

# Positive and Negative Control of Bacteriophage Lambda DNA Replication

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How is an origin of replication turned on? How is an origin turned off? We have asked these questions about the control of DNA replication in lytic growth and lysogenization of the temperate coliphage  $\lambda$ .

On one hand, for active replication,  $\lambda$  must take over the replication machinery of its host. With the exception of some specific initiator functions,  $\lambda$  appears to utilize most or all of the proteins needed for *Escherichia coli* DNA replication (for review, see Skalka 1977). On the other hand, if  $\lambda$  is to lysogenize successfully, then phage DNA synthesis must be turned off after an initial phase of active replication so that  $\lambda$  will not interfere with the normal replication of the host chromosome into which it integrates.

Genetic analysis has identified the elements carried by  $\lambda$  to ensure its own replication in the lytic cycle and to prevent this replication in lysogenization. Autonomous replication depends on the proteins encoded by  $\lambda$  genes *O* and *P* (Ogawa and Tomizawa 1968). Initiation occurs at a unique origin which lies near the *O* and *P* genes and which can be inactivated by *cis*-dominant mutations, called *ori*<sup>-</sup> (Schnös and Inman 1970; Dove et al. 1971; Stevens et al. 1971; Rambach 1973). These mutations affect an unusual nucleotide sequence that serves a dual role as a regulatory site and as part of the coding region of gene *O* (Furth et al. 1977; Furth and Yates 1978; Denniston-Thompson et al., this volume; Hobom et al., this volume). Both the expression of the *O* and *P* genes and the functional state of *ori* (Dove et al. 1969, 1971; Inokuchi et al. 1973) are controlled by the immunity repressor (*cI* protein) through its ability to shut off transcription from the promoter *p<sub>R</sub>* (see Fig. 1).

How do these elements work together to control  $\lambda$  DNA replication? The gene-*O* and -*P* polypeptides have recently been identified both in infected cells (Oppenheim et al. 1977; C. Epp and M. Pearson, pers. comm.) and among the products of  $\lambda$  DNA-dependent protein synthesis in vitro (Raab et al. 1977; Yates et al. 1977). Unfortunately, despite some success in carrying out specific  $\lambda$  DNA synthesis in concentrated lysates on cellophane disks (Klein et al. 1978), it has not yet proved possible to carry out a detailed biochemical analysis of the initiation of  $\lambda$  DNA replication in vitro.

Thus, we continue to rely on inferences from functional genetic tests in vivo and from autopsies of the molecular defects associated with mutations in the replication system.

In this paper, we draw together the recently published results from our laboratory and discuss the logic by which the  $\lambda$  replication system operates.

## Selective Replication of the $\lambda$ DNA Template

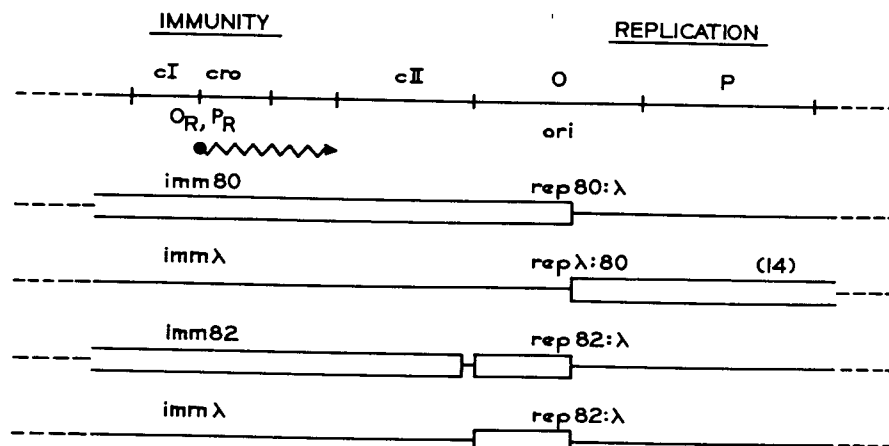
We propose that a chain of interactions among *ori*, gene-*O* protein, gene-*P* protein, and host proteins directs the host replication machinery to function on a  $\lambda$  DNA template.

Interactions in vivo between mutant alleles of  $\lambda$  gene *P* and alleles of gene *dnaB* (Georgopoulos and Herskowitz 1971) and of several additional host genes (Georgopoulos 1977; Saito and Uchida 1977; Sunshine et al. 1977) have been interpreted to show interactions between the gene-*P* protein and *E. coli* replication proteins. Wickner (this volume) and Klein et al. (1978, and pers. comm.) have now found direct biochemical evidence that the *P* and *dnaB* proteins interact. Cooperative function of the  $\lambda$  gene-*O* and -*P* proteins was inferred by Tomizawa (1971) on the basis of suppression of a mutation in gene *O* by certain alleles of gene *P*. We have extended this observation to show that the gene-*O* protein carries in its carboxyterminal region a determinant for interaction with *P*. Finally, we have found evidence that the amino-terminal segment of the gene-*O* protein carries a determinant for the specific recognition of a target sequence in *ori* (Furth et al. 1978; Furth and Yates 1978).

## Specificity Determinants for $\lambda$ Replication

To identify specific molecular interactions that control  $\lambda$  DNA replication, we have analyzed functions that differ in specificity among the lambdoid phages. Lambda is but one of a group of viruses that share a similar organization of blocks of functionally related genes and that can exchange some of these blocks by genetic recombination (Dove 1971; Hershey 1971; Botstein and Herskowitz 1974). Complementation tests can reveal differences in the functional specificities of the replication proteins of the various lamb-

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**Figure 1.** Genetic structure of the immunity and replication control regions of  $\lambda$  and its hybrids with lambdoid phages  $\phi 80$  and 82. Straight lines indicate sequences derived from  $\lambda$ ; boxed areas indicate sequences derived from the other lambdoid phages. The normal transcription of the replication region is controlled by the promoter and operator, designated  $p_R$  and  $o_R$ , and occurs rightward as indicated.

doid phage types. A mutant phage defective in an essential replication gene is tested for its ability to grow in mixed infection with a helper phage of another type. If the mutant fails to replicate, then we infer that the two phage types differ both in the specificity of the protein product of the gene defined by the mutation and in a target site with which this protein interacts, directly or indirectly.

Comparisons among the lambdoid phages  $\lambda$ ,  $\phi 80$ , and 82 have permitted the identification of the *ori* and *P* interactions of the *O* protein. Neither  $\phi 80$  nor 82 can promote the replication of  $\lambda$  mutants defective in *O*, although both phages do promote the replication of  $\lambda P^-$  mutants (Dove 1968; Szpirer and Brachet 1970). Furthermore, both  $\phi 80$  and 82 must differ from  $\lambda$  in the structure of *ori* because neither recombinates productively with  $\lambda ori^-$  mutants to give wild-type  $\lambda$  progeny (see Moore et al., and Hobom et al., both this volume).

The specificity elements of gene *O* have been dissected by constructing hybrids between  $\lambda$  and 82 or  $\phi 80$ . We designate a hybrid between phages X and Y, within the replication region, as *repX:Y*. The set of hybrids exchanged within gene *O* is diagrammed in Figure 1 (see Furth et al. 1978). In each case approximately half of the  $\lambda$  *O* gene has been substituted by heterologous sequences from the related phage. The *ori* region, as defined by *ori^-* mutations, lies just to the left of the border between the heterologous segments of gene *O*.

#### $\lambda$ - $\phi 80$ Replication Hybrids

If the segment of the  $\lambda$  *O* gene present in a hybrid phage is part of an essential hybrid replication gene, then the introduction of an amber chain-termination mutation into this portion of the gene should render the phage dependent on a nonsense suppressor for growth. When we constructed *Oam* derivatives of *rep80:lambda* or *rep lambda:80* hybrids, we found that the phages

acquired the amber phenotype; each failed to grow in a nonsuppressing host but could grow normally in a host carrying an amber suppressor. Thus, each of these phages appears to have a hybrid *O* gene which remains essential for replication.

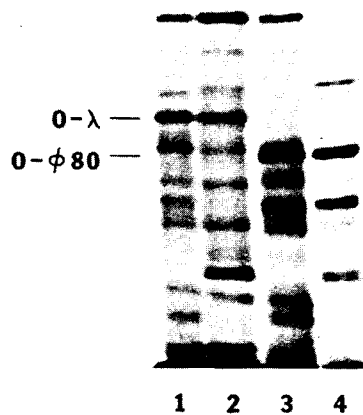
Analysis of proteins synthesized in vitro from *rep80:lambda* and *rep lambda:80* DNA templates shows directly that each encodes a hybrid polypeptide in place of that encoded by the *O* gene of  $\lambda$ . These hybrid polypeptides have been identified by showing that *Oam* mutations eliminate their synthesis, unless a suppressor tRNA fraction is added to the in vitro system. They have been compared to their parental counterparts with respect to size (Fig. 2) and with respect to their methionine-labeled tryptic peptides.

The *O*-like polypeptide of  $\phi 80$  differs from *O* of  $\lambda$  both in size and in the composition of its amino-terminal tryptic peptide. The corresponding polypeptide encoded by *rep80:lambda* carries the amino-terminal tryptic peptide of  $\phi 80$ , but the size determinant of  $\lambda$ . The polypeptide encoded by *rep lambda:80* displays the reciprocal configuration. A summary of the structures of the *O*-related polypeptides encoded by these and other phages of different *rep* genotype is presented in Figure 3 (Furth and Yates 1978).

Although *rep80:lambda* and *rep lambda:80* hybrid phages encode polypeptides related to the  $\lambda$  gene-*O* product and necessary for replication, the hybrid gene products cannot be freely interchanged with other elements in the  $\lambda$  replication system. Each of the hybrid gene-*O* proteins displays some of the functional characteristics of the *O*-related protein of  $\phi 80$ . The restrictions on successful combinations of elements give evidence for specificities of interaction between the elements of the replication system.

#### *ori* and *O* Interact

Interaction between *ori* and *O* has been inferred from complementation tests in which different donors



**Figure 2.** Gene-*O* proteins of *rep* $\lambda$ , *rep*80: $\lambda$ , *rep* $\lambda$ :80, and *rep*80 phages. Autoradiograph of [<sup>35</sup>S]methionine-labeled polypeptides produced when the DNAs of  $\lambda$ ,  $\phi$ 80, and two  $\lambda$ - $\phi$ 80 hybrid phages are used as templates for in vitro protein synthesis. The polypeptides were separated by electrophoresis on 12% polyacrylamide gels in the presence of SDS (Laemmli 1970). Migration was from top to bottom. The mobility of *O*- $\lambda$  corresponds to a polypeptide molecular weight of  $34,500 \pm 500$ , and the mobility of *O*- $\phi$ 80 corresponds to a molecular weight of  $31,000 \pm 500$ . Templates: (1) *lcI857Sam7* (*rep* $\lambda$ ); (2) *limm80hy42* (*rep*80: $\lambda$ ); (3) *lcI857 rep* $\lambda$ :80; (4)  $\phi$ 80 (*rep*80). (Reprinted, with permission, from Furth and Yates 1978.)

of gene-*O* function were encouraged to promote the replication of *O*-deficient target phages carrying one or another *ori* region. The pattern of complementation between pairs of phages derived from  $\lambda$  and  $\phi$ 80 is summarized in Table 1 (Furth et al. 1978). We find that successful interaction between the *O*<sup>+</sup> donor and the defective *Oam* target phage to permit growth of the target phage is observed only when the two phages match in the segment of gene *O* that encodes the amino-terminal segment of the gene-*O* protein and also includes *ori*. In contrast, the donor and target phages need not match in the source of the

<i>O</i> Protein	Monomer MW	<i>ori</i> Specificity	<i>P</i> Specificity
$\lambda$ —————	34,500	$\lambda$	$\lambda, \phi 80$
$\phi 80$ [ ]	31,000	$\phi 80$	$\phi 80$
$\lambda:80$ — [ ]	31,000	$\lambda$	$\phi 80$
80: $\lambda$ [ ] —	34,500	$\phi 80$	$\lambda, \phi 80$
82: $\lambda$ [ ] —	36,000	82	$\lambda, \phi 80$

**Figure 3.** Summary of properties of gene-*O* proteins of  $\lambda$ ,  $\phi$ 80, 82, and replication-region hybrids. Structures of gene-*O* polypeptides are shown schematically to indicate segments derived from  $\lambda$  (line) and from  $\phi$ 80 or 82 (box). The amino-terminal segment is at the left. The *O* genes of phage 82 and of *rep*82: $\lambda$  hybrids appear to be indistinguishable. (Reprinted, with permission, from Furth and Yates 1978.)

**Table 1.** Specificity of *ori*-*O* Interaction

<i>O</i> <sup>+</sup> Donor	Replication of <i>Oam</i> target phage		
	<i>rep</i> $\lambda$ <i>Oam</i> ( <i>ori</i> $\lambda$ )	<i>rep</i> $\lambda$ <i>Oam</i> :80 ( <i>ori</i> $\lambda$ )	<i>rep</i> 80: $\lambda$ <i>Oam</i> ( <i>ori</i> 80)
<i>rep</i> $\lambda$	+	+	-
<i>rep</i> $\lambda$ :80	+	+	-
<i>rep</i> 80	-	-	+
<i>rep</i> 80: $\lambda$	-	-	+

carboxy-terminal segment of gene *O*. For example, a *rep* $\lambda$ :80 derivative carrying an *Oam* mutation can be helped to replicate by a *rep* $\lambda$  or a *rep* $\lambda$ :80 donor, but it cannot be helped by a *rep*80 or a *rep*80: $\lambda$  donor.

Further complementation tests reveal that in phage 82 and *rep*82: $\lambda$  hybrids the specificity of the *ori*-*O* interaction is distinct both from that of  $\lambda$  and from that of  $\phi$ 80. Thus, among  $\lambda$ ,  $\phi$ 80, and 82, this interaction displays stringent type specificity (Fig. 3).

What is the receptor function, determined by the target phage, with which the amino-terminal portion of the gene-*O* protein interacts? We argue that the receptor is a specific nucleotide sequence within *ori*, the regulatory site for the origin of replication. The strongest evidence for this claim comes from the demonstration that a small substitution of 82 DNA, limited to the *ori* region and part of the amino-terminal segment of *O*, confers the entire 82-type replication specificity to  $\lambda$ .

#### $\lambda$ -82 Replication Hybrids

A  $\lambda$ -82 hybrid phage carrying 82 DNA only within the replication region was isolated by repeated backcrossing of an *imm*82 *rep*82: $\lambda$  hybrid against appropriate derivatives of  $\lambda$ . Genetic analysis indicates that the *rep*82 substitution covers the amino-terminal half of gene *O*, but affects no other known  $\lambda$  structural gene (Furth et al. 1978) (Fig. 1). This portion of gene *O* also includes the sites of all of the *lori*<sup>-</sup> mutations which have been mapped accurately. Hybrids carrying both the *rep*82 substitution and a  $\lambda$ *Oam* mutation (*rep*82: $\lambda$ *Oam*) can be helped to replicate by phage 82 or by a  $\lambda$ *rep*82: $\lambda$  hybrid phage, but not by  $\lambda$ . Conversely, an *O*<sup>+</sup>  $\lambda$ *rep*82: $\lambda$  donor fails to promote the replication of a *rep* $\lambda$ *Oam* mutant (Table 2). Thus, the small *rep*82 substitution includes both the donor and the receptor specificity determinants for 82-type gene-*O* function. Because the only known elements covered by the substitution are *O* and *ori*, it seems very probable that *ori* contains a specific target sequence which is recognized by the amino-terminal segment of the gene-*O* protein.

DNA heteroduplex mapping demonstrates that the *rep*82 substitution is limited to less than 1% of the genome (Fig. 4). In a *rep* $\lambda$ /*rep*82 heteroduplex, one strand of the substitution loop in the replication region appears slightly longer than the other; the shorter

**Table 2.** Substitution of *ori* and *O* Specificities in *rep82*: $\lambda$ 

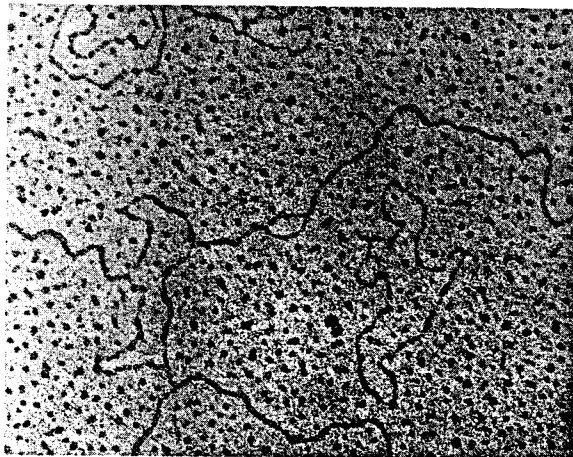
<i>O</i> <sup>+</sup> donor	Burst size of <i>Oam</i> target phage	
	$\lambda imm434 rep\lambda Oam205$	$\lambda imm434 rep82:Oam205$
	$\begin{array}{c} + \text{---} \times + \\ O \\ (ori\lambda) \end{array}$	$\begin{array}{c} + \text{---} \times + \\ O \\ (ori82) \end{array}$
None	0.1	0.2
$\lambda$ ( <i>rep</i> $\lambda$ )	20	0.4
$\lambda rep82:\lambda$	0.4	18
82 ( <i>rep82</i> )	0.4	20

The *O*<sup>+</sup> helper phages, at a multiplicity of infection (moi) 3–5 particles/cell, and the *Oam* target phages, at an moi of 0.2 particles/cell, were permitted to adsorb *E. coli* strain 594, which is nonpermissive for amber mutants. Infected complexes were diluted into broth and incubated at 37°C for 90 min to allow one cycle of phage growth. The helper- and target-type progeny phages were titrated separately by plating on appropriate lysogenic indicator strains. (Data from Furth et al. 1978.)

strand measures  $398 \pm 38$  bases, and the longer strand measures  $446 \pm 42$  bases. Because the *O*-82: $\lambda$  polypeptide is slightly larger than the *O*- $\lambda$  polypeptide (Fig. 3), we surmise that the longer strand of the substitution loop corresponds to 82 DNA. We conclude that the determinants of specificity of the *ori*-*O* interaction lie within an interval of fewer than 450 bp.

### *O* and *P* Interact

Does either the gene-*O* protein or *ori* also interact specifically with the product of replication gene *P*? Both  $\phi 80$  and 82 can contribute gene-*P* activity to  $\lambda P^-$  mutants (Dove 1968; Szpirer and Brachet 1970). However, the *P*-like function of  $\phi 80$  differs, at least subtly,



**Figure 4.** DNA heteroduplex showing *rep82* substitution. Heteroduplexes of  $\lambda b2/\lambda imm434 rep82:\lambda$  were prepared and analyzed as described by Davis et al. (1971). In the heteroduplex region shown here, the large substitution loop at the left corresponds to the *imm434/imm* $\lambda$  heterology, and the small substitution loop at the center of the field corresponds to the *rep* $\lambda/rep82$  heterology. A circular SV40 DNA molecule (5225 bp) is present at the right of the field. (Reprinted, with permission, from Furth et al. 1978.)

from that of  $\lambda$  because phages with the  $\phi 80$ -type *P* are able to replicate in some *E. coli* mutants (*groP*) in which the normal function of the  $\lambda$ -type gene-*P* protein is blocked (Georgopoulos and Herskowitz 1971; Szpirer 1972). DNA heteroduplex mapping and genetic mapping also indicate that the *P* genes of  $\lambda$  and  $\phi 80$  are partially heterologous (Fiandt et al. 1971; Furth et al. 1978).

Gene 14 of  $\phi 80$  (Sato 1970) appears to encode the *P*-like function of this phage; a *rep* $\lambda Pam$  mutant and a *rep* $\lambda:80$  hybrid phage carrying a *14am* mutation (*rep* $\lambda:8014am8$ ) fail to complement in mixed infection (Furth et al. 1978). A *rep* $\lambda Oam:80$  hybrid phage and the *rep* $\lambda Pam$  mutant do complement, confirming that the  $\phi 80$  gene-14 protein can function together with *O* of  $\lambda$ .

Surprisingly, a *rep* $\lambda Oam$  mutant and the *rep* $\lambda:8014am$  phage fail to complement, even though the gene-*P* protein of  $\lambda$  and the hybrid gene-*O* protein *O*- $\lambda:80$  should be produced in mixedly infected cells. Similarly, we find that wild-type  $\lambda$  fails to help a  $\phi 8014am$  mutant to replicate and that a *rep* $80:\lambda$  hybrid phage, which carries gene *P* of  $\lambda$ , fails to help the *rep* $\lambda:8014am$  hybrid (Furth et al. 1978). These complementation tests are summarized in Table 3. The two cases in which complementation fails imply that the gene-*P* protein of  $\lambda$  cannot substitute successfully for the gene-14 protein of  $\phi 80$  under conditions in which the only available gene-*O* protein has the carboxy-terminal segment characteristic of  $\phi 80$ .

We conclude that the gene-*O* and -*P* proteins interact (Tomizawa 1971) and that the carboxy-terminal segment of the gene-*O* protein carries a specificity determinant for this interaction. Structural analysis (see above) confirms that the gene-*O* protein of  $\lambda$  and its  $\phi 80$  counterpart differ in their carboxy-terminal segments. If the gene-*P* protein also interacts with a specific target sequence in the DNA molecule, this sequence must be conserved among  $\lambda$ ,  $\phi 80$ , and 82.

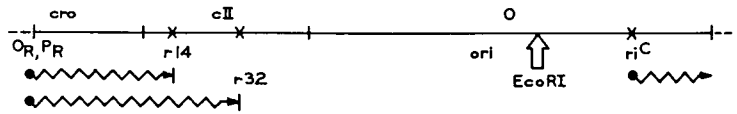
### Transcriptional Activation of *ori*

The presence of the gene-*O* and -*P* proteins and host replication proteins does not suffice to initiate replication of  $\lambda$  DNA. RNA synthesis in the vicinity of *ori* is also required. We call this effect *transcriptional activation* (Dove et al. 1969). Normally, the *ori* region is transcribed from the promoter *p<sub>R</sub>*, which can be regulated by the action of the *cI* repressor at *o<sub>R</sub>*. Repression, mutations which inactivate *p<sub>R</sub>*, and insertion mutations which introduce new transcription ter-

**Table 3.** Specificity of *O*-*P* Interaction

Type of gene- <i>P</i> protein	Type of gene- <i>O</i> protein			
	$\begin{array}{c} N \text{---} C \\ O-\lambda \end{array}$	$\begin{array}{c} N \text{---} \square \text{---} C \\ O-\lambda:80 \end{array}$	$\begin{array}{c} N \text{---} \square \text{---} C \\ O-80:\lambda \end{array}$	$\begin{array}{c} N \text{---} \square \text{---} C \\ O-\phi 80 \end{array}$
<i>P</i> - $\lambda$	+	-	+	-
<i>P</i> - $\phi 80$ (14)	+	+	+	+

**Figure 5.** Transcripts in the vicinity of *ori* studied for their capacity to activate the origin of replication. The insertions *r14* (IS1) and *r32* (IS2) were isolated by Brachet et al. (1970). In the absence of  $\lambda$  gene-*N* function, rightward transcription initiating at *p<sub>R</sub>* terminates at these insertions. The constitutive promoters *ri<sup>c</sup>D* and *ri<sup>c</sup>5b*, each of which maps at the position labeled *ri<sup>c</sup>*, are described by Dove et al. (1971). A site of cleavage of  $\lambda$  DNA by restriction endonuclease *EcoRI* is indicated.



minators between *p<sub>R</sub>* and *ori* (Fig. 5) all exert an epistatic block to DNA replication, which cannot be overcome merely by providing the gene-*O* and -*P* proteins from a helper phage (Thomas and Bertani 1964; Dove et al. 1971; Brachet and Green 1970; M. E. Furth et al., in prep.).

Does activation depend upon the transcription of a particular DNA sequence in *ori*? We have selected  $\lambda$  mutants (*ri<sup>c</sup>*) which can utilize the gene-*O* and -*P* proteins to replicate even when repressed. Four independent *ri<sup>c</sup>* mutants display constitutive transcription in the replication region as a consequence of new rightward promoters (Dove et al. 1971; Nijkamp et al. 1971). Two of these promoters, *ri<sup>c</sup>5b* and *ri<sup>c</sup>D*, map together within gene *O*, to the right of an *EcoRI* restriction endonuclease cleavage site located in that gene (Fig. 5) (M. E. Furth et al., in prep.). We have shown previously that this site delimits the essential *ori* sequence; a DNA fragment which includes the region between *p<sub>R</sub>* and the *EcoRI* site in gene *O* contains a functional  $\lambda$ -type replication origin (Furth et al. 1977). (Hobom et al. [this volume] have presented evidence that an additional segment of about 30 bp immediately to the right of the *EcoRI* site is required to give a maximal level of origin function.) Thus, both *ri<sup>c</sup>5b* and *ri<sup>c</sup>D* appear to lie entirely to the right of *ori*.

The transcript directed by the *ri<sup>c</sup>5b* promoter has been identified in vitro (M. E. Furth et al., in prep.). RNA synthesis starts approximately  $130 \pm 5$  bp to the right of the *EcoRI* site in gene *O* and proceeds to the right, away from *ori*. No transcription leftwards, towards *ori*, has been detected.

It seems that the requirement for local transcription to activate the replication origin can be completely uncoupled from all requirements for specific DNA sequence information in *ori*. To affirm this conclusion, we have asked whether the *ori* specificity of *rep82* can be reassorted with the transcriptional activation character conferred by *ri<sup>c</sup>5b* or *ri<sup>c</sup>D*. If *ri<sup>c</sup>* simply creates a new *ori* site for the  $\lambda$  replication system, then the recombinant *rep82:λri<sup>c</sup>* would display *ori* function with  $\lambda$ -type specificity. However, we find that a repressed *rep82:λri<sup>c</sup>* phage requires the 82-type gene-*O* protein in order to replicate and cannot utilize  $\lambda$ -type gene-*O* protein (Table 4). As one would expect, a reciprocal experiment shows that a repressed *repλri<sup>c</sup>* phage requires a gene-*O* protein capable of functioning with *ori* of  $\lambda$ . We conclude that the *ri<sup>c</sup>* character can be expressed when coupled either to the  $\lambda$ -type or to the 82-type *ori* region, and that the normal determinant in *ori* for interaction with the amino-terminal segment of

the gene-*O* protein still governs the specificity of the *ori*-*O* interaction.

## DISCUSSION

### A Chain of Interactions Controls $\lambda$ Replication

We have found that the proteins that are needed to turn on  $\lambda$  DNA replication function in cooperation with each other. The gene-*O* protein interacts both with a specific target sequence in the DNA molecule (at or near the origin of replication), and with the gene-*P* protein. Others have found evidence that the gene-*P* protein interacts with at least four host proteins (Georgopoulos and Herskowitz 1971; Georgopoulos 1977; Saito and Uchida 1977; Sunshine et al. 1977; Wickner, this volume; Klein et al. 1978). We can summarize the known interactions as a chain: *ori*-*O*-*P*-host proteins. Although we do not know the mechanism of initiation of  $\lambda$  replication, this chain would be sufficient to permit specific template selection and to channel essential host components to function in the replication of  $\lambda$  DNA.

In the specificity of its interaction with *ori*, the gene-*O* protein fits a major criterion for an *initiator*, as defined in the replicon model of Jacob et al. (1964). The amino-terminal half of the gene-*O* protein carries a specificity determinant for the interaction with *ori*. We have presented genetic and biochemical evidence that this portion of the protein differs in structure among coliphages  $\lambda$ ,  $\phi 80$ , and 82. The absence of detectable homology among these three phages in the part of gene *O* encoding the amino-terminal portion of

**Table 4.** Specificity of *ori*-*O* Interaction in Repressed *ri<sup>c</sup>* Phages

Helper phage	Yield/input target phage			
	$\lambda$ rep $\lambda$ ( <i>ori</i> $\lambda$ )	$\lambda$ rep $\lambda$ : <i>ri<sup>c</sup></i> ( <i>ori</i> $\lambda$ <i>ri<sup>c</sup></i> )	$\lambda$ rep82: $\lambda$ ( <i>ori</i> 82)	$\lambda$ rep82: $\lambda$ <i>ri<sup>c</sup></i> ( <i>ori</i> 82 <i>ri<sup>c</sup></i> )
$\lambda$ imm434 rep $\lambda$	1.3	57.0	0.7	0.5
$\lambda$ imm434 rep82: $\lambda$	0.9	0.7	0.7	23.0

The lysogenic *E. coli* strain Y10( $\lambda$ t11) was infected with *imm434* donor phages at an moi of 3–5 particles/cell and with *imm $\lambda$*  (*cIs857*) target phages at an moi of 0.2 particles/cell. Infected complexes were incubated at 37°C for 90 min. The yields of the helper and target phages were titrated by plating on strains Ymel( $\lambda$ ) and Ymel  $\lambda$ imm434, respectively. Results obtained with the mutants *ri<sup>c</sup>5b* and *ri<sup>c</sup>D* did not differ significantly and were therefore averaged. Data from Furth et al. (in prep.).

the protein and the stringent type specificity of the *ori-O* interaction show that the segment of the replication region containing these elements has diverged considerably among the lambdoid phages, giving individuality to each as a replicon.

The apparent separation of the *O* gene into two segments, each carrying a specificity determinant for a different interaction, may reflect the tertiary structure of the gene-*O* protein. It is possible that the gene-*O* polypeptide folds into two separate globular domains (see Kirschner and Bisswanger 1976). The amino-terminal domain might include a binding site for *ori* DNA, and the carboxy-terminal domain might include a binding site for the gene-*P* protein.

We do not know which, if any, of the host proteins can act directly at *ori* as a consequence of interactions mediated through the  $\lambda$  gene-*O* and -*P* proteins. However, it is interesting to note that the *dnaB*-gene product, which interacts with the gene-*P* protein, helps to direct the *dnaG* protein to initiate primer synthesis on some single-stranded phage DNA templates (McMacken et al. 1977; Meyer et al., this volume).

#### Transcriptional Activation

What is the function of rightward transcription of the replication control region in the initiation of  $\lambda$  DNA replication? Our studies of *ri<sup>c</sup>* mutants suggest that proximity to *ori* of the transcribed region is critical, but that the specific nucleotide sequence is not. We can imagine ways in which RNA synthesis in the vicinity of the origin of replication might control DNA replication by affecting the structure of *ori*, its intracellular location, or its interaction with other components of the replication apparatus (see Dove et al. 1969, 1971; M. E. Furth et al., in prep.).

The requirement for transcriptional activation gives  $\lambda$  a mechanism to turn off replication even after the initiation proteins have been synthesized. This is important during the lysogenization of *E. coli* by  $\lambda$ . Several rounds of autonomous DNA replication normally precede integration of  $\lambda$  into the host chromosome (Brooks 1965; Freifelder et al. 1975). This replication must be shut off at some time during lysogenization to prevent killing of the new lysogen. One mechanism which may contribute to the shutdown of the phage replication system is the decay of activity of the gene-*O* protein, which has a functional half-life of only a few minutes (Wyatt and Inokuchi 1974) and a chemical half-life of less than 3 minutes (C. Epp and M. Pearson, pers. comm.). However, it seems that transcription of the replication origin region also must be turned off (Ohashi and Dove 1976). The mutant phages  $\lambda ri^{c5b}$  and  $\lambda ri^{cD}$  kill the *E. coli* host during lysogenization, although lysis is not observed, and expression of the gene-*O* protein should be subject to normal repression. It appears that constitutive transcription near the phage replication origin during establishment of lysogeny in some way interferes with host DNA replication or cell division.

#### Positive and Negative Control

Positive control by the gene-*O* and -*P* proteins and negative control by the *cI* repressor serve complementary functions in regulating  $\lambda$  DNA replication. The action of the initiator proteins confers a high degree of individuality to the  $\lambda$  replicon and permits the phage to take over the replication machinery of its host. The requirement for local transcription to activate the replication origin provides the phage with a general mechanism to prevent the initiation of replication even in the presence of the specific positive regulators.

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