutant *cly-11* was isolated on *cya-408 rif-39*.

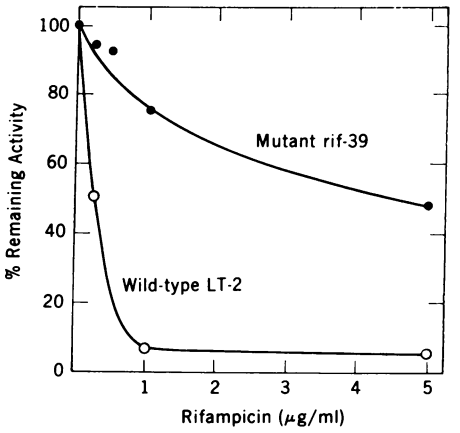

FIG. 1. The effect of rifampicin on RNA polymerase activity of wild-type LT-2 and mutant *rif-39*. The crude extract was prepared by grinding 2.5 g of frozen cells with 5 g of alumina, followed by extraction with buffer A of Berg *et al.* (21). The mixture was centrifuged at 10,000 × *g* for 10 min and the resulting crude extract was used for assay as sources of RNA polymerase and DNA. The assay was performed at 37°C for 10 min as described (21) with [^{3}H]-CTP (Schwartz 29 Ci/mol) as label, except that 20 mM potassium phosphate buffer (pH 7.8) was added to inhibit polynucleotide phosphorylase and no DNA was added. Rifampicin and the crude extract were incubated at 25°C for 10 min before being added to the reaction mixture. 100% activity represents 1400 ± 20 cpm. The control value, without ATP, GTP, and UTP, was 430 ± 10 cpm, and has been subtracted.

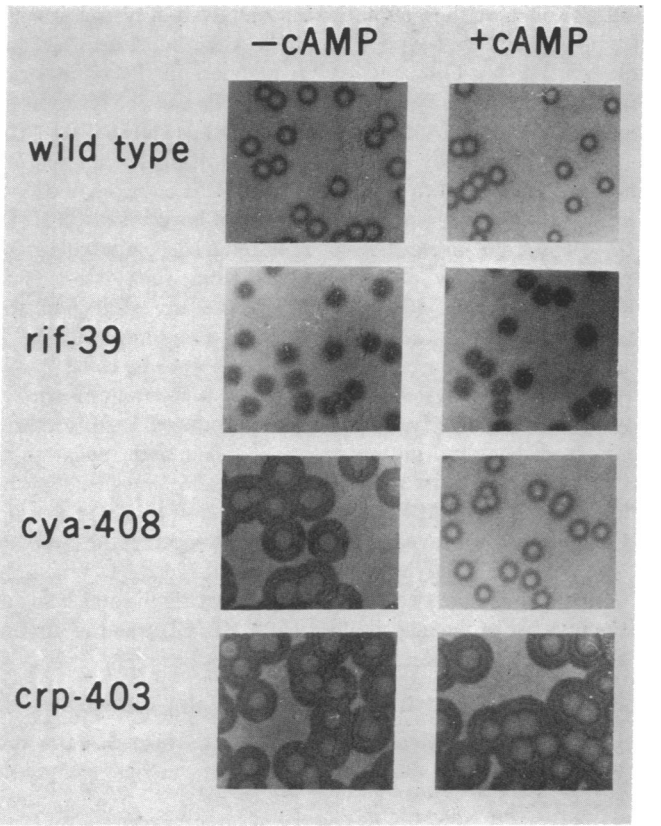

FIG. 2. Phage P22 plaque morphology on bacterial mutants.

Media

Nutrient broth (Difco) or minimal salts medium E (25) with 2% glucose was supplemented with 1.5% agar for plates. All phage were stored in nutrient broth or in T_2 buffer (26).

RESULTS

Bacterial mutants lysogenized at reduced frequency

During a study of *S. typhimurium* mutants with altered RNA polymerase, we observed that phage P22 forms clear plaques on certain mutants resistant to the antibiotic rifampicin. As we will show later, P22 forms clear plaques because these mutants are lysogenized at a much lower frequency than are wild-type cells. There are, thus, fewer lysogens growing in the center of the plaque to give it a turbid appearance.

This observation led us to consider how bacterial physiology might alter the frequency of lysogenization, and we examined mutants concerned with catabolite repression. We found that P22 also forms clear plaques on adenylate cyclase mutants (*cya* gene) and on cAMP-receptor protein mutants (*crp* gene). The plaque morphology of P22 plated on one example of each of the three classes of bacterial mutants on nutrient broth plates is shown in Fig. 2. Addition of cAMP (1 mM) to the plate restores the turbid plaque morphology on *cya* mutants, which cannot make cAMP, but not on the other classes.

In Table 1 we show a quantitative analysis of lysogenization frequency by wild-type P22 phage that substantiates our observations on clear and turbid plaques. In the *rif* and *cya* mutants, less than 5% of the infected cells become lysogens at low multiplicity of infection. Boyd (28) has shown that lysogenization frequency is dependent on multiplicity of infection and this is also shown clearly in Table 1. At either high or low multiplicity of infection, the frequency of lysogenization of the three classes of mutant bacteria is reduced compared to the wild type. Addition of 1 mM cAMP to an adenylate cyclase mutant (*cya-408*) substantially restores the ability of the phage to lysogenize, consistent with the observations in Fig. 2.

The effect of the bacterial mutations appears to be on the establishment of lysogeny, rather than on the maintenance of lysogeny. The rare lysogens that are produced from the three classes of bacterial mutants were analyzed and appear to be just as stable as the lysogens of the wild-type cells: they release phage at about the same rate and grow at the same rate. Furthermore, we have made (at 25°C) a lysogen of the temperature-sensitive adenylate cyclase mutant TA2314. When the temperature was raised to 42°C, the culture did not lyse and the number of phage produced (5×10^3/ml) was the same as that produced by a wild-type lysogen.

Phage mutants with increased ability to lysogenize

Phage mutants with a decreased ability to lysogenize the wild-type host bacteria and that, therefore, form clear plaques on them have been described in many phage systems including P22 (27, 29). We have isolated mutants of phage P22 which, by lysogenizing with increased frequency, counter-balance the defect in the mutant host bacteria and produce turbid plaques on them. These phage mutants were isolated as turbid-plaque formers on the different classes of bacterial mutants and also on the double mutant TA2311 (*rif-39 cya-408*). In the next section, we show that these phage mutations are all in the same gene, which we call the *cly* gene (for control of *ly*sogenization). Table 1 shows that the *cly* mutation of the phage causes an increased frequency of lysogenization, counterbalancing the alteration in the bacterial mutants, and this permits the mutant phage to produce a turbid plaque. The *cly* mutants lysogenize the wild-type bacteria at such a high frequency (99%) that they do not form plaques on this host and thus are defective mutants in the sense that none of them enters the lytic cycle.

Table 2 summarizes the results of an experiment with three different *cly* mutants on the various bacterial hosts at two temperatures. As seen in Table 2, the *cly-2* mutation (which is representative of the most frequent type of mutation found) has a temperature-dependent phenotype. At low temperature, a phage carrying this mutation behaves like wild-type phage; at high temperature, it lysogenizes all of the hosts with increased frequency. On those hosts where wild-type phage forms turbid plaques at 40°C, *Cly-2* forms no plaques. The *cly-3* mutation causes a higher frequency of lysogenization. The *cly-11* mutation results in an even more drastically altered function; at 40°C it is able to form plaques only on the doubly mutant host, *rif-39 cya-408*.

We have found that all of the *cly* mutations isolated fall into one complementation group. 10 mutants isolated as turbid-plaque mutants on *rif-39*, one mutant isolated on *cya-408* and one mutant isolated on the double mutant *rif-39 cya-408* were tested two by two for complementation by mixed infection of wild-type host at 40°C. Under these conditions, no phage by itself is able to grow and produce plaques. Under mixed infection conditions, there is still no phage growth, indicating that none of the mutants can supply the function missing in any of the other mutants. As a control, mixed infection with wild-type phage or with a mutant with an amber mutation in gene 2 (head-protein synthesis) (23) produced phage growth in all

TABLE 1. *Frequency of lysogenization*

	Phage per cell	Lysogenization among infected cells* (%)			
		Host bacteria			
		Wild type	*rif-39*	*crp-403*	*cya-408*
Phage					
P22	15	97	36	80	48 (81†)
P22	1.5	62	2	14	5 (22†)
Phage‡					
cly$^+$	1.5	59	7	11	4
cly-2	1.5	99	75	64	33

* The standard method (4, 27) was modified as follows. 10 min after addition of phage to exponentially growing bacteria in nutrient broth adsorption was complete (99%); the complexes were then diluted 1:10 into broth (at 37°C) containing sufficient P22 anti-serum (Cappel Laboratories, Downingtown, Pa.) to inactivate (99%) the remaining free phage. After 5 min, the mixture was diluted in T_2 buffer at 25°C, a sample was mixed with 10^8 cells of SU219 [*gal-50 hisD23* (HfrB2)] as indicator, and was plated with top agar on EMB-galactose (4). The plates with wild-type P22 were incubated at 37°C and those with *cly*$^+$ and *cly-2* at 25°C. After incubation for 1–3 days, the response of the indicator was scored as: (*a*) *lysogens*, appearing as large colonies with a halo of lysis of the indicator cells, (*b*) *lytic centers*, appearing as plaques on the indicator cells without a colony in the center, and (*c*) *uninfected cells*, appearing as large colonies without a halo of lysis. The results are reported as $a/(a + b)$. In most cases, 150–250 cells were scored.

† In the presence of 1 mM cAMP, which was present during both growth and dilution.

‡ P22 that carries mutations (m_3, affecting plaque morphology, and *sie-1*, abolishing superinfection exclusion) that do not affect lysogenization.

TABLE 2. *Lysogenization by cly mutants*

Bacterial host	Temp, °C	Plaque morphology*			
		Phage			
		Parent	*cly-2*	*cly-3*	*cly-11*
wild-type	25	turbid	turbid	0	0
	40	turbid	0	0	0
rif-39	25	clear	clear	turbid	turbid
	40	clear	turbid	turbid	0
crp-403	25	clear	clear	0	0
	40	clear	turbid	0	0
cya-408	25	clear	clear	0	0
	40	clear	turbid	0	0
rif-39 cya-408	25	clear	clear	clear	clear
	40	clear	clear	clear	turbid

* The nutrient-broth plates were incubated for 14–20 hr at 25°C or 40°C.

0 = no plaque.

cases. This shows that normal complementation occurs under these conditions and that the *cly* mutations are recessive to *cly*$^+$. The *cly* gene has been located in the region controlling phage immunity (27) in the order, c_3-c_2-c_1-*cly*-gene 12. This result will be reported in a separate study of this gene.

The preceding results show that the phage *cly* function is required for lytic growth on wild-type bacteria. We have determined that it is not required for lytic growth after induction by inducing wild-type bacteria, lysogenic for P22 *cly-3* or P22, by ultraviolet irradiation. The yields of phage were comparable, and both gave a 10^5-fold increase over unirradiated lysogens.

Other classes of bacterial mutants

We have observed that phage P22 forms clear plaques on the pyridoxine-requiring mutants *pdx-510* and TK 26. With both of these mutants, the addition of 0.03 mM pyridoxine to the nutrient broth plate (which already has sufficient vitamin B_6 for good growth) changed the plaque morphology to turbid, though 2 or 6 mM cAMP had no effect. P22 forms turbid plaques on protrotrophic revertants and transductants of both strains. Quantitative data on the frequency of lysogenization (done as in Table 1) confirm the observations on plaque morphology: *pdx-510* gave 2% lysogenization in unsupplemented nutrient broth and 29% with added pyridoxine. We have also observed that P22 forms clear plaques on a number of other classes of mutants and these are under further investigation.

DISCUSSION

The preferential utilization of a good energy source such as glucose to a poor energy source such as lactose is a result of catabolite repression (30), which is mediated through cAMP (31–34). The concentration of cAMP in the cell is an indicator of the degree of starvation of the bacteria for an energy source, and only when the cAMP concentration is sufficiently high can various operons for the utilization of poor energy sources be activated (9–12). This activation is accomplished through the binding of cAMP to a cAMP-receptor protein (13, 14), which can then bind to DNA at the promoter site (35–38) and activate RNA polymerase (38–41) to transcribe the operons concerned with utilization of poor energy sources. Thus, to transcribe the lactose operon, not only is inactivation of repressor by inducer necessary, but a sufficient concentration of cAMP, which indicates the absence of a better energy source, is required.

We have observed that bacterial mutants with a defective adenylate cyclase (*cya* mutants cannot make cAMP) are lysogenized at reduced frequency by phage P22. If cAMP is added to the bacteria, they can be lysogenized more efficiently. Bacterial mutants with a defective cAMP-receptor protein (*crp* mutants) are also lysogenized very poorly by P22 but, as expected, added cAMP does not restore efficient lysogeny. We interpret this result to mean that, in the wild-type bacteria, the cAMP-receptor protein with cAMP complexed to it is necessary for lysogenization. It is known in several bacterial viruses that the critical protein for the lysogenic response is the phage repressor. By analogy with the necessity of the cAMP-receptor protein–cAMP complex for activating the RNA polymerase at catabolite-repressible operons, we postulate that the transcription of certain phage operon(s) concerned with the synthesis of the phage repressor is activated, directly or indirectly, by the cAMP-receptor protein–cAMP complex. The host RNA polymerase is essential for the transcription of early operons in many phages (42), and our finding that a class of bacterial RNA polymerase mutants also is lysogenized inefficiently supports the idea that the host RNA polymerase plays a role in the lysis–lysogeny decision process. Our interpretation of these results is that a temperate virus, such as P22, uses the cAMP system of the bacteria for its own ends, to decide whether the energy supply is adequate for producing a burst of phage (the lytic response), or whether it is inadequate and thus favors integration into the bacterial chromosome as a dormant prophage (the lysogenic response).

The phage function affected by the *cya*, *crp*, and *rif* mutations appears to be required for the establishment of lysogeny, but not for its maintenance. In phage P22, two genes, c_2 and *mnt*, are required to maintain the prophage in the repressed state (43–45). Two other genes, c_1 and c_3, are required for the establishment of lysogeny, but are not required for its maintenance (43, 44). Stable lysogens of c_2 and *mnt* mutants cannot be formed, while stable lysogens of c_1 and c_3 can be formed, though at reduced frequency (27). We find that in the *rif*, *cya*, and *crp* mutants used here, wild-type P22 behaves like the c_1 or c_3 mutants, in that lysogens are formed at reduced frequency, but the lysogens that are formed are just as stable as lysogens of the wild-type host bacteria. It may be that expression of either the c_1 or the c_3 gene (or both) requires the combination of cAMP and cAMP-receptor protein, as well as the host polymerase. The mechanism in the phage that governs the decision between lysogeny and lysis is quite complicated (46, 47), and the exact point of action of the cAMP–cAMP-receptor protein complex is still unclear.

We have isolated phage mutants with increased ability to lysogenize. When these phages infect a wild-type cell, nearly every phage lysogenizes the host. When they infect the *rif*, *cya*, and *crp* mutants, an increased frequency of lysogenization is also observed. These *cly* mutations, which include some temperature-sensitive types, fall into one complementation group, thus indicating a single locus, which could be either a promoter or a structural gene. The wild-type *cly* gene function appears to be responsible for the prevention of lysogenization, and in this sense opposes the action of the c_1, c_2, and c_3 gene products that promote lysogenization.

In an attempt to establish the generality of the phenomenon of the effect of the cAMP system on phage decision, we have examined a different *Salmonella* phage, L (48). L phage also forms clear plaques on the three mutant strains. We have also communicated our results and interpretations to Dr. H. Eisen, who subsequently informed us that he repeated the findings with phage lambda. T. Grodzicker and R. Arditti have informed us that they made independent observations in *E. coli* on the cAMP system and lysogeny, and that they will publish their results, with Eisen, in a subsequent issue of *Proc. Nat. Acad. Sci. USA*. We suspect that this will be a general finding in temperate phages.

We have observed that phage P22 forms clear plaques on a number of other classes of bacterial mutants. Other bacterial control systems may influence the phage decision process, either directly or by acting through the cAMP system.

We are indebted to J. Roth, D. Botstein, D. Berkowitz, A. D. Kaiser, M. Chamberlin, H. Echols, H. Eisen, and D. Straus for strains and helpful discussions. This work was supported by USPHS grant AM 12092 to B.N.A. J.-S. Hong is a Postdoctoral Fellow of the American Cancer Society and G. R. Smith is a Postdoctoral Fellow of the Helen Hay Whitney Foundation.

1. Lwoff, A., A. S. Kaplan, and E. Ritz, *Ann. Inst. Pasteur Paris*, **86**, 127 (1954).
2. Lieb, M., *J. Bacteriol.*, **65**, 642 (1953).
3. Bertani, G., and S. J. Nice, *J. Bacteriol.*, **67**, 202 (1954).
4. Bertani, L. E., *Virology*, **4**, 53 (1957).
5. Christensen, J. R., *Virology*, **4**, 184 (1957).
6. Lwoff, A., *Bacteriol. Rev.*, **17**, 269 (1953).
7. Bertani, G., *Advan. Virus Res.*, **5**, 151 (1958).
8. Sanderson, K. E., *Bacteriol. Rev.*, **31**, 354 (1967).
9. Berkowitz, D., *J. Bacteriol.*, **105**, 232 (1971).
10. Yokota, T., and J. S. Gots, *J. Bacteriol.*, **103**, 513 (1971).
11. Perlman, R. L., and I. Pastan, *Biochem. Biophys. Res. Commun.*, **37**, 151 (1969).
12. Schwartz, D., and J. R. Beckwith, *The Lactose Operon*, ed. J. Beckwith and D. Zipser (Cold Spring Harbor Laboratory, 1970), p. 417.
13. Zubay, G., D. Schwartz, and J. Beckwith, *Proc. Nat. Acad. Sci. USA*, **66**, 104 (1970).
14. Emmer, M., B. de Crombrugghe, I. Pastan, and R. Perlman, *Proc. Nat. Acad. Sci. USA*, **66**, 480 (1970).
15. Perlman, R., B. Chen, B. de Crombrugghe, M. Emmer, M. Gottesman, H. Varmus, and I. Pastan, *Cold Spring Harbor Symp. Quant. Biol.*, **35**, 419 (1970).
16. Wehrli, W., F. Knusel, and M. Staehelin, *Biochem. Biophys. Res. Commun.*, **32**, 284 (1968).
17. Tocchini-Valentini, G. P., P. Marino, and A. J. Colvill, *Nature*, **220**, 275 (1968).
18. Ezekiel, D. H., and J. E. Hutchins, *Nature*, **220**, 276 (1968).
19. Yura, T., and K. Igorashi, *Proc. Nat. Acad. Sci. USA*, **61**, 1313 (1968).
20. Rabussay, D., and W. Zillig, *FEBS Lett.*, **5**, 104 (1969).
21. Berg, D., K. Barrett, and M. Chamberlin, *Methods Enzymol.*, (Academic press, New York, 1971), Vol. 21, p. 506.
22. Rao, R. N., *J. Mol. Biol.*, **35**, 670 (1968).
23. Lew, K. K., and J. R. Roth, *Virology*, **40**, 1059 (1970).
24. Smith, H. O., and M. Levine, *Virology*, **31**, 207 (1967).
25. Vogel, H. J., and D. M. Bonner, *J. Biol. Chem.*, **218**, 97 (1956).
26. Hershey, A. D., and M. Chase, *J. Gen. Physiol.*, **36**, 39 (1952).
27. Levine, M., *Virology*, **3**, 22 (1957).
28. Boyd, J. S. K., *J. Pathol. Bacteriol.*, **63**, 445 (1951).
29. Kaiser, A. D., *Virology*, **3**, 42 (1957).
30. Magasanik, B., *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 249 (1961).
31. Perlman, R., and I. Pastan, *Biochem. Biophys. Res. Commun.*, **30**, 656 (1968).
32. Ullman, A., and J. Monod, *FEBS Lett.*, **2**, 57 (1968).
33. Goldenbaum, P. E., and W. J. Dobrogosz, *Biochem. Biophys. Res. Commun.*, **33**, 828 (1968).
34. Perlman, R. L., and I. Pastan, *J. Biol. Chem.*, **243**, 3420 (1968).
35. Pastan, I., and R. Perlman, *Proc. Nat. Acad. Sci. USA*, **61**, 1336 (1968).
36. Silverstone, A. E., B. Magasanik, W. S. Reznikoff, J. H. Miller, and J. R. Beckwith, *Nature*, **221**, 1012 (1969).
37. Riggs, A. D., G. Reiness, and G. Zubay, *Proc. Nat. Acad. Sci. USA*, **68**, 1222 (1971).
38. de Crombrugghe, B., B. Chen, W. Anderson, P. Nissley, M. Gottesman, I. Pastan, and R. Perlman. *Nature New Biol.*, **231**, 139 (1971).
39. Varmus, H. E., R. L. Perlman, and I. Pastan, *J. Biol. Chem.*, **245**, 2259 (1970).
40. Arditti, R., L. Eron, G. Zubay, G. Tocchini-Valentini, S. Connaway, and J. Beckwith, *Cold Spring Harbor Symp. Quant. Biol.*, **35**, 437 (1970).
41. Zubay, G., D. A. Chambers, and L. C. Cheong, in *The Lactose Operon*, ed. J. R. Beckwith and D. Zipser (Cold Spring Harbor Laboratory, 1970), p. 375.
42. Calandar, R., *Annu. Rev. Microbiol.*, **24**, 241 (1970).
43. Levine, M., and H. O. Smith, *Science*, **146**, 1581 (1964).
44. Smith, H. O., and M. Levine, *Proc. Nat. Acad. Sci. USA*, **52**, 356 (1964).
45. Gough, M., *J. Virol.*, **2**, 992 (1968).
46. Levine, M., and C. Schott, *J. Mol. Biol.*, in press.
47. Reichardt, L. B., and A. D. Kaiser, *Proc. Nat. Acad. Sci. USA*, **68**, 2185 (1971).
48. Bezdek, M., and P. Amati, *Virology*, **31**, 272 (1967).