J. Mol. Biol. (1971) **59**, 127–150

Dual Control of Arabinose Genes on Transducing Phage λ*dara*

ROBERT SCHLEIF†, JACK GREENBLATT

Biological Laboratories
Harvard University, Cambridge, Mass. 02138, U.S.A.

AND

RONALD W. DAVIS‡

Chemistry Department
California Institute of Technology, Pasadena, Calif. 91109, U.S.A.

(*Received 14 December 1970, and in revised form 12 March 1971*)

A λ–φ80 hybrid derivative of the defective transducing phage φ80d*ara* (Gottesman & Beckwith, 1969) has most of its late genes replaced by *Escherichia coli* DNA containing an intact arabinose operon. In a cell lysogenic for this phage the arabinose operon on the phage responds normally to its regulatory gene *araC* and to the level of L-arabinose in the growth medium. However, as a consequence of transcriptional read-through from phage late genes to the arabinose operon, arabinose enzymes are also synthesized under the control of phage gene *Q* during growth of the phage in the absence of L-arabinose.

We also show that the gene *araC* has the same transcriptional orientation as the phage late genes and the arabinose operon genes *B*, *A* and *D*. Since monomers of phage lysozyme and L-arabinose isomerase are produced in comparable quantities under phage late control, it follows that if there is transcription termination at the end of the gene *araC* it is no more than 90% efficient.

1. Introduction

The L-arabinose metabolizing system of *Escherichia coli* contains four closely linked genes, *C*, *B*, *A* and *D*, and at least one unlinked gene involved in permeation (Gross & Englesberg, 1959; Englesberg *et al.*, 1962; Novotny & Englesberg, 1966). Since the inducibility by L-arabinose of genes *B*, *A*, *D* and the permease requires a functional *C* gene product (Sheppard & Englesberg, 1967), the system is considered to be positively controlled by the *araC* gene. The site of gene *C* product action has been determined by deletion mapping to be a region, called the operator and initiator, located between genes *C* and *B* (Englesberg, Sheppard, Squires & Meronk, 1969). Similar deletions were also used to show that transcription proceeds through genes *B*, *A* and *D* in that order (see Fig. 3).

In addition to the C^- mutations which result in pleiotropic uninducibility of genes *B*, *A*, *D* and the permease, *C* gene mutations, known as C^c, have also been found which result in constitutive functioning of all other genes in the system. It was

† Present address: Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154, U.S.A.

‡ Present address: Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Mass. 02138, U.S.A.

further found that in cells diploid for the arabinose region, the C^+ allele is dominant to C^c (Sheppard & Englesberg, 1966). The presently known data are consistent with a system in which gene *C* protein in the presence of L-arabinose acts at an initiator site to promote expression of arabinose operon genes, and in the absence of L-arabinose acts at an operator site to repress their expression. Consistent with this notion, a deletion of the *C* gene has been found which terminates in the promoter–operator region between genes *C* and *B* and appears to remove the repression site without affecting the activation site (Englesberg, Sheppard, Squires & Meronk, 1969; Englesberg, Squires & Meronk, 1969).

There is as yet no evidence to indicate whether control by the *C* gene regulatory protein is exerted at the level of transcription or translation. Also unclear is the relationship between the mechanism of *C* protein action and its genetically defined dual function. Questions of this type could at least be partly answered with studies of pure *C* protein *in vitro*. To aid searches for the *C* gene protein, and to facilitate some genetic studies of the operon, Gottesman & Beckwith (1969) have isolated an arabinose transducing $\phi 80$ phage, known as $\phi 80$*dara*. They have shown that the arabinose genes of $\phi 80$*dara* have replaced some phage late genes and that the inserted arabinose operon has the same transcriptional orientation as the late genes.

We have constructed a λ–$\phi 80$ hybrid derivative of the $\phi 80$*dara* that is both heat inducible and lysis defective. When this phage is in the lysogenic state in a strain deleted of chromosomal arabinose genes, the arabinose genes of the prophage are induced normally by L-arabinose in the medium. However, they are also induced to a comparable level following heat induction of the prophage in the absence of L-arabinose. We shall show that this is a result of the insertion of the entire arabinose operon into the phage late genes. In this position the operon retains its own control, but it is also controlled by the phage gene *Q* product, the inducer of phage late RNA and protein synthesis.

After repression has been removed, normal development of phage λ is under the positive control of gene *N*. During this time, gene *N* product is required for appreciable synthesis of *Q* gene product and the proteins involved in replication and recombination (Dove, 1966; Skalka, Butler & Echols, 1967; Taylor, Hradecna & Szybalski, 1967; Dambly, Couturier & Thomas, 1968; Radding & Echols, 1968). The protein product of phage gene *Q* then stimulates expression of phage late genes, most likely by acting at a single promoter located between genes *Q* and *S* (Toussaint, 1969; Herskowitz & Signer, 1970). In λ*dara*, RNA polymerase molecules which have begun late gene transcription under control of the *Q* product transcribe from phage late genes to the arabinose operon located further into the late gene region of the phage (see Fig. 3).

Here we shall also show that the *araC* gene product is synthesized under control of gene *Q* during growth of the λ*dara* phage. It then follows (provided the genes on the phage have the same order they normally have on the chromosome) that the *C* gene has the same transcriptional orientation as the arabinose operon structural genes *B*, *A* and *D*, and read-through into the structural genes is terminated neither at the end of the *araC* gene nor in the arabinose operator–initiator region. Although there is no *a priori* reason for a transcription stop signal to exist at the end of the *C* gene, in a similar situation there does appear to be some form of stop signal at the end of the *i* gene of the *lac* operon (Reznikoff, Miller, Scaife & Beckwith, 1969). It is possible that normal transcriptional termination at the end of the *C* gene is

abolished as a consequence of ph
N gene protein of phage λ exerts
tion of early phage RNA synth
evidence showing that it is not lik
acting at the end of the *C* gene.

2. M

All strains used were derived fro
λ*dara*, including the arabinose ope

E

Strain name	Genotype
RFS1	Hfr: *thi*
RFS73	F⁻: *thr leu lac thi s*
RFS424	F⁻: *araB424 thr leu*
RFS475	F′: *araB424* (*thr-leu*) *araB424*
RFS498	HfrH: *thi ara498 leu*
RFS518	F⁻: *leu498 thi lac s*
RFS568	su_{III}
RFS576	*gal T1*^{r} ($\lambda CI_{857}S_7$)/λ
RFS581	Hfr: *thi araD54* (λ*CI*
RFS605–624	su_I ($\phi 80$ *am-x*)
RFS626	su_I
RFS628, 629	F′: *araB424* (*thr-leu*) *lac thi* su_{II} *ara49* (λ*dara* $C^c{}_{81,87}CI$
JG28	Hfr: *thi ara498 leu4*
JG31	Hfr: *thi ara498 leu4* (λ*dara* $CI_{857}S_{t6}$
JG33	Hfr: *thi araD54* (λ*C*
JG39	Hfr: *thi araD54* (λ*dara* $CI_{857}S_{t6}$
JG62	F⁻: *thi lac* su_{II} *ara4* (λ*dara* $CI_{857}S_{t6}$
JG81, 87	F⁻: *thi lac* su_{II} *ara4* (λ*dara* $C^c{}_{81,87}CI$
JG103	Hfr: *thi ara498 leu4*

abolished as a consequence of phage growth. Indeed, it has been postulated that the *N* gene protein of phage λ exerts positive control by preventing premature termination of early phage RNA synthesis (Roberts, 1969). However, we shall present evidence showing that it is not likely that gene *N* inactivates a termination mechanism acting at the end of the *C* gene.

2. Materials and Methods

(a) *Bacteria*

All strains used were derived from *Escherichia coli* K12. The *E. coli* DNA on the phage λ*dara*, including the arabinose operon, is derived from *E. coli* B/*r*.

TABLE 1

Bacterial strains used

Strain name	Genotype	Comments
RFS1	Hfr: *thi*	Strain HfrH.
RFS73	F⁻: *thr leu lac thi* su_{II} *T1*^{r}	Strain C600.
RFS424	F⁻: *ara*B424 *thr leu lac thi* su_{II}	Strain 73 after nitrosoguanidine mutagenesis.
RFS475	F′: *araB424* (*thr-leu*)/*thr leu lac thi* su_{II} *araB424*	Episome from KLFI (Low, 1968) in strain RFS424 and homozygous for *ara*B424.
RFS498	HfrH: *thi ara498 leu498*	Deletes leucine and at least *araC*, *B* and *A* (Schleif, 1969).
RFS518	F⁻: *leu498 thi lac* su_{II} *ara498 T1*^{r}	*ara*498 from RFS498 transduced into RFS73 with phage P1, selecting for *thr*$^+$.
RFS568	su_{III}	From E. Signer. QD5003. Suppresses S_7 and *Q* mutations used.
RFS576	*gal T1*^{r} ($\lambda CI_{857}S_7$)/λ	From E. Signer. M5017. Heat inducible and lysis defective.
RFS581	Hfr: *thi araD54* ($\lambda CI_{857}ind^-$)	Heat inducible λ in RFS54 (Schleif, 1969).
RFS605–624	su_I (φ80 *am-x*)	CA5013 containing as lysogens φ80 amber mutants suppressible by su_I (Sato *et al.*, 1968).
RFS626	su_I	From Sato *et al.* (1968). CA5013.
RFS628, 629	F′: *araB424* (*thr-leu*)/*leu498 lac thi* su_{II} *ara498* (λ*dara* $C^c_{81,87}CI_{857}S_{ts8}$)	See below.
JG28	Hfr: *thi ara498 leu498* (φ80*dara*)	RFS498 transduced to *ara*$^+$ with φ80*dara* (Gottesman & Beckwith, 1969).
JG31	Hfr: *thi ara498 leu498* (λ*dara* $CI_{857}S_{ts8}$)	See below. (RFS566).
JG33	Hfr: *thi araD54* ($\lambda CI_{857}S_{ts8}h80$)	Heat inducible and lysis defective.
JG39	Hfr: *thi araD54* (λ*dara* $CI_{857}S_{ts8}$, $\lambda CI_{857}S_{ts8}h80$)	JG38 transduced to *ara*$^+$ with lysate made from JG31 using $\lambda CI_{857}S_{ts8}h80$ as helper phage.
JG62	F⁻: *thi lac* su_{II} *ara498 leu498* (λ*dara* $CI_{857}S_{ts8}$)	RFS518 transduced to *ara*$^+$ with lysate made from JG31 using $\lambda CI_{857}S_7$ as helper. Is a single lysogen (see below).
JG81, 87	F⁻: *thi lac* su_{II} *ara498* (λ*dara* $C^c_{81,87}CI_{857}S_{ts8}$)	Derived from JG62 (see below). Constitutive. (RFS577, 578).
JG103	Hfr: *thi ara498 leu498* (λ*dara* $CI_{857}S_7$)	See below. (RFS653).

(i) *JG31: HfrH* thi ara*498* leu*498* (λ*d*ara CI_{857} S_{t68})

This strain is a derivative of RFS498 which contains as a single lysogen a λ*dara* prophage which resulted from a cross between φ80d*ara* and $\lambda CI_{857}S_{t68}h80$. The strain was constructed as follows. Strain JG28, containing φ80d*ara* as a prophage, was grown in YT (Schleif, 1969) medium to about 2×10^8 cells/ml. The cells were concentrated 5-fold by centrifugation, mixed with an approximately equal number of $\lambda CI_{857}S_{t68}\,h80$ phage in 0·2 ml. of 0·01 M-$MgSO_4$, and incubated at 34°C for 15 min. After adding 1 ml. of YT medium, the cells were shaken at 42°C for 15 min and then at 34°C for 3 hr. After chloroform lysis, the bacterial debris was removed and the lysate was used to transduce RFS498 to ara^+ on minimal arabinose plates coated with 10^9 λCI_{60} phage. Among 100 transductant clones tested, most were abortive lysogens and only one, JG31, was a stable single lysogen at 34°C.

JG31 is ara^+ and temperature sensitive. Surviving cells at 42°C are ara^- and not immune to infection by phage λ. It is immune to phage λ, but not to φ80. It behaves after heat induction as a cell containing an S^- heat-inducible phage, but lysates contain no infectious phage and no arabinose transducing phage unless an infectious phage has been used as a helper in preparing the lysate. It segregates to ara^- at 34°C at a frequency of about 0·1%, and ara^- segregants lose both their immunity to phage λ and their temperature sensitivity. It follows that JG31 contains as a single lysogen the prophage λ*dara* $CI_{857}S_{t68}$, whose structural and behavioral properties are described in the Results section. This λ-d*ara* prophage has most probably not integrated at the φ80 attachment site since its curing rate is not increased by infection with φ80 (Maizels, personal communication).

(ii) *JG103: HfrH* thi ara*498* leu*498* (λ*d*ara $CI_{857}S_7$)

This strain was constructed in the same way as JG31, except that transduction was done on eosin–methylene blue-arabinose plates not coated with λCI_{60}, and was given the same tests with the same results as JG31. All kinetic experiments were done with both strains and yielded identical results.

(iii) *RFS628* and *RFS629: F′* (thr-leu) ara *B424*/leu thi lac su_{II} ara*498* (λ*d*ara $C^c_{81,87}$ $CI_{857}S_{t68}$)

These strains were constructed by mating RFS475 with JG81 and JG87 and selecting for ara^+ leu^+ merodiploids by plating on minimal arabinose agar containing only thiamine as a growth supplement. The strains JG81 and JG87 had first been derived from JG62 by mutagenesis with nitrosoguanidine and selection for resistance to D-fucose inhibition (Englesberg, Irr, Power & Lee, 1965). JG81 and JG87 have approximately fully induced levels of arabinose isomerase during growth in the absence of L-arabinose, but RFS628 and RFS629 have isomerase levels only several times basal under the same growth conditions. Furthermore, they readily segregate to a constitutive phenotype and are male because they plate the male-specific phage R17. It was necessary that the episome be $araB^-$ so that cells having lost the λ*dara* C^c could not grow in the preparation of inocula.

(b) *Bacteriophage*

TABLE 2

Bacteriophage used

Genotype	Comments
φ80d*ara*	Gottesman & Beckwith (1969).
φ80*am-x*	Amber mutants of φ80 in genes *1* to *19* (Sato *et al.*, 1968).
λCI_{60}	From J. Pero.
$\lambda CI_{857}S_7$	Heat inducible λ with amber mutation suppressible only by su_{III}. From E. Signer.
$\lambda CI_{857}S_{t68}h80$	Hybrid between λ and φ80 with host range of φ80. Absolute defective mutation t68 in *S* gene (Harris *et al.*, 1967). From M. Schwartz.

TABLE 2 (*continued*)

Genotype	Comments
λ*dara* $CI_{857}S_7$	This work.
λimm^{434}	Lambda except for phage 434 immunity region (Kaiser & Jacob, 1957).
λ$imm^{434}S_7$	From λimm^{434} × λ$CI_{857}S_7$.
λ$imm^{434}Q_{73}Q_{501}$	Q_{73} and Q_{501} are amber mutations in *Q* gene; grows only on su_{III}. From I. Herskowitz.
λ$imm^{21}S_7$	From λimm^{21} × λ$CI_{857}S_7$.
λb_5b_2	Immunity region of phage 21. From Jon Weigle.
λ$CI_{857}R_5$	From λCI_{857} × λR_5.
λ$N_7imm^{434}C_{17}byp$	Will grow without *N* gene function on non-suppressing host. (Hopkins, 1970).
λ$N_7imm^{434}C_{17}bypR_5$	From λ$N_7imm^{434}C_{17}byp$ × λR_5.
R17, P1	Indigenous to laboratory.

(c) *Growth media and antibiotics*

As in Schleif (1969), except where noted.

(i) *Eosin–methylene blue plates*

To 1 l. water: 2 g K_2HPO_4, 5 g NaCl, 1 g yeast extract, 10 g Tryptone, and 15 g agar were added. After autoclaving, 10 g sugar, 0·4 g eosin Y, and 65 mg methylene blue were added. Carbohydrate-utilizing colonies are red; carbohydrate non-utilizing are pink.

(ii) *Phage plates*

For phage λ. To 1 l. water: 5 g NaCl, 10 g Tryptone, and 10 g agar were added. Overlay agar contained 6·5 g agar/l. For an assay of φ80, only 0·5 g NaCl/l. was used.

(iii) *Rifampicin* (*Mann Research Laboratories*)

Just before each experiment, rifampicin was dissolved in yeast–tryptone at about 1 mg/ml. by rolling at 37°C. An $O.D._{475}^{1cm} = 17·5$ was judged to be 1 mg/ml. Rifampicin was used at a final concentration of 80 μg/ml. in all experiments. At 50 μg/ml., rifampicin blocked detectable growth of our strains in yeast–tryptone.

(iv) *Chloramphenicol*

(Chloromycetin; Parke, Davis & Co.) Just before its use, chloramphenicol was dissolved in yeast–tryptone at 1 mg/ml. It was used at a final concentration of 100 μg/ml.

(v) *Phage buffer*

To 1 l. water: 6×10^{-3} M-Tris · HCl (pH 7·5) at 25°C, 0·1 g $MgSO_4$, 4 g NaCl, 0·05 g gelatin were added.

(d) *Growth of cells*

Media were usually either supplemented M9 minimal salts or YT. Some lysogens of the λ*dara* phage are somewhat unstable with a curing rate about 10^{-3} per doubling, which is the same between 32 and 35°C. These were, therefore, maintained on minimal salts containing arabinose as long as possible so that cells having lost the λ*dara* prophage could not grow. (In other lysogens we have prepared in 498, the curing is less than 10^{-4}/doubling, and all lysogens in a *recA* strain showed an undetectably low curing rate of less than 10^{-5}/doubling.) Typically, purified cells for an experiment were grown overnight at 35°C (temp. were ±0·25 deg. C of those stated here) on M9 supplemented with thiamine, leucine and L-arabinose, and then in the morning washed twice by centrifugation before resuspending at 10^6/ml., in YT. The centrifugation steps were omitted if residual L-arabinose carried over into the YT would not interfere. Cells were then grown at 34°C to 2 to 4×10^8/ml. before infection or induction. For phage-infection experiments, the cells were chilled, centrifuged

and resuspended in 0·1 vol. of 0·01 M-$MgSO_4$ at 4°C. Phage were added at a multiplicity of 5 and allowed to adsorb for 20 min, after which time the mixture was diluted into prewarmed YT and grown at 34°C.

For phage induction experiments, the 500-ml. flask containing 100 ml. of cells was placed at 42°C in a shaking water bath. (The half-time for thermal equilibration was 45 sec.) After 10 min, ice was added to bring the water bath temperature to 35°C for the rest of the experiment. The temperature was lowered because we found that at 42°C the lysozyme and arabinose isomerase activities were unstable with half-lives of 12 and 20 min, respectively. Although arabinose isomerase was still somewhat unstable at 35°C, the half-life was about 60 min, long enough so as not to interfere significantly with the experiments. The timing of enzyme appearances was not affected if the flask was held at 42°C for the duration of the experiment.

To prepare induced cells for assay of rho factor, 1 l. of YT containing 3×10^8 cells/ml. at 34°C was shaken over a flame to raise the temperature to 42°C. This requires about 2 min and the temperature was not allowed to rise above 42·25°C. After 10 min at 42°C, the temperature was lowered to 34°C and shaking continued until the cells were ready to chill and harvest. A control flask was treated identically except that the temperature shift to 42°C was omitted. If rho factor was to be prepared from infected cells, the cells were handled as described above for infection experiments.

At times during the course of these experiments, the density of R17 in the laboratory became so great that standard aseptic procedures were inadequate. During these periods, a rabbit antiserum to R17 was included in all liquid media and in top agar at a concentration of $K = 0{\cdot}05$. Some λ plate stocks made under these conditions still contained 10% R17 which was then precipitated with antiserum. The presence of low levels of R17 (10^7/ml. in a λ stock of 10^{10}/ml.) and antiserum in growth media had no detectable effect upon the system studied here.

(e) *Preparation of phage stocks*

φ80 amber mutant stocks were prepared by ultraviolet induction of the corresponding KS strains and were titrated on strain CA5013. $\lambda CI_{857}S_7$ was prepared by heat induction of strain 576 and titered on strain 568. Other phage stocks were grown and titered on appropriate strains.

λ*dara* $CI_{857}S_{t68}$ was prepared by heat induction of strain JG39. The cells were harvested and frozen at −20°C, then thawed and broken by stirring gently for about 15 min. After treatment with 1 μg DNase (Worthington)/ml., bacterial debris was removed by centrifugation at 6000 *g* for 15 min. The phage were twice purified by centrifuging into preformed CsCl block gradients, $\rho = 1{\cdot}3$, 1·5, 1·7 g/cm³ in phage buffer, for 2 hr at 40,000 *g*. Finally, λ*dara* $CI_{857}S_{t68}$ was separated from its helper phage, $\lambda CI_{857}S_{t68}h80$, by centrifuging to equilibrium in CsCl at 40,000 *g* (20,000 rev./min) for 24 hr in the Spinco angle 40 rotor. Phage concentration was estimated by the O.D. at 260 nm.

(f) *Transduction*

Recipient *ara*⁻ cells were grown in YT to 5×10^8 cells/ml., then centrifuged and resuspended at 10^8/ml. in 0·01 M-$MgSO_4$. After shaking at 37°C for 1 hr, 10^8 cells were mixed with an appropriate number of φ80*dara* or λ*dara* phage. After 15 min at 35°C, the mixture was spread on a minimal plate containing arabinose as sole carbon source or on an eosin–methylene blue–arabinose plate. Transductant clones that appeared on the minimal plate after a day at 35°C tended to be abortive rather than stable transductants. However, when cells were spread on eosin–methylene blue–arabinose plates, dark *ara*⁺ transductants grew through the background of *ara*⁻ cells. 90% of the cells within a dark colony were *ara*⁺, whereas less than 10% of the cells within clear colonies, which also grew through the background, were *ara*⁺.

(g) *Marker rescue*

Cells of RFS498, 498 ($\lambda CI_{857}S_{t68}h80$) = JG 33 and 498 (λ*dara*) = JG31 were grown overnight in YT at 35°C. 0·1 ml. of cells was poured in 2·5 ml. of φ80 top agar on φ80 plates. After 20 min, drops of various φ80 amber mutants (Sato, Nishimune, Sato, Numich & Matsushiro, 1968) at 2×10^7/ml. in 10^{-3} M-$MgSO_4$, 5×10^{-4} M-$CaCl_2$, 10 mg/ml. gelatin,

were spotted on the plates. After th
at 40 cm from two 15-w reflector-ba
incubated at 35°C for 12 hr. The a
(λ*hy*80) and 498 (λ*dara*) due to w
mutations in a test gene and the p
λ*dara*. φ80 markers 1 to 15 were tes
found to be present. λ genes *O*, *P*,
λ*imm*^{434}, and were found to be pres

(i) *Arabinose isomerase*

This assay was done as previousl
10^{-3} M-2-mercaptoethanol was inc
longer than 60 min. Enzyme levels
media and, therefore, none was ex
number of isomerase monomers, w
& Lee, 1968), corrected for the fact
standard reaction mixture as we fir

(ii) *Lysozyme*

The lysozyme assay was adapte
turbidity decrease of a suspension
grown overnight in YT, then dilut
3×10^8/ml. The cells were centrifug
in 40 ml. of 0·1 M-Na–EDTA (pH
40 ml. of 0·01 M-potassium phosph
on ice for up to 4 hr.

The lysozyme to be assayed was
of 2-ml. samples chilled in ice wa
6000 *g* for 10 min. Up to 0·2 ml. of s
cells which had been prewarmed
0·4 to 0·8. The O.D. at 600 nm was
for 8 min, as was that of a parallel
concentration is proportional to the
as a function of time on semilogarit

Response is linear with lysozyme
10 μl of supernatant from strain
changes the O.D. from 0·6 to 0·1 in
of egg white lysozyme (Sigma; 20,
calibrated by taking the number of
strain RFS581 to be the same as th
at the time of lysis after u.v. indu
usually lie within 10% of each oth
usually lie within 25% of each oth

(iii) *Rho factor*

The rho factor content of cells wa
fering proteins. Cells of JG31, hea
overnight at −20°C. The cells wer
DNase (Worthington). The rho fa
saturation, then dialyzed and los
elution, the column fractions were
method of Roberts (1969). The fra
phosphate were pooled. Rho facto
DNA as RNA polymerase templat
the initial incorporation, the activit
nuclease.

were spotted on the plates. After the spots had dried, the plates were given a 3-sec exposure at 40 cm from two 15-w reflector-backed germicidal lamps (70 ergs/mm^2). The plates were incubated at 35°C for 12 hr. The absence of a clear spot on 498, but its presence on 498 (*λhy*80) and 498 (*λdara*) due to wild-type recombinants between infecting phages with mutations in a test gene and the prophage, indicated the presence of the test gene in the *λdara*. *φ*80 markers 1 to 15 were tested, but only late genes *1*, *2* and *3* on the left arm were found to be present. *λ* genes *O*, *P*, *Q* and *R* were also tested, using amber derivatives of *λimm*^{434}, and were found to be present in *λdara*.

(h) *Assays*

(i) *Arabinose isomerase*

This assay was done as previously described by Schleif (1969) with the exception that 10^{-3} M-2-mercaptoethanol was included in the assay mix when the incubation was for longer than 60 min. Enzyme levels were not changed if $MnCl_2$ was included in YT or M9 media and, therefore, none was explicitly added for most experiments. To calculate the number of isomerase monomers, we used the specific activity of pure isomerase (Patrick & Lee, 1968), corrected for the fact that we find isomerase to have 0·52 the activity in our standard reaction mixture as we find it to have in Lee's glycylglycine buffer.

(ii) *Lysozyme*

The lysozyme assay was adapted from Dambly *et al.* (1968). It measures the rate of turbidity decrease of a suspension of sensitized whole cells. Substrate strain RFS73 was grown overnight in YT, then diluted 1000 to 1 into 100 ml. of YT and grown at 37°C to 3×10^8/ml. The cells were centrifuged at 4°C, resuspended for 5 min at room temperature in 40 ml. of 0·1 M-Na–EDTA (pH 8·0), then centrifuged again at 4°C and resuspended in 40 ml. of 0·01 M-potassium phosphate, pH 6·8. The sensitized substrate cells were stored on ice for up to 4 hr.

The lysozyme to be assayed was prepared from cells by sonication (on an MSE sonicator) of 2-ml. samples chilled in ice water. The debris was then removed by centrifuging at 6000 *g* for 10 min. Up to 0·2 ml. of sonicated supernatant was mixed with 1 ml. of substrate cells which had been prewarmed to room temperature and adjusted to an O.D.$_{600}$ of 0·4 to 0·8. The O.D. at 600 nm was read on a Zeiss spectrophotometer at 1-min intervals for 8 min, as was that of a parallel cuvette containing only sensitized cells. The lysozyme concentration is proportional to the difference of the slopes when the O.D. values are plotted as a function of time on semilogarithmic paper.

Response is linear with lysozyme concentration over at least a 100-fold range. Typically, 10 *μ*l of supernatant from strain RFS581, taken 45 min after induction at 2×10^8/ml., changes the O.D. from 0·6 to 0·1 in 8 min. This was found to be about equivalent to 0·5 *μ*g of egg white lysozyme (Sigma; 20,000 units/mg). The lysozyme assay was approximately calibrated by taking the number of lysozyme molecules/cell at lysis after heat induction of strain RFS581 to be the same as the number directly measured by Black & Hogness (1969) at the time of lysis after u.v. induction of wild-type *λ*. Two assays of the same extract usually lie within 10% of each other, and two assays of a strain made on different days usually lie within 25% of each other.

(iii) *Rho factor*

The rho factor content of cells was estimated after it was partly purified to remove interfering proteins. Cells of JG31, heat-induced and uninduced, were harvested and frozen overnight at −20°C. The cells were then thawed, ground with alumina and treated with DNase (Worthington). The rho factor was precipitated by adding $(NH_4)_2SO_4$ to 50% saturation, then dialyzed and loaded onto phosphocellulose columns. After gradient elution, the column fractions were assayed for rho factor (Fig. 1(a)), according to the method of Roberts (1969). The fractions at the depression maximum eluting at 0·16 M-phosphate were pooled. Rho factor was quantitated by doing depression curves using *λ* DNA as RNA polymerase templates (Fig. 1(b)). Since the curves plateau at about 35% of the initial incorporation, the activity being measured is rho factor and not some unknown nuclease.

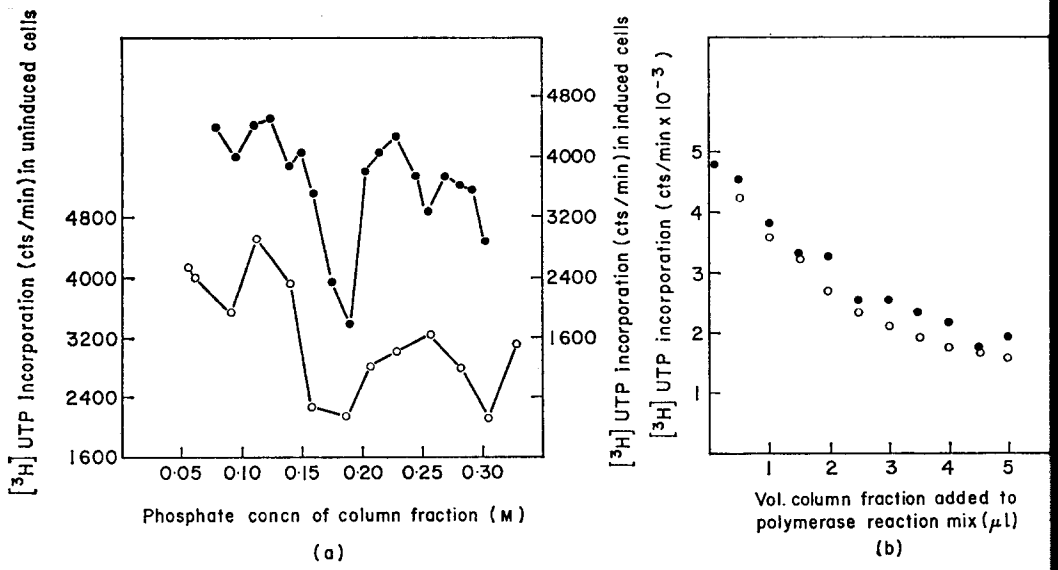

FIG. 1. (a) Effect of phosphocellulose column fractions on RNA polymerase activity using λ DNA as template. To a standard reaction (Roberts, 1969) containing 0·5 μCi of [^{3}H]UTP and 0·5 μg of RNA polymerase, purified according to the glycerol gradient technique of Burgess (1969), was added 5 μl. of a column fraction. [^{3}H]UTP incorporation into acid-insoluble material was determined after a 20-min incubation at 37°C. The position of maximum depression of activity elutes at 0·16 M-phosphate and is due to the rho factor. —●——●—, Extract from induced cells; —○——○—, extract from uninduced cells.

(b) Quantitation of rho factor. The 2 tubes containing maximum depressing activity (from (a)) were pooled, both from the induced cell column and the uninduced cell column. On the basis of an average absorbance of 0·5 at 280 nm of a 1 mg/ml. protein solution, each fraction contained about 200 μg protein/ml. To standard RNA polymerase reactions (Fig. 1(a)) were added increasing amounts of pooled column fraction, and [^{3}H]UTP incorporation was determined. (●) Pooled fractions from induced cells; (○) pooled fractions from uninduced cells.

(iv) *λ gene Q product made by N-independent phage*

The amount of *Q* product synthesized by an *N*-independent phage relative to a wild-type phage was estimated by the amount of lysozyme induced from a λ prophage following heteroimmune infection. These experiments were similar in execution to those described in Results. The superinfecting *N*-independent or wild-type phage possessed phage 434 immunity and had an inactive lysozyme gene by virtue of the $sus R_5$ mutation which had been crossed into it. The R^- derivative of the *N*-independent phage $\lambda N^- imm^{434} C_{17} byp$ leads to *trans* induction by *Q* of less than 10% of the amount of lysozyme induced by wild-type λ. It should be noted that the R^- wild-type induces lysozyme in *trans* to about 2% the level it is induced during growth of $\lambda CI_{857} R^+$. This result can be used to estimate that about 50 copies of the λ genome are used for late messenger transcription (see also the Results section).

(i) *Electron microscopy of heteroduplexes*

(i) *Heteroduplex preparation*

To a small test tube were added 5 μl. of 0·2 M-EDTA, 1 μl. of each phage at O.D.$_{260}$ = 10, 33 μl. of water, and 5 μl. of 1·0 N-NaOH. This alkaline condition lyses the phage and denatures the DNA. After 10 min, the solution was neutralized by the addition of 5 μl. 1·8 M-Tris · HCl, 0·2 M-Tris base and the DNA was allowed to renature by the addition of 50 μl. formamide. After 50% renaturation (requiring 1·5 hr), the DNA was used for electron microscopy. The preparation contains re-annealed parental duplexes and also

heteroduplexes which are double-s
DNA strands.

(ii) *Electron microscopy*

To 10 μl. of the heteroduplex p
1 mg cytochrome *c*/ml., 1 M-Tris (p
mide. This solution was layered on
EDTA (pH 8·5), and examined un
outlined by Davis, Simon & Davids
like structures, while single-strand
standards used to determine length
RFII DNA and φX174 single-strand

3. Re

(a) *Synthesis of ara*

This study began with the ob
the λ*dara* phage, was induced du
L-arabinose! These experiments
least chromosomal arabinose gen
The λ*dara* prophage itself is the
(Gottesman & Beckwith, 1969)
cross-over between these two pha
occurring within their regions of
the gene *exo*. As a result, it has th
and the lysis defect S_{t68} (Harris
JG31 contains no other prophage
of its construction and testing are

Strain JG31 can grow on L-ara
λ*dara* prophage contains arabinos
of L-arabinose, the basal level of
RFS1, the parent of JG31 cont
grown in the presence of L-arabi
and RFS1 are also comparable, a
like the chromosomal arabinose
the λ*dara* phage is sensitive to
operon has been induced for 15 m
of glucose reduces the rate of fu
tenfold for at least 15 minutes.
allele, and if this *C* gene is made
terized C^c mutations onto it, thes
region of λ*dara* behaves normall
mentioned here that the arabin
strain B/*r*, but the K12 and B/*r*
1969).

The presence of the thermolal
population of cells lysogenic for
irreversibly induced by heating
induction was essential for accu
enzymes. 15 minutes after the beg

heteroduplexes which are double-stranded only in regions of homology of the parental DNA strands.

(ii) *Electron microscopy*

To 10 μl. of the heteroduplex preparation was added 10 μl. of a solution containing 1 mg cytochrome *c*/ml., 1 M-Tris (pH 8·5), 0·1 M-EDTA, 45 μl. of water and 35 μl. formamide. This solution was layered onto a solution of 10% formamide in 0·01 M-Tris, 10 mM-EDTA (pH 8·5), and examined under the microscope in accordance with the procedure outlined by Davis, Simon & Davidson (1971). Double-stranded DNA appears as rigid rod-like structures, while single-stranded regions are more flexible and less distinct. The standards used to determine lengths of double- and single-stranded regions were φX174 RFII DNA and φX174 single-stranded DNA.

3. Results and Discussion

(a) *Synthesis of arabinose isomerase during phage growth*

This study began with the observation that arabinose isomerase, coded for by the λd*ara* phage, was induced during growth of the phage, even in the absence of L-arabinose! These experiments were done in strain JG31, which is deleted of at least chromosomal arabinose genes *C*, *B* and *A*, and contains λd*ara* as a prophage. The λd*ara* prophage itself is the product of a cross between the phage φ80d*ara* (Gottesman & Beckwith, 1969) and the λ–φ80 hybrid phage $\lambda CI_{857}S_{t68}h80$. The cross-over between these two phages was most likely a normal recombination event occurring within their regions of homology, lying between the attachment site and the gene *exo*. As a result, it has the heat inducibility CI_{857} (Sussman & Jacob, 1962) and the lysis defect S_{t68} (Harris, Mount, Fuerst & Siminovitch, 1967). The strain JG31 contains no other prophage with λ or φ80 immunity besides λd*ara*, and details of its construction and testing are found in the Materials and Methods section.

Strain JG31 can grow on L-arabinose as a sole carbon source and, therefore, its λd*ara* prophage contains arabinose genes *C*, *B*, *A* and *D*. When grown in the absence of L-arabinose, the basal level of arabinose isomerase is about the same as that of RFS1, the parent of JG31 containing a wild-type K12 arabinose region. When grown in the presence of L-arabinose, the fully induced levels of isomerase in JG31 and RFS1 are also comparable, about 300-fold above their basal level. Furthermore, like the chromosomal arabinose operon in *E. coli* strains, the arabinose operon on the λd*ara* phage is sensitive to catabolite repression. After the phage arabinose operon has been induced for 15 minutes by L-arabinose in the medium, the addition of glucose reduces the rate of further arabinose isomerase synthesis by more than tenfold for at least 15 minutes. The *C* gene on this phage is dominant to the C^c allele, and if this *C* gene is made C^c either by mutation *in situ* or by crossing characterized C^c mutations onto it, these are recessive in the C^+ allele. Thus, the arabinose region of λd*ara* behaves normally in the absence of phage growth. It should be mentioned here that the arabinose genes of λd*ara* are derived from the *E. coli* strain B/*r*, but the K12 and B/*r* arabinose operons are virtually identical (Schleif, 1969).

The presence of the thermolabile CI_{857} λ repressor allowed the prophages in a population of cells lysogenic for $\lambda CI_{857}S_{t68}$d*ara* to be quickly, simultaneously and irreversibly induced by heating cells to 42°C for ten minutes. This synchrony of induction was essential for accurate timing of the synthesis of phage-controlled enzymes. 15 minutes after the beginning of heat induction of the prophage, arabinose

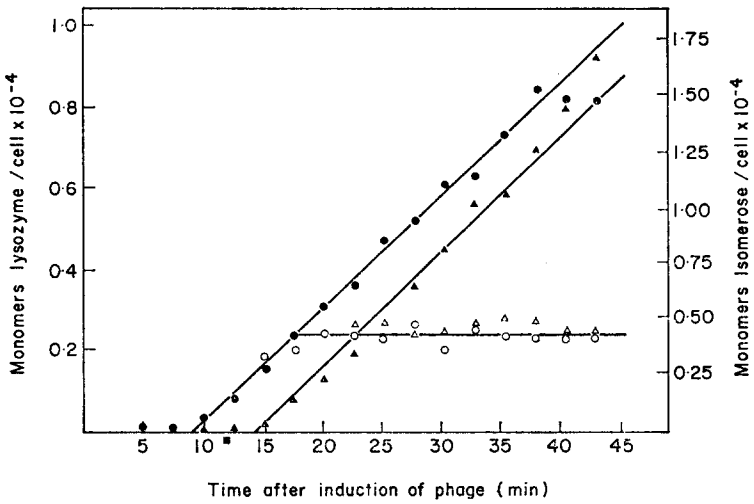

FIG. 2. Kinetics of arabinose isomerase and phage lysozyme production after heat induction of the λd*ara* phage in strain JG31. Cells were grown in YT medium to a cell density of 3×10^8/ml. The temperature was shifted to 42°C for 10 min and then to 34°C for the remainder of the experiment, with constant shaking maintained in a water bath. At the indicated times, 6-ml. samples were put into centrifuge tubes in ice water. 15 min after beginning induction, rifampicin was added to a parallel flask. At the end of the experiment, 1-ml. samples were sonicated and assayed for phage lysozyme and the remaining 5 ml. were concentrated tenfold by centrifugation and assayed for arabinose isomerase. Open symbols represent the results of assays on cells treated with rifampicin; ●—●, ○—○, lysozyme; ▲—▲, △—△, isomerase.

isomerase begins to appear at about 400 times its basal rate (Fig. 2). The synthesis is linear for at least two hours, although only the first 45 minutes are shown in the Figure. The kinetics of synthesis of phage lysozyme were also followed in the same experiment. Lysozyme begins to appear nine minutes after induction and it too is synthesized linearly for at least two hours.

Since many *E. coli* genes show a gene dosage effect, it might be expected that the rates of lysozyme and isomerase synthesis would increase in concert with λ DNA replication. Clearly this is not the case for the phage late genes, and so something other than the absolute number of genome copies must limit the total rate of synthesis of phage late proteins. The observed linear rate of lysozyme synthesis is not a peculiarity of the phage mutants used; the same kinetics are obtained with a non-defective heat inducible λ phage or a λd*ara* with a different mutation, S_7, in the *S* gene (Goldberg & Howe, 1969). Furthermore, a gene dosage effect cannot explain the high rate of isomerase synthesis shown in Figure 2, for the rate of *lac* repressor synthesis is increased only 20-fold following heat induction of *lac* transducing phage (Müller-Hill, Crapo & Gilbert, 1968), whereas the rate of isomerase synthesis is increased 400-fold following heat induction of λd*ara*.

In view of the very high constant rate of synthesis of arabinose isomerase after prophage induction and the timing of lysozyme and isomerase appearance, it seemed likely that the arabinose operon genes on the phage were being controlled as phage late genes. The five-minute lag between the initial appearances of lysozyme and isomerase could be explained as the time required for RNA polymerase to transcribe the DNA between these genes. This view is consistent with the location and orienta-

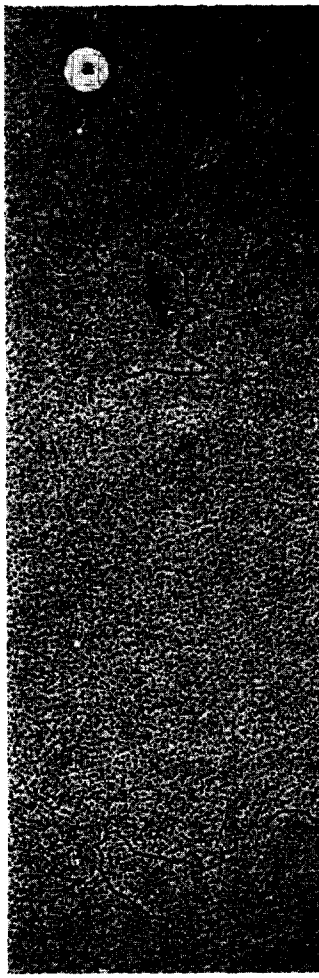

PLATE I. (a) λd*ara*/λ*h80* heterod
strand forms a region of non-hom
region composed of two unpaired
DNA is located on the right side o

(b) λd*ara*/λb_2b_5 heteroduplex. I
b_5 substitution (φ21 immunity sub
and serves to mark the right arm
The amount and location of λ DN
duplex DNA on both sides of the s
region to the right end of *E. coli*
exo-β homology region to the φ80
Lozeron & Szybalski, 1971). There
ment point. The right end of the I

The conditions of renaturation
only rarely formed the duplexes
Under these conditions, the closel
tures.

The grids were prepared for elec
platinum–paladium. DNA length
φX174 DNA. The λd*ara* and λφ80

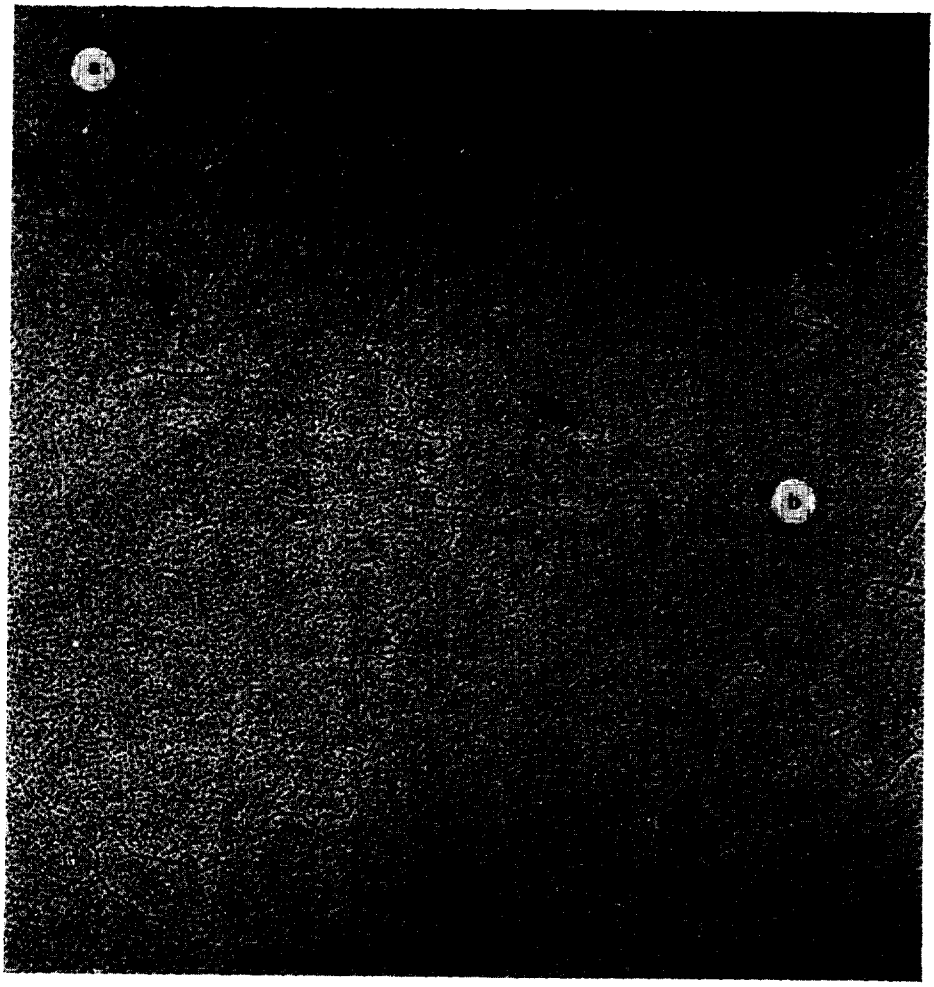

PLATE I. (a) λd*ara*/λ*h80* heteroduplex. In this heteroduplex, the *E. coli* DNA in the λd*ara* DNA strand forms a region of non-homology with the λ–φ80 hybrid strand. This is seen to be a single region composed of two unpaired single strands of DNA (between arrows). The right end of the DNA is located on the right side of the electron micrograph.

(b) λd*ara*/λb_2b_5 heteroduplex. In this heteroduplex, only λ DNA can form duplex regions. The b_5 substitution (φ21 immunity substitution) forms a small non-homology region (between arrows) and serves to mark the right arm of this heteroduplex and to locate precisely the immunity region. The amount and location of λ DNA in the λd*ara* DNA are determined by measuring the amount of duplex DNA on both sides of the substitution. The distance from the left end of the *exo*-β homology region to the right end of *E. coli* DNA is 0·079 ± 0·006 λ unit, whereas the distance from the *exo*-β homology region to the φ80 attachment site is known to be 0·052 unit (Fiandt, Hrodecna, Lozeron & Szybalski, 1971). Therefore, φ80 DNA must extend 0·027 unit to the left of the attachment point. The right end of the DNA is located on the right side of the electron micrograph.

The conditions of renaturation of the denatured DNA strands were such that λ and φ80 DNA only rarely formed the duplexes in the late homology seen by Fiant (personal communication). Under these conditions, the closely related phages 80 and 21 also only rarely form duplex structures.

The grids were prepared for electron microscopy by the formamide technique and shadowed with platinum–paladium. DNA lengths were calibrated with double-stranded and single-stranded φX174 DNA. The λd*ara* and λφ80 phage DNA's were prepared from strain JG39.

[*facing p. 136*

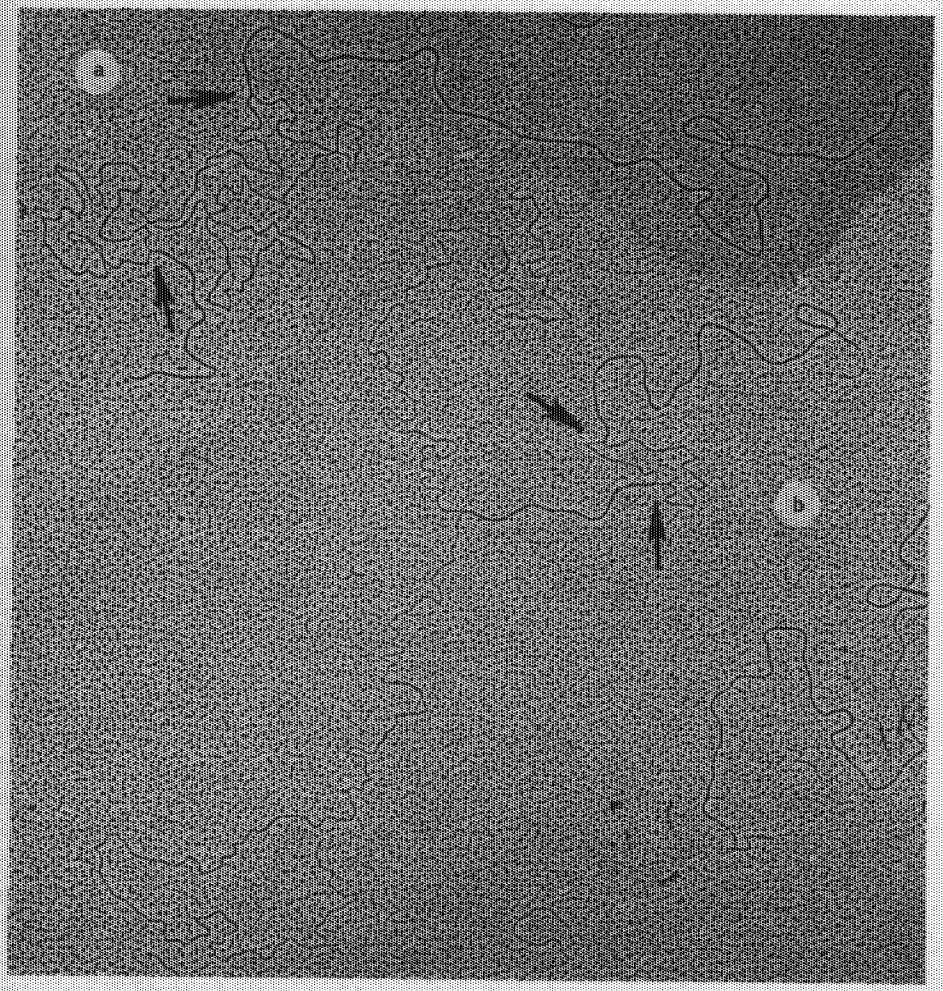

PLATE I. (a) *λdara/λh80* heteroduplex. In this heteroduplex, the *E. coli* DNA in the *λdara* DNA strand forms a region of non-homology with the λ–ϕ80 hybrid strand. This is seen to be a single region composed of two unpaired single strands of DNA (between arrows). The right end of the DNA is located on the right side of the electron micrograph.

(b) *λdara/λb$_3$b$_5$* heteroduplex. In this heteroduplex, only λ DNA can form duplex regions. The *b*$_5$ substitution (ϕ21 immunity substitution) forms a small non-homology region (between arrows) and serves to mark the right arm of this heteroduplex and to locate precisely the immunity region. The amount and location of λ DNA in the *λdara* DNA are determined by measuring the amount of duplex DNA on both sides of the substitution. The distance from the left end of the *exo-β* homology region to the right end of *E. coli* DNA is 0·079 ± 0·006 λ unit, whereas the distance from the *exo-β* homology region to the ϕ80 attachment site is known to be 0·052 unit (Fiandt, Hrodecna, Lozeron & Szybalski, 1971). Therefore, ϕ80 DNA must extend 0·027 unit to the left of the attachment point. The right end of the DNA is located on the right side of the electron micrograph.

The conditions of renaturation of the denatured DNA strands were such that λ and ϕ80 DNA only rarely formed the duplexes in the late homology seen by Fiant (personal communication). Under these conditions, the closely related phages 80 and 21 also only rarely form duplex structures.

The grids were prepared for electron microscopy by the formamide technique and shadowed with platinum–paladium. DNA lengths were calibrated with double-stranded and single-stranded ϕX174 DNA. The *λdara* and *λϕ80* phage DNA's were prepared from strain JG39.

[*facing p. 136*

tion of arabinose genes in the phage DNA (see next section) and with data (Toussaint, 1969; Herskowitz & Signer, 1970) indicating that there is only a single promoter for phage late genes located between genes *Q* and *S* (see Fig. 3).

We have also determined that the unlinked chromosomal gene coding for the arabinose binding protein (Schleif, 1969) is not turned on at all after heat induction of strain JG31. This implies that phage-induced synthesis of arabinose isomerase is not a consequence of titration of available repressor followed by escape from repression after replication of the λd*ara* genome (Epstein, 1967). Therefore, the experiments described in the following sections, including a physical and genetic study of the phage, were designed to test the hypothesis that the arabinose operon of λd*ara* is transcribed during phage growth as a consequence of transcriptional read-through from phage late genes.

(b) *Structure of the* λdara *phage*

The structure of the λd*ara* genome was determined genetically by marker rescue tests and physically by electron microscopy of heteroduplexes formed between λd*ara* DNA and the DNA of other testing phages. Both methods agreed and gave the structure shown in Figure 3. In the event producing φ80d*ara*, *E. coli* DNA containing the arabinose operon substituted for some of the late genes on the left arm of φ80 (Gottesman & Beckwith, 1969), and then in the cross between φ80d*ara* and λ*hy*80, φ80 genes from *exo* rightward were replaced by genes of λ origin, yielding the final λd*ara* phage containing regions of DNA of *E. coli*, of φ80, and of λ origin. The event producing the φ80d*ara* was unusual in that the inserted *E. coli* DNA was also inverted. In making the λd*ara* from φ80d*ara* and λ–φ80 hybrid, only a normal recombinational event in regions of homology was required.

In the marker rescue experiments, cells lysogenic for λd*ara* were infected with φ80 or λ*imm*^{434} containing amber mutations in various genes. These heteroimmune phage can grow on the lysogens only if they can replace their defective gene with a non-defective one from the prophage. By this method we found that λd*ara* contains only the first three φ80 genes on the left side, most likely corresponding to λ genes *A*, *W* and *B*. λd*ara* was also shown to possess λ genes *O*, *P*, *Q* and *R* by marker rescue and genes C_1 and *S* by the behavior of lysogenic cells.

With the electron microscope, single- and double-stranded DNA regions can be distinguished and measured. Therefore, the genome structure can be determined by examination of heteroduplexes formed between λd*ara* and other kinds of phage. Plate I shows the heteroduplex formed between DNA strands from λd*ara* and $\lambda b_2 b_5$. The characteristic region of non-homology between the λ and b_5 immunity regions identifies the right arm of the heteroduplex, and so the regions of λ homology between the two phages are determined. The Plate also shows the heteroduplex formed between λd*ara* and λ*hy*φ80. Together these observations also lead to the structure shown in Figure 3. The electron microscopy permits an accurate determination of the cross-over points, as indicated in the lower part of the Figure.

The excision event which produced the phage φ80d*ara* was unusual since the inserted arabinose operon of the phage is inverted from its original orientation with respect to the parent φ80 phage (Gottesman & Beckwith, 1969). In most transducing phage, the inserted *E. coli* DNA extends rightward or leftward from the phage *att* site. Electron microscopy indicates that the λd*ara* phage is also unusual in that the region of *E. coli* begins not at the φ80 *att* site but 3% of the phage genome to the

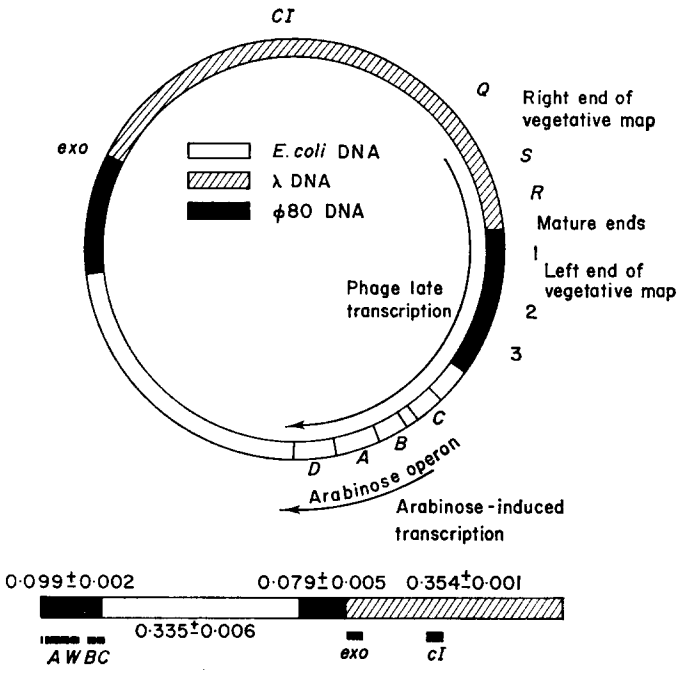

FIG. 3. The structure of the λd*ara* phage as determined by genetic and physical data. The origins of the various regions of phage DNA are noted as well as the locations of several λ genes. The phage late-gene transcription begins in the region between genes *Q* and *S*, proceeds across the mature ends of the molecule into the φ80 genes *1*, *2* and *3* and continues into the arabinose genes. The arabinose genes are drawn to scale except for the *C* gene which is drawn to a size corresponding to a 40,000 molecular weight protein. The location of the arabinose genes is assigned according to the 5-min transcriptional lag between the end of the lysozyme gene (*R*) and the end of the isomerase gene (*A*) on the basis of a transcription rate of 40 bases/sec (Manor *et al.*, 1969). At the higher rate of 65 bases/sec, the arabinose region would be located further into the *E. coli* DNA. The orientation of the arabinose region is known from the work of Gottesman & Beckwith (1969).

When the circular map, which represents the topology during phage growth, is opened at the mature ends, the linear rods represent the DNA as extracted from mature phage. This is shown in the bottom half of the Figure. The measured sizes of the DNA segments are shown along with the location and size of relevant λ genes. This linear form is to be compared with the electron micrographs in Plate I. The error flags indicate the interval which, at the 95% confidence level, contains the mean of the population from which the sample was drawn.

left of it (see Plate legend for data). The other end of the inserted *E. coli* DNA lies in what would correspond to λ gene *C*.

The distance from the late gene promoter, p'_R, located between genes *Q* and *S*, to the beginning of DNA of *E. coli* origin is about 18% of the length of λ. Therefore, time required for transcription of the DNA lying between the lysozyme gene, *R*, located close to the late promoter, and the isomerase gene, *araA*, located somewhere in the region of *E. coli* DNA, could be responsible for the five-minute lag between the times of lysozyme and isomerase appearance after phage induction. Assuming a transcription rate of 40 nucleotides per second (Manor, Goodman & Stent, 1969), the arabinose operon would be located as shown in Figure 3. This figure of 40 nucleotides per second is nearly the lower limit compatible with the physical structure,

and clearly the higher figure of
& Yuan, 1968), would locate the

(c) *Control of arabin*

Lambda late messenger synth
product (Skalka *et al.*, 1967; Ta
the arabinose operon in the λd*ar*
be possible to show that its induc
product. It was possible to dem
immune infection) originally used
requires a functioning *Q* product
infected with λ*imm*^{434} phage. Th
through a normal growth cycle,
then late proteins with *Q* produ
replication (Thomas, 1966; Gre
expression of other *N* mediated
(1966) work, we know that its l
gene product of the growing het
increase in the rate of isomeras
detectable stimulation of isomer
follows that *Q* product or someth
of λd*ara* arabinose isomerase.

Since genetic evidence indicat
positive control (Englesberg *et al*
the *Q* product could stimulate isom
promoter site where the *araC*

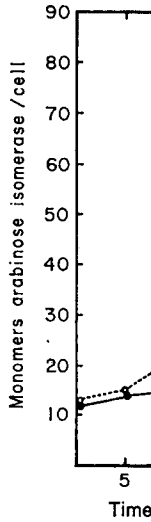

FIG. 4. The turn-on of arabinose ison
infecting phage λ*imm*^{434}*Q*$^+$ and the l
growth and phage infection were as des
of cells were pipetted into chilled centr
concentration of 0·01 M. Cells were
arabinose isomerase and phage lysozy

and clearly the higher figure of 55 nucleotides per second also suggested (Bremer & Yuan, 1968), would locate the arabinose operon further into the *E. coli* DNA.

(c) *Control of arabinose isomerase synthesis by phage gene* Q

Lambda late messenger synthesis is positively controlled by the phage gene *Q* product (Skalka *et al.*, 1967; Taylor *et al.*, 1967). If our suggestion is correct, that the arabinose operon in the λd*ara* phage is controlled as a phage late gene, it should be possible to show that its induction during phage growth requires functional *Q* gene product. It was possible to demonstrate this *Q* dependence by a technique (heteroimmune infection) originally used by Thomas (1966) to show that lysozyme synthesis requires a functioning *Q* product. In our experiment, cells lysogenic for λd*ara* were infected with λimm^{434} phage. The infecting heteroimmune 434 phage can proceed through a normal growth cycle, first inducing early proteins with *N* product, and then late proteins with *Q* product. The prophage, λd*ara*, is prevented from DNA replication (Thomas, 1966; Green, Gotchell, Henderschott & Kennel, 1967) and expression of other *N* mediated functions by its repressor. However, from Thomas' (1966) work, we know that its late genes should be turned on by the diffusible *Q* gene product of the growing heteroimmune phage. We have found a 10- to 15-fold increase in the rate of isomerase synthesis following $\lambda imm^{434}Q^+$ infection and no detectable stimulation of isomerase synthesis after $\lambda imm^{434}Q^-$ infection (Fig. 4). It follows that *Q* product or something under its control is required for the induction of λd*ara* arabinose isomerase.

Since genetic evidence indicates that both the *C* and *Q* proteins demonstrate positive control (Englesberg *et al.*, 1965; Dambly *et al.*, 1968), it is conceivable that the *Q* product could stimulate isomerase synthesis by acting at the arabinose operator-promoter site where the *araC* gene product normally acts. This seems unlikely

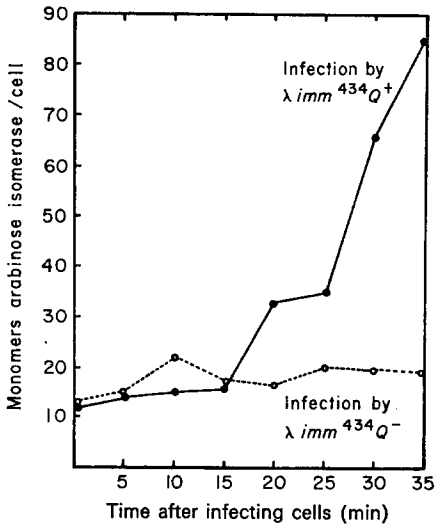

FIG. 4. The turn-on of arabinose isomerase in strain JG31 by the *Q* product of the heteroimmune infecting phage $\lambda imm^{434}Q^+$ and the lack of turn-on after infection with $\lambda imm^{434}Q^-_{73,501}$. Cell growth and phage infection were as described in Materials and Methods. At each time point, 25 ml. of cells were pipetted into chilled centrifuge tubes containing enough sodium azide to give a final concentration of 0·01 M. Cells were concentrated by centrifugation and assayed as usual for arabinose isomerase and phage lysozyme.

because, as has already been mentioned, the unlinked gene coding for the arabinose binding protein, also under gene *araC* control, is not induced during growth of λd*ara*.

Nevertheless, this possibility was directly excluded by an experiment in which $\lambda imm^{434}Q^+$ was used to infect a strain containing a chromosomal arabinose operon, genes *BAD*, and not lysogenic for λ*ara*. Arabinose isomerase synthesis was not stimulated after infection of this strain. Thus, the *Q* gene product is required for phage induction of the arabinose operon of λd*ara* and can only induce the operon by acting at a site specific to the phage, presumably the late gene promoter located between phage genes *Q* and *S*.

The strength of the λ late promoter can be estimated by comparing the synthesis rate of a single *araA* gene after heteroimmune infection of a λd*ara* lysogen with the synthesis of one *araA* gene induced by the presence of L-arabinose in the medium. In terms of a basal rate of isomerase synthesis of 1, the *Q* gene induced rate of isomerase synthesis per genome is 10 and the L-arabinose plus gene *C* protein induced rate of isomerase synthesis per genome is 300. Thus, a fully induced arabinose operon promoter is about 30 times as effective as the λ late gene promoter.

It also follows that, since the rate of isomerase synthesis under *Q* gene control is about 400 when the λd*ara* phage is replicating, about 400/10 = 40 copies per cell of the λ genome must be used as templates for late messenger synthesis during the development of phage λ. Consideration of the rates of phage lysozyme synthesis after induction and after heteroimmune infection of an R^+ lysogen by an R^- phage leads to the same conclusion (see Materials and Methods for data). Therefore, replicated genomes are of considerable importance in normal growth of phage λ. This calculation depends, of course, upon the correctness of the assumption that a prophage genome is transcribed at the same rate as a genome in the vegetative pool.

It should be noted that the pool of intracellular λ DNA contains about 50 genome equivalents ten minutes after infection (Carter & Smith, 1970). Since the rate of lysozyme synthesis is maximal beginning ten minutes after induction (Fig. 2), our estimation that 40 genome copies are used as templates for late protein synthesis is consistent with the actual amount of λ DNA present.

(d) *Synthesis of messenger RNA for lysozyme and isomerase*

Several predictions follow from the idea that the arabinose operon on the phage can be transcribed under control of the phage late gene promoter. We have already demonstrated control by the *Q* gene product, and the fact that after phage induction, lysozyme begins to appear five minutes before arabinose isomerase. Here we shall present evidence to show that the observed five-minute delay in isomerase appearance represents the time required for RNA polymerase molecules to travel from the lysozyme gene to the isomerase gene.

Once initiation of phage late messenger begins at the late gene promoter, each RNA polymerase molecule should transcribe first the *S* and *R* genes and later, the isomerase gene. Behind the first polymerase to cross the isomerase gene should be others which initiated messenger synthesis at later and later times. Consider the situation if, at the time the first polymerase molecules are crossing the isomerase gene, we block with rifampicin further initiation of late messenger synthesis, but allow all partially completed messengers to be fully completed and translated. Then, the train of polymerases (with the earliest molecules to initiate being at the end of the isomerase gene and the latest to initiate being near the late gene promoter) will

sweep across the lysozyme and
the lysozyme gene promptly sir
genes *Q* and *S*, but pass the isor
experiment are shown in Figur
pectations, lysozyme synthesis
continues for seven minutes aft

One artifactual explanation o
could be a long delay in the acqu
of the isomerase polypeptide. Th
protein synthesis promptly halt
a final concentration of 100 μg
beginning of heat induction at t
to appear. Further increase in i
isomerase level remained consta

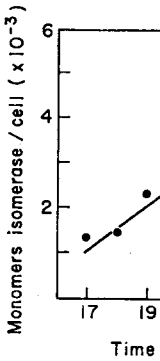

FIG. 5. Isomerase synthesis after
same as that described in Fig. 2 with
induction, chloramphenicol was adde

isomerase appearance cannot b
isomerase subunits. The observ
the messenger segment coding

The experiment with rifampic
initial polymerase initiations ne
initiations near the isomerase g
sustain synthesis of arabinose i
Nevertheless, this experiment d
merase initiations begin simulta
genes *Q* and *S* and at a second pl
time upstream from the arabino
phage late promoter exists whi
kowitz & Signer, 1970). The po
of RNA polymerase molecules m
then be activated by the pass
between genes *Q* and *S*. Our dat

An important consequence of

sweep across the lysozyme and isomerase genes. The end of this train should pass the lysozyme gene promptly since it is located quite close to the promoter between genes *Q* and *S*, but pass the isomerase gene five minutes later. The results of such an experiment are shown in Figure 2. It can be seen that, in agreement with our expectations, lysozyme synthesis stops within two minutes, while isomerase synthesis continues for seven minutes after the addition of rifampicin.

One artifactual explanation of the delays in the appearance of arabinose isomerase could be a long delay in the acquisition of isomerase enzymic activity after synthesis of the isomerase polypeptide. This possibility was excluded by showing that stopping protein synthesis promptly halts increase in isomerase activity. Chloramphenicol, at a final concentration of 100 μg/ml., was added to a culture 22·5 minutes after the beginning of heat induction at the time when isomerase activity was just beginning to appear. Further increase in isomerase activity ceased within 30 seconds and the isomerase level remained constant for 30 minutes (Fig. 5). Therefore, the delays in

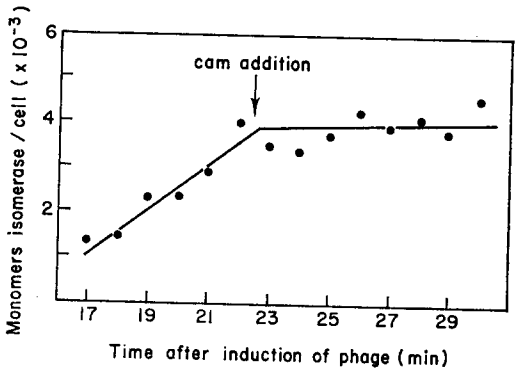

FIG. 5. Isomerase synthesis after addition of chloramphenicol (cam). This experiment was the same as that described in Fig. 2 with the exception that at 22·5 min after the beginning of heat induction, chloramphenicol was added.

isomerase appearance cannot be caused, for example, by slow aggregation of the isomerase subunits. The observed lags most likely represent delays in synthesis of the messenger segment coding for arabinose isomerase.

The experiment with rifampicin, shown in Figure 2, excludes the possibility that initial polymerase initiations near the lysozyme gene lead to subsequent polymerase initiations near the isomerase gene. Such adjacent secondary initiations could not sustain synthesis of arabinose isomerase for five minutes after rifampicin addition. Nevertheless, this experiment does not, by itself, rule out the possibility that polymerase initiations begin simultaneously at the phage late promoter located between genes *Q* and *S* and at a second phage late promoter located five minutes transcription time upstream from the arabinose operon. It is the data indicating that no second phage late promoter exists which rule out this possibility (Toussaint, 1969; Herskowitz & Signer, 1970). The possibility has been pointed out to us that a number of RNA polymerase molecules may bind to the late region of the λ DNA. These could then be activated by the passage of a polymerase which initiated in the region between genes *Q* and *S*. Our data and conclusions are compatible with such a model.

An important consequence of the idea that the lysozyme and isomerase genes are

both parts of a single-phage late gene operon is that the messengers coding for isomerase and lysozyme must be initiated at the same time, since they are, in fact, the same messenger. By adding rifampicin to a heat-induced culture at a given time after induction to block further initiation of messenger synthesis, and then allowing time for the completion and translation of all messenger initiated up to that time, it is possible to assay the total amount of messenger initiated up to the time of rifampicin addition. Then, by varying the time at which rifampicin is added, it is possible to learn the amount of messenger initiated up to any time.

The kinetics of initiation of lysozyme and isomerase messenger RNA are presented in Figure 6. Rifampicin was added to samples of cells at the indicated times after

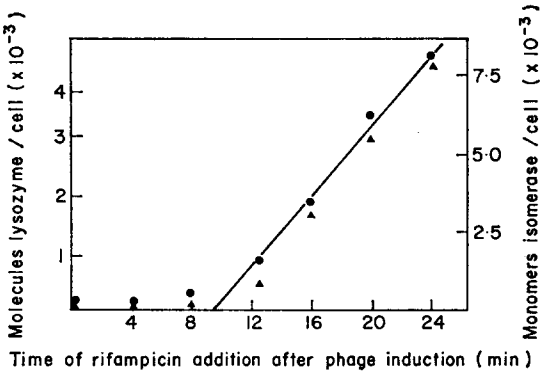

FIG. 6. Kinetics of initiation of messenger RNA for phage lysozyme and arabinose after heat induction of the λd*ara* phage in strain JG31. Cells were grown in YT medium at 34°C to a cell density of 3×10^8/ml. The temperature was shifted to 42°C for 10 min and then to 34°C for the remainder of the experiment. At the indicated times after beginning induction, samples were removed into flasks containing rifampicin and shaken at 34°C until the end of the experiment at 35 min. All samples were then chilled and assayed for lysozyme and isomerase. Phage lysozyme, (●); arabinose isomerase, (▲).

the beginning of heat induction and 20 minutes were allowed for expression of messenger in a sample before the same was chilled and enzyme assays were done. There were no detectable initiations until nine minutes after induction, and the subsequent messenger initiation kinetics for lysozyme and isomerase were identical within the limits of experimental accuracy.

Two independent types of experiments have indicated that the late genes of λ are transcribed under the control of a single promoter located between genes *Q* and *S* (Toussaint, 1969; Herskowitz & Signer, 1970). All of our data on the structure of the λd*ara* phage and on phage-controlled isomerase and lysozyme synthesis are consistent with this model. The five-minute delay in isomerase appearance represents the time required for RNA polymerase to transcribe the DNA between λd*ara* genes *R* and *araA* (Fig. 7).

The possibility has been mentioned (Herskowitz & Signer, 1970) that RNA polymerase molecules, which have initiated messenger synthesis at the late promoter, might transcribe the late gene region in segments. Unless evidence is found that RNA polymerase can terminate and then re-initiate synthesis of RNA without ever becoming sensitive to rifampicin, our experiments with rifampicin suggest that in

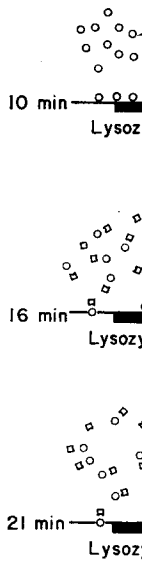

FIG. 7. Illustration of read-through
of RNA polymerase initiate transcript
between genes *Q* and *S* (10 min). The
5 min later and further initiation is bl
polymerase molecules to initiate tran
intervening DNA and are transcribing

λd*ara*, at least 8000 nucleotides o
E. coli DNA, are polymerized in

In any case, we believe that t
at the phage late promoter betw
phage late genes. At a rate of 40
minutes. Since we have shown t
nine minutes, there could be no
after induction. Consistent with
cellular infectious phage until ab
scription rate of 55 bases/second
considerable delay in assembly o

It has already been mentione
sensitivity to catabolite repressi
sensitive to catabolite repression.
that catabolite repression affect
elongation or translation of mess
messenger synthesis is initiated,
promoter, we should find that
insensitive to catabolite repressi
heat induction of the λd*ara* phag
arabinose isomerase, even though
is depressed at least ten-fold by

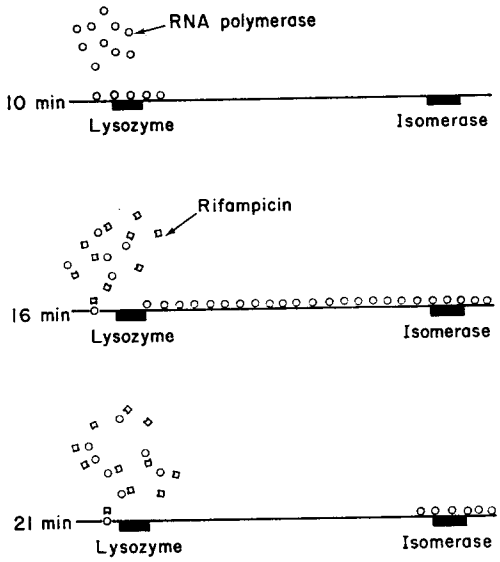

FIG. 7. Illustration of read-through from phage late genes into the arabinose operon. Molecules of RNA polymerase initiate transcription under *Q* gene control at the late gene promoter located between genes *Q* and *S* (10 min). The first polymerase molecules reach the arabinose operon about 5 min later and further initiation is blocked by rifampicin (16 min). After another 5 min, the last polymerase molecules to initiate transcription before rifampicion addition have negotiated the intervening DNA and are transcribing the arabinose operon (21 min).

λ*dara*, at least 8000 nucleotides of phage late messenger, from gene *R* to the region of *E. coli* DNA, are polymerized in one piece.

In any case, we believe that the majority of RNA polymerases initiate synthesis at the phage late promoter between genes *Q* and *S* and transcribe the entire set of phage late genes. At a rate of 40 bases/second, this process would require some ten minutes. Since we have shown that initiation of late messenger synthesis begins at nine minutes, there could be no tail protein (gene *J*) made until at least 19 minutes after induction. Consistent with this notion, we have found that there are no intracellular infectious phage until about 23 minutes after induction. At a higher transcription rate of 55 bases/second (Bremer & Yuan, 1968), there would have to be considerable delay in assembly of mature phage from a complete set of components.

It has already been mentioned that the arabinose operon of λ*dara* has normal sensitivity to catabolite repression. The phage control system is not likely to be sensitive to catabolite repression. Furthermore, it has been shown for the *lac* operon that catabolite repression affects only the rate of initiation and not the rate of elongation or translation of messenger RNA (Jacquet & Kepes, 1969). If isomerase messenger synthesis is initiated, not at the arabinose promoter but at the phage late promoter, we should find that the synthesis of arabinose isomerase has become insensitive to catabolite repression. As expected, adding glucose at any time after heat induction of the λ*dara* phage has no effect on the gene *Q* induced synthesis of arabinose isomerase, even though L-arabinose induced synthesis in the same medium is depressed at least ten-fold by glucose addition. This is additional evidence for the

view that arabinose operon messenger is initiated under gene *Q* control at a phage promoter, and suggests that RNA synthesis is not terminated and then re-initiated in the arabinose promoter region.

(e) *Transcriptional orientation of the gene* araC

Genetic work on the arabinose operon has established that the gene order is *CBAD* and that the structural genes *B*, *A* and *D* are read as a unit from *B* to *D* (Kessler & Englesberg, 1969). RNA polymerase molecules that have initiated messenger synthesis at the late gene promoter of λd*ara* and then transcribe the gene *araA* must also transcribe the intervening gene *araC*, providing then *C* still retains its normal position on the λd*ara* phage (see Fig. 3). Since gene *Q* induced isomerase synthesis continues for seven minutes after rifampicin addition and is not at all depressed by glucose addition, RNA synthesis is not terminated before the *C* gene and re-initiated at the arabinose promoter. It follows that the *C* gene must also be transcribed under gene *Q* control. However, the *C* gene *protein* would only be produced under gene *Q* control if the *C* gene has the same transcriptional orientation as the arabinose operon genes *B*, *A* and *D*.

Accordingly, an experiment to determine *C* gene orientation was designed with the object of detecting synthesis of gene *araC* product under gene *Q* control. It has already been mentioned that a cell of genotype $araC^c/C^+$, diploid for the arabinose region, behaves like a C^+ cell in exhibiting a basal isomerase level rather than a constitutive one (Englesberg *et al.*, 1965; Sheppard & Englesberg, 1966). Nevertheless, it is possible that an excess of C^c protein over C^+ protein could lead to dominance by the C^c phenotype. With this in mind, strains were constructed containing the $araC^+$ allele on an episome and an $araC^c$ allele on a λd*ara* prophage (see Materials and Methods for details of the construction of strains RFS628 and RFS629). As expected, the rate of isomerase synthesis was nearly basal in these strains in the absence of L-arabinose. By infecting these cells with the heteroimmune phage, $\lambda imm^{434}S_7$, containing the *S* gene lysis defect S_7 (Goldberg & Howe, 1969), it was possible to stimulate the late protein synthesizing system of the λd*ara* C^c prophage. This experiment is similar in principle to those described in section (c), and it should be recalled that replication of the λd*ara* prophage is prevented by λ repressor (Thomas, 1966).

The results of this experiment are presented in Figure 8. It can be seen that beginning about one hour after infection, arabinose isomerase begins to appear at a rate of about 100 times the basal rate. This is much above the rate of direct heteroimmune turn-on of the *araA* gene of the λd*ara* prophage that occurs as a result of read-through from phage late genes (Figs 4 and 8). The control in this experiment, JG62, $\lambda d araC^+$, is the same heteroimmune turn-on experiment shown in Figure 4. In the case of Figure 8, the isomerase assays were not done sensitively enough to show the direct *Q*-mediated turn-on shown in Figure 4. Since each cell still contains an equal number of C^c and C^+ genes, the partly constitutive rate of isomerase synthesis (fully constitutive is about 300 times basal level) observed implies that the prophage C^c gene is more active after infection than the episomal C^+ gene. The gene *araC* product can, therefore, be produced under gene *Q* control. It follows that the gene *araC* of λd*ara* is oriented in the same direction as the phage late genes and the arabinose operon structural genes.

This experiment may also provide information on how the *C* gene protein regulates

CONTRO

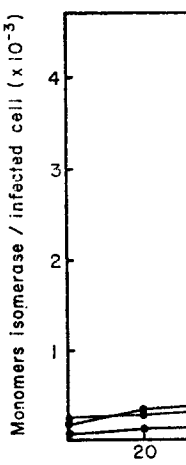

FIG. 8. Determination of the orien
operon chromosomal deletion lysogen
somal arabinose operon and containi
grown in YT medium to 3×10^8 cells/
in Materials and Methods. At interva
samples were put into centrifuge tub
samples were concentrated and assay
and shows that the large increase in th
is not, for example, due to accidental
ments, microscopic examination of th
elongated, a characteristic of cells gr
gone 3 to 4 doublings from the time c
initially uninfected was about 3% of t
distribution if the multiplicity of inp
tion in this experiment. Note the sc
Fig. 4.

arabinose operon expression. Th
dominant to gene C^+ protein, b
either subunit mixing or compet

(f) *Termination of I*

In the lactose system of *E.* c
operon and is transcribed in the
Beckwith, 1968). It has also be
or polarity in the translation at
we have shown that the regulat
as the arabinose operon genes, I
is formally analogous to that c
araC to *araA* does occur under t
seems a little surprising even th
stop signal would be advantage

One possibility is that the hig
fortuitous. It is true that, even m
of the lysozyme assay is only a
as many isomerase monomers as

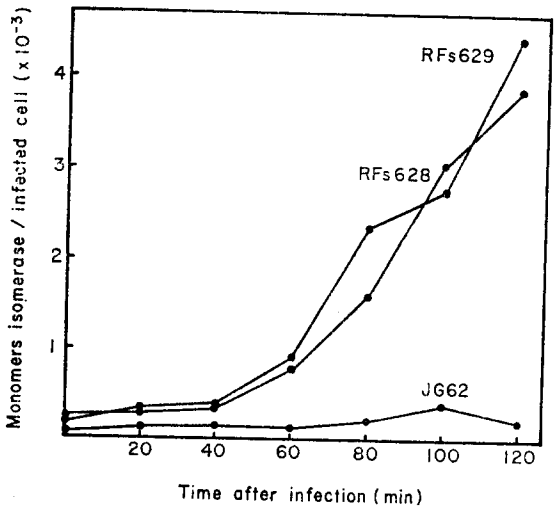

FIG. 8. Determination of the orientation of the gene *araC*. Cells of strain JG31, an arabinose operon chromosomal deletion lysogenic for *λdara*, and strains 628 and 629, deleted of the chromosomal arabinose operon and containing both a *λaraC*^{c} prophage and an F′–*araC*$^{+}$ episome, were grown in YT medium to 3×10^{8} cells/ml., concentrated, and infected with *λimm*$^{434}S_{7}$ as described in Materials and Methods. At intervals after the dilution into pre-warmed growth medium, 5-ml. samples were put into centrifuge tubes chilled in ice water, and at the end of the experiment the samples were concentrated and assayed for isomerase. Strain JG62 is a control for this experiment and shows that the large increase in the rate of isomerase synthesis observed in strains 628 and 629 is not, for example, due to accidental heat induction of their prophages. At the end of the experiments, microscopic examination of the cultures showed that approximately 50% of the cells were elongated, a characteristic of cells growing S^{-} mutants. Since uninfected cells could have undergone 3 to 4 doublings from the time of infection to the end of the experiment, the fraction of cells initially uninfected was about 3% of the cells. This would correspond to the zero term of a Poisson distribution if the multiplicity of input phage were 3·5, and thus we achieved about 70% adsorption in this experiment. Note the scale difference between this experiment and that shown in Fig. 4.

arabinose operon expression. The fact that an excess of gene C^{c} protein is partly dominant to gene C^{+} protein, but only after a substantial delay, is consistent with either subunit mixing or competition between C^{c} and C^{+} proteins.

(f) *Termination of RNA synthesis at the end of the* araC *gene*

In the lactose system of *E. coli*, the regulatory cistron, *i*, is in front of the *lac* operon and is transcribed in the same direction as the operon (Miller, Müller-Hill & Beckwith, 1968). It has also been shown that there is termination of transcription or polarity in the translation at the end of the *i* gene (Reznikoff *et al.*, 1969). Since we have shown that the regulatory gene, *araC*, is transcribed in the same direction as the arabinose operon genes, *B*, *A* and *D*, the arabinose system gene arrangement is formally analogous to that of the *lac* system. Nevertheless, read-through from *araC* to *araA* does occur under the positive control of the phage *Q* gene. This result seems a little surprising even though there is no obvious reason why a transcription stop signal would be advantageous at the end of a regulatory cistron.

One possibility is that the highly efficient read-through from *R* to *araA* is entirely fortuitous. It is true that, even making allowance for the fact that the standardization of the lysozyme assay is only accurate to within a factor of two, there are at least as many isomerase monomers as lysozyme monomers produced under phage control

(see Fig. 2). Even so, there could still be a 90% effective stop signal between genes *R* and *araA* if arabinose operon messenger is translated ten times as efficiently as phage lysozyme messenger. Such an unequal translation efficiency would not be unique since the RNA phage produce much more coat protein than gene *A* protein from the same messenger (Nathans, Oeschger, Polmer & Eggen, 1969), and the phage λ late messenger produces considerably more gene *E* protein than lysozyme (Hendrix, 1970). The only definite conclusion now possible is that, if there is a transcription stop at the end of the *C* gene, it is not likely to be more than 90% efficient, at least in cells growing λ.

That a transcription stop at the end of the *araC* gene might be inactive in cells growing λ is not unreasonable in view of what is presently known about the regulation of gene activity in phage λ. Current data support the hypothesis that the product of gene *N* stimulates early messenger synthesis by inactivating the transcription termination mechanism which requires rho factor (Roberts, 1969). (Rho factor is a protein which causes termination of RNA synthesis and release of the RNA chain from the DNA template when the RNA polymerase reaches specific sites on the DNA.) However, inactivation by the gene *N* product of all transcription termination mechanisms would lead to a depletion of free RNA polymerase molecules in the cell. Since we find that the lactose and arabinose operons are more than 50% fully inducible at any time after induction of phage λ, it is clear that at least some release and re-initiation of RNA polymerase is continuing in cells growing λ.

Nevertheless, it is possible that the gene *N* product of λ inactivates only one class of cellular termination mechanisms, and this is the class which terminates transcription at the end of the *araC* gene. We have done several experiments that render this explanation unlikely. First, rho factor was quantitated in cells growing λ by doing a partial purification and careful assay (see Materials and Methods). The amount of rho factor per cell is normal 30 minutes and one hour after heat induction of a λ lysogen and 10 minutes after infection by λCI_{857}. Since relatively small quantities of *N* gene product are needed for normal growth of λ (Schwartz, 1970), it is unlikely that we have purified rho away from *N* gene product, inhibiting its activity. It seems likely that rho is active *in vivo* in the same cells. Furthermore, we have found that RNA polymerase, partially purified from cells infected by λ, is still sensitive to rho factor.

Although these results make it unlikely that *N* product inactivates bacterial transcription stops, they cannot prove it. Therefore, more direct experiments were done to test whether read-through in λd*ara* can occur in the absence of *N* gene product or in the presence of a different *N* gene product.

Our direct test of an *N* gene function requirement for read-through provided no information. We used an *N*-independent phage of the type which bypasses its requirement for gene *N* function with a constitutive promoter mutation, C_{17}, located between genes *CI* and *O*, and a mutation of unknown function, *byp*, located between genes *P* and *Q* (Hopkins, 1970; Butler & Echols, 1970). Upon heteroimmune infection of a λd*ara* lysogen with this *N*-negative, *N*-independent phage, there was no detectable stimulation of isomerase synthesis from the prophage. However, the failure of the *N*-independent R^- phage to induce in *trans* detectable levels of lysozyme shows that this phage makes less than 10% the amount of *Q* product made by a wild-type λ (see Materials and Methods).

The best evidence we have obtained that *N* gene product is not needed to stimulate

read-through past *araC* in λd*ar*
effective in stimulating read-t
lysogen is similar after infecti
λ*imm*^{21}, having the *N* gene of
21 are different and do not co
seems unlikely that these differ
tion at the same stop signal at
that *N* gene function is not di

There is still the possibilit
stimulated by gene *Q* product
no reason to suspect that this
to separated strands of λ DNA
tion, that synthesis of late λ R
host cell RNA polymerase. Thu
host RNA polymerase and the
which might be incapable of r

The evidence can be summa
tion stop signal at the end of
more than 90% effective. Th
measurement of the amount of
control.

4. Sum

The synthesis of arabinose i
the phage λd*ara* is a consequ
genes to the arabinose operon.
phage, by the kinetics of lyso
initiation of arabinose isomera
this synthesis is under the con
repression. RNA polymerase r
araA also transcribe the regul
has the same structure as the
to have the same transcriptio
tural genes of the arabinose op
although this could be a result
part of the messenger than the
stop signal at the end of the *a*
efficient. Since read-through oc
N gene products of phage λ o
consequence of inactivation by
Implicit in these conclusions i
not been dismembered during
we feel this is unlikely since in
the same properties as a norm
appeared normal in gross map
normal recombinational event
this has not been explicitly sh

Due to the unverified struct

read-through past *araC* in λd*ara* is that the *N* products of phages λ and 21 are equally effective in stimulating read-through. Turn-on of arabinose isomerase in a λd*ara* lysogen is similar after infection either by λ*imm*^{434}, having the *N* gene of λ, or by λ*imm*^{21}, having the *N* gene of phage 21. Since the *N* gene products of phages λ and 21 are different and do not complement each other (Couterier & Dambly, 1970), it seems unlikely that these different *N* gene products can both act to prevent termination at the same stop signal at the end of the *araC* gene. This experiment also implies that *N* gene function is not directly required for late protein synthesis by phage λ.

There is still the possibility that the phage late gene transcription machinery stimulated by gene *Q* product cannot read transcription stop signals, but there is no reason to suspect that this is the case. Indeed, we have shown, by hybridization to separated strands of λ DNA of *in vivo* RNA labeled 26 or 43 minutes after induction, that synthesis of late λ RNA is as resistant or sensitive to rifampicin as is the host cell RNA polymerase. Thus, λ late messenger synthesis uses at least part of the host RNA polymerase and there is not likely to be an entirely new RNA polymerase which might be incapable of recognizing bacterial stop signals.

The evidence can be summarized by concluding that either there is no transcription stop signal at the end of the gene *araC* or that an existing stop signal is not more than 90% effective. The resolution of this ambiguity must await a direct measurement of the amount of arabinose operon messenger synthesized under phage control.

4. Summary and General Discussion

The synthesis of arabinose isomerase that occurs at a high rate during growth of the phage λd*ara* is a consequence of transcriptional read-through from phage late genes to the arabinose operon. This conclusion is supported by the structure of the phage, by the kinetics of lysozyme and isomerase synthesis, and by the kinetics of initiation of arabinose isomerase and phage lysozyme messenger RNA. Furthermore, this synthesis is under the control of phage gene *Q* and is not sensitive to catabolite repression. RNA polymerase molecules which read-through from phage gene *R* to *araA* also transcribe the regulatory gene *araC* (assuming the operon on the phage has the same structure as the normal chromosomal operon), which we have shown to have the same transcriptional orientation as the phage late genes and the structural genes of the arabinose operon. The read-through appears to be highly efficient, although this could be a result of more efficient translation of the arabinose operon part of the messenger than the lysozyme part. Nevertheless, if there is a transcription stop signal at the end of the *araC* gene, then it is not likely to be more than 90% efficient. Since read-through occurs in the presence of either of the non-complementing *N* gene products of phage λ or phage 21, it seems unlikely that read-through is a consequence of inactivation by gene *N* of a stop signal at the end of the *araC* gene. Implicit in these conclusions is the assumption that the arabinose operon itself has not been dismembered during the construction of these phages. As explained before, we feel this is unlikely since in all properties tested, the operon on the phage retains the same properties as a normal operon. Furthermore, since the operon on the φ80 appeared normal in gross mapping, and the λd*ara* was derived from the φ80 by a normal recombinational event, we believe the operon to be normal. Nonetheless, this has not been explicitly shown.

Due to the unverified structure of the arabinose operon on the λd*ara* phage and

the unknown effect the growth of λ phage has upon it—in particular, the effect upon recognition of transcription stop signals—we do not know whether the properties of the arabinose operon on the phage reflect the properties of a normal chromosomal operon. If, however, in the normal operon, there is no transcription stop at the end of the gene *araC*, then read-through from *araC* could contribute significantly to the basal level of arabinose enzymes in the cell. However, the rate of reading of the *C* gene in a wild-type cell should be low since it is a regulatory gene, and thus, the contribution of read-through to the basal enzyme level should be small in absolute amount. For example, the basal level of arabinose isomerase is about 20 monomers per genome, and if the number of monomers of *C* gene protein were ten per genome, then read-through would contribute 50% of the basal level. Thus, there appears to be no mechanistic requirement for termination at the end of the *C* gene.

Some evidence on control of the arabinose operon ascribes a repressor function to *C* gene protein (Sheppard & Englesberg, 1966; Kessler & Englesberg, 1969). However, bound *C* protein apparently does not appreciably hinder an RNA polymerase molecule from crossing the operator region between the *C* gene and the structural genes. This is not unexpected since even the *lac* repressor, which binds very tightly to its operator, stops sometimes none and at most only 90% of the RNA polymerase molecules which have initiated synthesis before the operator from transcribing across the *lac* operator region (Reznikoff *et al.*, 1969). *C* protein need bind to DNA only a little less tightly not to impede the progress of polymerase at all.

The read-through data provide enough information to calculate that about 40 copies of the λ genome are used for late messenger synthesis. In addition, the λ late promoter is only about 2 or 3% as effective as a fully induced arabinose operon promoter. Either the late promoter itself is fairly weak or else the relatively weak late synthesis is a reflection of the fact that the capacity of the cell to synthesize λ late protein is saturated as early as ten minutes after induction. With respect to control of late protein synthesis, our data are fully consistent with the view that all λ late messenger is initiated at a single promoter located between genes *Q* and *S* (Toussaint, 1969; Herskowitz & Signer, 1970). The experiments with rifampicin imply that at least the first third of the late messenger is made in one piece. The fact that phage 21 gene *N* product, in conjunction with phage λ gene *Q* product, is just as effective in stimulating isomerase as is phage λ gene *N* product suggests that gene *N* function is not directly required for late protein synthesis.

Deletions and fusions involving the arabinose operon have been studied previously (Kessler & Englesberg, 1969; Englesberg, Sheppard, Squires & Meronk, 1969; Englesberg, Squires & Meronk, 1969). These deletions were found by making use of the fact that the growth of *araD*$^-$ cells is inhibited by the presence of L-arabinose in the medium unless arabinose isomerase (*A* gene product) or ribulokinase (*B* gene product) or both are absent (Englesberg *et al.*, 1962). It follows that no fusions of the type found in λ*dara* (in which the entire arabinose system, including the *C* gene, was left intact and placed under the control of the promoter of another operon) could have been found.

Deletions were found that cut into the *C* gene and, therefore, left the operon both uninducible and presumably not linked to an active operon. One example was found of a deletion ending within the operator region and fusing this region to the leucine operon of *E. coli*. This deletion had a slight increase in arabinose isomerase level upon derepression of the leucine operon. Possibly, no deletions were found fusing

the middle of the *C* gene to th
fusion would have contained suf
read-through past *araC*, that, e
cells could not grow.

The authors thank Professors V
criticism, M. Magazin for technica
cussions and Volker Vogt for RN
(R.S.) was supported in part by
(J.G.) was supported by Medical F

Black, L. W. & Hogness, D. S. (19
Bremer, H. & Yuan, D. (1968). *J.*
Burgess, R. (1969). *J. Biol. Chem.*
Butler, B. & Echols, H. (1970). *V*
Carter, B. J. & Smith, M. G. (197
Couterier, H. & Dambly, C. (1970
Dambly, C., Couturier, M. & Thon
Davis, R. W., Simon, M. & Davi
Grossman, vol. 21, p. 413. N
Dove, W. F. (1966). *J. Mol. Biol.*
Englesberg, E., Anderson, R. L.,
Boyer, H. (1962). *J. Bact.* **84**,
Englesberg, E., Irr, J., Power, J.
Englesberg, E., Sheppard, D., Squ
Englesberg, E., Squires, C. & Mero
Epstein, W. (1967). *J. Mol. Biol.*
Fiandt, M., Hrodecna, A., Lozeron
ed. by A. D. Hershey. Cold S
Goldberg, A. R. & Howe, M. (196
Gottesman, S. & Beckwith, J. R.
Green, M., Gotchell, B., Hendersch
58, 2343.
Gross, & Englesberg, E. (1959).
Harris, A. W., Mount, D. W. A., F
Hendrix, R. (1970). Ph.D. Thesis,
Herskowitz, I. & Signer, E. R. (19
Hopkins, N. (1970). *Virology*, **40**,
Jacquet, M. & Kepes, A. (1969). *E*
Kaiser, A. D. & Jacob, F. (1957).
Kessler, D. P. & Englesberg, E. (
Low, B. (1968). *Proc. Nat. Acad.*
Manor, H., Goodman, D. & Stent
Miller, J. H., Müller-Hill, B. & Be
Müller-Hill, B., Crapo, L. & Gilbe
Nathans, D., Oeschger, M. P., Pol
Novotny, C. P. & Englesberg, E.
Patrick, J. W. & Lee, N. (1968). *J*
Radding, C. M. & Echols, H. (196
Reznikoff, W. S., Miller, J. H., S
201.
Roberts, J. W. (1969). *Nature*, **22**
Sato, K., Nishimune, Y., Sato, M.
(1968). *Virology*, **34**, 637.
Schleif, R. (1969). *J. Mol. Biol.* **4**

the middle of the *C* gene to the leucine operon because cells having this type of fusion would have contained sufficiently high arabinose enzyme levels as a result of read-through past *araC*, that, even with the leucine operon repressed, the *araD*⁻ cells could not grow.

The authors thank Professors W. Gilbert, J. Watson and N. Davidson for advice and criticism, M. Magazin for technical help, I. Herskowitz, J. Pero and R. Hendrix for discussions and Volker Vogt for RNA polymerase purified from cells growing λ. One of us (R.S.) was supported in part by the Helen Hay Whitney Foundation, and one of us (J.G.) was supported by Medical Research Council of Canada Fellowships.

REFERENCES

Black, L. W. & Hogness, D. S. (1969). *J. Biol. Chem.* **244**, 1968.
Bremer, H. & Yuan, D. (1968). *J. Mol. Biol.* **38**, 163.
Burgess, R. (1969). *J. Biol. Chem.* **244**, 6160.
Butler, B. & Echols, H. (1970). *Virology*, **40**, 212.
Carter, B. J. & Smith, M. G. (1970). *J. Mol. Biol.* **50**, 713.
Couterier, H. & Dambly, C. (1970). *C. R. Acad. Sci., Paris*, **270**, 425.
Dambly, C., Couturier, M. & Thomas, R. (1968). *J. Mol. Biol.* **32**, 67.
Davis, R. W., Simon, M. & Davidson, N. (1971). In *Methods in Enzymology*, ed. by L. Grossman, vol. 21, p. 413. New York: Academic Press, in the press.
Dove, W. F. (1966). *J. Mol. Biol.* **19**, 187.
Englesberg, E., Anderson, R. L., Weinberg, R., Lee, N., Hoffee, P., Huttenhauer, G. & Boyer, H. (1962). *J. Bact.* **84**, 137.
Englesberg, E., Irr, J., Power, J. & Lee, N. (1965). *J. Bact.* **90**, 946.
Englesberg, E., Sheppard, D., Squires, C. & Meronk, F., Jr. (1969). *J. Mol. Biol.* **43**, 281.
Englesberg, E., Squires, C. & Meronk, F., Jr. (1969). *Proc. Nat. Acad. Sci., Wash.* **62**, 1100.
Epstein, W. (1967). *J. Mol. Biol.* **30**, 529.
Fiandt, M., Hrodecna, A., Lozeron, H. & Szybalski, W. (1971). *The Bacteriophage Lambda*, ed. by A. D. Hershey. Cold Spring Harbor Laboratory.
Goldberg, A. R. & Howe, M. (1969). *Virology*, **38**, 200.
Gottesman, S. & Beckwith, J. R. (1969). *J. Mol. Biol.* **44**, 117.
Green, M., Gotchell, B., Henderschott, J. & Kennel, S. (1967). *Proc. Nat. Acad. Sci., Wash.* **58**, 2343.
Gross, & Englesberg, E. (1959). *Virology*, **9**, 314.
Harris, A. W., Mount, D. W. A., Fuerst, C. R. & Siminovitch, L. (1967). *Virology*, **32**, 553.
Hendrix, R. (1970). Ph.D. Thesis, Harvard University, Cambridge, Mass.
Herskowitz, I. & Signer, E. R. (1970). *J. Mol. Biol.* **47**, 545.
Hopkins, N. (1970). *Virology*, **40**, 223.
Jacquet, M. & Kepes, A. (1969). *Biochem. Biophys. Res. Comm.* **36**, 84.
Kaiser, A. D. & Jacob, F. (1957). *Virology*, **4**, 509.
Kessler, D. P. & Englesberg, E. (1969). *J. Bact.* **98**, 1159.
Low, B. (1968). *Proc. Nat. Acad. Sci., Wash.* **60**, 160.
Manor, H., Goodman, D. & Stent, G. (1969). *J. Mol. Biol.* **39**, 1.
Miller, J. H., Müller-Hill, B. & Beckwith, J. R. (1968). *Nature*, **320**, 1287.
Müller-Hill, B., Crapo, L. & Gilbert, W. (1968). *Proc. Nat. Acad. Sci., Wash.* **59**, 1259.
Nathans, D., Oeschger, M. P., Polmer, S. K. & Eggen, K. (1969). *J. Mol. Biol.* **39**, 279.
Novotny, C. P. & Englesberg, E. (1966). *Biochim. biophys. Acta*, **117**, 217.
Patrick, J. W. & Lee, N. (1968). *J. Biol. Chem.* **243**, 4312.
Radding, C. M. & Echols, H. (1968). *Proc. Nat. Acad. Sci., Wash.* **60**, 707.
Reznikoff, W. S., Miller, J. H., Scaife, J. G. & Beckwith, J. R. (1969). *J. Mol. Biol.* **43**, 201.
Roberts, J. W. (1969). *Nature*, **224**, 1168.
Sato, K., Nishimune, Y., Sato, M., Numich, R., Matsushiro, A., Inokuchi, H. & Ozeki, H. (1968). *Virology*, **34**, 637.
Schleif, R. (1969). *J. Mol. Biol.* **46**, 185.

Schwartz, M. (1970). *Virology*, **40**, 23.
Sheppard, D. & Englesberg, E. (1966). *Cold Spr. Harb. Symp. Quant. Biol.* **31**, 345.
Sheppard, D. E. & Englesberg, E. (1967). *J. Mol. Biol.* **25**, 443.
Skalka, A., Butler, B. & Echols, H. (1967). *Proc. Nat. Acad. Sci., Wash.* **58**, 576.
Sussman, R. & Jacob, F. (1962). *C. R. Acad. Sci., Paris*, **254**, 1517.
Taylor, K., Hradecna, Z. & Szybalski, W. (1967). *Proc. Nat. Acad. Sci., Wash.* **57**, 1618.
Thomas, R. (1966). *J. Mol. Biol.* **22**, 79.
Toussaint, A. (1969). *Molec. Gen. Genet.* **106**, 89.

J. Mol. Biol. (1971) **59**, 151–168

Probing the Topo
Photosensitized Oxida

GIULIO JORI,
AN

Institute of Orga
Via Ma

(*Received 8 August 19*

The thiol function of the sing
selectively conjugated with
carbamyl group. Absorption,
that, in both cases, the intr
significant alterations of the
Irradiation of the dinitroph
specific photo-oxidation of h
to be the only potentially pho
group; their distance from th
about 5 Å. These two residue
to the structural stability of p
chains induced only limited
protein. On irradiation of the
oxidation of tryptophan-177,
parallel, there was a drastic
molecule. It is concluded tha
from that of tryptophan-177
not directly involved in the
intactness of its side chain i
sional structure of papain.

It is already well-established tha
chains is critical to the biological
of the most challenging problem
in proteins is the determination
within the three-dimensional net
nature and the importance of t
been made in this field by mean
Stryer, 1968). Because the phys
form their biological function is
the protein conformation in solut
graphic conformation.

Recent papers (Rippa & Pon
1970; Jori, Galiazzo, Marchiori
applied, photosensitized oxidatio