Reprinted from THE BACTERIOPHAGE LAMBDA (1971)
published by the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Printed in U.S.A.

Replication Control in Phage Lambda

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EARLY in the productive growth cycle following infection, λ DNA can be found in doubly branched circular structures (Ogawa et al., 1968). The positions of branch points in these molecules suggest that each molecule carries two replication forks moving away from a locus in the region of genes *O* and *P* (Schnös and Inman, 1970). These suggestions have been substantiated and refined by measurements of replication in deletion prophages in the presence of helper (Stevens et al., this volume). The replication process can be divided into a step creating a replication fork (initiation) and the subsequent movement of the fork along the DNA template (progression or movement).

Three λ genes (*N*, *O*, and *P*) are known to specify products that enter into the replication process. The host supplies the biosynthetic machinery for DNA precursors, and at least one gene product that is involved directly in replication of both the host chromosome and λ DNA beyond the precursor level (Hirota et al., 1968; Fangman and Feiss, 1969; Georgopoulos and Herskowitz, this volume).

When λ DNA is repressed, it does not replicate autonomously (Wolf and Meselson, 1963), even if the products of genes *N*, *O*, and *P* are supplied by a heteroimmune hybrid of λ (Thomas and Bertani, 1964; Russo et al., 1970). Repressor not only prevents the synthesis of the products of genes *N*, *O*, and *P*; it also blocks their action. The latter aspect of repression is to be called an epistatic block. A mutant, *t*11, which is defective in the transcription of the *x–O–P* region also seems not to replicate in the presence of helper. It has been proposed that transcription of the *x–O–P* region increases the reactivity of λ DNA for replication (Dove et al., 1969). This proposed coupling would provide sufficient basis for the epistatic block exerted by repressor on λ DNA replication.

The biochemical reactions involved in initiation and those involved in progression are not yet defined. Thus it is not possible at this stage to distinguish unambiguously between a block to initiation and a block early in the progression of replication forks from their origin.

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A provisional assay for the initiation step has been introduced by Shuster and Weissbach (1969). They have found that intracellular λ DNA molecules can be converted from a closed circular form to a nicked form, and that this conversion is dependent on active products of both gene *O* and gene *P*. A different assay, also proposed to detect nicking, has been developed by Freifelder and Kirschner (1971). Here, too, active *O* and *P* products are found to be necessary for the presumptive nicking reaction. These "nicking assays" may indeed measure the initiation reaction(s); it is possible, though, that discontinuities in the parental DNA strands are produced during the movement of a replication fork rather than during its creation.

A test for a block in progression of the replication forks has been introduced by Thomas and Mousset (1970). If a DNA molecule fails to replicate because progression is blocked, that defect should be expressed even when the DNA molecule has been joined in tandem to a helper DNA molecule which can replicate. Thomas and Mousset have found that the block to replication exerted by repressor can be bypassed if the repressed DNA molecule is joined in tandem to an actively replicating, heteroimmune phage DNA molecule. Thus it can be argued that the epistatic block exerted by repressor is directed against initiation rather than against progression of a replication fork. However, λ DNA replication can be bidirectional. Therefore the direction in which a fork moves can be reversed when a tandem helper drives λ replication. Thus a polarized block to progression will disappear in the tandem-helper configuration.

During active replication, at least early in productive growth, initiation events are rationed. The molecules visualized by Schnös and Inman (1970) never carry more than one replication fork on one side of the presumed origin of replication, even if they are almost fully replicated.

When λ DNA is repressed, replication seems to be blocked at the stage of initiation. Thus, the initiation step is involved in setting the maximal rate of autonomous λ DNA replication, early in productive growth, as well as the minimal rate (zero) under repression.

These facts and inferences are discussed more fully in Chapter 9. They can be viewed fruitfully in the framework of the replicon hypothesis (Jacob et al., 1963). In this hypothesis, a unit of replication (replicon) is defined as a segment of DNA whose replication is coordinately controlled by a unique initiation site; this site is acted upon by specific initiating substances. The replicon is recognized experimentally by the discovery of a unique, localized initiation site or of a gene product required in active form for initiation and not for progression.

The replicon model can be generalized in two different ways which are useful for this paper. First, the possibility should be explicitly stated that a replicon can have substructure. In the simple replicon, all sequence-specific events occur at initiation, and progression involves only the base-pairing rules that guide polymerization. In the more general replicon, subsidiary sequence-specific events intervene in progression. For example, if replication forks move in the discontinuous mode suggested by Okazaki et al. (1968),

the sites of switchbacks could be determined by recurrent DNA sequences (see Inman and Schnös, 1971). Such a situation can be uncovered either by the demonstration that these special events occur in special DNA sequences, or by the discovery that some gene products are involved in progression only at special sites in the replicon.

One can generalize the replicon model in a different direction by considering the possibility that both negative and positive control can be exerted over initiation. The direct action of λ repressor on λ DNA replication is a possible example of this case (Thomas and Bertani, 1964; Dove et al., 1969).

We shall present experiments designed to explore the control of λ DNA replication within this framework. In the discussion we shall aim to answer four concrete questions.

1. Does the λ DNA molecule contain one or more units whose replication is controlled positively at a unique site?
2. Do the products of any of the genes *N*, *O*, and *P* enter λ DNA replication only at initiation?
3. How does repressor exert its epistatic block to λ DNA replication?
4. Does the product of gene *N* engage in replication only by stimulating the production of other, diffusible gene products?

RESULTS

Lambda Mutants with a *Cis*-dominant Defect in Autonomous DNA Replication

If there is a single site that is necessary for λ DNA replication, then a mutation which inactivates that site will prevent the replication of the entire λ DNA molecule in a *cis*-dominant fashion. We have searched for mutants of this kind first by screening a large number of defective prophage mutants. The mutants *t*11 and *t*5 alone among mutants in the region of genes *N*, *O*, and *P* appear to have a *cis*-dominant defect in productive growth (Dove et al., 1969). As mentioned, the defect of *t*11 may be ascribed to a coupling between transcription in the *x–O–P* region and the reactivity of λ DNA for replication (Dove et al., 1969). This situation will be discussed fully in later sections.

The *cis*-dominant growth defect of *t*5 can be traced to the level of replication by the assay for λ DNA replication introduced by Stevens et al. (this volume). A defective lysogen is induced after infection with the helper $\phi 80\text{-}imm^{\lambda}$. After 60 min of incubation, DNA is extracted, denatured, and fixed on a nitrocellulose filter. The content of DNA from the region of λ from *att* to *exo* is assayed by hybridization with excess ^{3}H-labeled RNA from that region. The helper has no homology with λ in that region. As shown in Table 1, mutants carrying recessive defects in genes *O* and *P* such as *t*60 (O^-), *t*71 (O^-), and *t*31 (P^-) do not replicate in the absence of helper, but do replicate 3- to 7-fold in the presence of helper. In contrast, *t*5 does not replicate perceptibly even in the presence of helper.

TABLE 1. REPLICATION OF *t5* MEASURED BY RNA-DNA HYBRIDIZATION

Lysogen	Prophage defect*	*att–exo* [^{3}H]RNA hybridized (count/min/5 μg DNA)		
		Uninduced	UV induced	UV induced plus helper
Y10(*t5*)	*cis*-dominant growth defect; *O* defect	1770	1210	1420
Y10(*t60*)	recessive *O* defect	1560	1450	4100
Y10(*t71*)	recessive *O* defect	1690	1530	5310
Y10(*t31*)	recessive *P* defect	1590	1420	10600
Y10(λ+)	none	—	29500	—

* See Dove et al., 1969.

Replication of the *att–exo* segment of defective prophage was studied after UV induction in the absence and presence of helper $\phi 80imm^{\lambda}$cI857. In this helper, the λ region from *att* to *exo* has been substituted with a heterologous region from $\phi 80$ (Fiandt et al., this volume). A detailed description and justification of the method involved is given by Stevens et al. (this volume). The *att–exo* [^{3}H]RNA preparation used in these measurements was assayed for purity with the following results: less than 0.1% of the number of counts hybridizing to the *l* strands of λ or $\lambda imm^{\phi 80}$ (Szpirer et al., 1969, $\lambda att^{\lambda} imm^{\phi 80}$ hy-1) could hybridize with *r* strands of λ; only 2.2% of it could hybridize with *l* strands of the helper $\phi 80imm^{\lambda}$. In a hybridization assay utilizing 0.05 μg of denatured λ DNA fixed to a nitrocellulose filter and a standard amount of labeled RNA (3.36×10^4 count/min), 1605 count/min were bound under fixed conditions.

Cultures (40 ml) of the lysogenic strains were grown in nutrient broth, concentrated, and UV induced for 45 sec at 10 ergs/nm^2/sec in $\phi 80$ adsorption buffer (Stevens et al., this volume). Following induction, helper was adsorbed at multiplicity = 3 particles per bacterium for 5 min at 30°C. These adsorption mixtures were diluted into nutrient broth at 42°C and incubated for 60 min. Control cultures, with either the helper, or the helper plus the UV induction omitted, were carried along in parallel. DNA was extracted from these cells, denatured, and fixed to nitrocellulose filters. The amounts of labeled RNA hybridizing to 5 μg of total DNA under standard conditions is given. All of these values have been corrected for a background of 43 count/min, the amount of labeled RNA bound to a control filter containing 5 μg of *E. coli* DNA.

We have investigated whether the replication defect of *t5* can be attributed to a defect in transcription of the *x–O–P* region. This transcription was measured by hybridization of RNA labeled 24 to 26 min after UV induction of the lysogen Y10(*t5*). RNA levels for the *x–O–P* region are estimated from the difference between the amount of RNA hybridizing with the *r* strand of $\phi 80imm^{\lambda}$ and that hybridizing with the *r* strand of $\phi 80$ (Nijkamp et al., 1970; Bøvre and Syzbalski, 1971).

As shown in Table 2, the level of *x–O–P* RNA is the same for *t5* as for *t60* (recessive O^-P^+) and *t67* (recessive O^+P^-). Thus we cannot ascribe the *cis*-dominant replication defect of *t5* to a more primary defect in transcription.

The genetic structure of *t5* is complex; it fails to revert to wild type at a detectable frequency (Eisen et al., 1966). By deletion mapping we have located all the defects carried by *t5* within the region between the imm^{21} substitution and the *O*125–*O*205 interval. As shown in Table 3, *t5* gives wild-type λ recombinants with imm^{21} and with SA439, a *chlA* prophage

TABLE 2. TRANSCRIPTION OF *t5*, *t60*, AND *t67*

Lysogen	Phage-specific transcription (% of TCA-precipitable input)			
	$\phi 80imm^{\lambda}$ *r* strand	$\phi 80$ *r* strand	$\phi 80imm^{\lambda}$-$\phi 80$ *r* strands	Wild type *r* strand
Y10(*t5*)	0.41	0.23	0.18	2.18
Y10(*t60*)	0.34	0.17	0.17	2.49
Y10(*t67*)	0.35	0.19	0.16	1.28

The lysogens were grown as described by Stevens et al. (this volume) in 20 ml medium without histidine. When the absorbance at 575 nm was 0.35, the cultures were centrifuged, suspended in 7 ml $\phi 80$ adsorption buffer and irradiated in a 10-cm petri dish for 45 sec at a dose of 10 ergs/mm^2-sec. Cultures were then diluted into 33 ml of nutrient broth and incubated at 42°C. RNA was pulse labeled with ^{3}H-labeled uridine during the 24 to 26 min interval after irradiation. Conditions of labeling, hot phenol extraction, assay for TCA-precipitable material, and hybridization have been described by Bøvre and Szybalski (1969, 1971). The input of TCA-precipitable material ranges from 13 to 16×10^4 count/min. All values have been corrected for the number of count/min adhering to a filter without DNA—22 to 42 count/min.

deletion which terminates between *O*125 and *O*205. There are no essential genes between *att* and the left end of the imm^{21} substitution (Signer et al., 1969). Thus, all growth defects of *t5* must lie to the right of imm^{21} and to the left of the SA439 end point.

It appears that *t5* is (at least) a double mutant, with a component on each side of the *O*29 site. This judgment is based on the segregation of outside markers in crosses between *O* mutants and *t5*, as shown in Table 4. There seems to be no component of *t5* to the right of *O*8 or *O*125, but there are components both left and right of *O*29. (Additional evidence for a component to the left of *O*29 will be presented in Table 9.) These components are denoted *t5*a and *t5*b. We view these conclusions with caution because the crosses cannot be performed in reciprocal fashion since the imm^{21} and *Rts* characters cannot be introduced into *t5*. High negative interference makes it risky to assign an order to two markers that are very closely linked (Parkinson, 1968).

We have searched for mutants other than *t5* that create a *cis*-dominant defect in autonomous λ DNA replication. The selection method we have employed is a modification of that devised by Pereira da Silva and his colleagues to elicit λ mutants defective in replication. A lysogen carrying a temperature-inducible λ prophage defective in gene *N* will die at elevated temperatures. This phenomenon is called replicative killing, since prophages with an additional mutation in one or another of the elements controlling autonomous replication will survive under these conditions. Eisen et al. (1968) selected a number of independent prophage mutants in which replicative killing did not occur. The great majority of these were the result of recessive mutations in genes *O* and *P* (Pereira da Silva, personal communication). We have modified this selection by performing it under diploid conditions, with a $\lambda N7c$I857 prophage inserted into both the episome and the chromosome of strain 594gal^-su^-/F′*gal*8. As shown in Table 5, an episomal

TABLE 3. DELETION MAPPING OF THE REPLICATION REGION OF λ

	cI	imm^{434}	ri^cE	ri^cF	c17	c39	imm^{21}	*ti*12	*t*5	*t*60	*O*29	ri^c5b	*O*8	ri^cD	*O*125	*O*205	*P*3	*P*80
imm^{434}	−				+	+					+	+	+		+		+	+
imm^{21}	−		−	−		−		+	+	+	+	+	+	+	+		+	+
$imm^{\phi 80}$									−	−								
d*bio*R24-2	−		−	−					−	−	−	−	+	+	+		+	+
d*bio*R30h-2a	−		−	−					−	−	−	−	−	−	+		+	+
d*bio*E5a-20	−										−		−		−		−	+
SA297	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−		
SA439	+	+	+	+	+		+	+	+	+	+	+	+	+	+	−	−	−
SA443		same as SA439																

The substitution and deletion strains listed vertically have come from the following sources: imm^{434} (Kaiser and Jacob, 1957)—from A. D. Kaiser; imm^{21} (hybrid 1 of Liedke-Kulke and Kaiser, 1967)—from M. Liedke-Kulke; $imm^{\phi 80}$ (hybrid 1 of Szpirer et al., 1969)—from C. Radding; d*bio*R24-2, and d*bio*R30h-2a (Kayajanian, 1968 and 1970)—from G. Kayajanian; SA297, SA439, and SA443 (Adhya et al., 1968)—from D. Court.

The mutants ri^c are described in this work and in Dove et al. (1969). c17 is described by Pereira da Silva and Jacob (1968). *c*39 is a cII mutant described by Kaiser (1957). *t*5 and *t*60 are defective prophage mutants described by Eisen et al. (1966). *ti*12 is described in this paper. *O*29, *O*8, *O*125, *P*3, and *P*80 are amber mutants in genes *O* and *P*, isolated by Campbell (1961). *O*205 is an amber mutant in gene *O* isolated by Thomas et al. (1967).

With the exceptions noted here, the data in this table are given by Kayajanian (1970). We have determined the position of the ri^c mutants, *ti*12, *t*5, and *t*60 and the end points of the deletions SA297, SA439, and SA443 independently, by the methods described below.

All ri^c mutants have a clear-centered plaque phenotype, which cannot be separated by recombination from the replication-constitutive phenotype. To determine whether the normal allele for an ri^c mutation is present in a substitution or deletion strain, ri^cN7 or ri^cP3 phages were crossed with the substitution or deletion strain [if necessary, in the presence of $\phi 80su$III (Andoh and Ozeki, 1968) to suppress amber mutations] and nondefective recombinants were selected on 594. These recombinants were screened for phages which gave normal turbid plaques. Such presumed ri^+ phages were then tested directly for replication control by the protocol of Thomas and Bertani (1964).

The mutation *ti*12 renders the plaque very tiny. This character was mapped in a similar fashion by crossing *N7ti*12 by the deletion or substitution in question and selecting recombinants on 594. These recombinants were then screened for phages which could form normal plaques. Since large-plaque revertants arise in stocks of *ti*12, a cross is considered positive only if the level of large-plaque phages is far higher than this level of revertants.

The defective prophages *t*5 and *t*60 were rescued into phage particles by the helper $imm^{\phi 80}$ (hy-1 of Szpirer et al., 1969). A lysate of this sort is then adsorbed to the deletion lysogen in question and a second round lysate prepared. This cross lysate is then analyzed for λ recombinants by plating on a lysogen for $imm^{\phi 80}$.

The end points of the *chlA* deletion prophages SA297, SA439, and SA443 were determined by constructing $\phi 80su$III lysogens of these strains and infecting with $imm^{434}O$ amber mutants. Recombinants carrying the imm^{λ} character were selected on C600 ($imm^{434}Oam$) lysogens. These were then scored for am^+ by replicating onto 594($\phi 80$). In a positive response, more than 10% of the imm^{λ} recombinants were also am^+; in a negative response, less than 1% were.

TABLE 4. GENETIC STRUCTURE OF *t*5

Cross:	imm^{21}cI	*O*i	*Rts*2	Infecting parent
	$imm^{\lambda}tu$	*O*j	+	Prophage parent

Analysis of cross: Select O^+ recombinants and score the segregation of $imm^{21}c/imm^{\lambda}tu$ and of $ts/+$. If *O*i is to the right of *O*j, $imm^{21}c > imm^{\lambda}tu$ and $+ > ts$.

Prophage parent (*O*j)	Infecting parent (*O*i)	f(O^+)	*ts*	ts^+	imm^{21}	imm^{λ}	Conclusion
none	$imm^{21}cO8Rts2$	9×10^{-6}	—	—	—	—	—
none	$imm^{21}cO29Rts2$	3×10^{-6}	—	—	—	—	—
*t*5	none	none	—	—	—	—	—
$imm^{\lambda}tuO29$	none	1×10^{-6}	—	—	—	—	—
$imm^{\lambda}tuO205$	none	2×10^{-6}	—	—	—	—	—
$imm^{\lambda}tuO205$	$imm^{21}cO29Rts2$	1×10^{-4}	74	29	54	60	*O*29–*O*205
$imm^{\lambda}tuO29$	$imm^{21}cO8Rts2$	7×10^{-4}	14	86	99	1	*O*29–*O*8
*t*5	$imm^{21}cO8Rts2$	4×10^{-4}	108	190	90	0	*t*5–*O*8
*t*5	$imm^{21}cO29Rts2$	4×10^{-5}	203	104	77	24	*t*5a–*O*29–*t*5b

Recipient bacteria were grown in tryptone broth into exponential phase, centrifuged, UV induced for 60 sec at 10 ergs/mm²/sec, and aerated in broth at 37°C for 20 min. They were harvested into TM buffer (0.01 M trishydroxymethylaminomethane-HCl, 0.01 M $MgSO_4$, pH 8), and the infecting phage was adsorbed (multiplicity = 0.1 particle/bacterium) at 30°C for 10 min. The infected bacteria were then diluted into broth at 30°C. After 2 hr, phage growth was terminated by chloroform. O^+ recombinants were detected on 594 at 30°C whereas the total phage burst was scored on C600 at 30°C. The frequency of O^+ recombinants or revertants is designated f(O^+). Such O^+ plaques were then scored for their *ts* and immunity markers by replicating onto 594 at 42°C and onto 594(imm^{21}) at 30°C. The order of markers was inferred from the segregation of the *ts* and immunity characters as suggested in the analysis.

prophage does not cause replicative killing as efficiently as does a chromosomal prophage (although it does cause efficient loss of the episome).

This diploid version of the Pereira da Silva selection method preferentially elicits *cis*-dominant, replication-defective mutations in the chromosomal prophage. The distribution of mutant types among 40 independent mutants is shown in Table 5. Twenty of the mutants arose spontaneously and twenty were induced by nitrosoguanidine. Spontaneous mutant 12 from this series has been studied in great detail. When made N^+, this mutant grows very poorly and gives tiny plaques; for that reason it is called tiny 12 (*ti*12).

The ability of *ti*12 to replicate has been measured in two different ways. In Table 6, the extent of replication is measured by determining the ratio between unmodified and modified *ti*12 in the yield of phage particles issuing from a nonmodifying cell mixedly infected with helper and with host-modified *ti*12. Whereas with normal λ*c*60 this ratio is 70, it is only 7 with *ti*12. Thus, *ti*12 DNA is replicated poorly in the presence of helper.

In Table 7 the method of Stevens et al. (this volume) is used to compare the extent of replication of *ti*12 with that of a recessive *O* mutant. In the absence of helper, *O*8 does not replicate whereas *ti*12 increases 1-fold in 30 min. [In contrast, λ*c*I857 increases 20-fold in 20 min under these conditions (Stevens

Table 5. Diploid Selection for Replication-deficient λ

Distribution of 40 independent mutants	
Recessive O^-	4
Recessive P^-	6
x^- (fail to recombine with imm^{434})	10
N-suppressible polars	15
Others (including *ti*12)	5

Strain 594 F′*gal*8 was used to construct lysogens for *N*7c1857. If the prophage resides only on the episome the lysogen is called an "episomal haploid." If the prophage lies only on the chromosome, the lysogen is called a "chromosomal haploid." If the prophage lies on both the chromosome and the episome, the lysogen is called a "diploid." If each of these kinds of lysogen is exposed to 42°C for 120 min, the colony-titer (at 30°C) falls 8-fold for the episomal haploid, 25-fold for the chromosomal haploid, and 150-fold for the diploid. Most of the surviving colonies from the episomal haploid and from the diploid have lost the episome. Independent cultures of the diploid, 20 mutagenized by nitrosoguanidine and 20 not mutagenized, were grown overnight to 10^8 cells/ml and then plated at 42°C on eosin methylene blue galactose plates. The level of survivors was near 10^{-6}. Most surviving colonies were galactose negative. One survivor from each culture was purified and studied. *N*-suppressible polar mutants were O^-P^- under *N*-deficient conditions but O^+P^+ under *N*-positive conditions (see Brachet et al., 1970).

Table 6. Replication of *ti*12 Measured by Host-Specificity Labeling

	Yield/bacterium		Dilution of host modification	
Infecting λ	Helper	Lambda	Helper	Lambda
c1600	121	27	40	71
*N*7c1857*ti*12	146	2.1	46	7.1

C600 was mixedly infected with helper imm^{434}c1 (multiplicity = 5 particles/bacterium) and with either c1600 or *N*7c1857*ti*12 (multiplicity = 1 particle/bacterium). The infecting phages carried host modification of type P1. The phage yield per bacterium was analyzed on indicators immune either to λ or to imm^{434}. One pair of these indicators lacked P1 restriction and a second pair exerted this restriction. The dilution of host modification is the titer of a particular phage on an indicator lacking P1 restriction divided by the titer of that phage on an indicator possessing P1 restriction.

Table 7. Replication of *ti*12 Measured by RNA-DNA Hybridization

	att–exo [^{3}H]RNA hybridized (count/min/5 μg DNA)	
Conditions	594(c1857*ti*12)	594(c1857*O*8)
uninduced	2800	1910
induced 30 min, 42°C	6070	2040
induced 30 min, 42°C, φ80 imm^{λ} helper	3710	7750

The replication of the *att–exo* segment of λ DNA was measured in the same way, with the same labeled RNA preparation, as described in the legend to Table 1. In this case the lysogenic strains were grown, thermally induced, superinfected with φ80imm^{λ} as indicated (multiplicity = 3 particles/bacterium), and assayed for *att–exo* DNA as described by Stevens et al. (this volume).

Table 8. Transcription of the *x–O–P* Region by *ti*12

		Phage-specific transcription (% of TCA-precipitable input)				
Lysogen	Labeling time (min)	φ80imm^{λ} *r* strand	φ80 *r* strand	φ80imm^{λ}-φ80 *r* strands	Wild type *r* strand	Wild type *l* strand
594(c1857*ti*12)						
	2–3	2.48	0.09	2.37	3.81	11.7
	3–4	2.75	0.38	2.37	4.98	12.3
	10–12	1.79	1.58	0.21	13.3	—
594(c1857*O*8*P*3)						
	2–3	2.29	0.10	2.19	3.54	12.1
	3–4	2.54	0.35	2.19	4.66	12.6
	10–12	0.61	0.59	0.02	6.20	—

The conditions of cell growth, thermal induction, labeling, hot phenol extraction, TCA precipitation, and hybridization have been described by Bøvre and Szybalski (1969, 1971). The input of TCA-precipitable ^{3}H-labeled RNA in the hybridization experiments was 8–10 × 10^4 count/min. All values have been corrected for a blank hybridization without DNA on the filter (38–52 count/min).

et al., this volume).] In the presence of helper, *O*8 increases 3-fold, whereas *ti*12 increases only 1.3-fold—less well than in the absence of helper. Thus the mutant *ti*12 displays a 5- to 10-fold defect in autonomous DNA replication which is not compensated by a helper.

Again, we must determine whether this *cis*-dominant defect can be attributed to inadequate transcription in the *x–O–P* region. We have measured the transcription of c1857*ti*12 after thermal induction during the time intervals 2–3 min and 3–4 min, when *x–O–P* transcription is maximal (Stevens et al., 1970). In Table 8 we see that transcription in the *x–O–P* region (φ80imm^{λ}–φ80 *r* strands) is the same in *ti*12 as in the recessive replication-defective *O*8*P*3. Also, the total *r*-strand and *l*-strand transcription is normal in *ti*12. Thus, the *cis*-dominant replication defect in *ti*12 cannot be traced to a transcriptional defect.

The genetic structure of *ti*12 has been determined by deletion mapping and by measurement of recombination distances to imm^{21}, *t*5, *O*29, and *P*80. As shown in Table 3, *ti*12 lies in the same segment as *t*5—between imm^{21} and the *O*125–*O*205 interval. In Table 9, crosses 1–4, *ti*12 is mapped in the region between imm^{21} and *P*80. The recombination distances for the intervals imm^{21}–*ti*12–*O*29–*P*80 are in the ratio 1:0.3:4.7. In crosses 5–7, *ti*12 is mapped between imm^{21} and *t*5, with the intervals imm^{21}–*ti*12–*t*5a in the ratio 1:0.14.

Thus the defect in *ti*12 lies to the left of all known *O* mutants and is closely linked to *t*5a. We do not know whether *ti*12 is a point mutant.

The properties of *t*5 and *ti*12 are consistent with any of several possibilities. (1) The mutations could affect an unknown replication gene lying between cII and *O* and encoding a *cis*-acting gene product (Tessman, 1965; Lindahl, 1970). (2) The mutants could synthesize a defective *O* gene product which is nondiffusible and which blocks the replication of the DNA molecule

TABLE 9. MAPPING OF THE REGION *imm*^{21}–*ti*12–*t*5–(*O*29–*t*60)–*P*

	Cross	Diagram	Conditions	Results
	imm^{21}cI	*O*29		
1		*ti*$^+$ *ti*	Mixed infection; UV	f(*am*$^+$) = 6.5 × 10^{-3} *ti* = 1002 *ti*$^+$ = 379
	*N*7cI857 *ti*12			
	imm^{21}cI	*O*29*P*3		
2		*ti*$^+$ *ti*	Mixed infection; UV	f(*am*$^+$) = 4.2 × 10^{-3} *ti* = 452 *ti*$^+$ = 142
	*N*7cI857 *ti*12			
	imm^{21}cI	*O*29		
3		*ti*$^+$ *ti*	Mixed infection; no UV	f(*am*$^+$) = 8.7 × 10^{-4} *ti* = 479 *ti*$^+$ = 131
	*N*7cI857 *ti*12			
	imm^{21}cI	*P*80		
4		*ti*$^+$ *ti*	Mixed infection; no UV	f(*am*$^+$) = 3.6 × 10^{-3} *ti* = 101 *ti*$^+$ = 509
	*N*7cI857 *ti*12			
		*t*5		
5		*ti*$^+$ *ti*	Infection of Y10(*t*5); UV induction	f(*am*$^+$) = 1 × 10^{-2} *ti* = 790 *ti*$^+$ = 29
	*N*7cI857 *ti*12			
		*t*60		
6		*ti*$^+$ *ti*	Infection of Y10(*t*60); UV induction	f(*am*$^+$) = 1 × 10^{-2} *ti* = 460 *ti*$^+$ = 102
	*N*7cI857 *ti*12			
		*t*5		
7		*ti*$^+$ *ti*	Infection of Y10(*t*5); UV induction	f(*am*$^+$) = 1.3 × 10^{-3} *ti* = 2269 *ti*$^+$ = 310
	imm^{21}cI *ti*12			

TABLE 10. EXPRESSION OF *O* AND *P* GENES BY *ti*12

Recipient	Yield/bacterium of *imm*^{434}*O*29*P*3
594	0.01
594(*c*I857*N*7)	80
594(*c*I857*N*7*ti*12)	101

The recipient bacterial strain was grown in tryptone broth at 30°C and starved in TM buffer. After infection with *imm*^{434}*O*29*P*3 at a multiplicity of 0.1 particle/bacterium, the cultures were diluted into broth at 42°C. After 20 min at 42°C, they were shifted to 37°C and incubated a further 60 min before terminating phage growth with chloroform. The burst size of *imm*^{434}*O*29*P*3 was determined by plating on C600(λ).

encoding it. (3) The mutations could block progression of replication forks. (4) The mutants could be defective in an obligatory origin of replication which controls the replication of at least the *att–exo* segment of λ DNA.

In the case of *ti*12 we have eliminated possibility (2) by showing that this mutant can supply active *O* product in *trans*. As shown in Table 10, *ti*12 can complement *imm*^{434}*O*29*P*3 well.

Possibilities (3) and (4) cannot be tested rigorously until a direct analysis of the initiation process can be carried out with *t*5 and *ti*12. In the discussion, we shall treat possibility (1) in general fashion, and we shall give arguments that lead us to favor possibility (4).

The Specific Action of the Products of Genes *N* and *O* Can Be Bypassed by a Helper Joined to λ in Tandem

Consider a gene product, *R*, which is involved in λ DNA replication, and a lambdoid phage, φ, which cannot supply a product that will substitute for *R* after mixed infection. If *R* is involved in progression (for example, by acting at secondary switching sites in discontinuous replication), then phage φ will remain incompetent to help *R*-deficient λ even when joined to it in tandem. In contrast, if *R* is involved only in the creation of replication forks, phage φ will be able to help *R*-deficient λ if joined to it (Thomas and Mousset,

Crosses were done either by mixed infection (multiplicity = 5 particles/bacterium) of a permissive host, C600, or by superinfection of a permissive lysogen Y10(*t*5) or Y10(*t*60) (multiplicity = 0.1 particle/bacterium) followed by UV induction. Both UV induction and UV stimulation for recombination after mixed infection were accomplished by 60 sec irradiation at 10 ergs/mm^2/sec. Recombinants (*am*$^+$) were selected on 594, while the total yield was titrated on C600, and the frequency of recombinants, f(*am*$^+$), was calculated as the ratio of these two titers. Recombinants were then analyzed for plaque size, *ti* or *ti*$^+$.

TABLE 11. HELPING OF *N7cI857O29* BY *imm*^{82} IN TANDEM

Strain	Infecting phage	Phage yield/bacterium: *imm*^{82} prophage	Phage yield/bacterium: *N7cI857O29* prophage	Phage yield/bacterium: Infecting phage
A	none	88	40	—
B	none	250	65	—
A	*c*I60	120	50	12
A	*N7c*I72	109	46	0.7
B	*N7c*I72	220	63	1.3
A	*O29c*I	102	40	0.3
B	*O29c*I	400	58	0.6

Strain 594 lysogenic for *λimm*^{82}c*Its* was made lysogenic for *N7c*I857*O29* by infection with the latter phage. Two different isolates were used and called strains A and B. Each strain was grown in nutrient broth into exponential phase, centrifuged, starved in TM buffer, and infected as indicated with *c*I60, *N7c*I72, or *O29c*I (multiplicity = 0.3 particle/bacterium). The lysogens were then thermally induced by dilution into 42°C broth. After 30 min at 42°C, the cultures were shifted to 37°C for 60 min further incubation and then phage growth was terminated by chloroform. The phage yields were determined by plating on appropriate immune lysogenic indicators. The doubly defective *λN7O29* was distinguished by its very small plaques.

1970). As we have mentioned, this test would fail to detect a polarized block to progression.

We have investigated the roles of the products of genes *N* and *O* by a test of this kind. The hybrid *λimm*^{82} cannot compensate for mutations of λ in gene *N* or in gene *O* after mixed infection (Ohashi and Dove, unpublished; Simon et al., this volume). This finding is confirmed in the experiments shown in Table 11 in which a lysogen, carrying *λimm*^{8}c*I857O2cIts*^{2} and *N71λ9*, is first superinfected with either *λN7c*I or *λO29c*I and then thermally induced. The superinfecting *N*-deficient or *O*-deficient phage multiplies little if any; in contrast, the tandem *N,O*-deficient prophage multiplies extensively. Thus we conclude that the *N* and *O* products act in the creation, rather than the movement, of replication forks. Although the experiment cited provides explicit evidence for this point, the same conclusion can be drawn, less directly, from other work (Thomas and Mousset, 1970; Chapter 9).

An analogous investigation of the role of the *P* product is not yet possible, since no lambdoid phage is known to be totally deficient in λ-type *P* activity.

The Epistatic Block Exerted by Repressor on λ DNA Replication Does Not Require Direct Participation by Repressor

It has been established that λ repressor specifically blocks λ DNA replication, even if early phage gene products are supplied by a heteroimmune helper. It has been argued that this epistatic block prevents the creation of replication forks rather than their progression.

Does this block involve repressor itself, or is it mediated through repressible transcription? We have investigated whether mutations that prevent the

TABLE 12. REPLICATION OF *x13*

Conditions	*att–exo* [^{3}H]RNA hybridized (count/min/5 μg DNA): W3102(cI857*x*13)$_2$	*att–exo* [^{3}H]RNA hybridized (count/min/5 μg DNA): 594(cI857*O8*)
not induced	1960	1910
induced 30 min, 42°C	1470	2040
induced 30 min, 42°C, *ϕ80 imm*$^{\lambda}$ helper	1950	7750

The assay of the replication of the *att–exo* segment of λ DNA has been described in the legend of Table 1. The *x*13 results have been divided by 2, since the lysogen carrying this prophage has two prophage copies per host chromosome (W. F. Stevens, unpublished data).

transcription of the *N–int* or *x–O–P* region of λ DNA also create a *cis*-dominant replication defect. The mutant *t*27, which prevents the transcription of the *N–int* operon, can multiply normally in the presence of helper. The mutant *t*11, however, which inactivates transcription of the *x–O–P* operon, is defective in multiplication in a *cis*-dominant fashion (Dove et al., 1969).

In this study we directly measured DNA replication in the presence of helper, using a mutant, *x*13, which shows the same transcriptional defect as *t*11 (Eisen et al., 1968; Nijkamp et al., 1970). A lysogen carrying *c*I857*x*13 is induced in the presence of helper *ϕ80imm*$^{\lambda}$, and the increase in DNA from the *att–exo* segment of the induced prophage is measured by the method of Stevens et al. (this volume). As shown in Table 12, with helper *x*13 is unable to replicate at all whereas the recessive mutant *O*8 increases about 3-fold in 30 min.

The block to replication caused by *x*13 can be bypassed by a tandem helper (Roberts, 1969). Thus this block may also involve initiation.

In principle, the behavior of *x*13 and *t*11 in regard to replication can be explained in any of the ways considered to explain the behavior of *t*5 and *ti*12 (see first section of Results). However, *x*13 and *t*11 also display a transcriptional defect, whereas *t*5 and *ti*12 do not. Whatever the final interpretation of the behavior of *t*5 and *ti*12 may be, it is economical to consider the transcriptional defect of *x*13 and *t*11 to be primary, and the replication defect to be secondary. This decision is justified further by an independent example in which the transcriptional activity of the *x–O–P* region determines λ replication activity (next section).

Mutants Transcribed Constitutively in the *x–O–P* Region Are Not Sensitive to the Direct Replication Block Exerted by λ Repressor

If the epistatic block to λ DNA replication exerted by λ repressor is mediated by *x–O–P* transcription, it should be lost in mutants that transcribe this region constitutively: Packman and Sly (1968), Pereira da Silva and Jacob (1968), and Ptashne and Hopkins (1968) have shown that *λc*17 and *λv1v*3 are insensitive to this replication block. Lambda *v*2 remains sensitive to the dominant action of repressor on λ DNA replication.

We have isolated four independent mutants insensitive to the block exerted by λ repressor (Dove et al., 1969). These mutants have satisfied two conditions: (1) multiplication in a λ lysogen in the presence of helper *λimm*^{434}; (2) plaque formation on a lysogen for *λimm*^{434}. We call these mutants *ri*^{c} (replication constitutive). Two, *ri*^{c}E and *ri*^{c}F, fail to recombine with SA297 (Table 1), and can express genes *O* and *P* constitutively. They may comprise new promoters lying in the *x* or *y* region. Two others, *ri*^{c}D and *ri*^{c}5b, lie within gene *O* (Table 3 and below), and can express only gene *P* constitutively. Both direct the constitutive synthesis of *r*-strand RNA from the *x–O–P* region (Nijkamp, Szybalski, and Dove, in preparation). These may therefore comprise new promoters lying within gene *O* and directing constitutive transcription to the right.

We have suggested that these *ri*^{c} mutants escape the replication block of λ repressor by virtue of this promoter activity. It is possible to maintain that the promoter activity is adventitious, and that the actual basis of the escape from repressor control is an alteration in an origin for replication or in the *O* and *P* products. The first alternative, suggested by H. Echols, has not been ruled out. The second alternative is ruled out by the finding that *ri*^{c}5b*O29P3* retains its replication-constitutive phenotype (in the presence of helper) in a nonsuppressing host (Table 13). Here, all active *O* and *P* products must be encoded by the wild-type helper.

Deletion mapping (Table 3) establishes that *ri*^{c}5b lies in the same segment as *O*29 and *ti*12—to the right of *imm*^{21} and to the left of *O*8. The mutant *ri*^{c}D lies in the same deletion segment as *O*8—to the right of *O*29 and to the left of *O*125. The positions of *ri*^{c}5b and *ri*^{c}D can be estimated by multiple-factor crosses, and they both lie significantly to the right of *O*29 by these measurements (Table 14). Thus, both these *ri*^{c} mutants seem to lie within gene *O*.

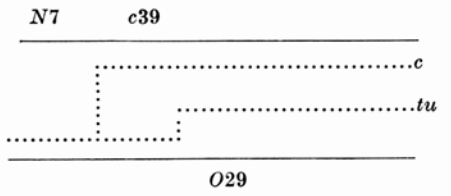
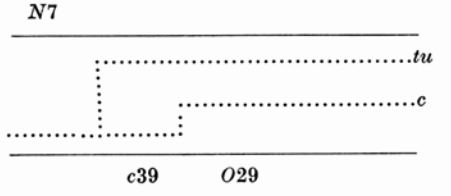
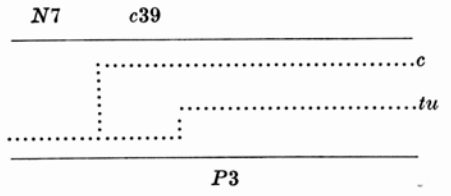
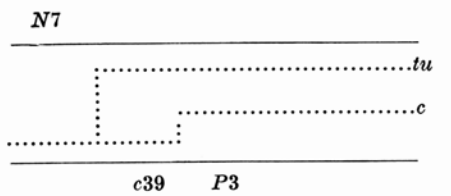
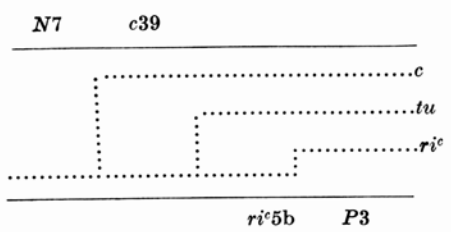
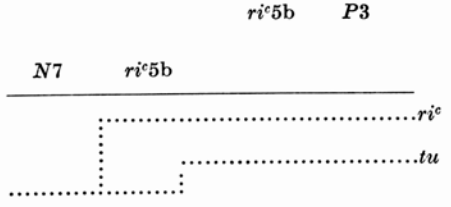
Recall that the selection of *ri*^{c} mutants entails plaque formation on a lysogen for *λimm*^{434}. Whereas a phage inactive in gene *P* can make plaques

TABLE 13. THE REPLICATION-CONSTITUTIVE PHENOTYPE OF *ri*^{c}5b WHEN IT IS DEFECTIVE IN GENES *O* AND *P*

Test phage	Recipient	Total phage yield/bacterium	Multiplication of	
			test phage	prophage
*c*I60	W3350(*λind*$^{-}$)*su*$^{-}$	111	1.2	0.64
*c*I60	Y10(*t5*)*su*$^{+}$	77	1.8	—
*c*I*O*29*P*3	W3350(*λind*$^{-}$)*su*$^{-}$	121	1.4	0.44
*c*I*O*29*P*3	Y10(*t5*)*su*$^{+}$	87	1.5	—
ri^{c}5b*O*29*P*3	W3350(*λind*$^{-}$)*su*$^{-}$	99	20	—
ri^{c}5b*O*29*P*3	Y10(*t5*)*su*$^{+}$	74	14	—

The recipient was grown in tryptone broth into exponential phase, centrifuged, starved in TM buffer, and infected with helper *imm*^{434}cI (multiplicity = 2.7 to 3.1 particles/bacterium) and the phage to be tested (multiplicity = 0.3 to 0.5 particles/bacterium). The infected cells were diluted into broth and permitted to grow for 90 min at 37°C, when phage growth was terminated by chloroform. The phage yields were determined by plating on appropriate immune lysogenic indicators.

TABLE 14. MAPPING OF THE REGION *N7–O29–(ri*^{c}5b, *ri*^{c}D)*–P3*

Cross	Diagram	Results
1		*c*: 1074 *tu*: 136 *c39-O29/N7-c39* = 0.13
2		*tu*: 726 *c*: 105 *c39-O29/N7-c39* = 0.15
3		*c*: 903 *tu*: 373 *c39-P3/N7-c39* = 0.41
4		*tu*: 491 *c*: 234 *c39-P3/N7-c39* = 0.48
5		*c*: 256 *tu*: 59 *ri*^{c}: 65 *c39-ri*^{c}*5b/N7-c39* = 0.25 *c39-P3/N7-c39* = 0.48 *ri*^{c}*5b-P3/N7-P3* = 0.17
6		*ri*^{c}: 314 *tu*: 66 *ri*^{c}*5b-P3/N7-P3* = 0.17

We have isolated four independent mutants insensitive to the block exerted by λ repressor (Dove et al., 1969). These mutants have satisfied two conditions: (1) multiplication in a λ lysogen in the presence of helper *λimm*^{434}; (2) plaque formation on a lysogen for *λimm*^{434}. We call these mutants *ri*^{c} (replication constitutive). Two, *ri*^{c}E and *ri*^{c}F, fail to recombine with SA297 (Table 1), and can express genes *O* and *P* constitutively. They may comprise new promoters lying in the *x* or *y* region. Two others, *ri*^{c}D and *ri*^{c}5b, lie within gene *O* (Table 3 and below), and can express only gene *P* constitutively. Both direct the constitutive synthesis of *r*-strand RNA from the *x–O–P* region (Nijkamp, Szybalski, and Dove, in preparation). These may therefore comprise new promoters lying within gene *O* and directing constitutive transcription to the right.

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TABLE 13. THE REPLICATION-CONSTITUTIVE PHENOTYPE OF *ri*^{c}5b WHEN IT IS DEFECTIVE IN GENES *O* AND *P*

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			test phage	prophage
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*c*I*O*29*P*3	W3350(*λind*$^{-}$)*su*$^{-}$	121	1.4	0.44
*c*I*O*29*P*3	Y10(*t5*)*su*$^{+}$	87	1.5	—
ri^{c}5b*O*29*P*3	W3350(*λind*$^{-}$)*su*$^{-}$	99	20	—
ri^{c}5b*O*29*P*3	Y10(*t5*)*su*$^{+}$	74	14	—

The recipient was grown in tryptone broth into exponential phase, centrifuged, starved in TM buffer, and infected with helper *imm*^{434}cI (multiplicity = 2.7 to 3.1 particles/bacterium) and the phage to be tested (multiplicity = 0.3 to 0.5 particles/bacterium). The infected cells were diluted into broth and permitted to grow for 90 min at 37°C, when phage growth was terminated by chloroform. The phage yields were determined by plating on appropriate immune lysogenic indicators.

TABLE 14. MAPPING OF THE REGION *N7–O29–(ri*^{c}5b, *ri*^{c}D)*–P3*

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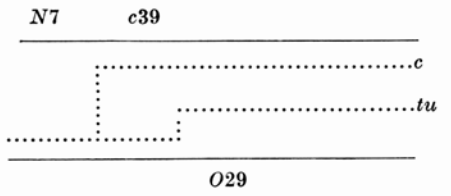
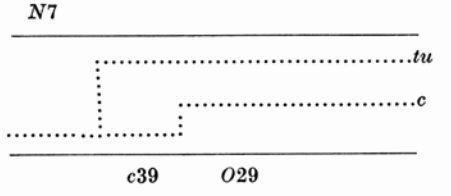
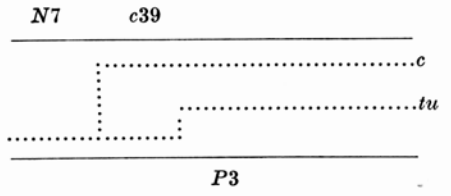
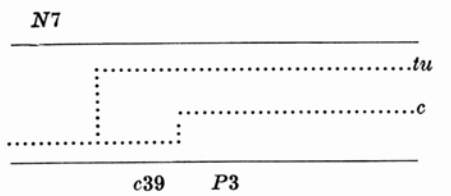
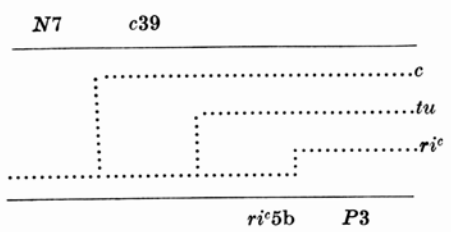
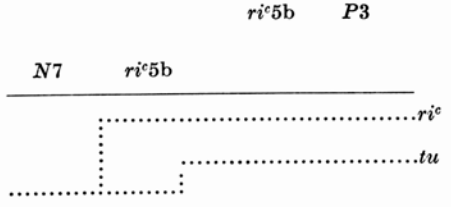
1		*c*: 1074 *tu*: 136 *c39-O29/N7-c39* = 0.13
2		*tu*: 726 *c*: 105 *c39-O29/N7-c39* = 0.15
3		*c*: 903 *tu*: 373 *c39-P3/N7-c39* = 0.41
4		*tu*: 491 *c*: 234 *c39-P3/N7-c39* = 0.48
5		*c*: 256 *tu*: 59 *ri*^{c}: 65 *c39-ri*^{c}*5b/N7-c39* = 0.25 *c39-P3/N7-c39* = 0.48 *ri*^{c}*5b-P3/N7-P3* = 0.17
6		*ri*^{c}: 314 *tu*: 66 *ri*^{c}*5b-P3/N7-P3* = 0.17

TABLE 14. (*continued*)

Cross	Diagram	Results
7	*N*7 *c*39 *c* *tu* *ri*^{c} *ri*^{c}D *P*3	*c*: 466 *tu*: 128 *ri*^{c}: 185 *c*39-*ri*^{c}D/*N*7-*c*39 = 0.27 *c*39-*P*3/*N*7-*c*39 = 0.27

Crosses were done either by mixed infection (5 particles of each kind per bacterium) of a permissive host, C600, with no UV stimulation, or by infection of a C600 lysogen carrying one parent as prophage, followed by UV induction (60 sec at 10 ergs/mm^2/sec). Crosses 1, 2, 3, 4, and 6 were done by this induction procedure; in each of these cases the parent listed below in the diagram was the prophage. Recombinants (*am*$^+$) were selected on 594 and scored by plaque morphology for *c*$^+$ (turbid), *c*39 (clear), or *ri*^{c} (clear centered).

under these conditions, one inactive in gene *O* cannot (Thomas, 1970). The *ri*^{c}5b and *ri*^{c}D mutations must lie in a dispensible part of the *O* gene and must not interfere severely with the normal transcription of the *x–O–P* region.

We have established, in two different ways, that the ability of λ DNA to replicate in the presence of helper is intimately connected with its ability to direct transcription of the *x–O–P* region. First, if *x–O–P* transcription is abolished by the *x*13 mutation, the DNA molecule cannot be replicated by a helper (previous section). Second, if *O–P* transcription is rendered constitutive by any of the mutations *o1o*3, *c*17, or *ri*^{c}, the DNA molecule is competent for replication even in the presence of λ repressor. Mutations that

TABLE 15. RECESSIVENESS OF *N7O29P3*

Input	Yield	
Helper phage/bacterium	Helper/bacterium	*N7O29P3*/bacterium
0.40	124	51
0.99	131	34
1.66	129	23

Nonsuppressing bacteria, 594, were grown in broth, starved in TM buffer, and mixedly infected with *N7O29P3* (multiplicity ca. 0.01 particle/bacterium) and *imm*^{434}cI (multiplicity ca. 1 particle/bacterium). The infected cells were diluted into broth. At 5 min, the surviving bacteria were measured by plating for colony formation. At 90 min, the cultures were treated with chloroform and titrated on C600(λ) to determine the burst of the helper and on C600(λ*imm*^{434}) to determine the burst of the λ. We calculated the multiplicity of helper, m particles per bacterium, from the fraction of surviving bacteria, f, assuming the Poisson term $f = P(O) = e^{-m}$. The yields/bacterium were calculated from the fraction of helper-infected bacteria, which was given by the initial bacterial titer corrected for noninfected (surviving) bacteria.

make the *N–int* region inactive or constitutive for transcription have no perceptible effect on the capacity of the DNA molecule to be replicated.

This positive coupling between *x–O–P* transcription and replication competence could arise from either of the following. (1) Transcription could permit synthesis of a gene product necessary for replication and not available from the helper. (2) Transcription could modify the *x–O–P* DNA segment either in structure or in location within the cell, thereby activating it for the initiation process.

We shall discuss a general argument against the first kind of explanation in the Discussion section. Here, we test an explicit form of it by determining whether λ*N7O29P3* can be helped to multiply by λ*imm*^{434} after mixed infection. As shown in Table 15, such helping does occur. We conclude that all known replication gene products can be supplied to λ DNA by λ*imm*^{434} helper.

The *N* Product Plays an Indirect Role in Stimulating λ DNA Replication

The product of gene *N* acts to stimulate the transcription of several regions of λ DNA, including that of the *x–O–P* region (Heinemann and Spiegelman, 1970; Brachet et al., 1970; Herskowitz and Signer, 1970b; Kumar and Szybalski, 1970). Its role in stimulating λ DNA replication could therefore be indirect (through production of diffusible gene products), semidirect (through stimulation of *x–O–P* transcription per se), or direct (through participation in the machinery that carries out replication). We have performed experiments to determine whether the role of *N* product in DNA replication is indirect.

The hybrid λ*imm*^{21} fails to provide a function that can substitute for λ *N* product in promoting the transcription of any of the regions of λ DNA which are under *N* control (Franklin, research article in this volume; Herskowitz and Signer, 1970b; Brachet et al., 1970; Couturier and Dambly, 1970). Yet this hybrid synthesizes *O* and *P* products in sufficient quantity to complement λ*OP* double mutants very well (Table 16). If the replication

TABLE 16. THE ABILITY OF *imm*^{21} TO HELP *N*7 TO MULTIPLY

Expt.	Test phage	Induced prophage helper	Yield/bacterium	
			Test phage	Helper
A	*N7*cI72	*imm*^{434}	53	33
A	*N7*cI72	*imm*^{21}	54	54
B	*N7*cI72	*imm*^{21}	87	74
B	c*IO29P3*	*imm*^{21}	90	57

Lysogens 594(*imm*^{434}) and 594(*imm*^{21}) were grown in tryptone broth at 37°C into exponential phase, centrifuged, resuspended in TM buffer, and irradiated for 60 sec at 10 ergs/mm^2-sec. They were then permitted to grow in broth for 20 min at 37°C and harvested again into TM. The test phage *N7*cI72 or c*IO29P3* was adsorbed (multiplicity = 0.1 particle/bacterium) and the complexes were diluted into 37°C broth. Phage growth was terminated by chloroform after 90 min further incubation. The yields of prophage types and superinfecting types were determined on appropriate immune indicators. These experiments were carried out by Mr. John F. Lehman.

defect of λN mutants is due only to a deficiency in O and P products, then λN mutants should be helped efficiently by λimm^{21}. They are not helped efficiently by λimm^{82}, which is unable to provide either an N product or an O product for λ (Table 11).

This prediction is tested in the experiment described in Table 16. We see that $\lambda N7$ is helped to multiply as well by imm^{21} as it is by imm^{434}. Thus, the requirement for N product in λ DNA replication can be fully satisfied by a helper which provides high levels of O and P product.

We might have expected that the deficiency in x–O–P transcription in the absence of a functional N product would inactivate such λ DNA for replication. In fact, the level of x–O–P transcription is not zero under these conditions. In the discussion we shall consider the quantitative relationship between x–O–P transcription and competence for replication.

DISCUSSION

In the introduction we postulated that the control of λ DNA replication can be analyzed fruitfully in terms of a generalized replicon hypothesis. To pursue this analysis we have performed experiments designed to answer four concrete questions. We shall discuss these questions in the light of our experimental results.

1. Does the λ DNA molecule contain one or more units whose replication is controlled positively at a unique site?

In Results, first section, we presented evidence that the replication of the *att–exo* segment of λ DNA is inactivated, in a *cis*-dominant fashion, by the localized mutations *t*5 (Table 1) and *ti*12 (Tables 6 and 7). Neither of these mutations influences x–O–P transcription perceptibly (Tables 2 and 8). Stevens et al. (this volume) have shown that SA297 (del*y-chlA*), but not SA439 (del*O*205-*chlA*), is also deficient in *att–exo* replication in the presence of helper. Schnös and Inman (1970) have found that there is a preferred starting locus for bidirectional λ DNA replication in the O–P region.

For *ti*12 (but not for *t*5) we have eliminated the trivial possibility that the *cis*-dominant replication defect is due to an altered form of the O product (Table 10).

For SA297, *t*5, and *ti*12 the replication defect could be ascribed to a hitherto-unknown *cis*-acting gene lying between genes *c*II and O (mapping—Tables 3, 4, and 9). In Fig. 1 we diagram the basis for a strong argument against the existence of any *cis*-acting replication gene in λ. First, the fact that SA439 can replicate (in the presence of helper) shows that there can be no *cis*-acting replication gene to the right of *O*205 (line 2). Second, the fact that repressed *ri*^{c}D can replicate in the presence of helper whereas repressed $\lambda+$ cannot (lines 4–6) requires that any *cis*-acting replication gene lie to the right of *ri*^{c}D. These two facts together require that any *cis*-acting replication gene lie within gene O. We have shown that the products of genes N, O, and P can all act in *trans* (Table 15) and that *ri*^{c}5b retains its replication-constitutive phenotype when it is defective in genes O and P (Table 13).Thus

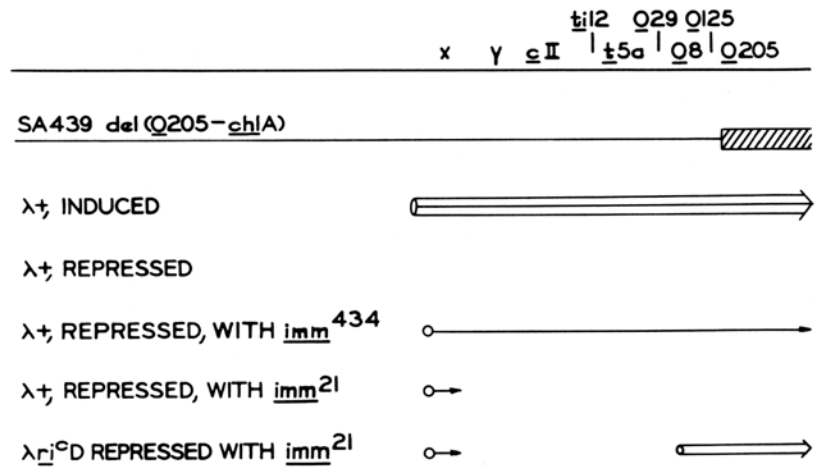


FIGURE 1. Argument against the existence of any *cis*-acting replication gene in λ. The order of sites in the x–O–P region of λ is shown on the top line. The *chlA* deletion SA439 is deficient in genetic markers from *O*205 to the right, yet it can be replicated in the presence of helper $\phi 80imm^{\lambda}$ (Stevens et al., this volume). The patterns of transcription in the x–O–P region presumed to exist for induced $\lambda+$ (wild type), repressed $\lambda+$, repressed $\lambda+$ in the presence of imm^{434} or imm^{21} helper, and repressed λ *ri*^{c}D in the presence of imm^{21} helper are shown in order below. Among all five of these cases, only induced $\lambda+$ and repressed λri^cD are able to replicate extensively in the presence of helper.

one cannot attribute the replication defect of SA297, *ti*12, or *t*5 to any unknown *cis*-acting gene product.

Two sorts of structural block can be adduced to explain the replication defects of SA297, *ti*12, and *t*5. One is that an essential initiation site for replication of the *att–exo* segment has been inactivated. Another is that the movement of replication forks toward the *att–exo* segment is blocked by mutant sequences in these strains (Herskowitz and Signer, personal communication). Because SA297 is a deletion strain, it is difficult to maintain that it has acquired a blocking signal.

Schnös and Inman (1970) and Stevens et al. (this volume) have shown that λ DNA replication can be bidirectional. The mutants *t*5 and *ti*12 each can form circles efficiently after prophage induction, as judged by curing experiments (Eisen et al., 1966; Inokuchi, unpublished experiments). Therefore these mutants would have to block the movement of replication forks in both directions along λ DNA in order to prevent the replication of the *att–exo* segment. They would have to straddle or else inactivate directly an essential initiation site.

Thus the properties of *ti*12 and *t*5 give evidence that the entire DNA molecule is controlled by a site or sites in the *c*II–O region. In these terms, the λ DNA molecule must be described as one bidirectional replicon or two divergent replicons. Below we shall argue that the initiation(s) in the *c*II–O

region are controlled directly or indirectly by positive action of at least the *O* product and by negative action of the repressor.

The fine structure of the *ti*12–*t*5–*O*29 segment (Tables 3, 4, and 9) can be interpreted in the following extreme way. Since *ti*12 and *t*5 recombine (rarely) to give wild type, and since they each prevent replication in both leftward and rightward directions from the obligatory origin, then there must be a nucleotide sequence between these mutations which controls both leftward and rightward replication. The minimum size of this region is estimated to be about 20 nucleotide pairs on the basis of the recombination frequencies in Table 9 and the physical distance, 800 nucleotide pairs, between imm^{21} and the end point of d*bio*E5a-20 (Fiandt et al., this volume). This region may represent a unitary origin for both leftward and rightward replication. Indeed Inman (personal communication) has suggested a model in which bidirectional replication can be initiated in one sequence-specific event.

In this interpretation, one can assign the component *t*5a to this unitary origin and the component *t*5b to the *O* gene itself, thereby accounting for both the *cis*-dominant replication defect and the *O* defect of *t*5. This assignment is strictly a simplifying assumption at this stage because the individual components of *t*5 have not been studied separately. The assignment of *ti*12 and *t*5a to a unitary bidirectional origin is also hypothetical; they must be shown to affect the initiation process itself to justify that assignment rigorously.

No statement can be made as to whether the λ replicon(s) have substructure—subsidiary sequences that guide the progression of replication forks. Were there to be such substructure, however, the secondary signals could not be λ specific. Transducing phage DNA in which the *A–att* region or the *att–N* region has been replaced by host DNA is fully capable of λ-driven replication.

2. Do the products of any of the genes *N*, *O*, and *P* enter λ DNA replication only at initiation?

In Results, second section, we adduced evidence that the specific requirement for *N* and *O* products in λ multiplication could be bypassed by a tandemly associated helper (Table 11). This evidence is subject to the caveat that the tandem-helper test of Thomas and Mousset will not detect a polarized block to progression.

We conclude that at least the *O* product acts only in the creation of replication forks. As we shall argue below, the action of the *N* product at initiation is only indirect, through its stimulation of the expression of genes such as *O*.

Since replication forks are created only in the *c*II–*O* region, as argued above, we infer that the *O* product must influence that region of λ DNA. The limited action of the *O* product confirms the statement that λ DNA is composed of one (or two) replicons controlled in the *c*II–*O* interval. Again, no statement can be made concerning the substructure of the replicon(s). The *P* product and any of the host gene products implicated in λ replication remain candidates for action either at initiation or at secondary sequences; alternatively, any of them could participate in blind polymerization, during the movement of replication forks.

3. How does repressor exert its epistatic block to λ DNA replication?

In the next two sections of Results we have shown that the *cis*-dominant replication defect of repressed λ can be mimicked in the absence of repressor

by mutants such as *x*13, which are unable to direct intense *x–O–P* transcription (Table 12). Furthermore, mutants which are transcribed constitutively in the *O–P* region are able to replicate in the presence of λ repressor, when *O* and *P* products are supplied (Table 13).

The positive coupling between *O–P* transcription and replication competence cannot be ascribed to a *cis*-acting replication gene (Fig. 1). It must be due to an effect of transcription per se on the initiation process. We call this transcriptional activation (Dove et al., 1969).

We conclude that repressor exerts its direct negative control over initiation by preventing transcriptional activation of the *c*II–*O* region. The function served by this specific control over initiation seems to be related to the process of lysogenization. As summarized in Chapter 6, λ DNA is usually replicated before prophage integration. Since λ replication *in situ* can lead to replicative killing of a lysogen (Eisen et al., 1968; Table 5), a newly formed lysogen must be protected from continued action of the λ replication system. This would be accomplished by the action of repressor in deactivating the *c*II–*O* region for initiation. Direct evidence that this aspect of replication control is important during lysogenization by O^+P^+ phages has been found by Ohashi and Dove (in preparation).

The quantitative aspects of the relationship between *x–O–P* transcription and competence for replication are summarized schematically in Fig. 2. The regions of λ DNA transcribed under various conditions are shown. The number of parallel lines making up an arrow indicates the relative intensity of transcription.

In the presence of imm^{434} helper, but not imm^{21} helper, the transactivation of gene *P* can be detected (Thomas, 1970; Herskowitz and Signer, 1970b). This transactivation may involve a secondary promoter in the *x* region (Thomas, personal communication). We would expect that the level of *x–O–P* transcription from repressed λ+ in the presence of imm^{434} helper would be no greater than the level of *x–O–P* transcription from induced *x*13, where a high level of *N* product and the putative secondary promoter in *x* would both exist. As measured by hybridization with the *r* strand of $\phi 80imm^{\lambda}$ (Table 2), this level is in the range of 0.04% of the total RNA labeled by a 2-min pulse (Nijkamp et al., 1970). The corresponding level for repressed λ+ is at least one order of magnitude lower (Taylor et al., 1967). For induced N^- this level is in the range of 0.2–0.4% (Nijkamp et al., 1970). For repressed *c*17 and ri^cD it is in the range of 0.15% (Nijkamp, Szybalski, and Dove, in preparation). For induced λ+ it ranges from 0.6 to 2.0% (Nijkamp et al., 1970). Thus the transcription of the *x–O–P* region is adequate for replication competence when it reaches a level in the range of 0.2%.

We cannot provide any strong evidence to permit a choice between the two alternative molecular mechanisms proposed for transcriptional activation. Transcription could alter the *c*II–*O* region of λ DNA either by changing its structure or by translocating it into a location of the cell where it can be acted upon.

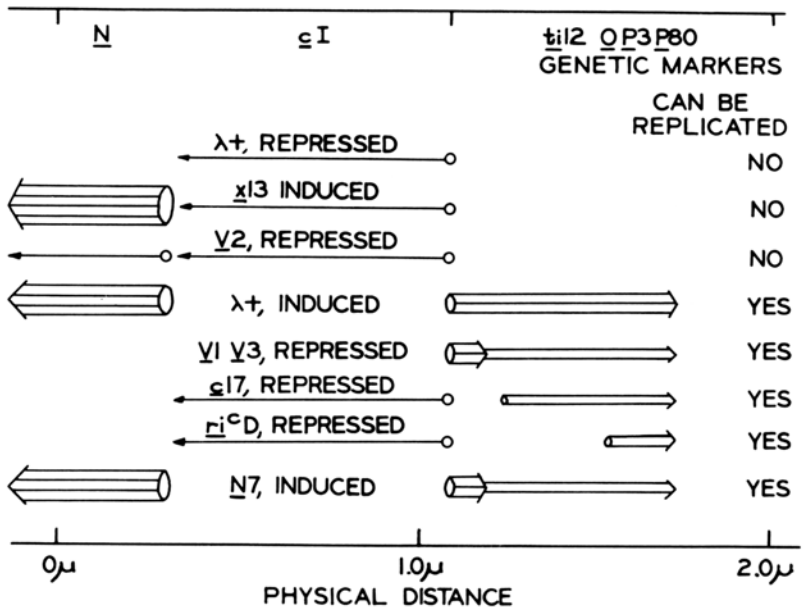


FIGURE 2. The relationship between transcription activity and competence for replication of λ DNA. The order of sites in the *N*–*c*I–*O*–*P* region is shown at the top. The physical distances from the left end of imm^{21} estimated for this region are shown at the bottom, on the basis of the left and right end points of the imm^{21} and imm^{434} substitutions and the end point of the d*bio*E5a-20 substitution between *P*3 and *P*80 (Fiandt et al., this volume). The transcription patterns for a number of situations are shown schematically, along with the ability of each kind of DNA molecule to participate in replication in the presence of a helper. Primary transcription data for repressed λ+ are from Taylor et al. (1967); for induced *x*13 from Nijkamp et al. (1970); for repressed *v*2 from Kumar and Szybalski (1970); for induced λ+ from Nijkamp et al., (1970); for repressed *v*1*v*3 from Kumar and Szybalski (1970); for repressed *c*17 and ri^cD from Nijkamp, Szybalski, and Dove (unpublished); and for induced *N*7 from Nijkamp et al. (1970) and Heinemann and Spiegelman (1970). The information on the replication capacity for each kind of λ DNA comes from sources given in the text.

The positions of ri^c5b and ri^cD lead us to favor the translocation mechanism. From the mapping data in Table 14, and from the distance of about 800 nucleotide pairs between imm^{21} and the *P*3–*P*80 interval (Fiandt et al., this volume), one can estimate that these ri^c mutants lie about 200 nucleotide pairs (0.07 μ) to the right of the *ti*12–*t*5 interval. We consider it plausible that translocation can operate over such a distance, since DNA in solution is rigid over these dimensions (Hays et al., 1969) and the cell is large compared to 0.07 μ.

Induced *x*13 and repressed *v*2 are not competent for replication, although at least the former directs intense transcription. Since this transcription occurs in a part of the DNA molecule about 1 μ from the *c*II–*O* region, it may well be too far away to lead either to a perturbation of DNA structure or to translocation of the *c*II–*O* segment. A similar situation holds in regard to transcription of late λ genes known to be induced by imm^{434} or imm^{21} helper (Thomas, 1970). This transcription may well start at a site between

genes *Q* and *S* (Herskowitz and Signer, 1970a), which is about 2 μ from the *c*II–*O* region.

The translocation of DNA segments suggested here may be equivalent to "membrane binding" of λ DNA (Salivar and Gardinier, 1970; Hallick et al., 1969; Sakakibara and Tomizawa, this volume). Should this be so, our analysis would require that "membrane binding" is caused by transcription or translation per se rather than by the action of a λ gene product (see Aronson, 1966, and Rouvière et al., 1969).

4. Does the product of gene *N* engage in replication only by stimulating the production of other, diffusible gene products?

In the final section of Results we showed that the helper imm^{21} could direct the replication of *N*-deficient λ as well as could the helper imm^{434}. If imm^{21} is unable to supply any *N* function for replication in the same way as it is unable to supply such a function for λ transcription, then the *N* product must not be directly involved in λ replication. As discussed above, *x*–*O*–*P* transcription under *N*-deficient conditions is adequate for transcriptional activation; presumably it is not adequate for saturating levels of *O* and *P* gene products.

SUMMARY

The experiments and arguments presented here give evidence that: (1) The λ DNA molecule is composed of one bidirectional or two divergent replication units controlled by a site (or sites) in the *c*II–*O* region. (2) This replication unit (or units) is controlled positively at the initiation stage by at least the *O* product. (3) The *N* product acts in replication only by controlling the levels of diffusible products such as that of gene *O*. (4) Repressor exerts a specific negative control over initiation by repressing the transcription of the *x*–*O*–*P* region. (5) Transcription of the *x*–*O*–*P* region stimulates the initiation process not by leading to the synthesis of a protein, but by modifying the structure or cellular location of that segment of DNA.

We have given provisional arguments to support the following: (1) Both *ti*12 and *t*5a lie in a unitary origin of replication controlling both leftward and rightward replication. (2) Transcription of the *x*–*O*–*P* region activates this origin by changing its cellular location rather than by perturbing its structure.

Acknowledgments

We thank a number of colleagues who have contributed suggestions to this work, including P. Brachet, R. W. Davies, and H. Echols. We are grateful for the support and interest of Dr. W. Szybalski, in whose laboratory one of us (W. F. S.) has been a guest. I. Herskowitz and E. Signer have contributed uniquely to this manuscript by their stringent criticism.

The work has been supported by a Program Project Grant (CA-07175) to the McArdle Laboratory from the National Institutes of Health. Mrs. Carol McLeester and Mr. Yak Saturen have provided expert technical assistance in all the experiments.

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