

THE LINEAR INSERTION OF A PROPHAGE INTO THE CHROMOSOME
OF E. COLI SHOWN BY DELETION MAPPING^{1, 2}

Naomi C. Franklin, William F. Dove³ and Charles Yanofsky
Departments of Biological Sciences and Biochemistry,
Stanford University, Stanford, California

Received January 25, 1965

Evidence bearing on the relationship of a prophage to the chromosome of its host bacterium has been lucidly reviewed by Hayes (1964), who considered that a clear decision between attachment and insertion of prophage could not then be made. Just prior to Hayes' summation, Campbell (1962) had formulated a largely theoretical model which would allow for the linear insertion of phage genetic material into a bacterial chromosome, and would account for the permuted gene sequence of the prophage as compared to vegetative phage. The permuted gene sequence had been indicated by comparisons of the gene order in phage recombination experiments and in crosses between bacteria lysogenic for genetically marked λ prophages (Calef and Licciardello, 1960). Evidence in support of the model was subsequently supplied by Campbell (1963) in studies of the segregation patterns of lysogenic heterogenotes, and by Rothman (1965) in studies of P1 cotransduction of galactose and λ markers.

We describe in this paper observations which substantiate Campbell's model by showing that deletions in particular lysogenic bacteria

¹Supported by grants from the National Science Foundation and the United States Public Health Service.

²W. F. D. would like to acknowledge an independent proposal of similar experiments by Mr. R. Freedman.

³Public Health Service Postdoctoral Fellow. Present address: McArdle Memorial Laboratory, University of Wisconsin.

simultaneously eliminate bacterial genes and segments of a prophage genome. The missing phage segments are overlapping, allowing sequencing of the prophage genes.

The prophage used is a hybrid with the immunity of $\phi 80$ and the host range of λ . Like the $\phi 80$ prophage (Matsushiro, 1963), it resides in E. coli K12 close to the genes which determine the structures of several enzymes essential for the synthesis of tryptophan (Yanofsky, 1960). Mutations of E. coli K12 to T1-resistance ($T1^R$) not infrequently result from deletions which extend into the tryptophan (tryp) operon for varying distances (Yanofsky and Lennox, 1959). $T1^R$ mutants isolated from lysogenic bacteria are also either tryp⁺ or tryp^{deletion}, and in addition may lack different amounts of prophage genetic material.

MATERIALS AND METHODS

Bacteria: E. coli K12 #1485, a non-lysogenic prototroph; a T5-resistant mutant, 1485T5^R (T5 resistance also confers resistance to T1, $\phi 80$ and $\phi 80h$, but not to λ); a T1-resistant mutant, 1485T1^R (T1 resistance also confers resistance to $\phi 80$ but not to $\phi 80h$ nor to λ); lysogenic derivatives of these bacteria.

E. coli K12 #173101, non-permissive for the λ sus mutants used (Campbell, 1961).

Phages: Virulent phages T1 and T5.

Transducing phage Plkc = P1 (Lennox, 1956).

Temperate phage λ wild type, obtained from Dr. D. K. Fraser, and several of its suppressor-sensitive (sus) mutants (Campbell, 1961): sus A (= sus 11), sus F (= sus 96b), sus I (= sus 2), sus J (= sus 6), sus K (= sus 24), sus L (= sus 63), sus N (= sus 7) and sus R (= sus 5).

Temperate phage $\phi 80$ (Matsushiro, 1963). From a sample kindly sent by Dr. Matsushiro, a single plaque was isolated and high titer stocks were prepared by Dr. R. L. Somerville in this laboratory. A spontaneous mutant $\phi 80h$ was selected from $\phi 80$ by its ability to plate on 1485T1^R.

Temperate phages λ and $\phi 80$ differ in immunity specificity (i) and in ability to adsorb to various bacteria (h), as well as in plaque type, serological properties, density and other characteristics. Nevertheless, recombination between $\phi 80$ and λ occurs (R. L. Somerville, personal communication; Signer, 1964). From 1485 doubly lysogenic for $\phi 80$ and λ a lysate was obtained after UV induction which contained infective phages with different bouyant densities and recombinant characteristics of λ and $\phi 80$. By CsCl density gradient centrifugation of the lysate, a phage type was recovered with a density of 1.510 g/ml (slightly denser than λ , considerably denser than $\phi 80$), which was able to form plaques on 1485(λ)Tl^r. This phage was therefore recombinant for the immunity determinant of $\phi 80$ and the host range determinant of λ , and is designated $i^{\phi 80} h^{\lambda}$. A clear mutant of this hybrid, $i_c^{\phi 80} h^{\lambda}$, was used to test immunity to $\phi 80$ in bacteria which are Tl^r. Bacterium 1485 lysogenized with $i^{\phi 80} h^{\lambda}$ was the parental stock for the present studies.

The extent of genetic homology between λ and $i^{\phi 80} h^{\lambda}$ was scanned by performing crosses of $i^{\phi 80} h^{\lambda}$ with various λ_{sus} mutants. Hybrid $i^{\phi 80} h^{\lambda}$ gave evidence of containing sus A⁺, B⁺, C⁺, E⁺, F⁺, N⁺, P⁺ and R⁺. That the whole left hand arm (sus A through h, Fig. 1) of the hybrid stemmed from λ is suggested by the fact that $\phi 80$ itself cannot contribute functions of sus A⁺, B⁺ or C⁺ in complementation tests with λ sus⁻ for these alleles, whereas $i^{\phi 80} h^{\lambda}$ can. Since $\phi 80$ can provide sus N⁺, P⁺ and R⁺, as shown in complementation and recombination tests, it is likely that the right-hand arm of $i^{\phi 80} h^{\lambda}$ stems from $\phi 80$.

Media: Tryptone broth: 10 g Bacto tryptone, 5 g NaCl, 1 liter H₂O; 12.5 g Bacto agar added for plates; 7 g Bacto agar added for soft agar top layer.

Minimal medium (Vogel and Bonner, 1956) with 0.2% glucose and 0.2% acid hydrolyzed casein (= MCA), supplemented when desired with 20 γ /ml L-tryptophan, solidified with 1.5% Bacto agar.

Induction and superinfection of lysogenic bacteria: Bacteria were grown in MCA + tryptophan or in broth to a concentration of 3×10^8 cells/ml, UV-irradiated (broth cultures are transferred to saline before irradiation), diluted 2X into broth with superinfecting phage, diluted further 0-100X after adsorption of the superinfecting phage, and allowed to lyse during 2 hours at 37°C with aeration. The lysates were sterilized with chloroform. For superinfection with λ , the bacteria must be starved by aerating in 0.01 M MgSO_4 at 37°C for 40 minutes before UV irradiation.

RESULTS

Initially it was observed in 1485($\underline{i}^{\phi 80} \underline{h}^{+\lambda}$) that Tl^{r} mutants which simultaneously suffered deletion of tryp genes of the bacterium also occasionally became defective lysogens: the bacteria were still immune to $\phi 80$; however, few if any infective phage particles were released. A similar observation has been made in E. coli B($\underline{i}^{\lambda} \underline{h}^{+\phi 80}$) (Signer, in preparation). This situation was investigated further.

Tl^{r} mutations occur spontaneously in E. coli at a frequency of 10^{-6} to 10^{-7} . A large number of Tl^{r} mutants were isolated, some of which required tryptophan. To assure that each mutant was the result of an independent mutational event, the following procedure was employed. A series of broth culture tubes was inoculated with 1485($\underline{i}^{\phi 80} \underline{h}^{+\lambda}$) to a concentration of 10^3 cells/ml. These were grown with shaking at 37°C to 4×10^8 cells/ml. All these initial sub-cultures were shown to produce infective phage in quantity. From each of twenty individual cultures, about 10^9 bacteria were spread on broth-agar with 10^9 T1 phages. Of the Tl^{r} survivors, those mutated at the T1-receptor gene adjacent to tryp (as opposed to the T1-T5-receptor gene unlinked to tryp) were recognized by their small colony size. These were purified by streaking twice, and were tested for their ability to produce infective phage. They were also tested for immunity to $\phi 80$ by spot testing with $\underline{i}^{\phi 80} \underline{h}^{+\lambda}$. The $\text{Tl}^{\text{r}} \underline{tryp}^-$

colonies were located by their ability to grow when replicated to MCA agar (tryptophan-free); they constitute about 5% of the Tl^R mutants. Tl^R mutants are considered to represent independent mutational events if they arise in different subclones, or if they differ from the other Tl^R 's from the same tube in any of the characteristics tested (Table I).

TABLE I

Number of independent isolates from 1485($i^{\phi 80}_h^{+\lambda}$) which were Tl^R and distinguishable by the characteristics given.

	<u>tryp</u> ⁺	<u>tryp</u> ⁻
active lysogenic	15	3
immune defective	15	19
not immune	0	3

From the independent Tl^R mutants of 1485($i^{\phi 80}_h^{+\lambda}$), 33 isolates, immune-defective or non-immune, were selected for further study of the amount of prophage material that was retained. The following properties were examined.

1) Rare production of infective phage. The parental strain and each mutant clone was grown in MCA + tryptophan to 2×10^8 cells/ml, UV-irradiated, mixed 1:1 with broth, shaken at 37°C for 3 hours, and sterilized with chloroform. Although no lysis was evident, some Tl^R clones did produce infective phages in small amounts (about 10^{-3} X normal yield of $i^{\phi 80}_h^{+\lambda}$). The plaques formed on 1485 were weakly centered, indistinguishable from those of $i^{\phi 80}_h^{+\lambda}$, indicating normal phage growth. Nevertheless, lysogenic clones could not be derived from these plaques. A search for more defective phage types by plating on UV-induced 1485(λ) was not successful. Lysates containing the non-lysogenizing phages were able to transduce the tryp A gene (10^{-3} transductants per plaque forming phage); the transductants, though initially immune to $i^{\phi 80}_c^{+\lambda}$, became sensitive after restreaking. These sensitive tryp⁺ clones did not yield high-frequency transducing lysates, even if superinfected with $\phi 80$, indi-

cating that transduction by lysogenization cannot occur with these lysates.

2) Ability to contribute the $h^{+\lambda}$ gene. The parental culture and the $T1^r$ mutants were UV-irradiated and superinfected with $\phi 80h$ at a multiplicity of infection (moi) = 5. Lysates were tested for presence of the $h^{+\lambda}$ allele from $i^{\phi 80}h^{+\lambda}$ prophage by spot testing or plating on 1485 $T5^r$, which can adsorb phage with $h^{+\lambda}$ phenotype, but not $h^{\phi 80}$.

3) Ability to contribute $i^{\phi 80}$. The parental strain and its $T1^r$ mutants were UV-induced and superinfected with λ at moi = 3, or with sus^- mutants of λ (see 4). Lysates were tested for the presence of infective phage with the $i^{\phi 80}$ gene by plating on 1485(λ).

4) Ability to contribute sus^+ alleles of λ sus^- A $^-$, F $^-$, I $^-$, J $^-$, K $^-$, L $^-$, N $^-$, or R $^-$. Strain 1485($i^{\phi 80}h^{+\lambda}$) and its $T1^r$ mutants were UV-irradiated and superinfected at moi = 1 with each of several sus^- mutants of λ . Lysates were assayed for the presence of sus^+ infective phage by plating on a non-permissive bacterium, W3101. These lysates were also tested for the presence of infective phage with $i^{\phi 80}$ by plating on 1485(λ).

The presence of sus^+ alleles in defective prophages can be screened easily by a quick spot test procedure (Campbell, 1964). Non-permissive W3101 is poured in soft agar onto broth-agar plates. The defective lysogenic bacteria to be tested are spotted over the surface. A series of λ sus^- mutants (10^8 phage/ml) is then spotted over the defective bacteria, and the plates are UV-irradiated with 1/8 inducing dose. Any infective sus^+ recombinant phages will be detected by lysis of W3101. Control spots are necessary on W3101 of each defective lysogenic bacterium alone, and of each λ sus^- , alone or with 1485; the last test is needed because growth of λ sus^- on permissive bacteria permits a certain accumulation of sus^+ revertants.

The characteristics of the defective $T1^r$ mutants of 1485($i^{\phi 80}h^{+\lambda}$) as shown by the above tests are given in Table II. On the basis of deletion endpoints, an unambiguous linear map can be derived for most of the

TABLE II
 Examination of 1485($\underline{i}^{\emptyset 80, +\lambda}$) and its T1^r mutants for phage and bacterial markers

T1 ^r mutant #	Infective phage average burst size	Markers detected or recovered											
		try ^p h ⁺	sus J ⁺	sus I ⁺	sus K ⁺	L ⁺	sus F ⁺	sus A ⁺	sus R ⁺	sus N ⁺	$\underline{i}^{\emptyset 80}$	by recombination	
1485($\underline{i}^{\emptyset 80, +\lambda}$)	4-20	+	++	+	+	+	+	+	+	+	+	+	+
T1 ^r mutant # 1	10^{-2}	-	+	+	+	+	+	+	+	+	+	+	+
3	10^{-3}	-	+	+	+	+	+	+	+	+	+	+	+
4, 5, 14, 31		-	+	+	+	+	+	+	+	+	+	+	+
10, 22		-	++	+	+	+	+	+	+	+	+	+	+
11, 23		-	++	+	+	+	+	+	+	+	+	+	+
12, 16		-	+	+	+	+	+	+	+	+	+	+	+
20, 28		+	++	+	+	+	+	+	+	+	+	+	+
32		+	+	+	+	+	+	+	+	+	+	+	+
13, 29, 33	$<10^{-5}$	-	+	+	+	+	+	+	+	+	+	+	+
25		+	+	+	+	+	+	+	+	+	+	+	+
24		-	-	+	+	+	+	+	+	+	+	+	+
27		-	-	+	+	+	+	+	+	+	+	+	+
7, 8		-	-	-	-	+	+	+	+	+	+	+	+
6, 17, 30		+	-	-	-	-	+	+	+	+	+	+	+
21		-	-	-	-	-	+	+	+	+	+	+	+
2		+	-	-	-	-	-	+	+	+	+	+	+
9		B ⁺ A ⁻	-	-	-	-	-	-	-	+	+	+	+
18		-	-	-	-	-	-	-	-	-	-	-	-
15		+	-	-	-	-	-	-	-	-	-	-	-
19, 26		-	-	-	-	-	-	-	-	-	-	-	-

prophage genes that were scored (Fig. 1).

That a defective prophage resulting from Tl^R mutation in 1435($i_{h^{80+\lambda}}$) is actually located on the bacterial chromosome close to Tl^R and residual

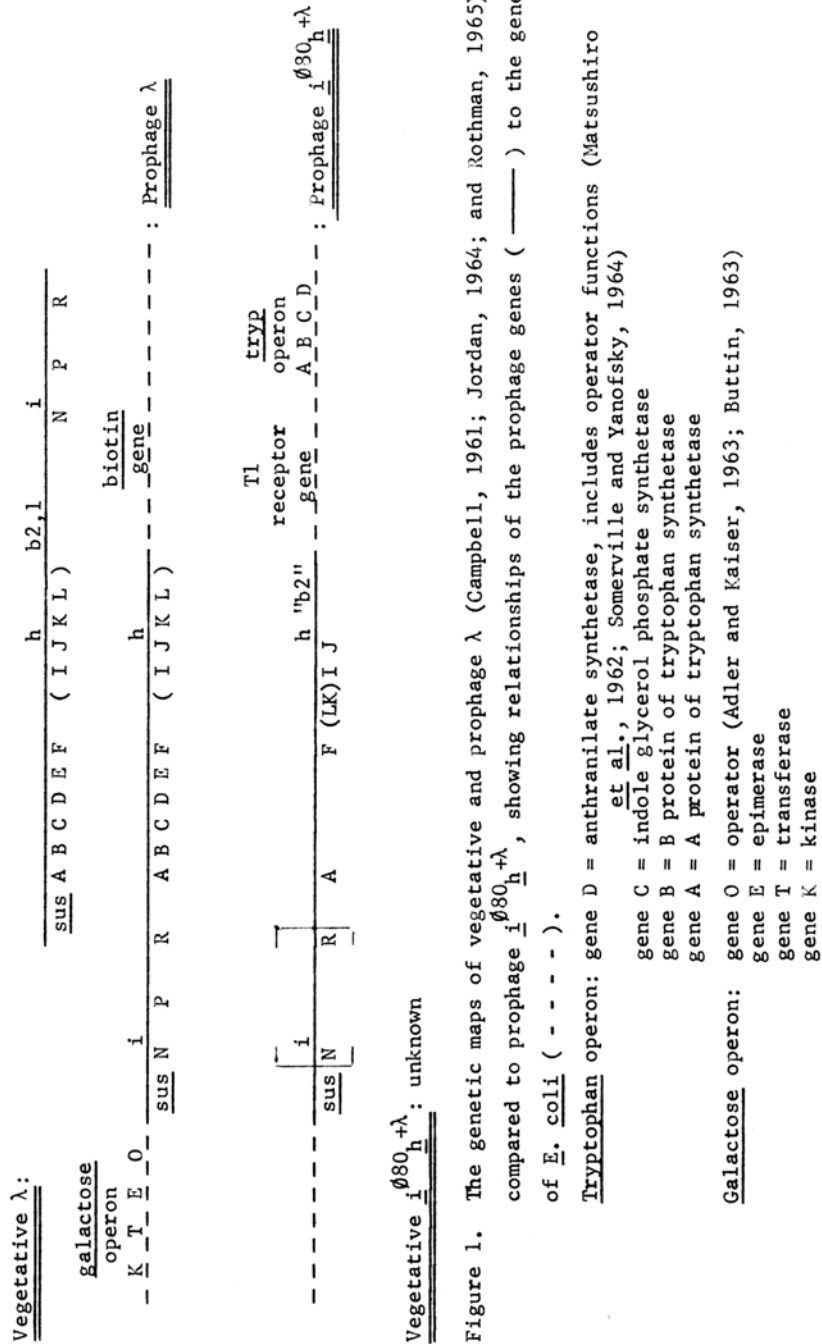


Figure 1. The genetic maps of vegetative and prophage λ (Campbell, 1961; Jordan, 1964; and Rothman, 1965) compared to prophage $i_{h^{80+\lambda}}$, showing relationships of the prophage genes (———) to the genes of *E. coli* (- - - -).

tryp genes could be confirmed by P1 transduction. Phage P1 grown on Tl^r defective #9, tryp B⁺A^{del}, was used to transduce a tryp B⁻ recipient (point mutant). The B⁺ transductants, selected by their ability to utilize indole in place of tryptophan, were of two types only: 10% B⁺A⁺Tl^s-Ø30^s, 90% B⁺A⁻Tl^rØ30^{immune}. Thus immunity to Ø30 was cotransduced with B⁺, in close linkage with tryp A^{del} and Tl^r. The absence of recombination between tryp A^{del}, Tl^r and immunity to Ø30 is one criterion for considering that these three were lost simultaneously by deletion.

The information in Table II does not permit us to distinguish between several of the more defective prophages: defective lysogenic Tl^r mutants #17, 21 and 30, which all contribute genes sus A⁺R⁺N⁺ and iØ30 upon superinfection with λ sus⁻, and mutants #9, 18 and 15 which do not contribute any of the above mentioned genes, although they show immunity to Ø30. Mutant #2 is intermediate between these two groups in that, unable to contribute sus A⁺ and immune to Ø30, it provides sus R⁺ and N⁺ poorly. When Tl^r mutants #17, 21 and 30 are superinfected with λ sus N⁻ or R⁻, substantially more i^λ sus R⁺ are recovered than i^λ sus N⁺, showing the closer linkage of sus N to i than sus R to i, as in phage λ itself. The order of sus R, sus N and i, however, is not given by these experiments.

DISCUSSION

A large proportion of the Tl^r mutants of 1435(i^{Ø30}h^{+λ}) become defective lysogens; i.e., immune to Ø30, but unable to yield infective phages in normal amount, if at all. When these defective prophages are tested for their ability to contribute i^{Ø30}, h^{+λ} and various sus⁺ alleles by recombination, it is found that the defective prophages lack varying amounts of their parental genome. [A partially deleted λ prophage has recently been described by Fischer-Fantuzzi and Calef (1964).] The deletions of i^{Ø30}h^{+λ} constitute a coherent series, allowing the interpretation that deletions of the Tl-receptor bacterial gene can extend for

varying distances into a prophage whose chromosomal location is adjacent to this gene. The deletions can terminate at various points within the prophage. Therefore, the prophage must be linearly inserted into the bacterial chromosome. This finding of linear prophage insertion is supported by recent work showing increased recombination frequencies between two bacterial markers after lysogenization with a prophage whose chromosome location lies between the two markers studied (Rothman, 1965; Signer, in preparation).

It was known previously that mutation to T1 resistance in E. coli occasionally results in the simultaneous loss of tryptophan-synthesizing ability (Anderson, 1946; Yanofsky and Lennox, 1959). Physiological and genetic studies of these T1^r tryp⁻ mutants indicated that deletions of the bacterial genes had occurred. In the present study it is seen that T1^r mutants may simultaneously lose prophage genes as well as tryp functions, supporting the interpretation that the prophage genes are also deleted. On the other hand, loss of tryp functions and prophage genes may occur independently in conjunction with mutation to T1 resistance, showing that tryp and prophage must lie on opposite sides of the T1-receptor gene. This gene order has been independently deduced (for prophage $\phi 80$) by transduction mapping with phage P1. The deletions show great variability in their points of termination on either side of the T1-receptor gene, though in K12 strains they rarely extend beyond the tryp operon on the one side, or beyond the prophage immunity gene on the other. Deletions extending into the prophage are about ten times more frequent than those affecting tryptophan prototrophy.

The coherent set of T1^r deletions entering the i ^{$\phi 80$} h^{+ λ} prophage allows mapping of that prophage in a manner analogous to the deletion mapping described by Benzer (1961) and Campbell (1961). The gene order is found (Fig. 1)/clearly the permutation of the vegetative λ gene order which has already been described for prophage λ by Campbell (1963) and by Rothman (1965). Although we do not know the order of genes in vege-

tative $\underline{i}^{\phi 30} \underline{h}^{+\lambda}$, we think it likely that it is the same as the order in vegetative λ . Certainly homology between λ and $\underline{i}^{\phi 30} \underline{h}^{+\lambda}$ is revealed by recombination between several of their genes.

Deletion mapping of prophage $\underline{i}^{\phi 80} \underline{h}^{+\lambda}$ can be used to order some of the λ genes which could not previously be ordered. The sus⁻ alleles I, J, K and L are all closely linked to h, all five being regularly replaced by gal in λ dg (Arber, Kellenberger and Weigle, 1957; Campbell, 1961). Results here show that the order of loci is (KL) I J h. This location for sus J is consonant with the finding that sus J mutants are deficient in antiserum-sensitive antigens of λ (W. F. Dove, unpublished).

Among the T1^r mutants, a class of defective lysogens is found in which the prophage retains the h^{+λ} gene and all sus genes tested, yet loses almost all ability to produce infective phage particles. A deletion of prophage genetic material at the h terminus is therefore indicated. This class of defective lysogen produces a few infective particles with an average burst size of 0.01, showing that an event of low efficiency is able to restore infectious phage. But the few phages produced, though apparently able to replicate normally, are unable to lysogenize. This behavior is ostensibly similar to that of the λ b2 mutant and the λ 1 mutant, both of which are associated with deletions of the λ chromosome (Kellenberger, Zichichi and Weigle, 1961). Furthermore, the terminal position of our deletions in apparent proximity to h is in accord with the known position of b2, linked to h (Kellenberger *et al.*, 1961), lying between i and h on the vegetative map of λ (Jordan, 1964). It appears, therefore, that a terminal prophage deletion may be the source of b2-type mutants.

The fact that prophages with terminal deletions are able to reconstitute replicating though defective structures is of importance to Campbell's (1962) model for lysogenization and induction of temperate prophages. This model predicts that in a lysogenic bacterium the prophage is bounded by duplicated regions which arose during an integra-

tional recombination between homologous regions of the phage and the parental bacterium. Recombination between such duplicated regions would allow escape of the prophage from the bacterial chromosome. A deletion of part of the member of the duplication proximal to the T1-receptor gene might account for reduced frequency of phage release. But it would not account for the release of an incomplete phage genome. Thus the incomplete phages would arise as exceptions to the above model, just as would transducing phage particles, which also are found in lysates of these terminally deleted strains.

The present experiments indicate that prophage fragments remaining after deletions even more drastic than the terminal deletion type can replicate after induction. Prophages which have suffered deletions extending beyond gene h are still, upon superinfection, able to contribute sus K⁺ or sus A⁺ with an efficiency near to that of a non-deleted prophage. Thus prophages deleted even beyond the terminus may be able to replicate normally.

When the deletion extends into sus A, however, none of the distal markers can be recovered, although the immunity function may still be in evidence. This may simply reflect limited homology between the distal prophage half, stemming from $\phi 30$, and that of the superinfecting λ test phage. But it is tempting to speculate that the boundary observed beyond prophage sus A has greater significance, since sus A marks an end of the vegetative phage.

Finally there is interest in the relationships of the prophage genes to the bacterial genes. In comparing λ and $i^{\phi 30}_h^{+\lambda}$ prophage maps (Fig. 1), it is seen that the biotin gene of E. coli bears the same relationship to the λ prophage as does the tryp operon to $i^{\phi 30}_h^{+\lambda}$. It is noteworthy that respective non-defective transducing phages can be recovered carrying these bacterial markers (Wollman, 1963; Matsushiro *et al.*, 1964), but not the galactose genes. Furthermore, the tryp and gal operons read in the same direction (right to left as written) relative to the order

of the adjacent prophage markers. If the prophages are considered as having a polarity, reading from early functions at the sus N end to late functions at the h end, then the prophage polarity is opposite, in both cases, to the operon polarity of the bacterial genes. The many implications of these relationships remain to be examined.

ACKNOWLEDGMENT

The work reported above is superficially far removed from the gene-enzyme relationships in Neurospora which first occupied me as a graduate student under Dave Bonner. His lack of inhibition fostered mobility in terms of experimental systems and approach. Yet the interest that he inspired in the examination of genetic phenomena is evident. A veteran of much excellent guidance through subsequent years of study, I still count myself most fortunate to have started in research with Dave, to have witnessed over many years his extraordinary quality and endearing nature. N. C. F.

REFERENCES

- Adler, J. and Kaiser, A. D., *Virology* 19, 117 (1963).
Anderson, E. H., *Proc. Natl. Acad. Sci. U.S.* 32, 120 (1946).
Arber, W., Kellenberger, G., Weigle, J., *Schweiz. Z. path. Bakt.* 20, 659 (1957).
Benzer, S., *Proc. Natl. Acad. Sci. U.S.* 47, 403 (1961).
Buttin, G., *J. Mol. Biol.* 7, 164 (1963).
Calef, E. and Licciardello, G., *Virology* 12, 81 (1960).
Campbell, A., *Virology* 14, 22 (1961).
Campbell, A., *Adv. Genetics* 11, 101 (1962).
Campbell, A., *Virology* 20, 344 (1963).
Campbell, A., in "The Bacteria", eds. I. C. Gunsalis and R. Y. Stanier, Academic Press, New York, p. 49 (1964).
Fischer-Fantuzzi, L. and Calef, E., *Virology* 23, 209 (1964).
Hayes, W., "The Genetics of Bacteria and Their Viruses", Blackwell Scientific Publications, Oxford (1964).
Jordan, E., *J. Mol. Biol.* 10, 341 (1964).
Kellenberger, G., Zichichi, M. L. and Weigle, J., *J. Mol. Biol.* 3, 399 (1961).
Lennox, E. S., *Virology* 1, 190 (1956).
Matsushiro, A., *Virology* 19, 475 (1963).
Matsushiro, A., Kida, S., Ito, J., Sato, K. and Imamoto, F., *Biochem. Biophys. Res. Comm.* 9, 204 (1962).

- Matsushiro, A., Sato, K. and Kida, S., *Virology* 23, 299 (1964).
Rothman, J. L., Ph.D. Dissertation, Massachusetts Institute of Technology (1965).
Signer, E. R., *Virology* 22, 659 (1964).
Somerville, R. L. and Yanofsky, C., *Bact. Proc.*, p. 88 (1964).
Vogel, H. and Bonner, D. M., *Microb. Genetics Bull.* No. 13, 43 (1956).
Wollman, E. L., *Compt. Rend. Acad. Sci. (Paris)* 257, 4225 (1963).
Yanofsky, C., *Bact. Revs.* 24, 221 (1960).
Yanofsky, C. and Lennox, E. S., *Virology* 8, 425 (1959).