J. Mol. Biol. (1978) **126**, 195–225

Specificity Determinants for Bacteriophage Lambda DNA Replication

I. A Chain of Interactions that Controls the Initiation of Replication

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(Received 7 April 1978)

The genetic elements which control autonomous DNA replication differ in functional specificity among coliphage λ, the coliphages φ80 and 82, and the *Salmonella* phage P22. Hybrid phages derived by genetic recombination between λ and each of these related phages have been used to define and to localize specificity determinants for DNA replication.

In λ-P22 hybrid phages (Hilliker & Botstein, 1976) the replication control elements segregate as an intact unit. By contrast, some viable λ-φ80 and λ-82 hybrid phages arise by recombination within the replication control region, in a small interval inside structural gene *O*. From the properties of such hybrid phages, we infer that the *O* gene product of λ and the functionally equivalent proteins of φ80 and 82 each interact with a specific nucleotide sequence in the cognate *ori* site, the DNA target for control of the origin of replication. With respect to this interaction, both the *O* products and the receptor sequences within *ori* show stringent type specificity. The donor and receptor specificity determinants for the *ori–O* interaction lie within an interval of less than 400 base-pairs.

The *O* gene product also interacts with the product of replication gene *P* (Tomizawa, 1971). The *O–P* interaction displays limited type specificity; the P-like protein of φ80 can function together with the O protein of λ, but the P protein of λ cannot function with the O-like protein of φ80. The specificity determinants for the *O–P* interaction can be separated from those for the *ori–O* interaction.

We propose that a chain of interactions between *ori*, *O* product, *P* product, and replication functions of the bacterial host, *Escherichia coli*, controls specific template selection and the assembly of the essential replication apparatus in the initiation of λ DNA replication.

1. Introduction

After infection of *Escherichia coli* cells, the DNA of bacteriophage λ forms covalently closed circles and within minutes begins to replicate (reviews: Kaiser, 1971; Skalka, 1977). Replication forks diverge from a single region of the phage chromosome, the origin of replication (Schnös & Inman, 1970; Stevens *et al.*, 1971). An essential site at which initiation of replication is controlled, termed *ori*, can be defined genetically by *cis*-dominant mutations (*ori*$^-$) which lie within the origin region and which block λ replication (Dove *et al.*, 1969,1971; Rambach, 1973). The nucleotide sequence

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0022–2836/78/340195–31 $02.00/0

affected by these *ori*⁻ mutations has been determined (Denniston-Thompson *et al.*, 1977). However, it is not yet known by what mechanism the structure of this region determines its ability to function as an origin of replication. Once replication forks have been formed at the origin, they can progress freely until the parent molecule has been completely copied (Valenzuela *et al.*, 1976).

We wish to identify the specific molecular interactions that control initiation at the λ origin of replication. λ DNA replication depends on a number of components of the host "replication apparatus" including the products of genes *dna*B, *dna*E, *dna*G and *dna*Z. By contrast, λ does not utilize the products of host genes *dna*A and *dna*C, which appear to be essential for initiation of *E. coli* DNA replication (see Skalka, 1977). In addition to host proteins, the products of two phage genes, *O* and *P*, are absolutely required for autonomous replication (Brooks, 1965; Joyner *et al.*, 1966; Eisen *et al.*, 1966; Ogawa & Tomizawa, 1968).

The particular role played by each of the proteins involved in λ replication is not yet understood in detail. Some components, such as the DNA polymerase encoded by *dna*E, clearly act in chain elongation steps in DNA synthesis (Gefter *et al.*, 1971). The *dna*Z product also appears to participate in chain elongation (Filip *et al.*, 1974; Wickner, 1976). Both the *dna*B and *dna*G proteins participate in primer formation on some single-stranded phage DNA templates *in vitro* (McMacken *et al.*, 1977; Wickner, 1977), and probably are required for priming of short DNA chains ("Okazaki fragments") during progression of replication forks (see Alberts & Sternglanz, 1977). In some cases, the *dna*B and *dna*G proteins may also be required for primary initiation at the replication origin of a double-stranded DNA molecule (Kogoma, 1976; Zyskind & Smith, 1977; McMacken *et al.*, 1977). The *O* and *P* products are essential for a very early step in λ DNA replication, perhaps primary initiation (Freifelder & Kirschner, 1971; Shuster & Weissbach, 1969; Thirion, 1971), but may also be required continuously for fork progression (Takahashi, 1975, Klinkert & Klein, 1978).

In the absence of a precise biochemical analysis of initiation, we find it productive to divide the initiation process into two conceptually distinct steps: selection of the origin region of the template DNA molecule by a specific initiator function (Jacob *et al.*, 1963); and entry of the remaining components essential for an active replication fork. In this paper we focus on the contributions of the λ O and P proteins to the control of these two aspects of the initiation of DNA replication. We present genetic evidence that the O protein interacts specifically with a nucleotide sequence within the *ori* region. In agreement with Tomizawa (1971), we conclude further that the *O* and *P* gene products function co-operatively. The P protein also interacts with several *E. coli* replication proteins, including the *dna*B gene product (Georgopoulos & Herskowitz, 1971; Georgopoulos, 1977; Saito & Uchida, 1977; Sunshine *et al.*, 1977). We argue that, through this series of interactions, the O and P proteins channel components of the host replication apparatus to the λ replication origin, whence replication forks can then progress.

2. Materials and Methods

(a) *Media*

Tryptone broth was 1% Bacto-Tryptone (Difco), 0·5% NaCl. LB was tryptone broth supplemented with 0·5% (w/v) yeast extract (Difco) and 0·1% (w/v) glucose. Tris/Mg buffer, used for diluting phage and bacteria, was 0·01 M-Tris·HCl (pH 8·0), 0·01 M-$MgSO_4$. Phage suspension medium, used for storage of phage, was 0·006 M-Tris·HCl (pH 7·4),

0·07 M-NaCl, 0·001 M-$MgSO_4$, 0·005% (w/v) gelatin (Weigle *et al.*, 1959). Tryptone plate agar contained tryptone broth and 1·5% (w/v) agar (Difco). Top agar contained tryptone broth and 0·6% agar. Trypticase plate agar, used for titering of phages which make relatively small plaques, contained 1% trypticase peptone (BBL Division of Becton, Dickinson and Co.), 0·001% thiamine, 0·5% NaCl, and 1% agar. EMB-O plate agar contained 0·8% Bacto-Tryptone, 0·1% yeast extract, 0·05% NaCl, 0·04% eosin Y (Allied Chemicals), 0·0065% methylene blue, 0·02% maltose, and 1·5% agar. XGal plate agar, used as an indicator for β-galactosidase, was trypticase plate agar supplemented with 30 μg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside/ml (BaChem). Phages carrying the *lac*5 substitution form blue plaques on this medium (Ippen *et al.*, 1971).

(b) *Bacterial and bacteriophage strains*

The lambdoid phage types used in this work are listed in Table 1 A and B. All hybrid phages have λ host range unless otherwise specified. The *rep*P22, *rep*λ:80, and *rep*82:λ substitutions can be combined with any of the immunity types *imm*λ, *imm*434, *imm*21 and *imm*P22. Mutations of λ, φ80 and P22 are listed in Table 1 C. UAG nonsense mutations (amber) are indicated as am, and UGA nonsense mutations (opal) are indicated as op. Temperature-sensitive mutations are indicated as ts. Phages in which the replication control region derives partly from one parent phage and partly from the other are designated *rep*X:Y, as described in Results section (b) (i). Phage strains constructed for this report are described below and in Results. Phage stocks were prepared by the agar layer method on permissive hosts (Adams, 1959).

The map order of *O*am mutations was determined from crosses against deleted λ prophages (Dove *et al.*, 1971; Furth *et al.*, 1977; Furth, 1978). The position of *O*op261 was determined by 4-factor crosses as described by Dove *et al.* (1971).

All bacterial strains are derivatives of *E. coli* K12 (Table 2). Lysogenic derivatives of these strains were made by spotting the desired phage strain at about 10^8 phage/ml on a lawn of a permissive host and incubating overnight, or by infecting a non-permissive host at high multiplicity (5 to 10), diluting 50-fold into LB broth, and incubating for several hours. In either case the lysogens were identified by cross-streaking single colonies, obtained by overnight growth on tryptone or LB plate agar, against streaks of phages (10^9 phage/ml) of the appropriate immunity type on EMB-O agar plates. Streaks from immune colonies show no signs of lysis, whereas streaks from sensitive colonies show a zone of lysis (no growth or a dark color reaction) at the intersection with the streak of the test phage.

(c) *Phage titration*

Indicator bacteria were grown to saturation in tryptone broth supplemented with 0·2% (w/v) maltose in screw-capped flasks at 37°C, collected by centrifugation, resuspended in an equal volume of Tris/Mg buffer, and stored for up to 5 days at 4°C. Phage lysates were diluted in Tris/Mg buffer, and portions of 0·05 to 0·2 ml were mixed with several drops of the appropriate indicator strain. Adsorption was achieved by incubation at 37°C for 15 min. The mixture was distributed on a tryptone or trypticase agar plate in 3 ml of top agar, and incubated overnight.

(d) *Phage crosses*

Crosses were carried out, whenever possible, in a host strain permissive for both parent phages. The bacteria were grown to saturation overnight at 30°C or 37°C in tryptone broth supplemented with 0·2% maltose and 0·005% yeast extract, and were diluted 25-fold into fresh medium and grown to about 2×10^8 cells/ml. The cells were collected by centrifugation and resuspended in Tris/Mg buffer to a density of 4×10^8 or 5×10^8 cells/ml. Then 0·1 ml of the cell suspension was mixed with 0·1 ml of a mixture of the parent phages in Tris/Mg buffer, to give a multiplicity of infection of 3 to 5 particles/cell of each phage type. The mixtures were incubated for 15 min at 37°C for phage adsorption, diluted 10-fold with Tris/Mg buffer, and in some cases were subjected to u.v. irradiation at a dose of 150 to 250 erg/mm^2. Then 0·1 or 0·5 ml of the mixture was diluted into 5 ml of LB broth in a screw-capped tube (covered with foil if the mixture had been u.v.-irradiated),

TABLE 1

Bacteriophage strains

A. *Immunity and immunity-replication substitutions*

Strain	Replication type	Reference/source
λ	*rep*λ	Dove (1969)
λ*imm*434	*rep*λ	Kaiser & Jacob (1957)
λ*imm*21 (hy1)	*rep*λ	Liedke-Kulke & Kaiser (1967)
λ*imm*P22*c*2–5 *rep*P22 (hy25)	*rep*P22	Hilliker & Botstein (1976)
λ*imm*P22*c*1–7 *rep*λ (hy109)	*rep*λ	Hilliker & Botstein (1976)
φ80	*rep*80	Matsushiro (1963); H. Inokuchi
λ*imm*80hy41	*rep*80	Szpirer & Brachet (1970); J. Salstrom
λ*imm*80hy42	*rep*80:λ	Szpirer (1972); F. Blattner
λ*imm*80hy21–1	*rep*80	N. Franklin; P. Toothman
82*c*	*rep*82	Jacob & Wollman (1956)
λ*imm*82*c*	*rep*82:λ	Dove *et al.* (1971)

B. *Replication types*

Designation	Description	Reference
*rep*λ	*ori*λ (*O P*)λ	
*rep*P22	*ori*P22 (*18 12*)P22	Hilliker & Botstein (1976)
*rep*80	*ori*80 (*14 16* others?)80	Sato *et al.* (1968); Sato (1970)
*rep*80:λ	*ori*80 *O*80:λ *P*λ	Szpirer (1972); this work
*rep*82:λ	*ori*82 *O*82:λ *P*λ	Dove *et al.* (1971); this work
*rep*λ:80	*ori*λ *O*λ:80 (*14* others?)80	Toothman (1976); this work

C. *Mutations in essential genes*

Mutation	Description	Reference/source
(i) *λ genes*		
Nam7Nam53	Early regulatory function	Campbell (1961); Horskowitz (1971)
Oam905	Replication function	Toothman (1976)
Oam29, *Oam8*, *Oam125*		Campbell (1961)
Oam205		Thomas *et al.* (1967)
Oam1005	Suppressed by *su*III, not by *su*II	Furth *et al.* (1977)
Oop261		I. Horskowitz
Pam80	Replication function	Campbell (1961)
Pam3	Suppressed by *su*II, not by *su*III	Campbell (1961)
Qam117	Late regulatory function	Campbell (1961)
Sam7	Lysis function; suppressed by *su*III, not by *su*II	Goldberg & Howe (1969)
ori$^-$*ti12*	Origin of replication	Dove *et al.* (1971)
ori$^-$*r93*, *ori*$^-$*r95*, *ori*$^-$*r96*, *ori*$^-$*r99*		Rambach (1973)
(ii) *Other phage types*		
φ80 14am8	Replication function	Sato *et al.* (1968); P. Toothman
P22 *12*amN14	Replication function	Botstein *et al.* (1972); S. Hillikor
P22 *12*ts12·1		Levine & Schott (1971); S. Hillikor

D. *Other markers*

Mutation	Description	Reference
*imm*λ*c*Its857	Thermolabile repressor	Sussman & Jacob (1962)
*c*II2002	Establishment of repressor synthesis	Brachet & Thomas (1969)
lac5	Transducing phage carrying *lacZ*	Ippen *et al.* (1971)
b2	Deletion of nonessential region	Kellenberger *et al.* (1961)
h80	Host range of *φ80*	Signer (1964)

TABLE 2

Bacterial strains

Strain	Genotype	Reference
594	*su*° *gal*K *gal*T *str*A	Campbell (1965)
594*su*III	*su*III *gal*K *gal*T *str*A	Furth *et al.* (1977)
Ymel	*su*III *mel*	Yanofsky & Ito (1966)
C600	*su*II *thr leu lac ton*A	Appleyard (1954)
Q5151	C600 *gro*PA15	Georgopoulos & Herskowitz (1971)
K834	C600 *him*A	Miller & Friedman (1977)
K802	*su*II hsr^- hsm^+ *met gal*	Wood (1966)
CA5070	su_{UGA}	Sambrook *et al.* (1967)

Abbreviation used: *su*°, inability to support the growth of λ*am* and *op* mutants; *su*II, *su*III are amber suppressors inserting glutamine and tyrosine, respectively, at the UAG codon: su_{UGA} is an opal suppressor inserting tryptophan at the UGA codon; *str*A, streptomycin resistance. The other genetic symbols are those used by Bachman *et al.* (1976), or by the authors of the pertinent reference.

and incubated at 37°C for 90 to 120 min. Crosses were terminated by addition of a drop of $CHCl_3$. The lysates were then vortexed briefly and chilled on ice.

Crosses with λ*ori*$^-$ mutants were performed by superinfecting a lysogenic host, carrying a thermoinducible *ori*$^-$ prophage, with a test hybrid phage. Ymel (λ*Nam cIts857 ori*$^-$) lysogens were grown at 30°C, harvested, and resuspended in Tris/Mg buffer as for a standard cross. The superinfecting phage was added at a multiplicity of 3, and adsorption was achieved by incubation for 20 min at 30°C. The infected complexes were diluted into LB broth prewarmed to 42°C, incubated at that temperature for 20 min to induce the prophage, and then transferred to 37°C and incubated for 90 min.

(e) *Complementation tests for replication functions*

We tested the ability of a non-defective phage to provide an essential replication function to a defective mutant, or of 2 defective mutants to provide complementary functions to each other, by measuring the yield of each parent phage after one cycle of growth in a non-permissive host. The bacterial strain 594 was used as the non-permissive host, and 594*su*III, a derivative of 594 into which the amber suppressor *su*III was introduced by P1 transduction (Furth *et al.*, 1977), was used as a permissive control in many experiments. Cells were grown at 37°C, and phage were added for adsorption as described for phage crosses. If both phages carried a mutation in an essential replication gene, each was added at a multiplicity of infection of 3 to 5 particles/cell. If a mutant phage was tested for growth in the presence of a non-defective helper, the mutant was added at a multiplicity of infection of 0·2 particle/cell, while the helper phage was added at a multiplicity of infection of 3 to 5 particles/cell. At the end of the adsorption period, the mixture was diluted 10-fold by addition of Tris/Mg buffer, a portion of 0·1 ml was diluted into 5 ml of LB broth in a screw-capped test tube (growth tube), and a drop of $CHCl_3$ was added to the remainder of the Tris/Mg dilution tube to permit titration of unadsorbed phage. The growth tubes were incubated at 37°C for 90 min, a drop of $CHCl_3$ was added to each, and the lysates were vortexed and titrated for phage of each type. Data are presented as the yield per input phage, unless otherwise specified. Both output and input titers were corrected for unadsorbed phage. The efficiency of adsorption always exceeded 95% and usually exceeded 99%.

Immunity type was generally used as a selective marker to permit differential titration of a helper phage and a defective mutant, or of two defective mutants, by plating on appropriate lysogenic indicator strains. In some cases, one phage carried the *lac*5 substitution so that its plaques could be identified on XGal plates.

We take the results of phage growth experiments to provide a measurement of phage DNA replication, but not to provide a linear assay of the level of a given replication function. All data represent numerical averages of at least 2 experiments.

(f) *Phage constructions*

(i) rep*P2212amN14 with* imm*λ or* imm*434*

The phages *λimm*P22*rep* P2212amN14 (derived from λhy10) and *λc*I857 *rep*P2212ts12.1 (λhy105) were obtained from Dr S. Hilliker (Hilliker & Botstein, 1976). They were crossed at 30°C without u.v. irradiation. Recombinants with *imm*λ and *ts*$^+$ characters were selected on Ymel (*λimm*P22*rep* P2212amN14) at 42°C and were tested for the *am* character. The phage *λc*I857 *rep*P2212amN14 was then crossed with *λimm*434 *c*II2002, and *imm*434 *rep*P2212amN14 recombinants with and without the *c*II marker were selected on the *su*II *gro*PA15 host Q5151 (λ), which is permissive for *rep*P22 hybrid phages but not for *λP*$^+$ phages (Botstein & Herskowitz, 1974).

(ii) rep*80*:λ *and* rep*82*:λ *derivatives carrying replication gene mutations from* λ

A *P*am mutation (*P*am3 or *P*am80) was introduced into *λimm*80hy42 by crossing the hybrid phage with the desired *λP*am mutant in a permissive host, without u.v. irradiation. Recombinants with *imm*80 and carrying the *P*am allele were selected on Q5151(λ) (Georgopoulos & Herskowitz, 1971).

An *O*am mutation (*O*am125 or *O*am205) was introduced into *λimm*80hy42 by crossing *λimm*80hy42 *P*am3 with *λimm*434 *O*am in strain C600, without u.v. irradiation, and selecting for recombinants on the indicator strain Ymel(*λimm*434 *P*am3). *P*am3 is not suppressed by *su*III, the amber suppressor carried by Ymel. However, both *O*am125 and *O*am205 are suppressed by *su*III. Individual *imm*80 *P*$^+$ recombinants were tested for growth on the *su*° strain 594(*λimm*434 *rep*P2212amN14). Those recombinants which appeared to carry the *O*am allele were purified, and their structure was confirmed by a test cross with *λO*am29 and a back cross against *λO*am125 or *λO*am205. In the back cross, no *am*$^+$ recombinants were obtained. In the test cross, *imm*80 *O*$^+$ recombinants were obtained at high frequency, but no *imm*λ *O*$^+$ recombinants were found.

Derivatives of *λimm*82 carrying *O*am and *P*am mutations were constructed by analogous crosses.

(iii) rep*λ*:*80 hybrid phages*

A *repλO*am:80 hybrid phage was constructed by crossing *λc*I857 *O*am29 *P*am3 by *λimm*80hy21-1, which has the intact replication region of *ϕ*80, in the host strain K802, without u.v. irradiation. Putative *λc*I857 *repλO*am29:80 recombinants were obtained by plating the cross on strain Ymel(*ϕ*80), to select against both *imm*80 and *P*am3 characters. Ten recombinants were purified and tested further. None grew on the *su*° host 594(*ϕ*80), so each must retain the *O*am29 mutation. Because phages with the *ϕ*80-type *P* function can grow on a *gro*PA15 host, which restricts the growth of phages with λ-type *P* (Georgopoulos & Herskowitz, 1971; Toothman, 1976), we expected the *repλO*am:80 hybrids to grow on strain Q5151. Surprisingly, this was not observed. However, we found that the double mutant *λO*am29 *P*am3 also fails to form plaques on Q5151, even though this bacterial strain is permissive for *λP*am3. Thus, *O*am29 may prevent expression of the "π" phenotype. We therefore selected *am*$^+$ revertants from the putative *repλO*am29:80 hybrids, and found that all could then form plaques on the *gro*PA15 host. This confirms that these hybrids carry the *P* function of *ϕ*80. In contrast, none of 10 *am*$^+$ revertants selected in two steps (first on Ymel, then on 594) from *λc*I857 *O*am29 *P*am3 form plaques on the *gro*PA15 host. The presence of the *O*am29 allele was confirmed directly by a back cross against *λO*am29; no *am*$^+$ recombinants were observed.

A *repλ*:80*14*am8 hybrid phage was constructed by crossing *λc*I857 *P*am3 by*ϕ*80*14*am8 in strain K802 with u.v. irradiation (150 erg/mm^2). The construction again takes advantage of the ambivalent suppression pattern of *P*am3 on a host which is permissive for the amber mutation to be introduced into the hybrid phage. A putative *λc*I857 *repλ*:80*14*am8 recombinant was selected on Ymel(*ϕ*80). It was then purified on Q5151, both to confirm the

presence of the ϕ80-type *P* function and to confirm the absence of the ϕ80-type host range determinant (the *ton*A mutation renders Q5151 resistant to adsorption by ϕ80). The recombinant fails to grow on strain 594, and so must carry a nonsense mutation; the presence of *14*am8 was confirmed by a back cross.

The functional properties of the *rep*λ:80 hybrid constructed here are identical to those of a hybrid phage designated $\lambda imm434$ (*O P*)ϕ80 which was kindly provided to us by P. Toothman (Toothman, 1976). We assume that Toothman's hybrid also arose by recombination within gene *O*, although we have not confirmed this directly.

Each of the *rep*λ:80 hybrids reported here appears to carry ϕ80 DNA from a point within gene *O* rightwards, including the region of genes *Q*, *S* and *R*. None of the hybrids can form plaques on *E. coli* strain K834, a *him*A mutant, which for unknown reasons fails to support the growth of phages which have the *Q*, *S*, *R* region of ϕ80 (Miller & Friedman, 1977, and personal communication).

(iv) rep*82*:λ *hybrid phages with immunity different from* imm*82*

The replication specificity determinants were separated from the immunity specificity determinants of the hybrid phage $\lambda imm82$ in a cross of $\lambda imm434$cI *O*am29 by $\lambda imm82$ *P*am3 (see Results section (c) (iii)). The lysate was plated on 594(82) to select *imm*434*am*$^+$ progeny phage. These phage were shown to carry the 82-type *O* donor specificity and were designated $\lambda imm434$cI *rep*82:λ. The *O*am205 mutation was introduced from $\lambda imm80$hy42 *O*am205 in a u.v.-stimulated cross (150 erg/mm^2); *imm*434 progeny phage were selected on Ymel(ϕ80) and were screened for growth on 594(ϕ80) to determine the presence or absence of the *O*am allele. We then selected an *am*$^+$ recombinant from a cross of $\lambda imm434$cI *rep*82:λ*O*am205 by $\lambda imm434$ *O*am29, to ensure that the rightward segment of gene *O* in the *rep*82:λ hybrid phage would derive from λ.

Various immunity regions (X) were then coupled with the *rep*82 substitution by crossing $\lambda imm434$ *rep*82:λ by λimmX *O*am29 and selecting for *am*$^+$ recombinants of the desired immunity type. The phage used for heteroduplex analysis of the *rep*82 substitution, $\lambda imm434$ *c*II2002 *rep*82:λ, was obtained by crossing $\lambda imm434c^+$ *rep*82:λ with $\lambda imm434$ *c*II2002 *rep*P2*212*amN14 and selecting for clear-plaque recombinants on the *su*° host 594. The substitution of DNA from phage 82 in this hybrid phage should be confined between the sites of *c*II2002 and *O*am205.

(v) *Introduction of* lac*5* *into* λimm*80hy42 and* λimm*82* O*am and* P*am derivatives*

A derivative of $\lambda imm80$hy42 carrying the *lac5* substitution and the mutation *S*am7 was obtained from Bill Williams. This phage was crossed by $\lambda imm80$hy42 *O*am205 and by $\lambda imm80$hy42 *P*am80, without u.v. irradiation. $\lambda plac5$ *imm*80hy42 *O*am or *P*am recombinants were identified by formation of blue plaques on XGal plate agar on a bacterial host, K802, which suppresses *O*am205 and *P*am80 but not *S*am7. The presence of the *O*am or *P*am allele was shown by failure to form plaques on a *su*$^-$ host, and was confirmed by a back cross. The *lac5* substitution was introduced into $\lambda imm82$ derivatives by analogous crosses.

(g) *Heteroduplex mapping of* rep*82 substitution*

Phages used to provide DNA for heteroduplex mapping were grown and purified as described by Furth & Yates (1978, accompanying paper). DNA of phages $\lambda b2$ and $\lambda imm434$ *c*II2002 *rep*82:λ was mixed, denatured, and annealed, and prepared for electron microscopy by the formamide technique as described by Davis *et al.* (1971). Molecules were observed in the Hitachi H-500 electron microscope at 50 kV.

The *b*2 deletion loop and the *imm*λ/*imm*434 substitution loop served to orient the left and right ends of heteroduplex molecules, and to determine the position of the *rep* substitution. An internal length standard was provided by a mixture of single and double-stranded simian virus 40 (SV40) DNA circles, generously supplied by Dr Janet Mertz.

Measurements were made by projecting photographic negatives onto a glass screen and tracing with a Numonics digitizer. We thank Dr Ross Inman for allowing us the use of this equipment. Ten heteroduplexes were measured; for eight of them an SV40 standard was measured in the same field. The distance from *imm*434 to *rep* and the lengths of the

shorter and longer strands of the *rep* substitution loops were then calculated for each molecule, using SV40 as 5225 base-pairs in length (Fiers *et al.*, 1978; Reddy *et al.*, 1978). The mean values and the standard error of the means were calculated by ordinary procedures.

3. Results

In order to analyze specific interactions involved in the control of λ DNA replication, we have taken advantage of natural variation among the lambdoid phages. The replication control regions of λ and of several related temperate bacteriophages are functionally distinct. The functional differences are revealed by tests of the ability of a helper phage of one type to promote replication of mutant phages of another type which are defective in an essential replication gene. If the helper phage fails to provide a gene product which can support the replication of a particular mutant, then the two phage types must differ both in the specificity of the product of the gene defined by this mutation (donor specificity), and in a target site with which the gene product interacts, either directly or indirectly (receptor specificity).

(a) *λ-P22 hybrids define the replication region*

The replication control region of bacteriophage λ lies between genes controlling prophage immunity and expression of early functions, and gene *Q*, which regulates expression of late functions (Fig. 1). The genome of *Salmonella* phage P22 is organized similarly to that of λ (Dove, 1971; Botstein *et al.*, 1972), and viable hybrids can be obtained from crosses between these two phages (Gemski *et al.*, 1972; Botstein & Herskowitz, 1974).

Hilliker & Botstein (1976) constructed λ-P22 hybrids in which the immunity (*imm*) and/or the replication (*rep*) control functions of λ are replaced by those of P22. They

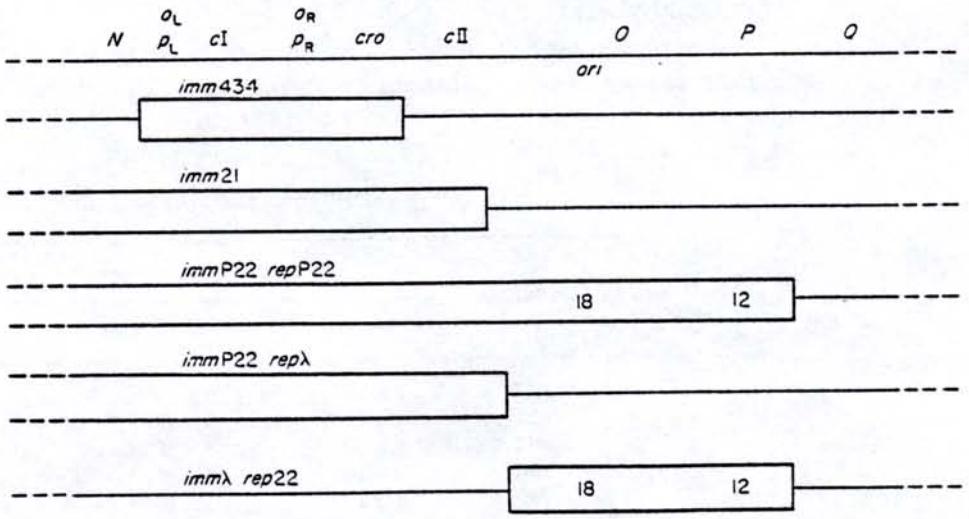

FIG. 1. Control region of bacteriophage λ genome, and substitutions in several hybrid phages. Segments derived from λ are indicated by lines, and heterologous segments derived from related phages are indicated by boxes. Gene *cI* encodes a repressor which blocks leftward and rightward transcription from promoters p_L and p_R, respectively, by binding to operators o_L and o_R. The specificity of *cI* and its associated operators determines the immunity type (*imm*). The products of *cro* and *cII* regulate the expression of *cI* and other genes. The products of *N* and *Q* are positive regulators for early and late transcription, respectively. Genes *O* and *P* and the *ori* site serve essential functions in autonomous DNA replication, and constitute the *rep* region of the phage.

found that hybrids carrying the replication genes (*18* and *12*) of P22 fail to promote the replication of λ mutants defective in *P*. Similarly, λ fails to help the replication of a hybrid carrying P22 replication genes and defective in *12*. The type specificity observed in these tests led Hilliker and Botstein to infer that the replication proteins of λ and P22 can function only with the respective replication origin. We have extended their study of λ-P22 hybrids to prove that the λ and P22 replication origins differ, and that all known replication elements, including *ori*, segregate as a unit.

In order to test whether *ori* differs between λ and P22, we determined whether the wild-type alleles corresponding to λ*ori*⁻ mutations could be rescued by genetic recombination from various λ-P22 hybrids. λ*ori*⁻ mutants have been obtained by selection among *E. coli*(λ*N*⁻ cIts) lysogenic cells for those which survive prophage induction but which remain able to express λ replication genes *O* and *P* (Dove *et al.*, 1971; Rambach, 1973). The *cis*-dominant *ori*⁻ mutations block an early step in λ replication, even in the presence of a wild-type helper phage (Inokuchi *et al.*, 1973). The *ori*⁻ mutations studied here lie in a tightly linked cluster inside gene *O* (Furth *et al.*, 1977). Nucleotide sequence analysis reveals that several of the *ori*⁻ mutations (*r*93, *r*96, *r*99) are small deletions of 24, 15, and 12 base-pairs, respectively, while one (*ti*12) is a transversion (Denniston-Thompson *et al.*, 1977).

Five different λ*ori*⁻ mutants were crossed against λ*imm*P22 *rep*P22, λ*imm*λ *rep*P22, and λ*imm*P22 *rep*λ hybrids (Fig. 1). We conclude that all of the λ*ori*⁻ mutations lie outside the region of the *imm*P22 substitution, and are covered by the *rep*P22 substitution. First, consider recombination in the interval between *imm* and *ori* in crosses of the *imm*λ *ori*⁻ mutants by an *imm*P22 *rep*λ or an *imm*P22 *rep*P22 hybrid (Table 3). In each case, wild-type λ recombinants are obtained efficiently from the cross of the *ori*⁻ mutant by the *imm*P22 *rep*λ hybrid, at approximately the same frequency as from a cross by an *imm*21 *rep*λ control. By contrast, no wild-type λ recombinants are observed from crosses of any of the *ori*⁻ mutants by the *imm*P22 *rep*P22 hybrid.

Complementary evidence for heterology between the λ and P22 *ori* regions comes from crosses of λ*ori*⁻ mutants by an *imm*λ *rep*P22 hybrid (Fig. 1, Table 4). The *ori*⁻

TABLE 3

Mapping λ ori⁻ *mutations with* imm*P22 hybrids*

ori⁻ allele	λ*ori*⁺ recombinants per cell		
	*imm*21 *rep*λ	*imm*P22 *rep*λ	*imm*P22 *rep*P22
*ti*12	2×10^{-2}	2×10^{-2}	$<10^{-6}$
*r*93	8×10^{-3}	8×10^{-3}	$<10^{-6}$
*r*95	6×10^{-3}	8×10^{-3}	$<10^{-6}$
*r*96	5×10^{-3}	7×10^{-3}	$<10^{-6}$
*r*99	1×10^{-2}	1×10^{-2}	$<10^{-6}$

Ymel(λ*N*am cIts857 *ori*⁻) lysogens were superinfected by λ*imm*21cI *rep*λ, λ*imm*P22c1–7 *rep*λ, or λ*imm*P22c2–5 *rep*P22, as described in Materials and Methods. The yield of *imm*λ *ori*⁺ recombinants was determined by titering each cross lysate at 30°C on the indicator strain Ymel-(λ*imm*P22c⁺ *rep*P22), which is immune to both *imm*P22 and *imm*21 phages. The yield of the superinfecting phage type was determined by titering each lysate at 37°C on Ymel(λ), and ranged from 40 to 130 phage/infected cell. The total yield of λ*N*am *ori*⁻ phage could be determined for the mutant *ti*12, which forms small plaques at 39°C, and ranged from 1·5 to 4 phage/cell.

TABLE 4

Mapping λori⁻ *mutations with a* rep*P2212am hybrid*

ori⁻ prophage	Fraction *am*⁺ recombinants		
	*λrepλP*am	*λrepP2212*am	
	30°C	30°C	39°C
λ*N*am7 *ti*12	0·15	$\leq 2\times10^{-6}$	0·03†
λ*N*am7*N*am53 *r*93	0·10	$\leq 5\times10^{-7}$	0·02†
λ*N*am7*N*am53 *r*95	0·10	$\leq 5\times10^{-7}$	$\leq 5\times10^{-7}$
λ*N*am7*N*am53 *r*96	0·11	$\leq 1\times10^{-7}$	$\leq 1\times10^{-7}$
λ*N*am7a*N*m53 *r*99	0·15	$\leq 4\times10^{-7}$	$\leq 4\times10^{-7}$
λ*N*am7*N*am53 *ori*⁺	0·11	0·04	0·04

Ymel(λ*N*am *c*Its857 *ori*⁻) lysogens were superinfected by λcIts857 *repλP*am80 or λcIts857 *rep*P22*12*am*N*14 as described in Materials and Methods. Recombination was stimulated by u.v. irradiation of infected complexes (200 erg/mm²) prior to thermal induction of the prophage. The total phage yield was determined by titering the cross lysates at 39°C on strain Ymel. The yield of *am*⁺ recombinants was determined by titering on 594. The fraction of *am*⁺ recombinants is the ratio of these 2 titers.

† At 39°C on trypticase plates, λ*N*⁺ *ti*12 recombinants form small plaques, and λ*N*⁺ *r*93 recombinants form very minute plaques on strain 594. None of the other *ori*⁻ mutants form visible plaques at 39°C. At 30°C only λ*am*⁺ *ori*⁺ recombinants form plaques on 594.

phages carry amber nonsense mutations in gene *N*, while the *imm*λ repP22 hybrid is *N*⁺, but carries an amber mutation in gene *12*, within the *rep*P22 substitution. In a control cross in which the *N*am parent is *ori*⁺, recombination in the interval between the *N*am and *12*am sites generates wild-type λ progeny (*am*⁺ *ori*⁺). Similarly, in crosses involving leaky *ori*⁻ mutants, capable of forming minute plaques at 39°C, *am*⁺ *ori*⁻ recombinants can be observed. However, no wild-type recombinants are obtained in crosses with any of the five *ori*⁻ mutants. In each case the fraction of such recombinants is at least 10^5-fold lower than in a control cross of the λ*N*am *ori*⁻ phage by a λ*N*⁺ *repλP*am phage.

The *rep*P22 substitution covers all known markers in the replication region. No wild-type (*am*⁺) recombinants, above the background level of revertants, are observed in marker rescue tests of a λ*imm*434 *rep*P22*12*am hybrid phage with any of a set of λ*O*am and *P*am alleles (Table 5), including one (*O*am905) which lies to the left of the *ori*⁻ mutations (Furth *et al.*, 1977).

TABLE 5

Extent of rep*P22 substitution*

Fraction *am*⁺ recombinants					
*O*am905	*O*am29	*O*am1005	*O*am125	*P*am80	*Q*am117
$\leq 1\times10^{-6}$	$\leq 2\times10^{-6}$	$\leq 1\times10^{-6}$	$\leq 2\times10^{-6}$	$\leq 1\times10^{-6}$	3×10^{-2}

Crosses between λ*imm*434 *rep*P22*12*am14 and each of a series of λcIts857 *am* mutants were carried out in strain Ymel. Total progeny phage were titered on Ymel or K802, and *am*⁺ progeny were titered on 594. The fraction of *am*⁺ phage among the total progeny is the ratio of the 2 titers. (≤) Means that this ratio does not differ significantly from the fraction of *am*⁺ revertants among the progeny of a control infection with only one of the *am* phages.

These results support the conclusion that the λ and P22 replication origins differ, and that the replication control regions of λ and P22 can be exchanged as intact units (Hilliker & Botstein, 1976).†

(b) *Dissection of the replication region*

While the λ-P22 hybrids help to delimit the replication control region, studies with these phages do not show which of the replication proteins of λ and P22 interact directly with the cognate replication origin. Evidence for specific interactions between components of the replication control system comes from studies of hybrid phages in which part of the replication control region derives from λ and part from a related coliphage. Because the genomes of phages φ80 and 82 are partially homologous to λ in the vicinity of genes *O* and *P* (Fiandt *et al.*, 1971; Simon *et al.*, 1971), we have been able to analyze the segregation of type-specific replication elements in λ-φ80 and λ-82 hybrids.

In contrast to P22, coliphages φ80 and 82 can help λ mutants defective in *P* to replicate. However, neither φ80 nor 82 can help λ mutants defective in *O* (Dove, 1968; Dove *et al.*, 1971; Szpirer & Brachet, 1970). We report here that viable hybrids can be produced by recombination within gene *O* between λ and φ80 or 82. Such a recombinant synthesizes a hybrid O protein, with the amino-terminal segment characteristic of one phage type and the carboxyl-terminal segment characteristic of the other (accompanying paper). The functional specificities of these hybrids suggest that the O protein acts as an adaptor between the replication origin and the P protein. In its amino-terminal segment the O protein carries a specificity determinant for interaction with *ori;* in its carboxyl-terminal segment it carries a specificity determinant for interaction with P protein.

(i) rep*80:λ and* rep*82:λ phages hybrid for gene* O

From crosses of λ by φ80, recombinants can be isolated which have the immunity region of φ80 and gene *Q* of λ (Szpirer & Brachet, 1970). Such recombinants can carry the *P–Q* region of λ. if that region bears a "bypass' mutation which renders expression of *Q* independent of λ *N* gene product (Szpirer, 1972). Recombinants with gene *P* of λ, such as λ*imm*80hy42 (Fig. 2), fail to grow on a *gro*P *E. coli* host which blocks λ DNA replication (Georgopoulos & Herskowitz, 1971; Szpirer, 1972). By contrast, recombinants with the intact replication region of φ80, such as λ*imm*80hy41 (Fig. 2), can grow on the *gro*P host. Genetic recombination to produce these two classes of λ-φ80 hybrids occurs within regions of homology between λ and φ80, as revealed by electron microscopy of heteroduplex DNA molecules (Fiandt *et al.*, 1971; R. Monnat and W. Szybalski, unpublished observations).

L. Lambert and R. Thomas (unpublished observations) have found that the recombination event which gave rise to λ*imm*80hy42 occurred within λ gene *O*. They observed that λ*imm*80hy42 is unable to recombine with λ*O*am29 to give λO^+, but can recombine with λ*O*am8. They showed further that, like φ80, λ*imm*80hy42 fails to help λ *O*-defective mutants to replicate. We have confirmed and extended these observations.

† The data show that neither a *rep*λ:P22 nor a *rep*P22:λ hybrid replication control region has been obtained in a viable phage. Although we attribute the absence of such recombinants to lack of homology between λ and P22 in the replication control region, we cannot rigorously exclude the possibility that some homology exists, but that segments of the hybrid replication control region in such recombinants would be incompatible.

The relative map positions of seven nonsense mutations in gene *O* have been determined (Materials and Methods). We find that λ*imm*80hy42 fails to recombine with the three leftmost O^- mutations to give λO^+, but efficiently yields wild-type (am^+) recombinants with each of the four distal O^- mutations (Table 6; Fig. 3). A hybrid with an intact φ80 replication region, λ*imm*80hy41, recombines productively with the four distal O^- mutations only at much lower frequency. The level of λO^+ recombinants is reduced still further if the *imm*80 phage carries an amber mutation in φ80 gene *14* (see below).

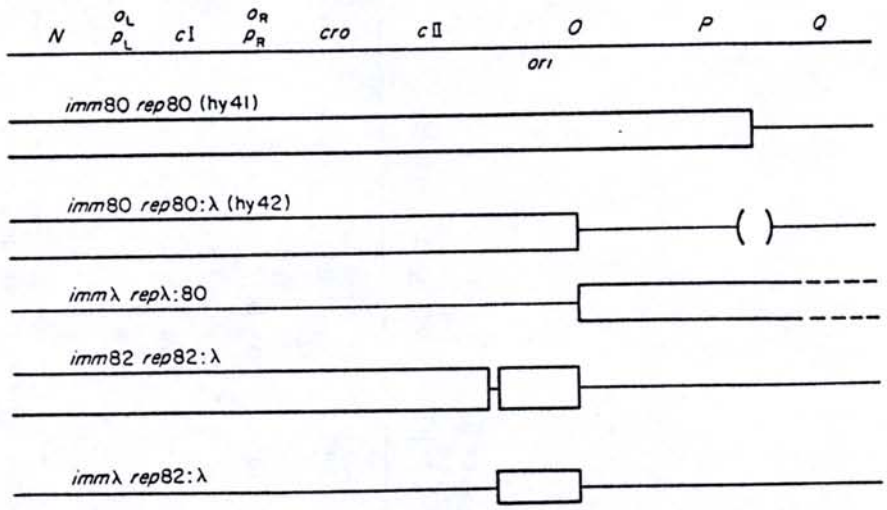

FIG. 2. Control region of bacteriophage λ genome, and substitutions in λ-φ80 and λ-82 hybrid phages. The genetic elements and nomenclature are described in the legend to Fig. 1. Segments derived from λ are indicated by lines, and heterologous segments derived from φ80 or 82 are indicated by boxes. Parentheses show the position of a *byp* deletion which renders expression of *Q* independent of *N* function in λ*imm*80hy42 (Szpirer, 1972; R. Monnat and W. Szybalski, unpublished observations). Broken lines indicate the tentative conclusion that the *Q* region of *repλ*:80 phages is derived from φ80. Substitutions in *rep*80:λ, *rep*82:λ and *repλ*:80 phages end within gene *O* (see Fig. 3).

The λ/φ80 junction in the replication region of λ*imm*80hy42 maps near to the sites of a number of λ*ori*$^-$ mutations, within gene *O* (Furth *et al.*, 1977). We find that no wild-type λ recombinants are obtained from crosses of the λ*ori*$^-$ mutants by λ*imm*80hy42 (fewer than 10^{-6} λ*ori*$^+$ recombinants per infected cell in crosses analogous to those of Table 3). Thus, the *ori* region, as defined by these mutants, segregates with the proximal portion of *O* in λ-φ80 hybrid phages.

λ*imm*80hy42 has part but not all of λ gene *O*. What, if any, function does the residual portion of λ gene *O* serve for replication of this hybrid phage? We consider two general possibilities: (1) λ*imm*80hy42 does not require λ *O* gene function, either because *P* function alone is sufficient for replication of this phage, or because the phage has an intact φ80 function which obviates the requirement for *O;* or (2) λ*imm*80hy42 has a hybrid *O* gene (φ80:λ), encoding a protein with amino-terminal characteristics of a φ80 protein and carboxyl-terminal characteristics of λ O protein. Data presented in this and the accompanying paper support the second possibility.

TABLE 6

Mapping of gene O *in* rep*80*, rep*80:*λ, *and* rep*82:*λ *hybrids*

Hybrid phage	Fraction O^+ recombinants						
	*O*am905	*O*op261	*O*am29	*O*am1005	*O*am8	*O*am125	*O*am205
*imm*80hy42 (*rep*80:λ)	$\leq 2\times10^{-7}$	$\leq 2\times10^{-6}$	$\leq 2\times10^{-7}$	6×10^{-3}	4×10^{-2}	2×10^{-2}	5×10^{-2}
*imm*80hy41 (*rep*80)	$\leq 5\times10^{-7}$	$\leq 3\times10^{-6}$	$\leq 5\times10^{-7}$	1×10^{-4}	2×10^{-4}	6×10^{-5}	7×10^{-4}
*imm*80 *rep*80*14*am8	$\leq 2\times10^{-7}$		$\leq 9\times10^{-7}$	3×10^{-6}	1×10^{-6}	1×10^{-6}	2×10^{-5}
*imm*82 *rep*82:λ*P*am3	2×10^{-5}		8×10^{-5}	6×10^{-3}		1×10^{-2}	2×10^{-2}
82c	2×10^{-4}		2×10^{-4}	3×10^{-2}		9×10^{-2}	1×10^{-1}

Standard crosses with u.v. irradiation (250 erg/mm²) were carried out between λcIts857 O^- mutants and various λ-φ80 and λ-82 hybrid phages. The host strain was Ymel or CA5070 (for *O*op261). The fraction of O^+ recombinants was calculated as the ratio of the yield of λcIts857 O^+ progeny, determined by titering on 594(φ80*14*am8) or on 594(82), to the total yield of λcIts857 progeny phage, determined by titering on Ymel(φ80), Ymel(82), or, for crosses involving *O*op261, CA5070(φ80). (≤) Means this fraction did not differ significantly from the reversion level of the test allele.

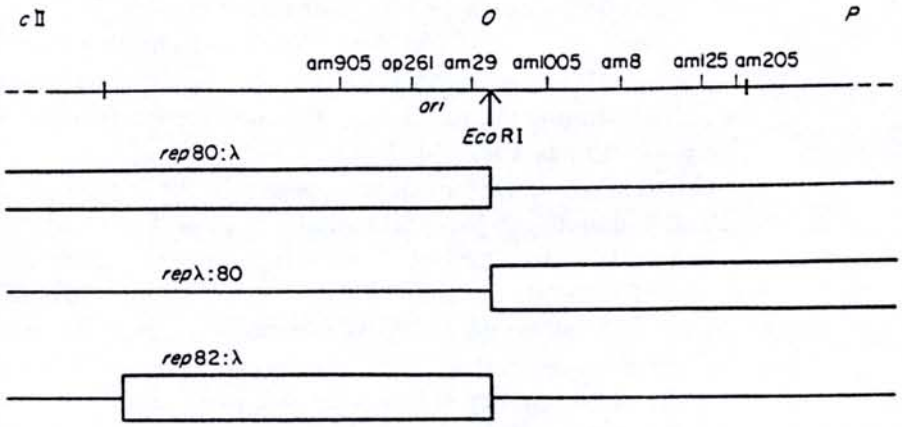

FIG. 3. Map of gene *O*. The positions of nonsense mutations in *O* are shown approximately to scale, based on recombination frequencies and available data on the sizes of amber peptides (*O*am1005, *O*am125 and *O*am205). The structures of the hybrid *O* genes of *rep*80:λ (λ*imm*80hy42), *repλ*:80 and *rep*82:λ hybrid phages were determined as described in the text (see Tables 6 and 7; Figs 5, 6 and 7). Segments derived from λ are indicated by lines, while heterologous segments derived from φ80 or 82 are indicated by boxes. Transcription and translation of *O* proceed from left to right. The arrow marks an *Eco*RI restriction endonuclease cleavage site. The *ori* region lies near, but to the left of the *Eco*RI site (Furth *et al.*, 1977). The size of the O polypeptide (Yates *et al.*, 1977), and the position of a possible site for the initiation of translation of *O* (Denniston-Thompson *et al.*, 1977; Schwartz *et al.*, 1978) suggest that the *O* coding sequence spans the interval between approximately 80·0±0·1 and 81·9±0·1 on the λ physical map (940 base-pairs).

If the residual portion of λ gene *O* is part of a hybrid gene essential for replication of λ*imm*80hy42, then the introduction of an amber nonsense mutation into this portion of *O* should confer conditional lethality on the hybrid phage. We therefore constructed a λ*imm*80hy42 derivative carrying an amber mutation in *O*, and asked whether this phage depends on a nonsense suppressor for growth.

λ*imm*80hy42 *O*am205 was obtained by crossing λ*imm*80hy42 *P*am3 with λ*imm*434 *O*am205 (Fig. 4). The construction takes advantage of the ambivalent suppression pattern of *P*am3, which permits direct selection against *P*am3 on a host permissive for *O*am205 (for details see Materials and Methods). Some of the *imm*80 *P*am$^+$ recombinants should carry the λ *O*am mutation. It was observed that 15% of *imm*80 *P*am$^+$ recombinants require an amber suppressor for growth. Thus, the *O*am205 mutation must lie in a gene essential for growth of these recombinants. The fraction of putative *O*am recombinants is consistent with the relative map positions

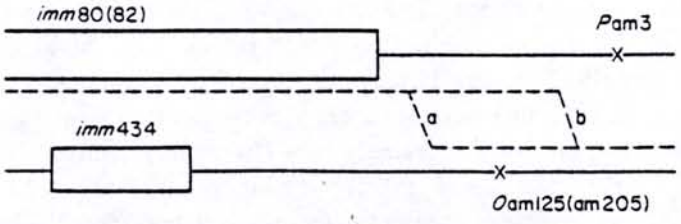

FIG. 4. Construction of *O*am derivatives of λ*imm*80hy42 and λ*imm*82. λ*imm*80 (or 82) *P*am3 and λ*imm*434 *O*am205 (or *O*am125) were crossed, and λ*imm*80 (or 82) *P*$^+$ recombinants were selected as described in the text. Exchanges in interval a should generate recombinants carrying the *O*am mutation, while exchanges in interval b should generate *O*$^+$ recombinants.

of the *imm*80 substitution, *O*am205, and *P*am3. Very similar results were obtained when *O*am125 was used in place of *O*am205.

In principle, the λ*imm*80hy42 *O*am205 hybrids might have acquired the entire λ *O* gene. However, test crosses reveal that the endpoint of the φ80 substitution remains within *O* (Materials and Methods section (f) (ii)). We therefore conclude that λ*imm*80hy42 has a hybrid *O* gene.

The replication regions of phages hybrid for gene *O* are designated *rep*X:Y, where X and Y denote the parental lambdoid phages from which the hybrid derives (Fig. 3). Mutant alleles are attributed to the appropriate parent. For example, the replication region of λ*imm*80hy42 *O*am205 is designated *rep*80:λ*O*am205.

Like φ80, lambdoid phage 82 fails to help to replicate λ *O*-defective mutants, but does promote replication of λ *P*-defective mutants (Dove, 1968). A λ-82 hybrid phage with the 82 immunity region, λ*imm*82, behaves like 82 in dominance tests with λO^- and P^- mutants (Dove *et al.*, 1971). The λ mutation *P*am3 was introduced into this hybrid phage. Thus, the replication control regions of λ and 82 must share some homologous sequences. Crosses of λ*imm*82 *P*am3 with λ*O*am mutants reveal that the 82 substitution ends within gene *O*, in the same interval (between *O*am29 and *O*am1005) as does the φ80 substitution in λ*imm*80hy42 (Table 6; Fig. 3. Note that the *P*am3 allele is not essential for mapping the end point of the substitution). Crosses of the parental phage 82 with λ*O*am mutants suggest that 82 is itself homologous to λ in part of gene *O*, distal to the site of *O*am29. Again, as in the case of φ80, the sites of the λ*ori*$^-$ mutations lie within the region of heterology between λ and 82 (Furth, 1978).

The *O*am205 mutation was introduced into λ*imm*82 by the same procedure used to construct λ*imm*80hy42 *O*am205 (Fig. 4). The λ*imm*82 *O*am205 recombinants are also found to depend on an amber suppressor for growth. Test crosses of λ*imm*82 *O*am205 with *O*am mutants confirm that the phage has a hybrid replication region *rep*82:λ.

(ii) repλ: *80 phages hybrid for gene* O

The demonstration that the *O* gene of λ*imm*80hy42 (*rep*80:λ) is hybrid suggested that it should be possible to isolate the reciprocal hybrid (*rep*λ:80). Toothman (1976) has isolated a hybrid phage which has the immunity region *imm*434 but the *P* function of φ80, as judged by the ability to replicate in a *gro*P bacterial host. We find that such hybrid phages differ from φ80 in *O* function, because they can help λ *O*-defective mutants to replicate (see Results section (c)(i)). In order to test whether gene *O* is hybrid, we constructed similar phages with immunity of λ and *P* function of φ80, carrying an amber mutation within either the λ or the φ80 portion of the *rep* region.

We first found that hybrid phages with *imm*λ and the *P* function of φ80 can arise from a recombination event in the interval to the right of the position of *O*am29. λ*c*I857 *O*am29 *P*am3 was crossed by a λ*imm*80 *rep*80 phage (Fig. 5(a)). Recombinants with *imm*λ and both *am*$^+$ characters, which would have the intact φ80 replication region, were extremely rare (frequency approx. 10^{-8}) and will not be discussed here. However, *imm*λ P^+ recombinants were obtained at a frequency of 10^{-4} on a host which is permissive for *O*am29 but not *P*am3. These recombinants retain the *O*am29 mutation, and have acquired the *P* function of φ80. Biochemical evidence that the *O* product of λ*rep*λ*O*am29:80 is hybrid is presented in the accompanying paper.

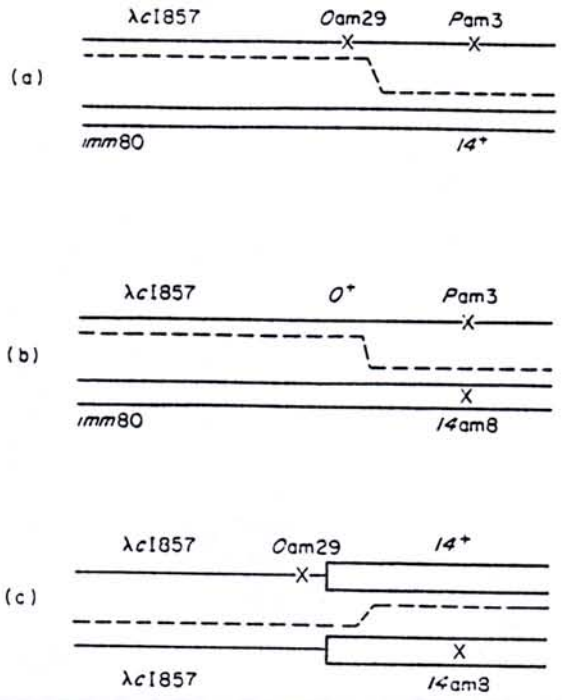

FIG. 5. Construction of *repλ*:80 hybrid phages. Broken lines indicate the contributions of each parent phage to the selected recombinants from crosses described in the text: (a) λcIts857 *repλO*am29:80; (b) λcIts857 *repλ*:80*14*am8; (c) λcIts857 *repλ*:80. The phages constructed in crosses (a) and (b) were used as the parents for cross (c).

To facilitate further analysis of *repλ*:80 hybrids, we next constructed one which carries an amber mutation in the segment of the replication region derived from φ80. Sato and co-workers (Sato *et al.*, 1968; Sato, 1970) have identified at least two φ80 cistrons (*14* and *16*) which seem to map congruently to λ replication genes. Crosses of φ80*14*am8 by λcI857 *repλO*am29:80 yield φ80*am*$^+$ recombinants at high frequency (data not shown). Thus, the *14*am8 mutation appears to lie in the region of the φ80 genome carried in the *repλO*am:80 hybrid. The construction of a *repλ*:80*14*am8 hybrid phage is detailed in Materials and Methods (Fig. 5(b)).

The φ80 substitution in λ*repλ*:80*14*am8 was mapped with respect to λ *O* and *P* nonsense mutations. Only the three leftmost *O* mutations give a high level of wild-type λ recombinants (Table 7; Fig. 3). These are the same three alleles which fail to recombine productively with λ*imm*80hy42. Thus, the phage λ*repλ*:80*14*am8 has a hybrid *O* gene and is the reciprocal of λ*imm*80hy42 (*rep*80:λ). Complementation tests (see section (d), below) indicate that the *14*am8 mutation inactivates a φ80 function equivalent to *P* of λ.

A *repλ*:80 hybrid free of amber mutations was selected from a cross of λ*repλO*am29:80 by λ*repλ*:80*14*am8 (Fig. 5(c)). The *am*$^+$ recombinant was used as a donor phage in tests of the type-specificity of hybrid O proteins (see below).

(c) *Segregation of* ori *with proximal segment of* O

(i) *Type-specificity of* O *function*

Phages with the hybrid replication regions *rep*80:λ and *rep*82:λ fail to help λ *O*-defective mutants to replicate in mixed infections (Dove *et al.*, 1971; Lambert &

TABLE 7

Mapping of gene O *in a* repλ*: 8014am hybrid*

Hybrid phage	Fraction O^+ P^+ recombinants							
	*O*am905	*O*op261	*O*am29	*O*am1005	*O*am8	*O*am125	*O*am205	*P*am3
$\lambda rep\lambda$:80*14*am8	8×10^{-3}	1×10^{-2}	1×10^{-2}	2×10^{-6}	3×10^{-6}	6×10^{-6}	7×10^{-6}	8×10^{-6}
$\lambda rep\lambda O$am29			$\leq 3\times10^{-7}$				4×10^{-2}	3×10^{-2}

Standard crosses with u.v. irradiation (250 erg/mm^2) were carried out in strain K802. All phages carried the cIts857 mutation. The fraction of O^+ P^+ recombinants was calculated as the ratio of am^+ progeny phage, determined by titering on 594, to total progeny phage, determined by titering on K802. The total yield in the cross involving *O*op261 was taken as the sum of the titers determined on K802 and CA5070.

Thomas, unpublished observations; Table 8). Because λ*O*am mutations can inactivate a function essential for growth of *imm*80 *rep*80:λ and *imm*82 *rep*82:λ hybrid phages, we argue that these phages must each have a gene equivalent in function to λ*O*, but type-specific. The type-specificity of hybrid *O* products can be defined further by determining for which mutant phage types replication can be promoted by a particular hybrid O protein.

TABLE 8

Type-specificity of O *function*

Helper phage	Yield per input test phage					
	*imm*80hy42 *rep*80:λ*O*am		*imm*82*c* *rep*82:λ*O*am		*imm*λ *rep*λ*O*am	
	su°	*su*III	*su*°	*su*III	*su*°	*su*III
*imm*80hy42 (*rep*80:λ)	14	11	0·5	23	0·1	12
*imm*80hy41 (*rep*80)	11	10	—	—	0·1	11
*imm*434 *rep*λ	0·1	10	0·4	24	12	19
*imm*434 *rep*λ:80	0·1	21	—	—	13	27
82*c*	0·2	60	33	141	0·6	76
None	0·1	103	0·7	312	0·1	66

The ability of helper phages to promote the replication of *O*am mutants was tested as described in Materials and Methods, section (e). The *su*° and *su*III bacterial hosts were 594 and 594*su*III, respectively. Each of the defective test phages carried the *O*am205 mutation. When the immunity types of the helper and the *O*am test phages differed, the yields of each type were determined by titering on appropriate lysogenic derivatives of Ymel. The *imm*80 and *imm*82 *O*am test phages also carried the *lac*5 marker, and were distinguished from helper phages of the same immunity type by titering on strain Ymel on XGal plates. The yield per input helper phage ranged from 40 to 110.

We find that λ*imm*80 *rep*80:λ*O*am205 can be helped to replicate by phages carrying *rep*80 or *rep*80:λ, but not by those carrying *rep*λ, *rep*λ:80, or *rep*82 (Table 8). Similarly, λ*imm*82 *rep*82:λ*O*am205 can be helped by phages carrying *rep*82 or *rep*82:λ, but not by those carrying *rep*λ or *rep*80 (Tables 8 and 9). Finally, both λ*rep*λ*O*am29 and λ*rep*λ*O*am29:80 can be helped by phages carrying *rep*λ or *rep*λ:80 (Table 10), but not by those carrying *rep*80 or *rep*80:λ (Table 11). Thus, *O* activity can be provided successfully to an O^- mutant only by a helper which is homologous to the mutant for the portion of gene *O* encoding the amino-terminal portion of its product. By contrast, the portion of *O* encoding the carboxyl-terminus of its product need not be homologous. Evidence for a type-specific interaction between the carboxyl-terminal segment of the O protein with the P protein is discussed below (section (d)).

The type-specificity observed for *O* function cannot be attributed to exclusion between various hybrid phages, because in a permissive host (carrying *su*III) the *O*am derivatives grow well in the presence of any helper (Tables 8 and 10). Furthermore, the *O*am mutations are not polar on expression of *P*; all of the *O*am derivatives of phages studied here can promote the replication of a *P*-defective phage, even when the *O*am phage itself is unable to replicate (Table 9).

TABLE 9

O × P *complementation of* repλ, rep*82:λ and* rep*80:λ phages*

	Test phage yield per infected cell					
	*repλO*am/	*repλP*am/	*rep*80:*λO*am/	*rep*80:*λP*am/	*rep*82:*λO*am/	*rep*82:*λP*am/
/*repλO*am	0·3	59/46	0·3/0·3	51/0·4	0·3/0·2	64/0·7
/*repλP*am		0·4	0·7/47	0·4/0·3	0·6/86	0·3/0·2
/*rep*80:*λO*am			0·3	53/19	0·4/0·2	
/*rep*80:*λP*am				0·4		
/*rep*82:*λO*am					0·3	64/33
/*rep*82:*λP*am						0·4

Complementation tests were carried out by mixed infection of strain 594 as described in Materials and Methods, section (e). All phages carried either *O*am205 or *P*am3. The yield per input phage was calculated separately for each *O*am and *P*am phage, except for control experiments in which both parents carried the same test allele. For each pair of test phages, the yield of the phage type listed in the top row is given above the slash, while that of the phage type listed in the first column is given below the slash. The *rep*80:*λ* phages carried *imm*80, and the *rep*82:*λ* phages carried *imm*82*c*. The *repλ* phages carried either *immλ*cIts857 or *imm*434cI. If the 2 test phages in a mixed infection differed in immunity type, their yields were determined separately by plating on appropriate lysogenic derivatives of C600. In infections involving two *imm*80 or two *imm*82 phages, one carried the *lac*5 marker, so that each could be distinguished by titering on C600 on XGal plates. The data represent the average yields per infected cell determined in 2 experiments in which the distinguishing markers of the *O*am and *P*am phages (*imm*434 or *immλ*, and presence or absence of *lac*5) were reversed.

TABLE 10

O *and* P *specificity of* repλ *and* repλ*:80 phages*

Helper phage	Yield per input test phage							
	*repλO*am29		*λrepλO*am29:80		*λrepλ*:80*14*am8		*ϕ*80*14*am8	
	su°	*su*III	*su*°	*su*III	*su*°	*su*III	*su*°	*su*III
*imm*434 *repλ*	15	17	29	50	43	84	0·1	15
*imm*434 *repλ*:80	11	27	16	22	26	12	58	57
None	0·02	160	0·02	270	0·02	80	0·1	740

The ability of *λimm*434cI6T *repλ* and *λimm*434cI6T *repλ*:80 to promote replication of *ϕ*80*14*am8 and of mutant λcIts857 *repλ* and *repλ*:80 derivatives was tested as described in Materials and Methods, section (e). The bacterial hosts were 594 (*su*°) and 594*su*III. The yield per input test phage was determined by titering on Ymel(*λimm*434). The yield per input helper phage was determined by titering on Ymel(*λ*) or Ymel(*ϕ*80), and ranged from 33 to 46.

(ii) *Nature of specificity determinants*

The studies of *O*am derivatives of phages carrying *repλ*, *repλ*:80, *rep*80:*λ*, or *rep*82:*λ* define specificity determinants for the interaction of a diffusible element, the O protein, with a receptor function. What is the nature of the receptor? It must be a target nucleotide sequence or, alternatively, a gene product which itself interacts specifically with a target sequence. Furthermore, it must differ among phages *ϕ*80, 82, and λ. The detailed genetic structure to the left of *O* is not known for *ϕ*80 and 82, so it is difficult to localize the receptor function. However, we show below that a substitution in λ of about 1% of the genome of phage 82, covering the regulatory site *ori*

TABLE 11

Donor specificity of imm*80* rep*80 and* imm*80* rep*80:λ phages*

Helper phage	Yield per input test phage					
	λrepλ *O*⁺ *P*⁺	*λrepλ*:80 *O*⁺ *14*⁺	*λrepλ* *O*am29 *P*⁺	*λrepλ* *O*⁺ *P*am3	*λrepλ*:80 *O*am29 *14*⁺	*λrepλ*:80 *O*⁺ *14*am8
*imm*80hy41 (*rep*80)	64	292	0·2	13	0·4	80
*imm*80hy42 (*rep*80:λ)	35	81	0·1	47	0·4	0·4
None	93	272	0·2	0·2	0·2	0·2

The ability of *λimm*80hy41 and *λimm*80hy42 to promote replication of mutant λcIts857 *repλ* and *repλ*:80 derivatives was tested by mixed infection in 594 (Materials and Methods, section (e)). Non-defective *repλ* and *repλ*:80 phages served as controls for the *O*am, *P*am and *14*am test phages. The yield per input test phage was determined by titering on strain K802(φ80). The yields per input helper phage were determined by titering on Ymel(λ), and averaged 91 for *λimm*80hy42 and 105 for *λimm*80hy41.

and the proximal portion of *O*, suffices to confer 82-type-specificity to both the donor and receptor components of *O* function.

(iii) *Localization of specificity determinants in* rep*82:λ hybrid phages*

The substitution of 82 DNA in *λimm*82 *rep*82:λ covers the sites of the two leftmost λ *O*am alleles. However, crosses of *λimm*82 *rep*82:λ*P*am3 by λ*O*am905 or λ*O*am29 yield *immλ am*⁺ recombinants at frequencies slightly above the reversion frequencies of the test alleles (Table 6). Heteroduplexes of λ and 82 DNA reveal a small segment of homology, in or near to gene *c*II, between heterologous segments of the immunity and replication control regions (Simon *et al.*, 1971). Recombination within this homologous segment might generate viable λ-82 hybrid phages containing 82 DNA only in the replication control region. We therefore asked whether recombinants with 82-type *O* function could be identified among the *imm*434 *am*⁺ progeny of a cross between *λimm*434 *O*am29 and *λimm*82 *rep*82:λ*P*am3 (Materials and Methods; Fig. 6). Five *imm*434 *am*⁺ recombinants were tested for their ability to help replication of *λrepλO*am205 and of *λimm*82 *rep*82:λ*O*am205. All five help replication of the *rep*82:λ*O*am hybrid, but fail to help the *repλO*am mutant (Table 12, column 1). Thus, these *imm*434 *am*⁺ recombinants have the *O* donor specificity characteristic of phage 82.

In order to test the *O* receptor specificity of putative *imm*434 *rep*82:λ hybrids, we introduced *O*am205 into these phages. The *O*am derivatives display 82-type receptor

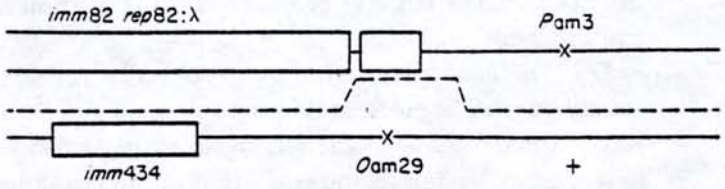

FIG. 6. Construction of *λimm*434 *rep*82:λ. *λimm*82*c rep*82:λ*P*am3 and *λimm*434cI *O*am29 were crossed, and *λimm*434cI *am*⁺ recombinants were selected as described in the text. Broken lines indicate the contributions of each parent phage to the selected recombinant.

TABLE 12

O specificity of imm*434* rep*82:λ*

Helper phage	Yield per input *O*am test phage 1		2	
	*immλ repλO*am	*imm*82 *rep*82:*λO*am	*imm*434 *repλO*am	*imm*434 *rep*82:*λO*am
*imm*434 *repλ*	23	0·6		
*imm*434 *rep*82:*λ*	0·4	21		
immλ repλ			17	0·2
*imm*82*c rep*82:*λ*			0·08	39
None	0·2	0·6	0·03	0·06

The ability of *repλ* and *rep*82:*λ* helper phages to promote the replication of *O*am205 derivatives of *repλ* and *rep*82:*λ* was tested by mixed infection in strain 594 (Materials and Methods, section (e)). The *immλ* phages carried *c*Its857 and the *imm*434 helper phages carried *c*II2002. The yield of each phage type was determined by titering on an appropriate lysogenic derivative of Ymel. The yield per input helper phage ranged from 40 to 51.

specificity; their replication can be promoted by *λimm*82 *rep*82:*λ* and *λimmλ rep*82:*λ*, but not by *λ* (Table 12, column 2).

We conclude that the 82 substitution in *λimm*434 *rep*82:*λ* includes both the *O* donor and receptor-specificity determinants. The following experiments localize this substitution on both the genetic and physical maps (Fig. 3).

Plaques of *immλ* or *imm*434 *c*I$^+$ *rep*82:*λ* hybrids are as turbid as those of corresponding *repλ* phages, indicating that the *rep*82 substitution does not interfere with expression of the repressor gene *c*I. Mutations in gene *c*II can be introduced into the hybrids, and confer the characteristic clear-plaque morphology. This suggests that the 82 substitution lies to the right of *c*II.

The *imm*P22 substitution covers most or all of *c*II, and extends into the sequence encoding a small RNA molecule, named *oop*, which is transcribed leftwards from a region near the right end of *c*II and to the left of *O* (Blattner & Dahlberg, 1972; Hayes & Szybalski, 1973; Roberts *et al.*, 1976; Schwarz *et al.*, 1978; C. Brady, S. Hilliker and M. Rosenberg, personal communication). We find that *imm*P22 *am*$^+$ recombinants can be isolated readily from a cross of *λimm*P22 *O*am29 by *λimm*434 *rep*82:*λ* (Table 13). The putative *imm*P22 *rep*82:*λ* recombinants have the 82-type *O* donor specificity (Table 14). The isolation of such recombinants strongly suggests that the *rep*82 substitution lies entirely to the right of the immunity control region. An alternative possibility is that P22 and 82 are homologous for some sequences in this region which differ between P22 and *λ*.

The frequency at which *imm*P22 *rep*82:*λ* recombinants arise is about 11-fold below that at which *imm*P22 *repλ* recombinants arise in a control cross (Table 13). This implies that the left end of the *rep*82 substitution lies considerably to the left of the site of *O*am29.

DNA heteroduplex mapping graphically reveals the limited extent of the *rep*82 substitution. The electron micrograph of Figure 7 shows a portion of a *λrepλ*/*λimm*434 *rep*82:*λ* heteroduplex. The larger substitution loop corresponds to the *imm*434/*immλ* heterology (Westmoreland *et al.*, 1969), while the smaller substitution loop corresponds to the *rep*82/*repλ* heterology. The length of the double-stranded segment between the *imm*434 and *rep*82 substitutions is 580$\pm$37 base-pairs, while the two single strands of

TABLE 13

Construction of λimm*P22* rep*82:λ*

		Fraction *am*+ recombinants among *imm*21 or *imm*P22 progeny		
		*imm*434 *repλ*	*imm*434 *rep*82:λ	Ratio *repλ*/*rep*82:λ
*imm*21	*O*am29	7×10^{-2}	1×10^{-2}	5·8
*imm*P22	*O*am29	7×10^{-2}	6×10^{-3}	11

λ*imm*21cI *O*am29 and λ*imm*P22c1–7 *O*am29 were crossed by λ*imm*434 cII2002 *repλ* and λ*imm*434 cII2002 *rep*82:λ in Ymel, without u.v. irradiation. The total yield of *imm*21 or *imm*P22 progeny phage was determined by titering on strain Ymel(λ*imm*434), while the yield of *imm*21 or *imm*P22 *am*+ recombinants was determined by titering on 594(λ*imm*434 *rep*P22*12*amN14). The fraction *am*+ among *imm*21 or *imm*P22 progeny was calculated as the ratio of these 2 titers. The fraction of *am*+ recombinants observed with *repλ* was then divided by the fraction observed with *rep*82:λ to yield the ratio shown in the final column. An *imm*P22 *rep*82:λ recombinant was tested for *O* donor specificity in the experiment of Table 14.

TABLE 14

O *specificity of* imm*P22* repλ *and* imm*P22* rep*82:λ hybrids*

Helper phage	Yield per input *O*am test phage *imm*434 *repλO*am205	*imm*434 *rep*82:λ*O*am205	*imm*P22 *repλO*am29
*imm*434 *repλ*			11
*imm*434 *rep*82:λ			0·01
*imm*P22 *repλ*	10	0·2	
*imm*P22 *rep*82:λ	0·2	16	
None	0·1	0·2	0·02

The ability of a helper phage to promote replication of *O*am recipients was tested by mixed infection in strain 594 (Materials and Methods, section (e)). All *imm*P22 phages carried c1–7. The *imm*434 helpers carried cII2002, and the *imm*434 *O*am test phages carried cI6T. Yields of *imm*434 and *imm*P22 phages were determined by titering on Ymel(λ*imm*P22c+ *rep*P22) and Ymel(λ*imm*434) respectively. The yield per input helper phage averaged 17 for *imm*P22 helpers and 12 for *imm*434 helpers.

the *rep*82/*repλ* loop measure 398±38 and 446±42 bases, respectively. The larger single-stranded segment probably derives from phage 82, because the molecular weight of the O protein encoded by the *rep*82:λ hybrid is slightly greater than that of the O protein encoded by λ (accompanying paper). Taking the right-hand end-point of the *imm*434 substitution as 79·1 on the standard λ physical map (Westmoreland *et al.*, 1969), we calculate that the *rep*82 substitution lies between 80·3±0·1 and 81·1±0·1, in good agreement with the measurements of Simon *et al.* (1971).

(d) *Specificity of interaction of* O *and* P *products*

Evidence for a specificity determinant for interaction with *P* function, associated with the carboxyl-terminal portion of the O protein, arises from studies of λ-φ80 hybrids. Complementation tests were designed to compare the functional defect of a φ80*14*− mutant with those of λ*O*− and λ*P*− mutants. A non-permissive (*su*°) host was

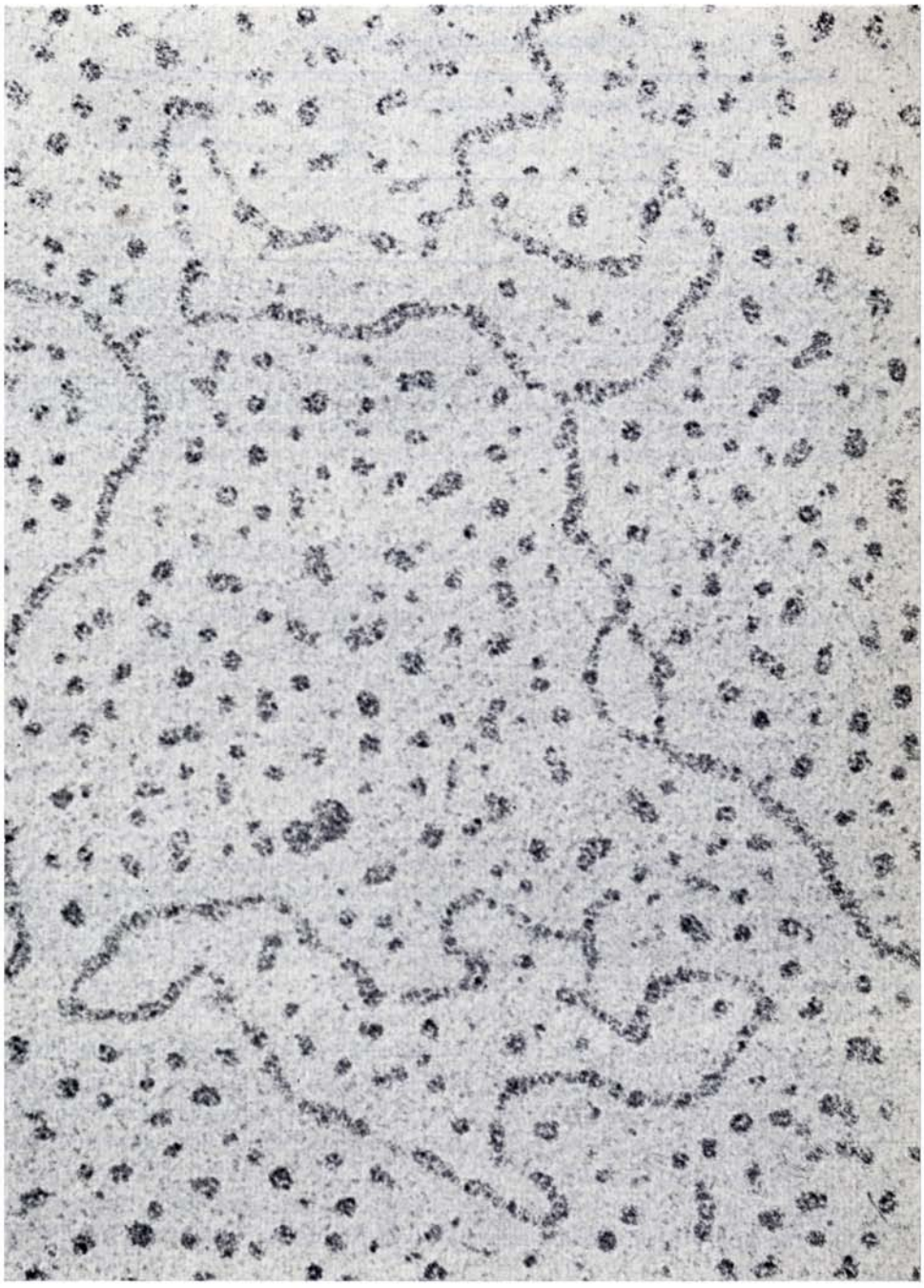

FIG. 7. DNA heteroduplex showing *rep*82 substitution. Heteroduplexes of *λb*2/*λimm*434 *rep*82:*λ* were prepared and analyzed as described in Materials and Methods. In the portion of a molecule shown here, the large substitution loop at the top corresponds to the *imm*434/*immλ* heterology, while the small substitution loop at the center of the field corresponds to the *repλ*/*rep*82 heterology. A circular SV40 DNA molecule is present at the bottom of the field.

TABLE 15

O × P *complementation of* repλ *and* repλ*:80 phages*

	Phage yield per infected cell							
	repλ *O⁻ P⁺*		*repλ* *O⁺ P⁻*		*repλ*:80 *O⁺ 14⁻*		*repλ*:80 *O⁻ 14⁺*	
	su°	*su*III	*su*°	*su*III	*su*°	*su*III	*su*°	*su*III
repλ O⁻ P⁺	0·1	48	21	26	0·08	78	0·05	113
repλ O⁺ P⁻			0·01	91	0·02	55	29	63
repλ:80 *O⁺ 14⁻*					0·02	56	68	63
repλ:80 *O⁻ 14⁺*							0·3	30

Complementation was tested by mixed infection in strain 594 and, as a control, in 594*su*III (Materials and Methods, section (e)). The mutant alleles were: *O*am29, *P*am3 and *14*am8. All phages carried *c*Its857. The total phage yield per infected cell was determined by titering on strain C600. In cases in which the yield per infected cell exceeded 20, a number of clones of progeny phage were tested to determine the distribution of the two mutant test alleles. This was done by scoring for growth on the *gro*PA15 host Q5151, or by crossing against the parent phages. In each case the 2 mutant phage types were represented about equally in the burst.

mixedly infected with amber derivatives of *repλ* and *repλ*:80 phages (Table 15). The hybrids carrying *repλO*am29:80 and *repλ*:80*14*am8 complement well. This shows that the mutations *O*am29 and *14*am8 lie in different cistrons (Benzer, 1957). However, we find that λ*repλ*:80*14*am8 and λ*P*am3 fail to complement (Table 15). From these data we conclude that gene *14* of φ80 is equivalent in function to *P* of λ.

Several surprising observations show that the λ P protein and the φ80 14 protein differ in functional specificity. We find that some combinations of *O* and *P* gene products do not permit replication. The λ P protein appears not to function effectively with the φ80 protein equivalent to 0, because a *repλ* helper phage fails to promote replication of φ80*14*am8 (Table 10). Similarly, the λ P protein cannot replace the φ80 14 protein for function with the hybrid O protein of a *repλ*:80 phage: an *imm*80 *rep*80:λ hybrid, which has *P* of λ, fails to promote the replication of λ*repλ*:80*14*am8 (Table 11), and the mutants λ*O*am29 and λ*repλ*:80*14*am8 fail to complement in mixed infection (Table 15). The λ P protein, therefore, appears unable to function with an O protein carrying the carboxyl-terminal segment characteristic of φ80. However, the φ80 14 protein can function effectively with each of the several types of O protein. We conclude that the limited type-specificity observed for *P* function results from constraints on the interaction between the O and P proteins, and is unrelated to the type-specificity of interaction of the O protein with its target nucleotide sequence in *ori*.

4. Discussion

Several phage and host components must be assembled together in order to initiate DNA synthesis at the origin of λ DNA replication. In this study we have examined specific interactions between those components which are determined by the phage. Our major experimental tools are hybrid phages in which elements of the λ replication control region are substituted by equivalent functions from other lambdoid phages.

The replication control elements *O*, *P* and *ori* lie close together in the λ genome. In hybrid phages derived by genetic recombination between λ and the *Salmonella* phage P22, these elements appear to segregate as an intact unit (Hilliker & Botstein,

1976). We have strengthened this conclusion by showing that the region of heterology between λ and P22 covers the sites of a number of *ori*⁻ mutations, and of the leftmost nonsense mutation in *O*.

In contrast to λ-P22 hybrid phages, some λ-φ80 and λ-82 hybrids can be shown to contain replication control elements derived from each parent phage. The recombination events giving rise to such hybrids have occurred within gene *O*. The O protein of these recombinants is itself hybrid (accompanying paper). Functional analysis of hybrid O proteins permits us to identify some of the interactions involved in the initiation of phage DNA replication (Fig. 8). The type-specificity of these interactions is summarized in Table 16.

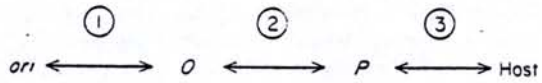

FIG. 8. Chain of interactions in the initiation of bacteriophage λ DNA replication. ① Interaction between the amino-terminal segment of the O protein and *ori*; ② interaction between the carboxyl-terminal segment of the O protein and the P protein; ③ interactions between the P protein and components of the *E. coli* replication apparatus.

We find that the *O* gene product interacts both with the *ori* site in the phage chromosome 1, and with the product of gene *P* 2. The target sequence which determines the receptor specificity of the DNA molecule lies within an interval of about 400 base-pairs which includes the positions of known *ori*⁻ mutations. The donor specificity determinant for the interaction with *ori* and a separate specificity determinant for interaction with P protein are localized, respectively, to the amino-terminal and carboxyl-terminal segments of the O polypeptide.

(a) ori–O *interaction*

The major conclusion of this work is that the product of gene *O* specifically interacts with a nucleotide sequence in the vicinity of the λ origin of replication. It is helpful to consider this interaction in terms of type-specific donor and receptor activities involved in *O* function. In practice these activities are defined by the ability of an *O*⁺ phage to promote the replication of an *O*⁻ phage; the donor specificity of the *O*⁺ phage must correspond to the receptor specificity of the *O*⁻ phage if the latter is to replicate. Our data show that both the *O* donor and *O* receptor activities of each of the lambdoid phages λ, φ80 and 82 are entirely type-specific (Table 16).

(i) O *donor specificity*

The donor specificity for *O* function resides in the amino-terminal segment of the O protein. Although the O protein encoded by *rep*80:λ and *rep*82:λ hybrid phages has the carboxyl-terminal segment characteristic of λ, neither of these hybrid phages can promote replication of a *repλO*⁻ phage. Conversely, a *repλ*:80 donor, which encodes an O protein with the amino-terminal portion characteristic of λ and the carboxyl-terminal portion characteristic of φ80, can successfully provide *O* function to a *repλO*⁻ mutant, but fails to promote replication of an *O*⁻ phage with the receptor specificity of φ80. The 82-type donor specificity can be conferred to λ by the introduction of a small substitution of DNA from 82. This substitution appears to be

TABLE 16

Summary of type-specificity of O protein interactions

Replication type	Structure of O protein		Interaction with *ori*			Interaction with *P*	
	Amino-terminal segment	Carboxyl-terminal segment	*ori*λ	*ori*80	*ori*82	*P*λ	*P*φ80 (*14*)
*rep*λ	λ	λ	+	−	−	+	+
*rep*80	φ80	φ80	−	+	−	−	+
*rep*λ:80	λ	φ80	+	−	−	−	+
*rep*80:λ	φ80	λ	−	+	−	+	+
*rep*82	82	82	−	−	+	not tested	
*rep*82:λ	82	λ	−	−	+	+	+

confined within the amino-terminal half of gene *O*. Thus, the donor type-specificity of *O* function does not depend on the product of any other known phage gene. The degree to which the O protein differs among λ, φ80, and 82 is discussed in the accompanying paper.

(ii) O *receptor specificity*

The receptor with which the O protein interacts must be either a specific target sequence within the phage DNA molecule, or another protein which itself interacts with such a sequence. We argue that the receptor almost certainly is a component of *ori*, the regulatory site for control of the replication origin. The determinant for receptor type-specificity is contained within the region covered by the *rep*82 substitution, and so lies within or very near to gene *O*, in an interval of about 400 base-pairs between 80·3±0·1 and 81·1±0·1 on the λ physical map. The receptor for *O* function, therefore, is closely linked to known mutations in *ori*, which lie within *O* at 80·9±0·2 (Denniston-Thompson *et al.*, 1977). All indispensable components of *ori* lie to the left of a site, located at 81·0±0·1, that is cleaved by the restriction endonuclease *Eco*RI (Furth *et al.*, 1977). This *Eco*RI site was mapped between the positions of *O*am29 and *O*am1005, the same interval within which recombination can occur to generate λ-80 and λ-82 phages hybrid for gene *O* (Fig. 3). Both φ80 and 82 appear to have an *Eco*RI site located within this interval (Denniston-Thompson *et al.*, 1977). The receptor for *O* function must lie to the left of this site.

The nucleotide sequence has been determined for a region of 110 base-pairs immediately to the left of the *Eco*RI site in *O*. This region includes the positions altered in *ori*⁻ mutants (Denniston-Thompson *et al.*, 1977), but we do not know whether it includes the specific target for interaction with O protein.

In order to test the hypothesis that the O protein binds to *ori*, it will be necessary to purify the protein. This task may prove difficult because the *O* gene product has a functional half-life of only a few minutes (Wyatt & Inokuchi, 1974).

(b) O–P *interaction*

Tomizawa (1971) has suggested that the *O* and *P* products of λ function together in a complex, because some alleles of *P* can suppress a temperature-sensitive mutation in *O*. Our data show that the λ P protein and the equivalent protein encoded by gene *14* of φ80 can each function with the O protein encoded by *rep*λ and *rep*80:λ phages, in which the carboxyl-terminal segment of *O* derives from λ. However, it appears that the λ P protein is not compatible with the O protein encoded by a *rep*80 or a *rep*λ:80 phage, in which the carboxyl-terminal segment of *O* derives from φ80 (Table 16). We propose that the P protein interacts directly with the carboxyl-terminal portion of the O protein, and that λ P protein cannot function if this portion of the O protein has φ80-type characteristics.

The specificity of interaction between the *O* and *P* products does not influence the specific recognition of *ori* by the O protein. If the *P* product also interacts with the DNA molecule, its site of action must not differ significantly among λ, φ80 and 82.

(c) P–*host interaction*

The isolation of mutants of *E. coli* in which λ fails to replicate, and the discovery that some mutations in gene *P* permit λ to overcome the block imposed by these mutant hosts, have led to the conclusion that the *P* product interacts with several

host replication proteins (Georgopoulos & Herskowitz, 1971; Georgopoulos, 1977; Saito & Uchida, 1977; Sunshine *et al.*, 1977). The replication of phages with the φ80-type *P* function is not restricted in some hosts in which λ replication is prevented by a *gro*P character in gene *dna*B (Georgopoulos & Herskowitz, 1971; Toothman, 1976). However, phages with the φ80-type *P* still require the *dna*B function in order to replicate, since they fail to replicate at the restrictive temperature in a host carrying a temperature-sensitive mutation in *dna*B (A. Strathern and I. Herskowitz, personal communication; our unpublished results). Some of the λ-φ80 hybrid phages which are able to replicate in the *gro*P hosts have the *ori* region and the proximal segment of *O* from λ (*rep*λ:80). This implies that the type-specificity of the interaction between *ori* and the amino-terminal portion of the O protein does not influence the interaction between P protein and the *dna*B product. We do not know whether or not the carboxyl-terminal segment of the O protein participates in the interaction with host proteins.

(d) *Chain of interactions in initiation*

We have shown that a chain of specific interactions connects elements which control λ DNA replication. This chain is sufficient to account both for specific template selection and for entry of the essential apparatus for autonomous replication.

Template selection depends on a specific interaction between the amino-terminal portion of the O protein and *ori*. The target sequence which determines the receptor specificity of *ori* lies within the segment of gene *O* which determines the donor specificity of the O protein. This overlap represents an extreme case of close genetic linkage of interacting elements, a situation which may facilitate the evolution of the lambdoid bacteriophages (Hershey, 1971; Botstein & Herskowitz, 1974).

The entry of the remainder of the replication apparatus depends on interactions between the carboxyl-terminal segment of the O protein and the P protein, and between the P protein and host factors. These interactions permit phage λ to parasitize *E. coli* for DNA replication by channeling host replication proteins to the phage replication origin, which differs in structure from that of the host.

We thank Doug Berg, Fred Blattner, Carol Gross and Ira Herskowitz for their continued interest, Martha Howe for a close reading of the manuscript, and Janet Mertz for assistance with DNA heteroduplex mapping. Waclaw Szybalski informed us of unpublished data on the structure of λ*imm*80hy42. Strains used for this work were generously provided, prior to publication of their description, by Sandra Hilliker and Penny Toothman. Our research is supported by Program-Project grant CA-07175 from the National Cancer Institute to the McArdle Laboratory (to W.F.D.). One author (M.E.F.) has been supported by a predoctoral fellowship from the National Science Foundation and training grant T32 CA-09135 from the National Cancer Institute.

REFERENCES

Adams, M. H. (1959). In *Bacteriophages*, p. 592, Interscience, New York.
Alberts, B. & Sternglanz, R. (1977). *Nature (London)*, **269**, 655–661.
Appleyard, R. (1954). *Genetics*, **39**, 440–452.
Bachman, B. J., Low, K. B. & Taylor, A. L. (1976). *Bacteriol. Rev.* **40**, 116–167.
Benzer, S. (1957). In *The Chemical Basis of Heredity* (McElroy, W. D. & Glass, B., eds), pp. 70–93, John Hopkins Press, Baltimore.
Blattner, F. R. & Dahlberg, J. E. (1972). *Nature New Biol.* **237**, 227–240.
Botstein, D. & Herskowitz, I. (1974). *Nature (London)*, **251**, 584–589.
Botstein, D., Chan, R. K. & Waddell, C. H. (1972). *Virology*, **49**, 268–282.

Brachet, Ph. & Thomas, R. (1969). *Mutat. Res.* **7**, 257–260.
Brooks, K. (1965). *Virology*, **26**, 489–499.
Campbell, A. (1961). *Virology*, **14**, 22–32.
Campbell, A. (1965). *Virology*, **27**, 329–339.
Davis, R. W., Simon, M. N. & Davidson, N. (1971). In *Methods in Enzymology* (Grossman, L. & Moldave, K., eds), vol. 21, pp. 413–428, Academic Press, New York.
Denniston-Thompson, K., Moore, D. D., Kruger, K. E., Furth, M. E. & Blattner, F. R. (1977). *Science*, **198**, 1051–1056.
Dove, W. F. (1968). *Annu. Rev. Genet.* **2**, 305–340.
Dove, W. F. (1969). *Virology*, **38**, 349–351.
Dove, W. F. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 297–312, Cold Spring Harbor Laboratory, New York.
Dove, W. F., Hargrove, E., Ohashi, M., Haugli, F. & Guha, A. (1969). *J. Genet. (Japan)*, **44**, 11–22, (suppl. 1).
Dove, W. F., Inokuchi, H. & Stevens, W. F. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 747–772, Cold Spring Harbor Laboratory, New York.
Eisen, H. A., Fuerst, C., Siminovitch, L., Thomas, R., Lambert, L., Pereira da Silva, L. & Jacob, F. (1966). *Virology*, **30**, 224–241.
Fiandt, M., Hradecna, Z., Lozeron, H. A. & Szybalski, W. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 329–354, Cold Spring Harbor Laboratory, New York.
Fiers, W., Contreras, R., Haegeman, G., Rogiers, R., Van de Voorde, A., Van Heuverswyn, H., Van Herreweghe, J., Volckaert, G. & Ysebaert, M. (1978). *Nature (London)*, **273**, 113–120.
Filip, C. C., Allen, J. S., Gustafson, R. A., Allen, R. G. & Walker, J. R. (1974). *J. Bacteriol.* **119**, 443–449.
Freifelder, D. & Kirschner, I. (1971). *Virology*, **44**, 223–225.
Furth, M. E. (1978). Ph.D. thesis, University of Wisconsin.
Furth, M. E. & Yates, J. L. (1978). *J. Mol. Biol.* **126**, 227–240.
Furth, M. E., Blattner, F. R., McLeester, C. & Dove, W. F. (1977). *Science*, **198**, 1046–1051.
Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. & Barnoux, C. (1971). *Proc. Nat. Acad. Sci., U.S.A.* **68**, 3150–3153.
Gemski, P., Baron, L. S. & Yamamoto, N. (1972). *Proc. Nat. Acad. Sci., U.S.A.* **69**, 3110–3114.
Georgopoulos, C. P. (1977). *Mol. Gen. Genet.* **151**, 35–40.
Georgopoulos, C. P. & Herskowitz, I. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 553–564, Cold Spring Harbor Laboratory, New York.
Goldberg, A. R. & Howe, M. (1969). *Virology*, **38**, 200–202.
Hayes, S. & Szybalski, W. (1973). *Mol. Gen. Genet.* **126**, 275–290.
Hershey, A. D. (1971). *Carnegie Inst. Year Book*, **70**, 3–5.
Herskowitz, I. (1971). Ph. D. thesis, Massachusetts Institute of Technology, Cambridge.
Hilliker, S. & Botstein, D. (1976). *J. Mol. Biol.* **106**, 537–566.
Inokuchi, H., Dove, W. F. & Freifelder, D. (1973). *J. Mol. Biol.* **74**, 721–727.
Ippen, K., Shapiro, J. A. & Beckwith, J. R. (1971). *J. Bacteriol.* **108**, 5–9.
Jacob, F. & Wollman, E.-L. (1956). *Ann. Inst. Pasteur*, **91**, 486–510.
Jacob, F., Brenner, S. & Cuzin, F. (1963). *Cold Spring Harbor Symp. Quant. Biol.* **28**, 329–348.
Joyner, A., Isaacs, L. N., Echols, H. & Sly, W. S. (1966). *J. Mol. Biol.* **19**, 174–186.
Kaiser, A. D. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 195–210, Cold Spring Harbor Laboratory, New York.
Kaiser, A. D. & Jacob, F. (1957). *Virology*, **4**, 509–521.
Kellenberger, G., Zichichi, M. L. & Weigle, J. (1961). *J. Mol. Biol.* **3**, 399–408.
Klinkert, J. & Klein, A. (1978). *J. Virol.* **25**, 730–737.
Kogoma, T. (1976). *J. Mol. Biol.* **103**, 191–197.
Levine, M. & Schott, C. (1971). *J. Mol. Biol.* **62**, 53–64.
Liedke-Kulke, M. & Kaiser, A. D. (1967). *Virology*, **32**, 465–474.
Matsushiro, A. (1963). *Virology*, **19**, 475–482.
McMacken, R., Ueda, K. & Kornberg, A. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 4190–4194.

Miller, H. I. & Friedman, D. I. (1977). In *DNA Insertion Elements, Plasmids and Episomes* (Bukhari, A. I., Shapiro, J. & Adhya, S., eds), pp. 349–361, Cold Spring Harbor Laboratory, New York.
Ogawa, T. & Tomizawa, J.-I. (1968). *J. Mol. Biol.* **38**, 217–225.
Rambach, A. (1973). *Virology*, **54**, 270–277.
Reddy, V. B., Thimmappayer, B., Ohar, R., Subramanian, K. N., Zain, B. S., Ghosh, P. K., Celma, M. L. & Weissman, S. M. (1978). *Science*, **200**, 494–502.
Roberts, J. W., Roberts, C. W., Hilliker, S. & Botstein, D. (1976). In *RNA Polymerase* (Losick, R. & Chamberlin, M., eds), pp. 707–718, Cold Spring Harbor Laboratory, New York.
Saito, H. & Uchida, H. (1977). *J. Mol. Biol.* **113**, 1–25.
Sambrook, J. F., Fan, D. P. & Brenner, S. (1967). *Nature (London)*, **214**, 452–453.
Sato, K. (1970). *Virology*, **40**, 1067–1069.
Sato, K., Nishimune, Y., Sato, M., Numich, R., Matsushiro, A., Inokuchi, H. & Ozeki, H. (1968). *Virology*, **34**, 637–649.
Schnös, M. & Inman, R. B. (1970). *J. Mol. Biol.* **51**, 61–73.
Schwarz, E., Scherer, G., Hobom, G. & Kössel, H. (1978). *Nature (London)*, **272**, 410–414.
Shuster, R. C. & Weissbach, A. (1969). *Nature (London)*, **223**, 852–853.
Signer, E. R. (1964). *Virology*, **22**, 650–651.
Simon, M. N., Davis, R. W. & Davidson, N. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 313–328, Cold Spring Harbor Laboratory, New York.
Skalka, A. M. (1977). In *Current Topics in Microbiology and Immunology*, vol. 78, pp. 201–237, Springer-Verlag, Berlin.
Stevens, W. F., Adhya, S. & Szybalski, W. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 515–533, Cold Spring Harbor Laboratory, New York.
Sunshine, M., Feiss, M., Stuart, J. & Yochem, J. (1977). *Mol. Gen. Genet.* **151**, 27–34.
Sussman, R. & Jacob, F. (1962). *C. R. H. Acad. Sci.*, **254**, 1517–1519.
Szpirer, J. (1972). *Mol. Gen. Genet.* **114**, 297–304.
Szpirer, J. & Brachet, Ph. (1970). *Mol. Gen. Genet.* **108**, 78–92.
Takahashi, S. (1975). *J. Mol. Biol.* **94**, 385–396.
Thirion, J. P. (1971). *Ann. Inst. Pasteur*, **120**, 453–465.
Thomas, R., Leurs, C., Dambly, C., Parmentier, D., Lambert, L., Brachet, Ph., Lefebvre, N., Mousset, S., Porcheret, J., Szpirer, J. & Wauters, D. (1967). *Mut. Res.* **4**, 735–741.
Tomizawa, J.-I. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 549–552, Cold Spring Harbor Laboratory, New York.
Toothman, P. (1976). Ph. D. thesis, University of Oregon, Eugene.
Valenzuela, M. S., Freifelder, D. & Inman, R. B. (1976). *J. Mol. Biol.* **102**, 569–581.
Weigle, J., Meselson, M. & Paigen, K. (1959). *J. Mol. Biol.* **1**, 379–386.
Westmoreland, B. C., Szybalski, W. & Ris, H. (1969). *Science*, **163**, 1343–1348.
Wickner, S. (1976). *Proc. Nat. Acad. Sci., U.S.A.* **73**, 3511–3515.
Wickner, S. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 2815–2819.
Wood, W. B. (1966). *J. Mol. Biol.* **16**, 118–133.
Wyatt, W. M. & Inokuchi, H. (1974). *Virology*, **58**, 313–315.
Yanofsky, C. & Ito, J. (1966). *J. Mol. Biol.* **21**, 313–334.
Yates, J. L., Gette, W. R., Furth, M. E. & Nomura, M. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 689–693.
Zyskind, J. W. & Smith, D. W. (1977). *J. Bacteriol.* **129**, 1476–1486.