

## Hybrids of Bacteriophages $\lambda$ and $\phi 80$ : A Study of Nonvegetative Functions

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Hybrids of phages  $\lambda$  and  $\phi 80$  were made by selecting for recombination between the genes for host range ( $h$ ) and immunity ( $i$ ). The parental origin,  $\lambda$  or  $\phi 80$ , of markers in the  $h-i$  region of the hybrids was determined; and the following order of markers was deduced:  $h$  ( $att$ ,  $int$ )  $exo$  ( $\beta$ ,  $N$ ). All the genetic elements involved in the specificity of the chromosomal site of attachment of  $\lambda$  and  $\phi 80$  appear to be located in the above region. The different types of hybrids obtained with respect to the genes for exonuclease and  $\beta$  protein provide genetic evidence that exonuclease and  $\beta$  protein are distinct polypeptides rather than polymeric forms of the same peptides.

### INTRODUCTION

The chromosomes of the temperate bacteriophage  $\lambda$  and  $\phi 80$  include a large central segment, between genes  $h$  and  $N$ , which is not required for vegetative development (Kellenberger *et al.*, 1961; Jordan, 1964). This segment is involved in prophage integration and excision and in recombination (for reviews, see Echols and Joyner, 1968; Signer, 1968). It contains the structural genes for exonuclease (Radding, *et al.*, 1967) and for protein  $\beta$  (Manly *et al.*, 1968), which are two of the few  $\lambda$  proteins which have been highly purified (Radding and Shreffler, 1966; Little *et al.*, 1967; Radding, unpublished results on  $\beta$  protein).

The present report, which is an extension of an earlier study (Radding *et al.*, 1967), provides data on the genetic elements in this region, their order, and the nature of the proteins they specify. The general approach has consisted of isolating hybrids of bacteriophage  $\lambda$  and the related phage

$\phi 80$  (Matsushiro, 1963; Signer, 1964), and then determining, for each of several properties, whether it was derived from the  $\lambda$  or the  $\phi 80$  parent.

### MATERIALS AND METHODS

#### *General*

$\lambda$ - $\phi 80$  hybrids were obtained by selecting recombinants between gene  $h$  (host range) and the immunity character ( $i$ ). All of them were in fact recombined between gene  $h$  (host range) and gene  $N$ . The parental origin of the characters  $att$ ,  $int$ ,  $exo$ ,  $\beta$ ,  $red$ , and the phenotype  $Loc$ , was determined as described below. The parental origin of markers in the hybrids will be indicated by a subscript  $\lambda$  or  $80$ .

As already noted, the genetic segment between  $h$  and  $N$  is not essential for vegetative development. The left part of the segment is the "homology region" (Campbell, 1962),  $att_{\phi}$ , which we will call  $att_{\lambda}$  or  $att_{80}$  according to whether it is derived from  $\lambda$  or from  $\phi 80$ . This section of the genome is deleted at least partially in the density mutant b2 (Kellenberger, *et al.*, 1961; Campbell, 1965). Genes involved in prophage integration and excision ( $int$ ) (Zissler, 1967; Gingery and

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Echols, 1967; Gottesman and Yarmolinsky, 1968) and in recombination (*red*) (Echols and Gingery, 1968; Signer and Weil, 1968) have been located between *att<sub>φ</sub>* and *N*. Integration involves recognition between the *att<sub>φ</sub>* region of the phage chromosome and a region (*att<sub>B</sub>*) of the bacterial chromosome located between *gal* and *bio* in the case of *att<sub>λ</sub>* and near *try* in the case of *att<sub>80</sub>*. A reciprocal recombination takes place in this region. It is mediated by a phage-directed Int system specific for *att<sub>λ</sub>* or *att<sub>80</sub>* according to its origin. Prophage excision, following either induction or heteroimmune superinfection, also involves recognition of two *att* regions and operation of the Int system. When a lysogen survives prophage excision, it is said to be "cured" of its prophage.

In this work we identify the specificity (*att<sub>λ</sub>* or *att<sub>80</sub>*) of the *att* region of a prophage by determining whether curing occurs following heteroimmune superinfection with a phage of the specificity of curing of  $\lambda$  (*int<sub>λ</sub>*). Conversely we identify the specificity of the Int system (*int<sub>λ</sub>* or *int<sub>80</sub>*) of a phage by testing whether or not it cures lysogens with the specificity of attachment of  $\lambda$  (*att<sub>λ</sub>*) (see Signer, 1968). It has not been shown whether the gene involved in the specificity of the Int mechanism and identified by its alleles *int<sub>λ</sub>* or *int<sub>80</sub>* coincides with the gene previously identified by *int<sup>-</sup>* mutations.

One expects a hybrid which has derived its *att* region and its *int* region from the same parent to lysogenize at a normal frequency at the corresponding site of the bacterial chromosome. The site of integration defines the phenotypes *Loc<sub>λ</sub>* and *Loc<sub>80</sub>*. A hybrid will be considered *Loc<sub>λ</sub>* or *Loc<sub>80</sub>* according to whether its lysogens transduce Gal (and not Try) or Try (and not Gal).

### Materials

**Bacterial strains.** All the strains used are derivatives of *Escherichia coli* K12.

CA 161 (Brenner), and CA 5013 are derivatives of Hfr H. These permissive strains have, respectively, the suppressors *su<sub>IR</sub><sup>+</sup>* and *su<sub>I</sub><sup>+</sup>*.

W3350 (Weigle; ref.: Campbell and Balbinder, 1958) is a nonpermissive (*su<sup>-</sup>*) strain with two galactose mutations (*gal<sub>1</sub> gal<sub>2</sub>*).

594 (Campbell, 1961) is a streptomycin-resistant derivative of W3350. Both  $\lambda$  and  $\phi$ 80 adsorb and grow on all these strains.

C600 (Appleyard, 1954), which does not adsorb phage  $\phi$ 80, is *su<sub>IR</sub><sup>+</sup>*.

CR63 does not adsorb  $\lambda$  wild type ( $\lambda h_{\lambda}^{+}$ ). A host range mutant of  $\lambda$  ( $\lambda h_{\lambda}$ ) and  $\phi$ 80 adsorb normally on this strain. A  $\lambda$  resistant,  $\phi$ 80 lysogenic derivative of the *su<sup>-</sup>* strain W3101 (Lederberg, 1960) was used to distinguish *h<sub>80</sub><sup>+</sup>N<sup>+</sup>i<sub>λ</sub>* recombinants from *h<sub>80</sub><sup>+</sup>N i<sub>λ</sub>*.

N100 (Meselson via Yarmolinsky) is a recombination-deficient (*rec<sup>-</sup>*), streptomycin-resistant derivative, of the *su<sup>-</sup>*, *gal<sup>-2</sup>* strain W3102. Two lysogenic derivatives of this strain were used here: N100 ( $\lambda c_{857}int^{-}susA_{11}$ ) (Gottesman and Yarmolinsky, 1968), which carries a suppressor-sensitive, thermoinducible  $\lambda$ ; and N100(434hyd30), which carries a defective *gal<sup>+</sup>*-transducing prophage (Campbell, 1964).

QR14 (Signer and Weil, 1968) is a *su<sup>+</sup>* cryptic strain with a host range marker *h<sub>λ</sub>* on its prophage. Upon infection with a *red<sup>+</sup>* phage with host range *h<sub>λ</sub><sup>+</sup>*, recombination will rescue *h<sub>λ</sub>* from the cryptic prophage, thus allowing growth on strain CR63.

**Phage.** Our  $\lambda$  wild-type has been described before (Thomas, 1966). Suppressor-sensitive (*sus*) mutants were provided by Campbell (1961).

$\lambda susN_7^+susN_{53}$  was obtained from B. Egan and D. Hogness.

$\lambda b2cI$  is a clear mutant of  $\lambda b2$  (Zichichi and Kellenberger, 1963).

21-1-b2 from Brenner, a hybrid between phage 21 and  $\lambda b2$ , is isogenic with  $\lambda b2$  except for the region specifying immunity 21.

The  $\phi$ 80 used in Brussels to prepare *hy1* to 4 is the "Cambridge strain," also called  $\phi$ 80-1 (Brenner, via Prell).

The strain used in Ann Arbor to prepare the hybrids of series *a* was obtained from R. Somerville.

### Methods

(i) *Isolation of the  $\lambda$ - $\phi$ 80 hybrids.* *Hy 1* to 4 have been described previously (Radding *et al.*, 1967). They were obtained from crosses between  $\lambda$  and  $\phi$ 80 in strain CA 5013. The lysates were plated on C600( $\lambda$ ) to select

for  $h_{\lambda}^{+}$  and  $i_{80}$ , and the hybrids were purified by conventional methods. Hy a1 to a24 were isolated from crosses between  $\lambda$  susN<sub>7</sub>susN<sub>53</sub> and  $\phi 80$ , followed by a selection for  $h_{\lambda}^{+}$  and  $i_{80}$ ; all these hybrids were N<sup>+</sup>. We also used two hybrids  $h_{80}^{+}$ : hy5, isolated at the Institut Pasteur, was sent to us by E. Signer; hy 6 was isolated by S. Mousset, using selection on strain CR63 (80).

(ii) *Curing of strains lysogenic for the hybrids.* A lysate of phage 21-1-b2, which cures strains lysogenic for  $\lambda$  but not for  $\phi 80$ , was spotted on a background of each lysogen. Colonies (100 in each case) isolated from the center of the turbid lysis area were tested for the loss of prophage immunity.

(iii) *Curing by the hybrids.* The ability of the hybrids to cure strains lysogenic for  $\lambda$  or a related phage was tested in two ways:

1. Using the technique of Gottesman and Yarmolinsky (1968): strain N100 ( $\lambda$ C<sub>857</sub>int<sup>-</sup>susA<sub>11</sub>), which contains a thermo-inducible prophage, is killed at 42° whereas cured cells survive. The ratio of cells plating at 42° to the total count (at 32°) is thus a measure of the curing frequency.

2. Lysates of the phage to be tested were spotted on a background of strain N100 (434hyd30). Colonies were reisolated from the center of the lysis area, by spreading on EMB gal medium. Curing of the strain results in a change from Gal<sup>+</sup> to Gal<sup>-</sup>.

(iv) *Determination of the phenotype Loc<sub>λ</sub> or Loc<sub>80</sub>.* To test the location of the phage on the bacterial chromosome, three lysogenic strains were isolated following infection of the gal<sup>+</sup> try<sup>+</sup> strain CA 161 with each phage. These strains were UV-induced and the lysates titrated for the ability to transduce gal<sup>+</sup> or try<sup>+</sup>, using as recipients the gal<sup>-</sup> strain W3350 and the try<sup>-</sup> strain Ca 2441. Gal transducers were classified as Loc<sub>λ</sub>, Try transducers as Loc<sub>80</sub>.

v) *Exonuclease and β protein.* Exonuclease and β protein were assayed as previously described (Radding, 1966; Radding and Shreffler, 1966). The nuclease assay is the same for the  $\lambda$  enzyme and the  $\phi 80$  DNase (Radding *et al.*, 1967), which has also been shown to be an exonuclease (Pricer and Weissbach, 1967). Immunodiffusion was

used to determine the parental origin *exo<sub>λ</sub>* or *exo<sub>80</sub>* of the gene for exonuclease, as well as to detect qualitatively altered forms of exonuclease. The immunodiffusion assay was also used to score the hybrids as β<sup>+</sup> or β<sup>-</sup>. The assay determined the presence of the β gene contributed by the  $\lambda$  parent. An assay for a presumed homologous protein (see Discussion) from  $\phi 80$  does not yet exist.

(vi) *Density of the hybrids and rescue of b2<sup>+</sup> in crosses with λ b2.* Because of the very low frequency of recombination between  $\lambda$  and  $\phi 80$ , recombination between  $\lambda$  and a  $\lambda/\phi 80$  hy is expected to take place almost exclusively between the  $\lambda$ -derived part of the hybrid and the homologous part of the  $\lambda$  parent. Thus, in a cross between a hybrid  $h_{\lambda}^{+}i_{80}$  and b2i<sub>λ</sub> the recovery of b2<sup>+</sup> i<sub>λ</sub> (and b2i<sub>80</sub>) recombinants might be expected to be a measure of the distance between the site of the  $\lambda$ - $\phi 80$  crossover and the limit of deletion b2 rather than between immunity and b2 (see Fig. 1). If the  $\phi 80$ -derived arm of the hybrid overlaps the deletion, one would not expect to find a sizable proportion of recombinants. The hybrids were crossed with  $\lambda$ b2c at multiplicities of 5 each in strain CA5013 (procedure, see Thomas, 1966). The lysates were adjusted to a density of 1.49 with CsCl, then fractionated according to the densities of the particles by centrifuging for 20 hours at 23,000 rpm (Spinco SW39). Fractions were collected and titrated for phage of i<sub>λ</sub> (on a strain lysogenic for  $\phi 80$ ) and for phage of i<sub>80</sub> (on a strain lysogenic for  $\lambda$ ).

(vii) *Recombination with the marker susJ<sub>6</sub>.* Hybrids were crossed with  $\lambda$ susJ<sub>6</sub> (procedure, Thomas, 1966), and the frequency of the  $\lambda$ sus<sup>+</sup> recombinants was measured by plating on the su<sup>-</sup> strain 594( $\phi 80$ ). The frequency of these recombinants was taken as a measure of the distance between the  $\lambda$ - $\phi 80$  crossover and the marker J<sub>6</sub> (rather than between immunity and J<sub>6</sub>; see (vi) above).

(viii) *Spot test for red<sup>+</sup> activity.* Red<sup>+</sup> activity was tested using the method of Signer and Weil (1968) on a mixture of strains QR14 and CR63. This test is based on recombinational rescue from the cryptic strain QR14 of an h<sub>λ</sub> marker which allows growth on the strain CR63. It can thus be used

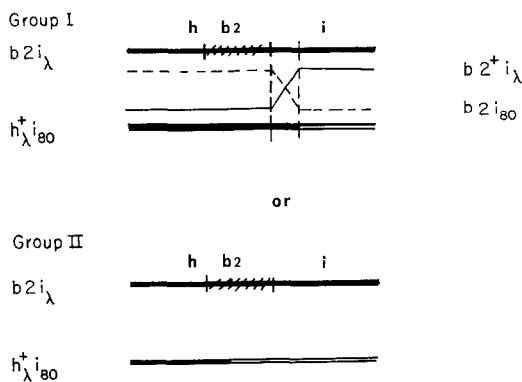


FIG. 1. Interpretation of crosses between  $b2i_\lambda$  and  $\lambda/80$  hybrids.

only for phage with the host range marker  $h_\lambda^+$ , which do not absorb to CR63;  $\phi 80$  and the hybrids with the marker  $h_{80}^+$  grow by themselves on CR63 without requiring rescue of an  $h_\lambda$  marker from the cryptic strain.

## RESULTS

### *Att and Int Specificities of the Hybrids*

Eighteen of the nineteen hybrids  $h_\lambda^+i_{80}$  can be classified into two principal groups (Table 1).

The 9 hybrids of group I were cured from their lysogens by phage 21-1-b2, which has the curing specificity of  $\lambda$ . These hybrids cured lysogens with the specificity of attachment of  $\lambda$ . They were thus classified as  $att_\lambda$  and  $int_\lambda$ . As expected from this genetic constitution, the seven hybrids of group I which were tested in specialized transduction experiments were located near *gal* ( $Loc_\lambda$ ).

The 9 hybrids of group II and one hybrid  $h_{80}^+i_{434}$  (hy7 derived from hy6) were not cured from their lysogens by phage 21-1-b2, and they did not cure lysogens with the specificity of attachment of  $\lambda$ . Thus they were classified, by exclusion, as  $att_{80}$  and  $int_{80}$ . In agreement with the inferred genetic constitution, the four hybrids of this group which were tested in specialized transduction experiments integrated near *try* ( $Loc_{80}$ ). The two hybrids  $h_{80}^+i_\lambda$  (hy5 and hy6) were not cured by 21-1-b2. They transduced Try but not Gal; they are thus  $Loc_{80}$  and, by inference,  $att_{80}int_{80}$ .

Hy a24 is unique. We were unable to isolate lysogens when this hybrid was used in single infection. However, it did integrate in mixed infection with  $\lambda$  or  $Loc_{80}i_\lambda$  (hy5). All the strains lysogenic for  $i_{80}$  (56/56; 50/50) were also lysogenic for  $i_\lambda$ . They did not segregate single lysogens for  $\phi 80$ . Two double lysogens of each type were tested for the transducing abilities of their lysates. The lysogens obtained with  $\lambda$  as helper transduced Gal, whereas those obtained with  $Loc_{80}i_\lambda$  as helper transduced Try. The behavior is like that of  $\lambda b2$  and suggests that hy a24 may also have a structural abnormality. In fact, whereas all the other hybrids tested have a density at least as great as that of  $\lambda$ , hy a24 has a lower density, close to that of  $\lambda b2$ . Also in mixed infection with  $\lambda b2$ , we obtained only abortive lysogeny.

Hybrids of the type  $att_\lambda int_{80}$  might be expected to be deficient in integration unless helped by a phage which is  $int_\lambda$ . In contrast to hy a24, a hybrid  $att_\lambda int_{80}$  should integrate frequently without concomitant integration of the helper; in other words it should make single lysogens when helped. We did not observe any hybrid of this type.

### *Nature of the Exonuclease of the Hybrids*

As reported previously, the  $\lambda$  and  $\phi 80$  exonuclease can be distinguished immunologically (Radding *et al.*, 1967). This is done by titration of antiserum to  $\lambda$  exonuclease, or by the immunodiffusion assay. In Plate I, photograph no. 14 shows the reaction of crude extracts of induced lysogens of  $\lambda$  and  $\phi 80$ . The antiserum was anti-exonuclease, anti- $\beta$  protein (Radding and Shreffler, 1966). Extracts of  $\lambda$  show the  $\alpha$  precipitin band associated with exonuclease, and the  $\beta$  precipitin band which identifies the  $\beta$  protein. Extracts of  $\phi 80$  show neither precipitin band (cf. next section below). The exonuclease of hybrids which are  $Loc_{80}$  was like that of  $\phi 80$  [Radding *et al.*, (1967) and Plate II, hybrids a10, a11, and a12]. Among the hybrids which are  $Loc_\lambda$ , some made exonuclease like the  $\lambda$  enzyme [Radding *et al.* (1967), and Plates I and II, hybrids 1 and a7]; the other  $Loc_\lambda$  hybrids made qualitatively altered exonuclease (Plate I, hybrid 2, a20 and a22). When com-

TABLE 1  
 PROPERTIES OF THE  $\lambda/\phi 80$  HYBRIDS

Phages	Curing by 21-1-b2 <sup>a</sup>	Curing of		Transduction <sup>d</sup>		Exonuclease <sup>e</sup>		$\beta$ protein <sup>e</sup> precipitin reaction	Recombination <sup>f</sup> (%)	
		N100 ( $\lambda_{C857}$ int <sup>-</sup> sus A <sub>II</sub> ) <sup>b</sup> (%)	N100 (434 dg) <sup>c</sup>	Gal (%)	Try (%)	Units/mg	Precipitin reaction		With $\lambda b2c$	With $\lambda$ sus J <sub>6</sub> II
$\lambda$	22/100		+	$7.6 \times 10^{-5}$	—	12	+	+	9	(with $\lambda$ sus N7c <sup>+</sup> ) 6
<i>Group I</i>										
hy 1	37/100	4.2	+	$5.5 \times 10^{-4}$	—	12	+	—	12	6.6
a7	58/100					7	+ or *	—		
2	32/100	1.6	+	$5.9 \times 10^{-4}$	—	17	*	—	9	4.2
3	39/100	2.3	+	$7.6 \times 10^{-4}$	—	19	*	—	14	5.0
a20	42/100	1.8	+	$1.5 \times 10^{-3}$	—	12	*	—	13	5.0
a21	44/100	3.4	+			11	*	—		5.2
a22	24/100	2.4	+	$1.6 \times 10^{-3}$	—	10	*	—	6	4.8
a23	29/100	3.9	+			10	*	—		5.4
a18	21/100	3	+	$5.3 \times 10^{-3}$	—	1.6	*	—	8	4.0
<i>Group II</i>										
4	0/100	$2.3 \times 10^{-3}$	—	—	$2.9 \times 10^{-5}$	23	—	—	<0.1	3.2
a1	0/100	$8.8 \times 10^{-3}$	—			11	—	—		2.6
a2	0/100	$1.4 \times 10^{-2}$	—			11	—	—		3.4
a3	0/100	$1.2 \times 10^{-3}$	—			14	—	—		3.2
a4	0/100	$7.5 \times 10^{-3}$	—				—	—		3.0
a5	0/100	$3 \times 10^{-3}$	—	—	$9 \times 10^{-6}$		—	—		2.0
a6	0/100	$8 \times 10^{-3}$	—				—	—		2.6
a8	0/100	$9 \times 10^{-3}$	—	—	$6.7 \times 10^{-6}$		—	—		2.6
a9	0/100	$8.5 \times 10^{-3}$	—	—	$6.2 \times 10^{-6}$		—	—		2.8
hy a24		$6 \times 10^{-3}$							<0.1	2.4
$\phi 80$	0/100	$7.5 \times 10^{-3}$	—	—	$7 \times 10^{-6}$	32	—	—	<0.4	$3.4 \times 10^{-3}$
hy 5	0/100		—	—	$6.2 \times 10^{-6}$	5	—	+		
hy 6	0/100		—	—	$1.7 \times 10^{-5}$					
hy 7	0/100	$7.5 \times 10^{-3}$	—	—	$8.1 \times 10^{-5}$					

<sup>a</sup> Curing of strains lysogenic for the hybrids by 21-1-b2: see Methods (ii).

<sup>b</sup> Curing of N100 ( $\lambda_{C857}$  int<sup>-</sup> sus A<sub>II</sub>) by the hybrids: see Methods (iii). The frequency of spontaneous curing of this strain was  $1.4 \times 10^{-3}$ . Curing of this strain by 21-1-b2 was 24% (positive control).

<sup>c</sup> Curing of N100 (434 dg) by the hybrids: see Methods (iii). (+) indicates the presence of gal<sup>-</sup> colonies among the gal<sup>+</sup> population.

<sup>d</sup> Transduction of Gal and Try: see Methods (iv). (—) means that the frequency of gal<sup>+</sup> or try<sup>+</sup> was not higher than the frequency of spontaneous revertants (in the case of Gal transduction:  $5 \times 10^{-7}$ ; in the case of Try transduction:  $2.5 \times 10^{-6}$ ).

<sup>e</sup> Exonuclease and  $\beta$  protein were assayed as cited in Methods (v). Precipitin reactions were scored as (+) where present and qualitatively normal; (\*) where present and qualitatively altered (see Plates I and II); (—) where absent.

<sup>f</sup> Recombination with  $\lambda b2$  and  $\lambda$  sus J<sub>6</sub>: the figures give, respectively, the frequency of  $\lambda b2^+$  plus  $\phi 80b2$  recombinants, expressed in percent of the total yield, and the frequency of  $\lambda$  sus J<sup>+</sup> recombinants multiplied by two to account for the reciprocal recombinants, which were not scored.

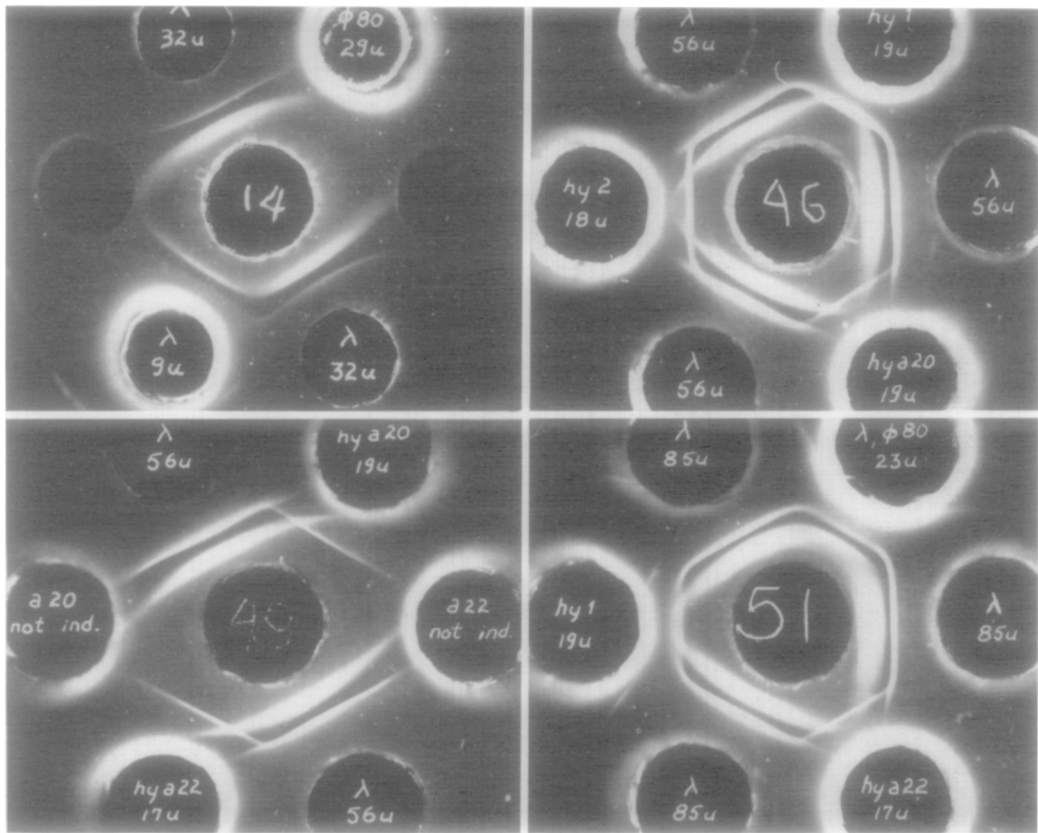


PLATE I. Immunodiffusion assay for exonuclease and  $\beta$  protein. The central well contained anti-serum to exonuclease and  $\beta$  protein prepared as described previously (Radding and Shreffler, 1966); the peripheral wells contained crude extracts of the indicated induced lysogens. The band near the central well is due to  $\beta$  protein, the farther band is due to  $\lambda$  exonuclease. In some cases, extracts of  $\lambda_{T11}$  lysogens (Radding and Shreffler, 1966) were used as the source of  $\lambda$  reference antigens. The units indicated in the labels of the peripheral wells refer to the total units of exonuclease present in the well. In photograph no. 49, the label, "not ind." means that the extracts used as controls were from lysogens which were not induced. In photograph no. 51, the well labeled " $\lambda$ ,  $\phi 80$ " contained an extract of a double lysogen of phage  $\lambda$  and phage  $\phi 80$ . In photograph No. 14, the extract in the upper left hand well is from  $\lambda$ .

pared with the  $\alpha$  precipitin band of a neighboring wild-type  $\lambda$  extract, the  $\alpha$  precipitin bands of these hybrids showed a strong spur, which indicates the loss of some antigenic groups. As a control, a double lysogen containing both  $\lambda$  and  $\phi 80$  was examined (Plate I, photograph No. 51). In this case the  $\alpha$  precipitin band was not altered. Thus the presence of the complete genes for both the  $\lambda$  and  $\phi 80$  exonuclease was not sufficient to produce the altered enzyme. Our interpretation is that the altered enzyme was produced by recombination within the structural gene(s) for exonuclease. A large propor-

tion of the hybrids selected for recombination between  $h$  and  $i$  produced altered exonuclease. A corollary of the above interpretation and the selection of hybrids for the markers  $h_{\lambda}^{+}$  and  $i_{\phi 80}$  is that *exo* is located to the right of *att* and *int* in the genetic map:  $h^{+}$  (*att int*) *exo i*.

#### $\beta$ and *N*

In confirmation of earlier results with a smaller number of hybrids (Radding *et al.*, 1967), none of the hybrids of the type  $h_{\lambda}^{+}i_{\phi 80}$  made the  $\beta$  protein,<sup>3</sup> whose synthesis is de-

<sup>3</sup> There may be weak cross-activity of the anti- $\beta$

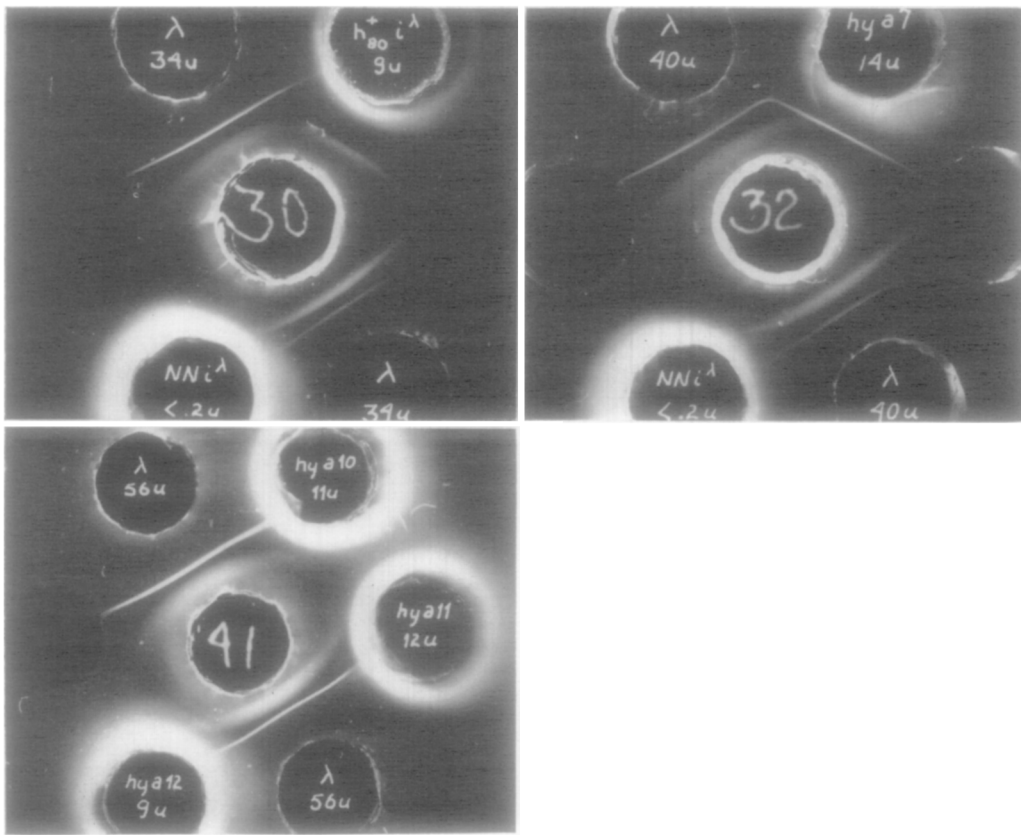


Plate II. Immunodiffusion assay is as described in the legend to Plate I. The wells labeled “NN  $\lambda$ ” contained extracts of the defective lysogen W3350 ( $\text{sus}N_7N_{64}\lambda$ ) (see Methods). The well labeled  $h_{80}^+i_{80}\lambda$  in photograph no. 30 contained an extract of hy5 (see Table I).

terminated by the  $\lambda$  parent (Table 1, Plates I and II). In contrast, hy 5, of the type  $h_{80}^+i_{80}\lambda$  made  $\beta$  protein (Plate II No. 30). In all cases examined the  $\lambda/80$  crossover appears to have occurred to the left of the genetic determinant(s) for  $\beta$  protein; in all group I hybrids, crossover occurred in or to the right of *exo*. Thus  $\beta$  maps to the right of *exo* (Fig. 2).

All the hybrids of the *a* series were obtained from a cross of  $h_{\lambda}^+N_7N_{53}i_{\lambda} \times h_{80}^+N_{80}^+i_{80}$  in which *h* and *i* were the markers selected in the progeny. All the  $h_{\lambda}^+i_{80}$  recombinants were  $N^+$ . Since the reversion frequency of the double *N* mutant is less than  $10^{-10}$ ,  $N^+$

serum with a corresponding protein made by  $\phi 80$  (cf. Plate I:  $\phi 80$ , photo 14; hy 2, photo 46, and Plate II: hy a10, hy a11, and hy a12, photo 41). But the reaction is weak and not reproducible.

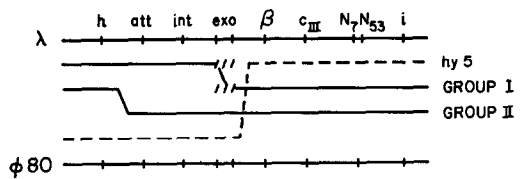


FIG. 2. Interpretation of crosses leading to  $\lambda/80$  hybrids (see Table 1). Only the relative order of markers is indicated. The order,  $\beta$ , *CIII*, *N* has been reported elsewhere (Manly *et al.*, 1968).

in the progeny must have come from the  $\phi 80$  parent. All (99%) of the  $h_{80}^+i_{80}\lambda$  progeny were  $N^-$ . The  $\lambda/80$  crossover was thus located in all cases to the left of *N*. This locates *exo* to the left of *N* and supports much other evidence that *N* is not the structural gene for exonuclease (Radding

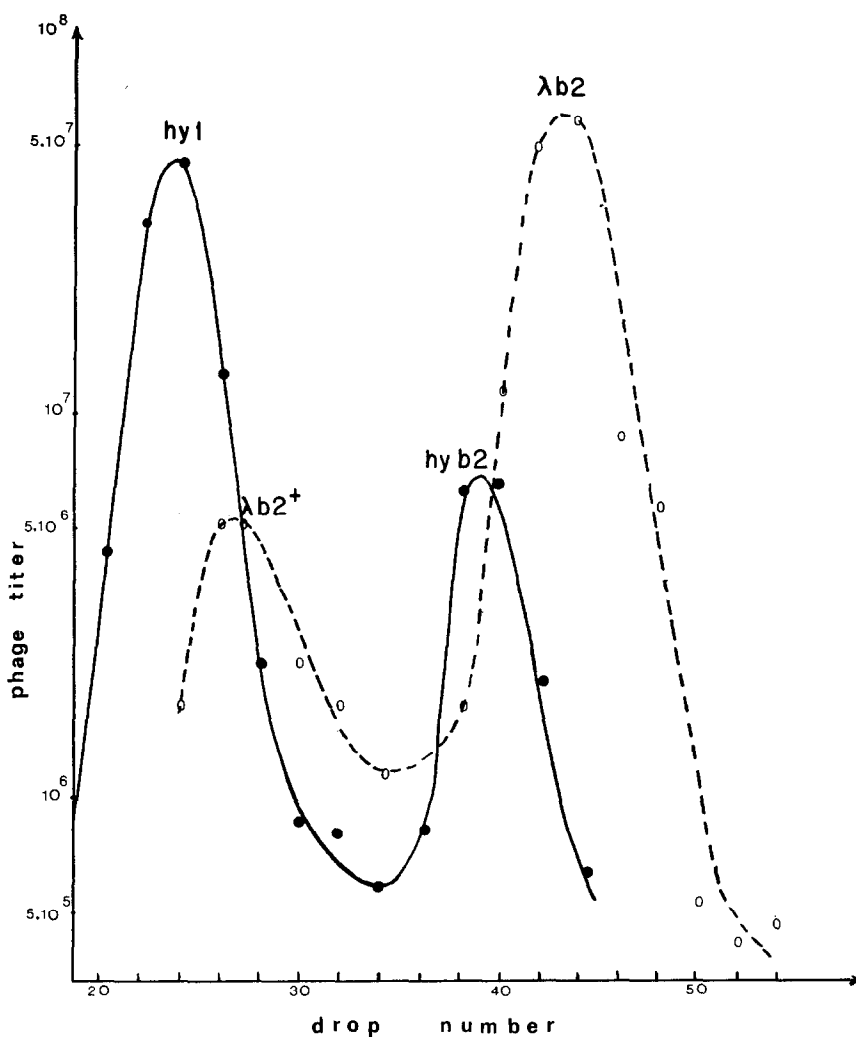


FIG. 3. Density gradient analysis of a cross between  $\lambda b2c$  and  $hy1$  (group I). A cross between  $\lambda b2c$  and the  $hy$  was performed and analyzed as described in Methods (vi). All tubes containing 2 drops from the  $CsCl$  gradient, were assayed separately. —, Phages of  $i_{80}$ ; ---, phages of  $i_{\lambda}$ .

*et al.*, 1967; Radding and Echols, 1968; Manly *et al.*, 1968).

#### Crosses with $\lambda b2$ and $\lambda susJ_6$

The results of crosses with  $\lambda b2$  and  $\lambda susJ_6$  confirm the relative locations of the crossover points in the different types of hybrids. The crossover point of group I hybrids (which are  $h_{\lambda}+Loc_{\lambda}i_{80}$ ) is to the right of  $b2$ : about 10%  $b2+i_{\lambda}$  and  $b2i_{80}$  recombinants were obtained in crosses of 6 such hybrids with  $\lambda b2$ . In contrast, a hybrid of group II (these hybrids are  $h_{\lambda}+Loc_{80}i_{80}$ ) gave less

than 0.2%  $b2+i_{\lambda}$  or  $b2i_{80}$  recombinants. (Figs. 3 and 4).

#### Recombination

The hybrids  $h_{\lambda}+i_{80}$  were all tested for the phage-determined activity (Red) which stimulates recombination (Signer and Weil, 1968; Echols and Gingery, 1968). They all show normal activity in a spot test. This does not determine whether their Red system derives from the  $\lambda$  or the  $\phi 80$  parent since, as might have been anticipated for a general recombination function, Red is in-



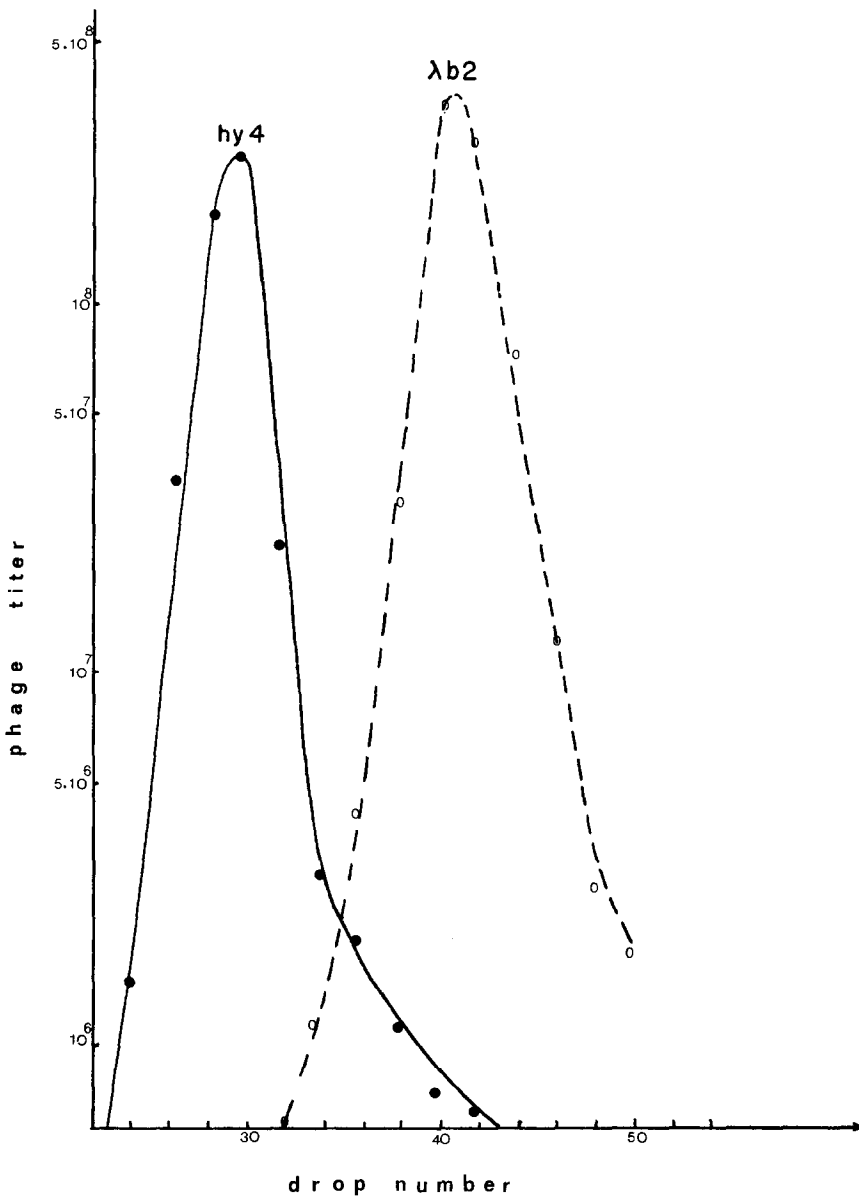


FIG. 4. Density gradient analysis of a cross between  $\lambda b2c$  and  $hy4$  (group II).

terchangeable between  $\lambda$  and  $\phi 80$ . (This is demonstrated by the fact that two *red<sup>-</sup>* derivatives of  $\lambda$  recombine 100 times more in the presence of  $\phi 80$ : Szpirer and Thomas, unpublished results.)

In addition, all the hybrids of group I gave 4.2–5.4% recombination (the percent of  $\lambda sus^+$  recombinants multiplied by two) with the marker  $susJ_6$ ; hybrids of group II gave only 2.0–3.4%. One may

wonder why there was more recombination between the crossover point of the hybrids of group I and  $b2$  than between the same point and  $J_6$ , which is farther to the left. This remains unexplained but may be related to the recent finding that two markers spanning the  $b2$  region give an abnormally high frequency of recombination if one parent is  $b2^+$  and the other  $b2$  (Echols *et al.*, 1968).

In a preceding paper (Radding *et al.*, 1967) the genetic marker  $t_{50}$  was used to map the  $\lambda/80$  crossover points. A strain lysogenic for  $\lambda_{50}$  (from Fuerst) was induced and superinfected with  $h_{\lambda^+i_{80}}$  hybrids. Since  $t_{50}$  was considered a defective mutation the  $i_{\lambda}$  progeny were scored as  $t_{50}^+i_{\lambda}$  recombinants, and their numbers were taken as measures of the distance between the crossover points of the hybrids and the markers  $t_{50}$ . In fact the choice of this marker was not a happy one, since it turned out not to be a real defective, but rather an *int*<sup>-</sup> mutant (personal communications from Fuerst, Signer, and Eisen). A strain lysogenic for  $\lambda_{50}$  thus produced very few phage following induction simply because the prophage was unable to excise properly. However, when the induced strain was superinfected with a  $\lambda/80$  hybrid of the proper *Int* specificity, prophage excision presumably occurred giving progeny  $t_{50}i_{\lambda}$  particles which plate normally. That  $i_{\lambda}$  particles were about 10% of the yield in crosses with hybrids of group I, and only 0.2% with hybrids of group II, is now easily understood since the hybrids of group I are *int* <sub>$\lambda$</sub>  (and can thus excise the prophage) whereas the hybrids of group II are *int*<sub>80</sub> (and cannot excise the prophage).

## DISCUSSION

### *Structure of the Hybrids*

In  $\lambda \times \phi 80$  crosses, recombinants occur at about one-thousandth the frequency found between the same markers in homoimmune crosses. The rarity of the hybrids might reflect a strong reduction of the absolute frequency of the recombinational events, due to poor pairing. In addition, the generation of viable hybrid structures might require complex and therefore infrequent rearrangements: this would be the case if the gene order in  $\lambda$  and  $\phi 80$  is not the same, or if certain functions are not interchangeable.

The hybrids have the host range of one parent and gene *N* from the other parent. Strictly speaking this means that there has been one or an odd number of genetic exchanges in the interval. However, since the region *J-N* is dispensable, there is no selective requirement for multiple exchanges in

this interval. For this reason (and keeping in mind the rarity of the exchanges), we will assume that the hybrids involve a single genetic exchange in the interval *J-N*. There may be additional crossover(s) outside this interval if this is required to produce a viable hybrid.

Another feature of these interspecific crosses is that recombination appears to occur preferentially at certain sites, as observed also by N. Franklin (personal communication). Recombination has not been observed between *N* and *i* (Radding *et al.*, 1967; and Results) in the examination of a large number of progeny observed by plating on a selective indicator. The detailed examination of markers in a small number of hybrids has revealed recombination to the left of *att*, and in or to the right of *exo*, but not between *att* and *int*, or between  $\beta$  and *N* (or *i*). In the case of every hybrid, *exo* was linked to *Loc*, but in about half of the hybrids, recombination occurred between determinants for exonuclease and  $\beta$  protein. *Exo* and  $\beta$ , however, are nearby or adjacent genes (Manly *et al.*, 1968). In spite of the structural change in the exonuclease of six of the hybrids (2, 3, a20-a23) none of these seem to have suffered a loss of enzymatic activity. Only one mutant, a18, for which an  $\alpha$  reaction was not observed, appeared to have low exonuclease activity. These results seem to underscore the special nature of the points at which recombination occurs between  $\lambda$  and  $\phi 80$ . If recombination occurred at random in the exonuclease genes, one might expect more variation in the immunologic and enzymatic activity of the hybrid enzymes.

### *Gene Order*

Assuming that the hybrids examined usually originated from a single recombination event with respect to the interval *h-N*, (see preceding section) and that the gene order in this segment is the same for  $\lambda$  and  $\phi 80$ , we deduce the following order of markers:

$$h \text{ (att, int) } exo \text{ (}\beta, N\text{)}$$

These results are consistent with the more complete order deduced from characteriza-

tion of nondefective  $\lambda$  *bio* mutants:

*h att int exo  $\beta$  C<sub>III</sub> N* (Manly *et al.*, 1968)

*The Role of the Genes in the Central Segment of the Chromosome*

Among the hybrids selected for recombination between *J* and *N*, some have the *att* region (defined by the ability to be cured by 21-1-b2) and the specificity of the *Int* mechanism (defined by the ability to cure  $\lambda$ -lysogens) of  $\lambda$  whereas others do not. This shows that the genetic markers involved in the specificity of integration are located between *J* and the  $\lambda/\phi 80$  crossover of the hybrids of group I. The fact that the hybrids usually lysogenize at a normal rate, and at one of the two expected locations (*Loc $\lambda$*  or *Loc $\phi 80$* ) strongly suggests that *all* the genetic elements involved in the specificity of integration are located in the above-mentioned segment. This implies that if one of the two loci at which *int*<sup>-</sup> mutations have been found is involved in the specificity of integration and excision, it is *int<sub>A</sub>* (which is indeed to the right of *att*) rather than *int<sub>B</sub>* (which is in the right arm of the chromosome) (Gingery and Echols, 1967). It may be added here that *int<sub>B</sub>* is apparently not involved in heteroimmune excision (S. Mousset, personal communication).

*On the Nature of Exonuclease and  $\beta$  Protein*

Previous experiments (Radding and Shreffler, 1966; Radding *et al.*, 1967) have indicated that exonuclease and  $\beta$  protein are not different polymeric forms of the same polypeptides but are different polypeptides which reversibly associate:  $\alpha + \beta \rightleftharpoons \alpha\beta$ . These studies strengthen this conclusion. The immunologic reactivity of exonuclease has been labeled  $\alpha$  (Radding and Shreffler, 1966), and that of the  $\beta$  protein is, of course,  $\beta$ . Among our hybrids the following types have been observed with respect to immunologic reactivity (see Table 1 and Plates I and II):

$\alpha^+\beta^-$  (e.g., hy1)

$\alpha^*\beta^-$  (e.g., hy2)

$\alpha^-\beta^-$  (e.g., hy a10)

$\alpha^-\beta^+$  (e.g., hy5)

Those strains that are  $\alpha^-$  have DNase activity which has been shown in one case to be indistinguishable from the  $\phi 80$  exonuclease (Radding *et al.*, 1967). Thus we can conclude that the presence of immunologically active  $\alpha$  protein is neither necessary nor sufficient for the formation of immunologically active  $\beta$  protein, and vice versa. From a functional point of view it is interesting that all these hybrids are capable to some extent, at least, of genetic recombination, which is the biological function of exonuclease (Signer *et al.*, 1968) and  $\beta$  protein (Radding, unpublished observations). These results also imply that  $\phi 80$  has a homologous  $\beta$  protein. Since  $\beta$  protein is required for recombination by phage  $\lambda$ , all of the  $\beta^-$  hybrids must have  $\beta$ -like activity inherited from the  $\phi 80$  parent. The hybrid systems, *exo $\lambda$  $\beta$  $\phi 80$*  and *exo $\phi 80$  $\beta$  $\lambda$*  may prove to be useful tools in exploring the physical and biological interaction of exonuclease and  $\beta$  protein.

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