JOURNAL OF BACTERIOLOGY, Feb. 1982, p. 694–699
0021-9193/82/020694-06$02.00/0

Vol. 149, No. 2

Physical Mapping and Cloning of Bacteriophage T4 Anti-Restriction Endonuclease Gene

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Received 20 July 1981/Accepted 23 September 1981

We have proposed that the ability of T4 to produce non-glucosylated progeny after a single cycle of growth on a *galU rglA rglB*$^+$ mutant of *Escherichia coli* is due to the inhibition of the *rglB*$^+$ function by a phage-coded, anti-restriction endonuclease protein. Based on this hypothesis, we screened T4 deletion mutants for failure to give a burst in this host. The absence of an *arn* gene in phage mutants lacking the 55.5- to 58.4-kilobase region is verified by their inability to protect secondary infecting non-glucosylated phage from *rglB*-controlled cleavage. A functional *arn* gene was cloned on plasmid pBR325, and the 0.8-kilobase insert DNA was shown to be homologous to the DNA missing in the *arn* deletion phage.

Non-glucosylated (Glu$^-$) T-even phage are restricted by wild-type *Escherichia coli*, unlike wild-type glucosylated (Glu$^+$) phage. This restriction is specific for hydroxymethylcytosine (HMC) DNA (cytosine residues of T4 DNA are replaced by HMC) and is controlled by two distinct *E. coli* genes, *rglA* and *rglB* (formerly r_6 and $r_{2,4}$ [20–22]). The *rglA*-coded activity appears to be located in the membrane (9) and recognizes sites in all Glu$^-$ T-even phage (20). In contrast, the *rglB*-coded activity is located in the cytoplasm (9, 12) and recognizes sites in T2 and T4 Glu$^-$ DNA but not in T6 Glu$^-$ DNA (20). These two activities seem to be endonucleolytic (9, 12) and to cleave a small number of sites (9) in the Glu$^-$ T4 genomes.

T-even phage grown in *galU* strains of *E. coli* are non-glucosylated since the glucose donor, uridine diphosphoglucose, is not synthesized in these mutants (13, 23, 27). It was reported earlier (13, 23) that although parental Glu$^-$ DNA is restricted in *galU* mutants, progeny Glu$^-$ DNA (from a Glu$^+$ phage infection) is not. This paradox was explained by the observation (10) that, early after infection, T4 synthesizes an anti-restriction endonuclease (*arn*) activity directed against *rglB* activity, permitting unrestricted Glu$^-$ progeny phage DNA replication in *galU* mutants.

In this study, the inability of T4 *arn* mutants to produce single-cycle progeny in a *galU* host was used to screen T4 deletion phage for the absence of *arn* activity. When coupled with the *imm* mutation (to permit normal injection by a superinfecting phage), the *arn* deletion phage failed to protect a secondary infecting Glu$^-$ phage from *rglB* restriction. A functional *arn* gene was cloned in pBR325 and shown to be homologous to the genetic region missing in the Arn$^-$ deletion phage. The *arn* gene is located in the *nrd*C-*rI* region (30) between 55.5 and 58.4 kilobases (kb).

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MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. Derivatives of *Escherichia coli* K-12: HR112 (F$^+$ *rglA rglB* λ$^+$), formerly K-12 r_6^- $r_{2,4}^-$ or K *rgl*$^-$ (21–23), was the permissive host for Glu$^-$ T4; U95 (*galU rglA rglB*$^+$) (23) and its derivative HR141 (*galU rglA rglB*), formerly U95 r_6^- $r_{2,4}^-$ (21), was the isogenic pair of *galU* strains used to screen T4 deletion mutants for the absence of *arn* function; DO100 [Δ(*lac-pro*) *rglA rglB*$^+$], the restrictive host for Glu$^-$ T4 used to clone and detect Arn$^+$ plasmids, was from D. Oliver; JC7729 (F$^-$ *rglA*$^+$ *rglB*$^+$ *recB21 sbcB15 his-327 trpE9829 leu lac rpsL32 thi*), a restrictive host lacking exonuclease V activity used in sucrose gradient analysis of Arn$^+$ and Arn$^-$ T4, was from A. J. Clark. *Escherichia coli* B strain B834 (*hsdS met galU56*), used to grow T4 *alc7* to produce cytosine-containing T4 DNA, was from L. Snyder. T4 bacteriophage strains: T4D was the wild-type phage: Δ*(39-56)12*, Δ*(63-32)1*, Δ*(63-32)7*, and Δ*(63-32)9* were from Homyk and Weil (14); Δ*2* and Δ*5* were from G. G. Wilson (Ph.D. thesis, Sussex University, Sussex, England, 1976); Δ*tk2* and Δ*farP13* were from D. Hall (5); *imm*$_2$*s* was from Vallée (28); and *alc7*, a multiple mutant of T4 (amE51 [gene 56], amC87 [gene 42], NB5060 [genes *denB-rII*], *alc7* [gene *alc*]) used for making cytosine-containing T4 phage, was from Wilson et al. (29). Plasmid pBR325 (5.4 kb) confers ampicillin, tetracycline, and chloramphenicol resistance to the host (2) and was received from John Watson.

Enzymes and chemicals. Restriction endonuclease *Eco*RI, T4 DNA ligase, and *E. coli* RNA polymerase holoenzyme were from New England Biolabs. DNA

polymerase was from Miles Laboratories, Inc. [α-^{32}P]ATP, 111 TBq/mmol, and phosphorus-32 (carrier free) were from New England Nuclear Corp. Ribonucleoside triphosphates and deoxyribonucleoside triphosphates were from Boehringer Mannheim Corp. Other chemicals and reagents were of the highest purity available.

Bacterial, phage growth, and other techniques. Bacterial and phage techniques were described by Adams (1). Preparation of ^{32}P-labeled HMC T4 DNA and sucrose gradient analysis of intracellular phage DNA were described earlier (9). T4 Δ5 *imm* double mutants were constructed by crossing T4 Δ5 and T4 *imm*$_{29}$*s* mutants at a ratio of 20:1. Since T4 *imm* mutants make larger plaques than do Δ5 or wild-type T4, 10 large plaques were picked, purified, and used to make stocks. The Imm phenotype was confirmed by the absence of superinfection breakdown of ^{32}P-labeled wild-type T4 DNA (11). The Arn$^-$ phenotype was verified by the inability to give a burst on a *galU rglA rglB*$^+$ host cell. We presume that the particular recombinant we used in this study is actually a T4 Δ5 *imm*$_{29}$*s* triple mutant, since this recombinant is Imm$^-$ at 30°C. T4 *imm* single mutants are deficient in immunity only at 37°C, unlike T4 *imm*$_{29}$*s* (28).

Nick translation and DNA-DNA hybridization. Plasmid pDH346 DNA carrying the T4 *arn* gene insert was nick translated with [α-^{32}P]ATP as described by Davis et al. (7) to produce a labeled probe. DNA hybridization was carried out as described by Denhardt (8). In brief, 1 μg of the unlabeled denatured T4 DNA bound to nitrocellulose filters was annealed with 0.025 μg (1.1 × 10^6 cpm/μg) of denatured ^{32}P-nick-translated plasmid DNA fragments. Phage DNA for hybridization experiments was isolated by phenol extraction from cesium chloride gradient-purified phage particles.

Isolation of phage and plasmid DNA. T4 cytosine-containing DNA was prepared from the T4 multiple mutant of *alc7* as described by Wilson et al. (29). Plasmid DNA was isolated from cultures grown in M9 (7) minimal medium (supplemented with 0.4% glucose and 0.2% Casamino Acids) by a modification of the method of Katz et al. (15) which gives a high yield of plasmid DNA with very little contamination of chromosomal DNA. Cultures were grown to 5 × 10^8 cells per ml, and plasmid DNA was amplified by growing the cultures in the presence of spectinomycin (300 μg/ml) for 16 h. Cells from 500-ml cultures were concentrated and washed in TE buffer (10 mM Tris-hydrochloride [pH 8.0]–1 mM EDTA). Washed cells were suspended in 9 ml of cold 25% sucrose buffered with 0.05 M Tris-hydrochloride (pH 8.0). A 1.8-ml amount of lysozyme (5 mg/ml in 0.25 M Tris-hydrochloride [pH 8.0]) was added and incubated on ice for 10 min with occasional shaking. At this point, 3.6 ml of 0.25 M EDTA was added, and the incubation on ice was continued for 10 min. A 14.4-ml amount of Brij lysis mix (1.0% Brij 58; 0.4% sodium dodecyl sulfate; 0.0625 M EDTA; 0.05 M Tris-hydrochloride, pH 8.0) was added, and the cells were incubated for 60 min on ice with gentle shaking. The lysate was cleared by centrifugation at 20,000 rpm for 60 min at 4°C in a 60 Ti rotor (Beckman Instruments, Inc.). The supernatant fluid was decanted carefully, and the volume was adjusted to 30 ml with TE buffer. For most of the experiments, a portion of the cleared lysate was treated with RNase (10 μg/ml) and then deproteinized with phenol. Phenol was removed by chloroform-isoamyl-alcohol (99:1) extraction, and the DNA, precipitated with ethanol and redissolved in TE buffer, was used without further purification. In some cases, covalently closed DNA was purified by equilibrium centrifugation after adding 31.5 g of CsCl (density, 1.64) and 5 ml of 7.5-mg/ml ethidium bromide to 30 ml of cleared lysate. The plasmid DNA was collected, freed of ethidium bromide by extraction with isopropanol, dialyzed against TE buffer, and concentrated by ethanol precipitation.

Cloning the *arn* gene. DNA cleaved with restriction endonucleases by the supplier's instructions was ligated with T4 DNA ligase at 12°C for 16 h in a buffer containing 0.05 M Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 20 mM dithiothreitol, and 1 mM ATP. An *Eco*RI partial digest of T4 cytosine-containing DNA was ligated to *Eco*RI-cleaved plasmid pBR325 DNA and used for transforming calcium-shocked (7) DO100 cells. Since the insertion of foreign DNA at the *Eco*RI site of pBR325 inactivates the chloramphenicol transacetylase gene, we screened the Amp^r Cam^s transformants for the *arn*$^+$ property which renders the *rglB*$^+$ restricting cells permissive for Glu$^-$ T4 phage. The purified transformants were grown in wells (fraction collection tray model no. 26107; Gilson Medical Electronics, Inc.) overnight at 37°C. A duplicating device with 25 prongs was used to transfer droplets to a set of three plates that had been seeded with either no phage (master plate) or 10^8 T4 Glu$^-$ or T4 Glu$^+$ phage particles in the soft agar. The transformants which failed to grow on both T4 Glu$^-$ and T4 Glu$^+$ plates were picked from the master plates and tested for efficiency of plating of T4 Glu$^-$ phage.

RESULTS

Strategy for mapping the T4 *arn* gene. T-even phage-coded glucosyltransferases transfer the glucose moiety from uridine diphosphoglucose to HMC residues of the phage DNA (6). Since *galU* mutants of *E. coli* fail to synthesize uridine diphosphoglucose, progeny phage grown in these mutants lack the glucosyl modification of HMC residues in their DNA (13, 22). The growth properties of T4 on two *galU* host strains are shown in Table 1. The burst sizes of T4 in *galU rglA rglB*$^+$ and *galU rglA rglB* host cells were similar when the progeny phage were assayed on a nonrestricting host. T4 forms plaques, however, on only *galU rglA rglB* and not on *galU rglA rglB*$^+$ cells (13, 21, 23, 27). To explain why the replicating HMC DNA is immune to restriction, it has been suggested that both *rglA*- and *rglB*-restricting DNase activities are localized in the host membrane (29). However, Dharmalingam and Goldberg (9) have shown that only *rglA* may be membrane bound; the *rglB* activity has a cytoplasmic location (12). An additional observation which explains the lack of restriction of HMC DNA in *galU* mutants is that, early after infection, T4 makes an anti-restriction endonuclease protein (Arn) which inhibits *rglB* activity (10). Therefore, in such

TABLE 1. Growth properties of wild-type T4 in *galU rglA rglB*$^+$ and *galU rglA rglB* strains

Host strain[a] genotype	Efficiency of plating[b]	Burst size[c]
galU rglA rglB$^+$	$<10^{-6}$	170
galU rglA rglB	1	130

[a] Host strains used were U95 and HR141.

[b] The efficiency of plating is the plaque count obtained relative to the plaque count on *E. coli* HR112 *rglA rglB*.

[c] In burst size experiments, log phase cells (2×10^8/ml) were infected with 0.1 T4 phage per cell, incubated at 37°C for 10 min, and then diluted 2,000-fold. The diluted samples were aerated for 2 h and phage released by the addition of chloroform. Burst size is calculated from the number of phage plaques at 2 h after infection compared with the number of infective centers at 10 min after infection measured on HR112 *rglA rglB*.

cells, progeny HMC DNA can replicate since the *rglA* product (located in the membrane) is also inactive against the replicating HMC DNA.

If we presume that the *arn* gene is nonessential for glucosylated T4 phage growth, we could screen for T4 *arn* mutants since such mutants would liberate only two or less phage per infected cell in *galU*-restricting strains, whereas in *galU rglA rglB* mutants, a normal burst would be obtained. Therefore, we screened available T4 mutants which have deletions in different parts of the genome to locate the *arn* gene.

Physical mapping of the *arn* gene. The burst sizes of various T4 mutants deleted in nonessential regions of the genome are shown in Table 2. The ratio of burst sizes on *galU rglA rglB*$^+$ and *galU rglA rglB* host cells was close to 1.0 for T4 wild type and the deletions Δ*(39-56)12*, Δ*(63-32)1*, Δ*(63-32)7*, and Δ*(63-32)9*. In contrast, Δ*farP13*, which covers the region extending from 48.4 to 65 kb clockwise from the *r*IIA-*r*IIB junction (Fig. 1), gave a burst size ratio of 0.01. This result implies that the region of DNA missing in the Δ*farP13* mutant codes for an activity which overcomes *rglB* restriction. To further localize the presumptive *arn* gene, we tested shorter deletions in the same region. Deletion Δ*tk2* (49.5 to 60 kb) gave a burst size ratio of 0.03 and must also lack the *arn* gene. However, Δ*2* (deleted from 49.4 to 55.5 kb) gave a ratio of 1.3. These results show that at least part of the *arn* gene is located between the *r*II-distal ends of Δ*2* (55.5 kb) and Δ*tk2* (60 kb). The absence of *arn* gene expression in Δ*5* (deleted from 53 to 58.4 kb; ratio, 0.028) indicates that at least a segment of the *arn* gene is in the 2.9-kb region extending from 55.5 to 58.4 kb, the region between the *r*II-distal ends of Δ*2* and Δ*5*.

Analysis of the *arn* gene expression. Expression of the *arn* gene in T4 wild-type phage-infected cells inhibits the host *rglB* restriction activity so that superinfecting Glu$^-$ T4 DNA remains uncleaved (10). In cells infected with T4 *imm* mutants, the superinfecting Glu$^-$ T4 DNA is uncleaved (Fig. 2A), confirming the earlier observation (10). The *imm* mutation (11, 28) is needed to alleviate the superinfection exclusion property of the primary infecting phage. We constructed T4 Δ*5 imm* double mutants (as described in Materials and Methods) to study expression of the *arn* gene. In T4 Δ*5 imm*-infected cells, the secondary infecting Glu$^-$ DNA is cleaved into fragments (Fig. 2B), demonstrating that the *arn* gene is not expressed by T4 Δ*5 imm* mutants.

Molecular cloning of the *arn* gene. To clone the *arn* gene, an *Eco*RI partial digest of cytosine-containing T4 DNA was ligated to the single *Eco*RI site in plasmid pBR325. Amp^r Cam^s transformants of the restrictive host *E. coli* DO100 were screened for their ability to permit the growth of T4 Glu$^-$ phage. The plasmid DNA isolated from two such transformants had an insert of about 0.8 kb in length. DNA from one transformant, plasmid DNA pDH346, was further characterized (Table 3). This recombinant DNA hybridized extensively with wild-type T4 DNA as well as with Δ*2* DNA but not with Δ*5* or to any significant extent with Δ*farP13* DNA. These results limit the location of the *arn* gene to the 55.5- to 58.4-kb segment of the T4 map.

DISCUSSION

The physical deletion mapping data show that the *arn* gene or a portion essential for the expression of *arn* is located between the *r*II-distal endpoints of Δ*2* and Δ*5*. That this 2.9-kb region covers the entire *arn* gene is shown by the hybridization data, using the cloned T4 DNA

TABLE 2. Burst size[a] of T4 and T4 deletion mutants

Phage strain	Burst size in *galU rglA rglB*$^+$	Burst size in *galU rglA rglB*	*rglB*$^+$/*rglB* burst size ratio
T4 wild type	200	140	1.42
T4 Δ*(39-56)12*	17	23	0.74
T4 Δ*(63-32)7*	16	19	0.84
T4 Δ*(63-32)9*	27	47	0.70
T4 Δ*(63-32)1*	14	30	0.47
T4 Δ*farP13*	2	140	0.01
T4 Δ*tk2*	1	30	0.03
T4 Δ*2*	68	53	1.3
T4 Δ*5*	3	107	0.028

[a] Burst sizes were determined as described in Table 1, footnote *c*.

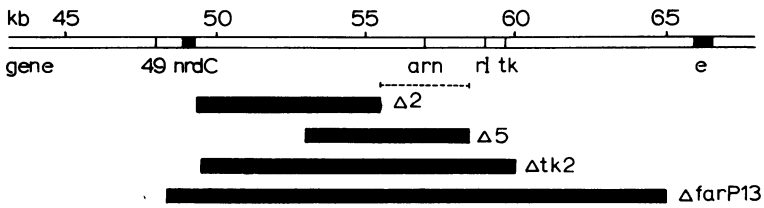

FIG. 1. Map location of the *arn* gene on the T4 genome. The distance clockwise from the *r*IIA-*r*IIB junction, the arbitrary 0 point (30), is given in kilobases at the top of the figure. Location of the *arn* gene (-----) was determined as described in the text. The positions of genes *e* and *nrd*C were taken from Wood and Revel (30). The positions of Δ*2*, Δ*5*, and *r*I genes are based on the heteroduplex data of G. Wilson (Ph.D. thesis). The position of genes 49 and *tk* as well as the endpoints of Δ*tk2* and Δ*farP13* were determined from the recombination frequencies published by Chace and Hall (5). Their recombination data was converted to map lengths in base pairs, using the four-parameter switch function of Stahl et al. (24). We used 166 kb as the total length of T4 DNA (14) instead of the value 200 kb used by Stahl et al. (24). The total map length of T4 is taken as 2,000 map units.

from pDH346. This 0.8-kb T4 DNA fragment contains the functional *arn* gene since clones derived from restrictive cells containing pDH346 are nonrestricting and the plasmid DNA does not hybridize with Δ*5* DNA, indicating a lack of significant homology to T4 DNA beyond the endpoints of the Δ*5* deletion. This observation limits the *arn* gene to the 55.5- to 58.4-kb region covered by Δ*5*.

In an attempt to determine whether the *arn* gene is expressed from its own promoter or from a vector promoter, we looked for promoters on the isolated 0.8-kb insert by R-loop analysis (3, 25). pDH346 DNA was cut with *Eco*RI, and both fragments were transcribed with *E. coli* RNA polymerase holoenzyme. After 2 and 3 min of transcription, more than 95% of 450 vector DNA molecules had more than one R-loop per molecule, whereas a similar number of 0.8-kb T4 DNA fragments showed none. Incubations of up to 8 min also showed no R-loops associated with the T4 insert. This suggests to us that, in the plasmid, *arn* is not associated with its own promoter; however, additional work will be needed to establish this point.

The mapping data show that the *arn* gene is located in the 10-kb region between *nrd*C and *r*I. This was the largest segment of the T4 chromo-

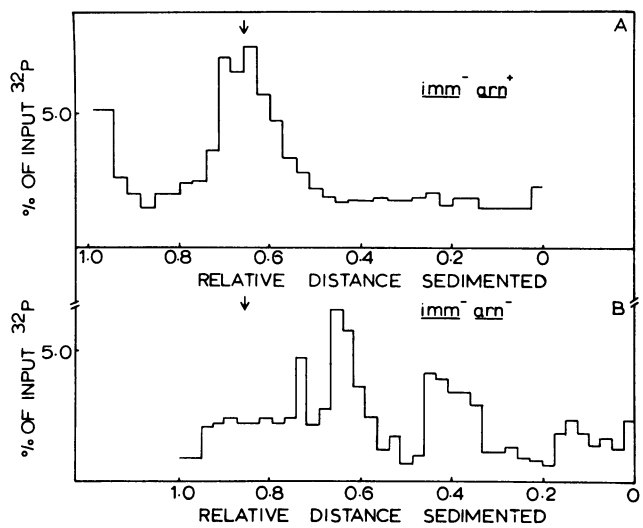

FIG. 2. Restriction of superinfecting Glu⁻ T4 DNA by cells previously infected with phage T4 missing the *arn* gene. Strain JC7729, a restricting host lacking exonuclease V activity, was preinfected with (A) T4 $imm_{2}s$ or (B) T4 Δ*5*, $imm_{2}s$ at a multiplicity of 4.0 and superinfected at 5 min with ^{32}P-labeled Glu⁻ T4 phage at a multiplicity of 1.0. At 5 min after secondary infection, cells were lysed, and the DNA was analyzed by neutral sucrose gradients as described previously (9–11). The arrows indicate the position of ^{3}H-labeled marker T4 DNA. For experiments A and B, sedimentation was for different times; therefore, the graphs have been aligned at the position of the T4 ^{3}H marker DNA.

TABLE 3. Hybridization of pDH346 plasmid DNA with DNA isolated from wild-type T4 and deletion phages

Source of phage DNA	% Hybridization[a]
T4 wild type	10.0
T4 Δ2	8.6
T4 Δ5	0.0
T4 Δ*farP13*	0.84

[a] DNA-DNA hybridization was done as described in the text, with α-^{32}P-labeled plasmid pDH346 DNA and a 40-fold excess of phage DNA. Of the input DNA, 10% equals 2,878 cpm.

some (about 7% of the T4 genome) which contained no known genes. One gene, adenine methylase (*dam*), in the closely related phage T2, is mapped (4) in the region corresponding to this portion of T4 DNA (16). The only other gene reported to map in this region is *su*30 (18); however, the data for this claim has not yet been published. It is interesting that the five genes located in this region are functionally related in the sense that all genes are involved in the DNA metabolism of the phage and, more specifically, *nrd*C, *tk*, and *dam* are all nucleotide-modifying enzymes.

Since the original description of phage-coded activities that prevent T7 and T4 restriction (10, 26), this phenomenon has been reported for T3, T5, and bacillus phage NR2 (for review, see reference 17). More recently, the direct interaction of the T7 antirestriction protein with the host-restricting enzyme has been nicely demonstrated (19). However, the *arn* gene is unique in that it is a second protection mechanism apart from the glucose modification. Furthermore, the *arn* gene seems to be useful only for the glucosylated phage under special circumstances, i.e., in hosts where phage-mediated glucosylation is not possible. We have shown earlier that one *rglB* restriction site is located in the *arn* gene itself (11). Therefore, the *arn* gene function is blocked when Glu$^-$ T4 DNA enters a restricting host. Since T4 deletion mutants lacking this gene grow normally as long as the phage DNA is glucosylated, the *arn* gene appears to be nonessential for the development of T4. Though average burst sizes in the permissive host vary among different deletion mutants (Table 2, column 3), there is no obvious correlation between this variation in burst size and the presence or absence of the *arn* gene.

At this point, it is unclear of what use the *arn* gene might be to wild-type T4 or, for that matter, to the Glu$^-$ T4 in a restricting host. The existence of an alternative (possibly more primitive) protection mechanism which is useful only for a glucosylated phage in a host where glucosylation is not possible invites evolutionary speculation. However, this might be more useful when the regulation of *arn* gene expression and the interaction of the *rglB* enzyme with the *arn* protein is clearer.

ACKNOWLEDGMENTS

We thank Judith Reichler for her help in the electron microscopy of the R-loops.

This work was supported by a Public Health Service grant from the National Institutes of Health and by a grant from the National Science Foundation.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. **Bolivar, F.** 1978. Construction and characterization of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique *Eco*RI sites for selection of *Eco*RI generated recombinant DNA molecules. Gene **4**:121–136.
3. **Brack, C.** 1979. Electron microscopic analysis of transcription: mapping of initiation sites and direction of transcription. Proc. Natl. Acad. Sci. U.S.A. **76**:3164–3168.
4. **Brooks, J., and S. Hattman.** 1973. Location of the DNA-adenine methylase gene on the genetic map of phage T2. Virology **55**:285–288.
5. **Chace, K. V., and D. H. Hall.** 1975. Characterization of new regulatory mutants of bacteriophage T4. II. New class of mutants. J. Virol. **15**:929–945.
6. **Cohen, S. S.** 1968. Virus induced enzymes, p. 44. Columbia University Press, New York.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
8. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
9. **Dharmalingam, K., and E. B. Goldberg.** 1976. Mechanism, localisation and control of restriction cleavage of phage T4 and λ chromosomes *in vivo*. Nature (London) **260**:406–410.
10. **Dharmalingam, K., and E. B. Goldberg.** 1976. Phage-coded protein prevents restriction of unmodified progeny T4 DNA. Nature (London) **260**:454–456.
11. **Dharmalingam, K., and E. B. Goldberg.** 1979. Restriction *in vivo*. IV. Effect of restriction of parental DNA on the expression of restriction alleviation systems in phage T4. Virology **96**:404–411.
12. **Fleischman, R. A., J. L. Campbell, and C. C. Richardson.** 1976. Modification and restriction of T-even bacteriophages. *In vitro* degradation of deoxyribonucleic acid containing 5-hydroxymethylcytosine. J. Biol. Chem. **251**:1561–1570.
13. **Hattman, S., and T. Fukasawa.** 1963. Host-induced modification of T-even phages due to effective glucosylation of their DNA. Proc. Natl. Acad. Sci. U.S.A. **50**:297–300.
14. **Homyk, T., Jr., and J. Weil.** 1974. Deletion analysis of two non-essential regions of the T4 genome. Virology **61**:505–523.
15. **Katz, L., D. T. Kingsbury, and D. R. Helinski.** 1973. Stimulation by cyclic adenosine monophosphate of plasmid deoxyribonucleic acid replication and catabolite repression of the plasmid deoxyribonucleic acid-protein relaxation complex. J. Bacteriol. **114**:577–591.
16. **Kim, J.-S., and N. Davidson.** 1974. Electron microscope heteroduplex study of sequence relations of T2, T4, and T6 bacteriophage DNAs. Virology **57**:93–111.
17. **Krüger, D. H., and C. Schroeder.** 1981. Bacteriophage T3 and bacteriophage T7 virus-host cell interactions. Microbiol. Rev. **45**:9–51.
18. **Krylov, V. N.** 1972. A mutation of T4B phage, which

enhances suppression of ligase mutants with *r*II mutations. Virology **50**:291–293.
19. **Mark, K., and F. W. Studier.** 1981. Purification of the gene 0.3 protein of bacteriophage T7, an inhibitor of the DNA restriction system of *Escherichia coli*. J. Biol. Chem. **256**:2573–2578.
20. **Revel, H. R.** 1967. Restriction of nonglucosylated T-even bacteriophage. Properties of permissive mutants of *Escherichia coli* B and K12. Virology **31**:688–701.
21. **Revel, H. R., and C. P. Georgopoulos.** 1969. Restriction of nonglucosylated T-even bacteriophages by prophage P1. Virology **39**:1–17.
22. **Revel, H. R., and S. E. Luria.** 1970. DNA-glucosylation in T-even phage; genetic determination and role in phage host-interaction. Annu. Rev. Genet. **4**:177–192.
23. **Shedlovsky, A., and S. Brenner.** 1963. A chemical basis for the host-induced modification of T-even bacteriophages. Proc. Natl. Acad. Sci. U.S.A. **50**:300–305.
24. **Stahl, F. W., R. S. Edgar, and J. Steinberg.** 1964. The linkage map of bacteriophage T4. Genetics **50**:539–552.
25. **Stüber, D., H. Delius, and H. Bujard.** 1978. Electron microscopic analysis of *in vitro* transcriptional complexes: mapping of promoters of the coliphage T5 genome. Mol. Gen. Genet. **166**:141–149.
26. **Studier, F. W.** 1975. Gene 0.3 of bacteriophage T7 acts to overcome the DNA restriction system of the host. J. Mol. Biol. **94**:283–295.
27. **Symonds, N., K. A. Stacey, S. W. Glover, J. Schell, and S. Silver.** 1963. The chemical basis for a case of host-induced modification of phage T2. Biochem. Biophys. Res. Commun. **12**:220–222.
28. **Vallée, M., and O. deLapeyrière.** 1975. The role of the genes *imm* and *s* in the development of immunity against T4 ghosts and exclusion of superinfecting phage in *Escherichia coli* infected with T4. Virology **67**:219–233.
29. **Wilson, G. G., V. I. Tanyashin, and N. E. Murray.** 1977. Molecular cloning of fragments of bacteriophage T4. Mol. Gen. Genet. **156**:203–214.
30. **Wood, W. B., and H. R. Revel.** 1976. The genome of bacteriophage T4. Bacteriol. Rev. **40**:847–868.