GENE STRUCTURE AND FUNCTION IN THE h_{III} REGION OF BACTERIOPHAGE T4

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1. INTRODUCTION

FROM the strain of *Escherichia coli* K12S, a strain KS/4 can be isolated as a survivor from a plating of K12S with a large excess of T4D wild type phage (Edgar, 1958). The latter when plated on KS/4 give faint turbid plaques (ghost plaques) with a low efficiency of plating, *i.e.* less than 10 per cent. of the phage give a plaque. However, mutants (h) of the phage may be isolated which give clear plaques on KS/4 with close to the same efficiency of plating as on the sensitive strain K12S or S/6. These h mutants may be distinguished from wild type (h^+) on appropriate mixed bacterial platings. For example on mixtures of S/6 to K/4 in the ratio 5:1 the h mutants give clear plaques and the wild type h^+ produce turbid plaques with a clear edge. Thus the use of this mixed indicator allows the h mutants to be treated as a morphological marker for experimental purposes.

Using these procedures Edgar (1958) isolated eleven independent h mutants which gave clear plaques on the mixed indicator. From crossing the latter in all possible combinations he concluded that they occupied six closely linked sites. Further by crossing three of them to $m41$ (a mutant giving minute plaques) and $tu45$ (a mutant giving turbid plaques) Edgar showed that they occupied a region on linkage group III between $m41$ and $tu45$. The recombination frequencies based on the relatively small progenies scored from the crosses did not allow the h mutants to be arranged in an unambiguous linear order.

The clustering of the h loci on a relatively short segment of linkage group III and the suggestion that there is "phenotypic mixing" between h and h^+ characters (Edgar, 1958) similar to that found by Novik and Szilard (1951) in phage T2, which might be adopted as a test of functional allelism (Streisinger and Franklin, 1956), makes these mutants potentially useful for investigating the relationship between gene structure and function. The present paper extends the type of analysis of structure and function carried out by Benzer (1955) on the r_{II} region in phage T4B to the h_{III} region of phage T4D.

2. MATERIAL AND METHODS

Phages. The h mutants used were those of Edgar's earlier work. Mutants of the $tu45$ region which were also used were kindly supplied by Dr A. Doermann and described by Doermann and Hill (1953). The phage stocks, prepared by the method

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of Chase and Doermann (1958), were sterilised by the addition of chloroform followed by centrifugation and filtration.

Bacteria. *Escherichia coli* strain B was used as host for crosses. Strains S/6, a derivative of B (Doermann and Hill, 1953), and KS/4 were used as indicator strains for plating.

Crosses. Crosses were performed in the manner described by Chase and Doermann (1958) except that anti-serum was not used. Unadsorbed phage was determined by assay after shaking a sample from the adsorption mixture with chloroform. In no case was there more than 0.1 per cent. unadsorbed parental phage in the progeny of the crosses. In all crosses considered later in this paper the total parental multiplicities varied between 12 and 18 and in no case does the parental input ratio vary significantly from 1 : 1.

3. RESULTS

(i) Mapping the *h* mutants

Five of the original *h* mutants (*h*₄₁, *h*₄₃, *h*₄₉, *h*₅₀ and *h*₅₂ of Edgar, 1958) were crossed together in all combinations and in view of their heat sensitivity, described later, all precautions were taken to avoid exposing the phages to temperatures above 5° C. longer than was absolutely necessary to perform the crosses. Similar precautions were exercised with their progeny. In these as in all other crosses described in this paper sufficient progeny were scored from each cross so as to include at least 100 recombinants.

Two recombinant genotypes are expected from these crosses, wild type and the reciprocal double *h* mutant, and while the phenotype of the first of these is known we have no reason to expect the double *h* mutant to be lethal, or to be like a single *h* or even wild type in phenotype on the indicator strains. Its phenotype was therefore determined as follows. From two of the crosses *h*₄₁ × *h*₄₃ and *h*₄₁ × *h*₄₉ twenty wild type recombinants were backcrossed to a known wild type strain. All the progeny were wild type. Had the double *h* mutant a wild phenotype approximately half these backcrosses would have segregated single *h* mutant recombinants. Unless the double *h* mutant is lethal it must, therefore, have a single *h* phenotype on the indicator strains. To distinguish between these two possibilities fifty *h* progeny from each of the crosses *h*₄₁ × *h*₄₃, *h*₄₃ × *h*₄₉ and *h*₄₃ × *h*₅₀ were backcrossed to each of the two parents of the cross. Among the fifty *h* progeny from each of these three crosses 3, 2 and 2, respectively, failed to give wild type recombinants in either of the two backcrosses, although sufficient progeny were scored from the latter to give less than one chance in a thousand of missing the wild type recombinant (due to the vagaries of sampling) if present. We can conclude, therefore, that these anomalous *h*'s are in fact the double *h* mutants and further phenotypic classifications described later confirm this conclusion. Furthermore, the frequency with which the double *h* recombinant is recovered in these and later experiments is that expected from equality with the much larger samples of the wild type reciprocal recombinant. Since the

method of estimating the frequency of the double *h* recombinant class is clearly unsuitable for large scale determinations, twice the frequency of the wild type recombinant class has been used for mapping purposes. These frequencies allow the five *h* mutants to be ordered in the unambiguous linear sequence shown in fig. 1. This sequence has been confirmed in a number of ways. Firstly the five *h* mutants have been crossed to *tu45a* where both recombinant classes, namely *tu45h* and wild type can be scored on the indicator strains. These crosses gave the recombination frequencies given in fig. 1 and they are compatible with the previously determined linear sequence. Secondly the *tu45h* recombinant stocks obtained in this way were crossed to a different *h* mutant. This provides a simple qualitative test of the positions of

FIG. 1.—Linkage map of the *hIII* region of phage T4D. All recombination frequencies are based on more than 100 recombinants from equal parental impact crosses with total multiplicities between 12 and 16.

the two *h* mutants relative to *tu45*. For example if *hA* is crossed to *hB tu45* and *hA* lies outside the *hB-tu45* region then the majority of *h*⁺ recombinants will be non-turbid since turbids could only arise by simultaneous crossovers in both the *hA-hB* and *hB-tu45* regions. On the other hand, if *hA* lies between *hB* and *tu45* then the relative frequencies of turbid and non-turbid *h*⁺ recombinants will be reversed. In this way *hA* and *hB* may be ordered relative to *tu45*. This test again confirms the order of the *h* mutants given in fig. 1.

(ii) Possible allelism

On small samples of progeny Edgar (1958) found that four *h* mutants (*h44*, *h45*, *h46* and *h47*) failed to give wild type recombinants when crossed to *h41*, suggesting possible allelism. This tentative conclusion has been confirmed in so far as progenies have been examined large enough to rule out frequencies of wild type recombinants as low as 10^{-3} at which frequency the presence of reversions to *h*⁺ in the parental stocks makes higher resolutions impossible (Jinks, 1961).

(iii) Adsorption of h_{III} mutants to KS/4

From the approximate rates of adsorption of h_{43} and wild type to KS/4 (Edgar, 1958) it appears that the difference between the two is quantitative rather than qualitative, there being a measurable adsorption of the wild type phage. In a more detailed comparison the adsorption curves for h_{41} and wild type have been determined. The adsorptions were carried out at 35°C . using 2×10^7 mixture of h_{41}

FIG. 2.—Graphs of the log percentage unadsorbed phage of mutant h_{41} and wild type and the progeny of a cross between h_{41} and wild type against time in hours using 2.8×10^8 bacteria of strain KS/4 at 35°C . The graphs for the mutant (clear plaques) and wild type phage (turbid plaques) in the progeny of the cross are identical, hence there is "phenotypic mixing" for adsorption to KS/4.

and wild type in equal parts and 2.8×10^8 log phase KS/4. At appropriate intervals the unadsorbed phage was assayed as for the crosses. Plotting the logarithm of the percentage of unadsorbed phage against time for h_{41} and wild type phage in the mixture gives the graph in fig. 2. Both are curvilinear, an initial period of rapid adsorption being followed by a period of almost no adsorption. The approximate rate of adsorption during the first period is 0.8 ± 0.29 per cent. per minute for wild type and 2.8 ± 0.47 per cent. for h_{41} . This rate becomes virtually zero after about 8 per cent. of wild type and 80 per cent. of h_{41} phages have been adsorbed.

The fate of the adsorbed fraction of the phage appears to be similar for both wild type and mutant although the number of phage released per bacterium, *i.e.* the burst size, is 240 for wild type but only 130 for the mutant under the condition used for making the crosses. Before, however, paying too much attention to the absolute values obtained we must consider the heat sensitivity of the *h* mutants which would distort both their apparent rate of adsorption and their burst size as estimated here.

(iv) *Heat sensitivity*

All the *h_{III}* mutants and *tu₄₅* mutants *a*, *b*, *c* and *d* of Doermann and Hill (1953) are inactivated at a faster rate than wild type phage at temperatures above 25° C. Since this difference in rate of inactivation is greatest at 45° C. all comparisons were made at this temperature. Apart from this no further attempts were made to find experimental conditions which maximised this difference as it was in general sufficient for our purposes.

The heat inactivations were invariably carried out in screw cap glass containers using 5 ml. of approximately 5×10^6 phage per ml. in EHA broth. Samples of 0.1 ml. were removed at appropriate intervals and diluted in chilled broth, the dilutions having been previously estimated from pilot experiments to give between 200 and 400 surviving phage per 0.1 ml. plated. Whenever possible the control wild type phage was mixed with the mutant in 1:1 proportions before inactivation and the inactivated mixture plated on an indicator strain on which both mutant and wild type could be distinguished, *i.e.* 5:1 S/6 and KS/4 for *h*/+ mixtures and S/6 for *tu₄₅*/+ mixtures. Occasionally, because of difficulties of classification, an independent wild type control was used.

In general the inactivations were exponential for as far as 10^{-3} survival which more than covered the range over which we were primarily interested. Hence the logarithms of the percentage survival plotted against time are more or less straight lines (fig. 3). The graphs given in fig. 3 are based on as many as ten independent consistent inactivations and never less than two and each point on each occasion was based on a count of more than 100 survivors.

Somewhat more uniform slopes were obtained by inactivating single cycle stocks instead of the higher titre laboratory stocks produced by multiple cycling. The additional precisions which resulted from the single recycling was not considered worth the effort for the investigations in hand although it is clearly of importance in considering the mechanism of inactivation.

The log rates of inactivation of the *h_{III}* mutants fall between 2.62 and 96.13 times the rate for wild type, the extreme mutants being *h₄₈* and *h₅₀* respectively. Mutant *h₄₈* is included in these comparisons because it represents the lower extreme of heat sensitivity. It is not, however, included in the map given in fig. 1. Its exact location has yet

to be determined although it is within 1 per cent. recombination frequency of *h*₄₃ and it maps to the right of *h*₄₁, *h*₄₉ and *h*₅₀.

The turbid mutants of the *tu* region are about average in their sensitivity, giving rates of inactivation relative to wild type from 14.75 to 27.78. None of the other turbid loci in phage T4D, *i.e.* *tu*₄₁, *tu*₄₂, *tu*₄₃ and *tu*₄₄ of Doermann and Hill (1953) is more heat sensitive than wild type. Thus the only sensitive turbids are those which adjoin the sensitive *h*_{III} region.

(v) *The nature of the heat inactivation*

The heat inactivated phage are killed in the sense that they fail to give a "plaque" on an indicator strain of sensitive bacteria under the normal plating conditions. However, there are many stages between the successful attachment of a phage particle to a sensitive bacterium and the release of sufficient viable phage progeny to produce a plaque and a phage may be classified as dead as a result of its failure to complete any one of these stages following the heat treatment.

To obtain some indication of the stage at which this failure occurred heat inactivated phage were compared for their plaque forming and bacterial killing ability. A stock of *h*₄₁ was heat inactivated in the usual way for $\frac{1}{2}$, 1, 2, 3 and 4 hours at 45° C. and at each time interval assayed for its plaque forming ability. At the same time intervals samples of the phage were assayed for their ability to kill bacteria by adding 8×10^8 of the treated phage to 4×10^8 bacteria at 35° C.

Both plaque forming ability and bacterial killing ability fell at identical exponential rates with increasing time of exposure of the phage to 45° C. Hence a phage which is dead on the basis of its failure to produce a plaque is also dead in the sense that it fails to kill a sensitive bacterium.

(vi) *Phenotypic mixing*

In phage T2 the elements of the phage particle responsible for the specific adsorption of phage host cells are produced in a pool by the joint action of all the phage within the same bacterium. The specificity of the elements is determined by the *h* functional unit but the elements become associated almost at random with the phage genetic material during the maturation of the phage particles prior to their release from the host cell (Streisinger and Franklin, 1956). This phenomenon is known as "phenotypic mixing".

To test for a similar behaviour in T4D progeny of crosses between the *h*_{III} and *tu*₄₅ mutants and wild type were compared with their parents for their ability to adsorb to KS/4. The results for one such cross and its parental controls, *i.e.* *h*₄₁ $\times$ *h*⁺ are given in fig. 2. The log adsorption curve of the progeny is clearly intermediate between that of its two parents both in the initial period of rapid adsorption and in the

level of adsorption at which further adsorption virtually ceases, namely, approximately 60 per cent. But what is more important both the h_{41} and h^+ phage particles in the progeny while distinguishable by their plaque morphology on the indicator mixture, had identical rates of adsorption in spite of the enormous difference in rate of adsorption of the h_{41} and h^+ parents. There is, therefore, phenotypic mixing for this character.

The technical simplicity of the heat inactivations plus the exponential form of the inactivation curves which would facilitate quantitative comparisons led to an investigation of phenotypic mixing for this character. The heat inactivation curves of the progeny from crosses between the heat sensitive h_{III} and tu_{45} mutants and wild type are given in fig. 4. Although parental controls were included in all these comparisons only the curve for wild type and the most and least sensitive mutants are included in the fig. 4 for the sake of clarity. In no cross is there a significant difference between the rate of inactivation of the mutant and wild type phage in the progeny, the relative frequency of the two genotypes remaining constant throughout the inactivations. Furthermore, the curves for the progeny are intermediate between those of their parents. We have, therefore, phenotypic mixing for heat sensitivity.

The quantitative aspects of the inactivation curves reveal two different situations. For h_{41} and the tu_{45} mutants the curves are essentially biphasic with an initial slope comparable with that of the mutant parent and a final slope with a back extrapolate around 70 per cent. approximating to wild type. The inactivation curves of the remaining progenies, that is those crossing h_{43} , h_{49} , h_{50} and h_{52} by wild type, are on the other hand triphasic. The initial slope is again like that of the mutant parent, similarly the final slope with a back extrapolate around 25 per cent. approximates to that of wild type. There is, however, a class of phage in the progenies with a sensitivity which is intermediate between that of its mutant and wild type parents. This latter fraction constitutes some 50 per cent. of the total having a back extrapolate around 70 per cent. thus coinciding with that of progenies from crossing h_{41} and tu_{45} to wild type.

FIG. 3.—Graphs of the log percentage survival of mutants and wild type phage against time in hours at 45° C.

(vii) *The removal of phenotypic mixing*

A critical test of the phenotypic mixing explanation of the behaviour of the progeny of crosses between h_{III} and tu_{45} and wild type phage is provided by the results of recycling these progenies in a ten-fold excess of a bacterial host so that virtually all infections are by single phage particles. Under these conditions the mutant phage particles can only become associated with gene products of their own genes

FIG. 4.—Graphs of the log percentage survival of the progenies of crosses of the h_{III} and tu_{45} mutants to wild type against time in hours at 45°C . In all cases the graphs for the mutants and wild types in the progenies are identical, hence there is phenotypic mixing for this character. The graphs for the wild type and the two extreme mutants used in these crosses are included for comparison. The mutants fall into two classes on the basis of these graphs. (1) Including h_{41} and the tu_{45} mutants are basically biphasic and (2) including all the remaining h_{III} mutants are triphasic.

during maturation and similarly for the wild type phage. Hence, the mutant phage particles should emerge from the recycling with the heat sensitivity of the original mutant parent of the cross and the wild type phage with that of the original wild type parent. This is in fact the result obtained on recycling all the progenies described in the last section, thus supporting the explanation based on phenotypic mixing.

(viii) *Functional tests within the h_{III} - tu_{45} region*

The heat sensitivity of the h_{III} and tu_{45} mutants and the phenotypic mixing for this character can be used to test whether the same or different functions are affected in the various mutants. Basically the qualitative test consists of making a mixed infection with a pair of mutants and examining the progeny for the existence of a heat-stable,

i.e. phenotypically wild type, fraction. The progeny of such mixed infections are of course already available from the crosses made to map the *h_{III}* and *tu₄₅* mutants.

FIG. 5.—Graphs of the log percentage survival of the progenies of crosses between pairs of complementing mutants (X) of the *h_{III}-tu₄₅* system against time in hours at 45° C. The upper curve of each graph is that expected if there is complementation and the lower curve if there is no complementation. For two of the crosses the progeny of the corresponding coupling cross is included (●) for comparison. These show that the *cis* and *trans* configurations give identical results if the mutants are functionally independent, *i.e.* if they complement.

An independent quantitative expectation for the size of the heat stable fraction can be obtained from the behaviour of the progenies from a mixed infection of each mutant with wild type during heat inactivation as described earlier and graphed in fig. 4. If we denote by $S_1(t)$ the fraction surviving as a function of time at 45° C. of the progeny from

a mixed infection of a mutant and wild type and by $S_2(t)$ the corresponding survival curve for a second mutant, then if independent functions are affected by the two mutants, the heat survival curve for

FIG. 6.—Graphs of the log percentage survival of the progenies of crosses between pairs of non-complementing mutants (X) of the *hIII-tu45* system against time in hours at 45°C. The upper curve in each graph is that expected if there is complementation and the lower curve if there is no complementation. For two of the crosses the corresponding coupling cross is included (●) for comparison. These show the lack of identity of the *cis* and *trans* configurations if the mutants affect the same function, *i.e.* if they do not complement.

the progeny from a mixed infection of the two mutants should be simply the product $S_1(t) \times S_2(t)$. If the mutants affect the same function and do not interact the survival of the progeny should be the same as the parents given in fig. 3.

For each pair of mutants, therefore, there are two expectations for the rate of inactivation of their progeny from a mixed infection, one assuming that they affect the same function and the other that they affect different functions. In figs. 5 and 6 the inactivation curves

TABLE 1

Classification of the functional relationships of the h_{III} and tu_{45} mutants based on the inactivation curves in figs. 5 and 6. A plus indicates functional independence of the two mutants and a minus functional dependence, i.e. complementing and non-complementing pairs respectively.

	h_{41}	h_{49}	h_{52}	h_{50}	h_{43}	tu_{45a}	tu_{45c}
h_{41}		+	+	+	+	+	+
h_{49}			—	—	+	—	—
h_{52}				—	—	—	—
h_{50}					—	—	—
h_{43}						+	+
tu_{45a}							—
tu_{45c}							

observed are compared with these two expectations individually for each pair of mutants. The results are quite unambiguous, the inactivation curves for the progenies following one or other of the expected curves for every cross. The qualitative conclusions from these graphs are given in table 1, where a plus indicates functional independence between the two mutants.

h_{41} and its alleles (h_{44} , h_{45} , h_{46} and h_{47}) affect a different function, or belong to a different cistron (Benzer, 1955) from all the other heat

FIG. 7.—Linear complementation map of the h_{III} - tu_{45} mutants.

sensitive mutants. The remaining mutants, however, do not constitute a single functional group or cistron and mutant h_{43} appears to affect a different function from h_{49} and the tu_{45} mutants although like the latter group it does not appear to affect a different function from h_{50} and h_{52} . Clearly no unambiguous subdivision of this group of mutants into independent functional units is possible. It is, however, possible to draw a linear "complementation map" of the type used in presenting similar data from enzymatic mutants of *Neurospora* (see Fincham, 1959; Beadle, 1960; Catcheside, 1960, for review) but such a complementation map (fig. 7) is not colinear with the genetic map (fig. 1).

The data from the heat inactivations of the progenies of pairs of mutants also provide additional evidence of phenotypic mixing for heat sensitivity. Thus in the progenies of two h_{III} or tu_{45} mutants there is

always a small fraction of wild type recombinants and in the progenies of an *h_{III}* and a *tu45* mutant all four phenotypes in the progeny can be classified. It is a simple operation therefore to confirm that all the genotypes in a single progeny have identical heat sensitivity. Similarly it is easily shown that following a single recycling of these progenies in a ten fold excess of bacterial host cells the phage particles re-emerge with the heat sensitive characteristic of their individual genotypes.

(ix) *Cis-trans comparisons*

Another test of the functional independence of pairs of mutants is provided by comparisons of the heat inactivation curves of progeny from mixed infections with the same pair of mutants in the coupling (or *cis*) and repulsion (or *trans*) configurations. For mutants involving different functions, the curves should be essentially identical, otherwise the survival should be much greater with the coupling configuration. Indeed in the latter case the coupling cross might be expected to behave like that between a single mutant and wild type (*e.g.* fig. 4).

As a preliminary to these comparisons, it was necessary to isolate the coupling combinations of pairs of the *h_{III}* and *tu45* mutants. This had already been done on a limited scale to establish the *h* phenotype of the double *h* mutants which arose as recombinants during the mapping experiments. To obviate the need for some hundred backcrosses to isolate a single coupling combination a selective technique was sought. Stocks of *h_{III}-tu45* mutants in coupling were readily obtained as recombinants from the corresponding repulsion crosses on the basis of their morphology on the mixed indicator. Stocks of these double mutants had heat sensitivities which were the product of those of their component single mutants. On the assumption that this relationship would extend to stock of pairs of *h_{III}* mutants in coupling the phenotypically *h* plaques from the progeny of repulsion crosses between pairs of *h_{III}* mutants were scored for heat sensitivity. One hundred *h* plaques from each such cross were isolated into E.H.A. broth and assayed before and after heat inactivation for one hour at 45° C. From each cross four or five of the isolates had survival rates at the end of the inactivation which were much lower than the remainder. These were built into stocks in the usual way and backcrossed to both parents to confirm their double mutant nature. In every case the isolates selected on their greater heat sensitivity proved to be double mutants on the backcross test. Indeed it was subsequently shown, in properly controlled experiments, that the double *h_{III}* mutant stocks had heat sensitivities which were exactly the product of the heat sensitivities of their component single mutants.

The double mutant stocks *h₄₁h₄₃*, *h₄₃tu45a*, *h₄₃h₅₀* and *h₄₉tu45a* were all crossed to wild type and the heat inactivation curves of their progenies from the mixed infections compared with those from the corresponding repulsion crosses. In every case our earlier conclusions concerning the functional relationships of the *h_{III}* and *tu45* mutants

(figs. 5 and 6, table 1) and the expectations derived from them were confirmed.

In principle the methods used in determining the functional relationships between the *h_{III}* and *tu₄₅* mutants are directly applicable to the detection of "partial complementation" of the type which is now well known in *Neurospora* (Fincham, 1959; Catcheside, 1960; and Beadle, 1960). That is, the production of a proportion of heat-stable phage progeny by a pair of mutants in mixed infection which is less than that expected on the basis of complete functional independence of the mutants. And indeed some of the apparently non-complementing pairs of mutants, e.g. *h₄₉ × h₅₀*, *h₅₀ × h₅₂* and *h₄₉, h₅₀* and *h₅₂ × tu_{45a}* in fig. 6 and table 1 do in fact appear to give a small proportion of heat-stable phage progeny. But the heat inactivation curves for these combinations do not differ significantly from the expectations based on the functional identity of the pairs of mutants involved. Furthermore, an alternative source of a small heat-stable fraction in the progenies would need to be eliminated before considering seriously an explanation based on partial complementation. This alternative source is the possibility of a small contribution of heat-stable elements to the pool within the mixedly infected bacterial cells by the small percentage of wild type recombinant phage particles produced by all the pairs of mutants involved in these functional tests. Clearly, until the magnitude of any such contribution by the wild type recombinants is known speculation on the existence of partial complementation is fruitless.

A peculiarity shown solely by the heat inactivations of the progenies of the coupling crosses is the slight, but significant, association between genotype and phenotype for heat sensitivity so that the wild type in a progeny are more heat-stable than the mutants in the same progeny, i.e. "phenotypic mixing" is incomplete. As a consequence the proportion of wild types in the surviving progeny increases during the course of the heat inactivations. While offering no rigorous proof of the origin of this peculiarity it could arise from the small proportion of bacterial host cells which are infected by only one parent during the crosses which produce the progenies used in the heat inactivations. These will inevitably lead to the production of a corresponding proportion of the progeny in which genotype and phenotype will be completely correlated for heat sensitivity. This correlation would remain undetected in the repulsion crosses where both parents have approximately the same heat sensitivity. When, however, the parents differ so enormously in heat sensitivity as do the double mutants and wild type in the coupling crosses even a small amount of correlation is sufficient to cause detectable disturbances.

(x) *The number of elements controlling heat sensitivity*

It is quite clear that in phage T₄D as in phage T₂h the elements of the phage particle responsible for their heat stability are produced in

a pool. And while the specificity of the elements is determined by the *h_{III}* and *tu₄₅* functional units in T₄D the elements become associated more or less at random with phage genetic material during the course of maturation. In T₂h it has been shown that at least two elements are associated with each phage particle, but the number may be larger (Streisinger, 1957). The determination of this number is based on a method of Hershey and depends on the agreement between the proportion of heat stable phage emerging from a mixed infection of different multiplicities of a sensitive and stable parent and the proportion expected on the assumption of 1, 2 or more elements per phage.

Only one multiplicity has been used in the experiments with phage T₄D but similar arguments may be used to arrive at an estimate of the minimal number of elements per phage.

The mixed infections between the heat sensitive mutants and the heat stable wild type gave progenies which fell into two classes (1) giving 65 to 75 per cent. and (2) giving 25 to 30 per cent. heat stable, *i.e.* phenotypically wild type, progeny (fig. 4). In both classes only about 25 per cent. of the phage progeny had the sensitivity of the mutant parent, the balance of around 50 per cent. in those of class (2) being intermediate in sensitivity. The mere existence of the intermediates with hybrid phenotypes proves that phage particles can acquire elements from both the mutant and wild type parental contributions to the pool within the mixedly infected host cells. There must, therefore, be more than one element per phage and the following considerations suggest that there are probably two. Thus if it is assumed that there are two elements per phage and further that the mutant and wild type phage particles in mixedly infected cells contribute elements equally to the pool from which the progeny phage draw at random during maturation, then, 25 per cent. will draw both elements from the wild type contribution, 25 per cent. both elements from the mutant contribution and 50 per cent. one element from each. So far the model makes no distinction between class (1) and (2) behaviour. To explain this class difference a further assumption is required, namely, that while only one wild type element per phage is required to give the wild type heat stability for progenies which belong to class (1) both elements must be wild type to achieve this stability in progenies of class (2).

In so far as the form of the heat inactivation curves of the complementing pairs of mutants are predictable from those of the individual mutants crossed to wild type (figs. 4, 5 and 6) they can provide no independent evidence for or against the estimate of two elements per phage.

4. DISCUSSION

All the *h_{III}* and *tu₄₅* mutants used in this investigation give recombinants on crossing, there is, therefore, no structural overlap between them. There is, however, functional overlapping although every one of the mutants will complement at least one other. The pattern

of complementation is not simple. One mutant *h41* and its alleles is clearly a distinct functional group or cistron in that it complements all other known mutants in the *hIII-tu45* system. No unambiguous classification of the functional relationships of the remaining mutants in the system is, however, possible, hence no subdivisions into cistrons can be made. All the complementation on which the classification of functional relationships has been attempted is equally efficient, that is, none is "partial" in the sense used in *Neurospora* (see Fincham, 1959; Catcheside, 1960; and Beadle, 1960, for reviews) although the existence of "partial complementation" between pairs of mutants classified as non-complementing cannot be ruled out on the evidence presented here. From the functional relationships a linear complementation map can be constructed (fig. 7) but this has no correspondence with the unambiguous linear order of the mutants established by recombination studies. Thus the simplicity of the relationship between gene function and structure in the *rII* region of phage T4 which led to the formulation of the cistron concept in phage (Benzer, 1955) is not repeated in the *hIII-tu45* system.

Since internal suppressors of the heat sensitivity of the *hIII-tu45* system which map within the *III* region throw further light on the organisation of this chromosome segment further discussion will be held over until these results are presented in a later paper (Jinks, 1961).

5. SUMMARY

Wild type phage T4D adsorbs very poorly to bacterial strain KS/4 and gives turbid ghost plaques on a mixed indicator of strains KS/4 and S/6. It is possible to isolate phage mutants designated *h*, which adsorb more readily to KS/4 and give clear plaques on the mixed indicator. Most of these mutants are located within a region of about 12 map units on the third linkage group between *m41* and *tu45*.

Mapping experiments have allowed ten of these mutants to be unambiguously ordered relative to each other and to *tu45*.

At 45° C. the *hIII* and *tu45* mutants are exponentially inactivated at rates varying from 2 to 100 times that of wild type. This property can be used to test whether the same or different functions are affected in the various mutants.

Qualitatively the test consists of determining whether or not a pair of mutants can produce heat-stable phage particles when mixedly infected into bacterial host cells. A quantitative test allows the proportion of heat stable phage produced to be compared with an expectation based on the proportion of heat stable phage which each mutant of a complementing pair produces when mixedly infected with wild type phage. Further evidence in some instances is provided by *cis* and *trans* comparisons.

All agree in the classification into complementing and non-complementing pairs. The results obtained, however, do not allow the mutants to be grouped into functional units or cistrons. It is possible

to draw a linear complementation map which is not colinear with the genetic map.

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