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INTERNAL SUPPRESSORS OF THE h_{III} AND *tu45* MUTANTS OF BACTERIOPHAGE T4

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1. INTRODUCTION

THE h_{III} and *tu45* mutants of phage T4 are a functionally inter-related system within which no simple grouping into independent units or cistrons is possible (Jinks, 1961). Nor can a complementation map be constructed which is co-linear with the order of the genes established by mapping experiments. All the mutants in this system have heat sensitivities which vary between twice and a hundred times that of wild type at 45° C. Heat stable reversions of the mutants, however, are present in all the high titre stocks produced by multiple cycling in host bacteria. The revertants can be selectively isolated and their properties, which are described in this paper, throw additional light on the organisation of the h_{III}-*tu45* segment of the phage genome.

The bacterial and phage strains used and the conditions of assay and crossing are identical with those used previously (Jinks, 1961).

2. RESULTS

(i) *Isolation of reversions of the* h_{III} *mutants*

Reversions of the h_{III} mutants which gave turbid plaques on the mixed indicator (5 parts of S/6: 1 part of KS/4) similar to those produced by wild type phage are present in all high titre stocks of the mutants produced by multiple cycling in host bacteria. Their frequency is sufficiently high (approximately 10^{-3} to 10^{-4}) for direct isolation of the revertants from high density platings. The revertants, however, like the wild types they resemble in plating morphology, are more heat stable than the h_{III} mutants from which they arise.

They can, therefore, be concentrated by heat inactivation at 45° C. As a preliminary, the sample to be heat inactivated is first recycled at a low multiplicity of approximately ten host bacterial cells per phage particle so as to remove " phenotypic mixing " and hence ensure that the heat sensitivities of the phage particles correspond with their genotype (Jinks, 1961).

In general the recycled stocks were heat inactivated to 10^{-4} survivors, the time required to achieve this at 45° C. being deduced from the inactivation curves. The survivors were plated on the mixed indicator where 1 to 50 per cent. gave turbid plaques, the

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remainder giving the typical clear plaque of an h_{III} mutant. The former were isolated and made into high titre stocks in the usual way. All the h_{III} mutants gave revertants under these conditions. To ensure the independence of the revertants used in the later studies only one was isolated from each recycled sample.

The relatively high frequency of revertants in the high titre stocks of the h_{III} mutants presumably reflects a certain amount of selection for the more heat stable particles during the multiple cycling at 35° C. To obtain more realistic estimates of the reversion rate single cycle stocks made from 4 hour h_{III} plaques were made at low multiplicities. Subsequent heat inactivations gave reversion frequencies of the order of 10^{-6} to 10^{-7}.

(ii) *Isolation of reversions of the* tu45 *mutants*

Revertants of the *tu45* mutants which are non-turbid on the indicator strain S/6 occur in the high titre stocks of these mutants with frequencies around 10^{-5}. These revertants with their wild type plating morphology also possessed the wild type heat stability and hence could be selectively concentrated in the manner described earlier for revertants of the h_{III} mutants. The frequency of revertants in single cycle stocks of the *tu45* mutants made from 4 hour plaques is around 10^{-7} and always lower than the frequency of h_{III} revertants (see later for detailed comparisons, *e.g.* table 2). The higher frequency of *tu45* revertants in the high titre stocks produced by multiple cycling at 35° C. presumably reflects some selective concentration during this process.

(iii) *Nature of the revertants*

In all, 129 revertants of h_{III} and *tu45* mutants have been crossed to wild type and in every cross the progeny contained a small proportion of the original mutant phenotype, *i.e.* h_{III} or *tu45*. It appears, therefore, that the reversions are due to linked suppressor mutations and not to true back mutations. If this is the case the suppressor mutations should appear in the progeny of the crosses between the revertants and wild type with the same frequency as the original mutant, the two being reciprocal recombinants. No novel phenotype is, however, present in these progenies. This leaves three alternatives for the phenotype of the suppressor mutants (1) they resemble the parents of the crosses which are wild type on the indicator strains used, (2) they are lethal, *i.e.* they do not form plaques on the indicator strains.

That the phenotype of two suppressors of h_{III} mutants is not like that of the h_{III} mutants themselves has been established as follows. The suppressed h_{III} mutants *h41 sug3* and *h41 suo1* (fig. 1) were crossed to wild type and 20 h_{III} recombinants isolated from their progenies. All 20 were backcrossed to a stock of *h41*. All the progenies of these backcrosses were *h41* in phenotype. If the suppressor mutants *sug3*

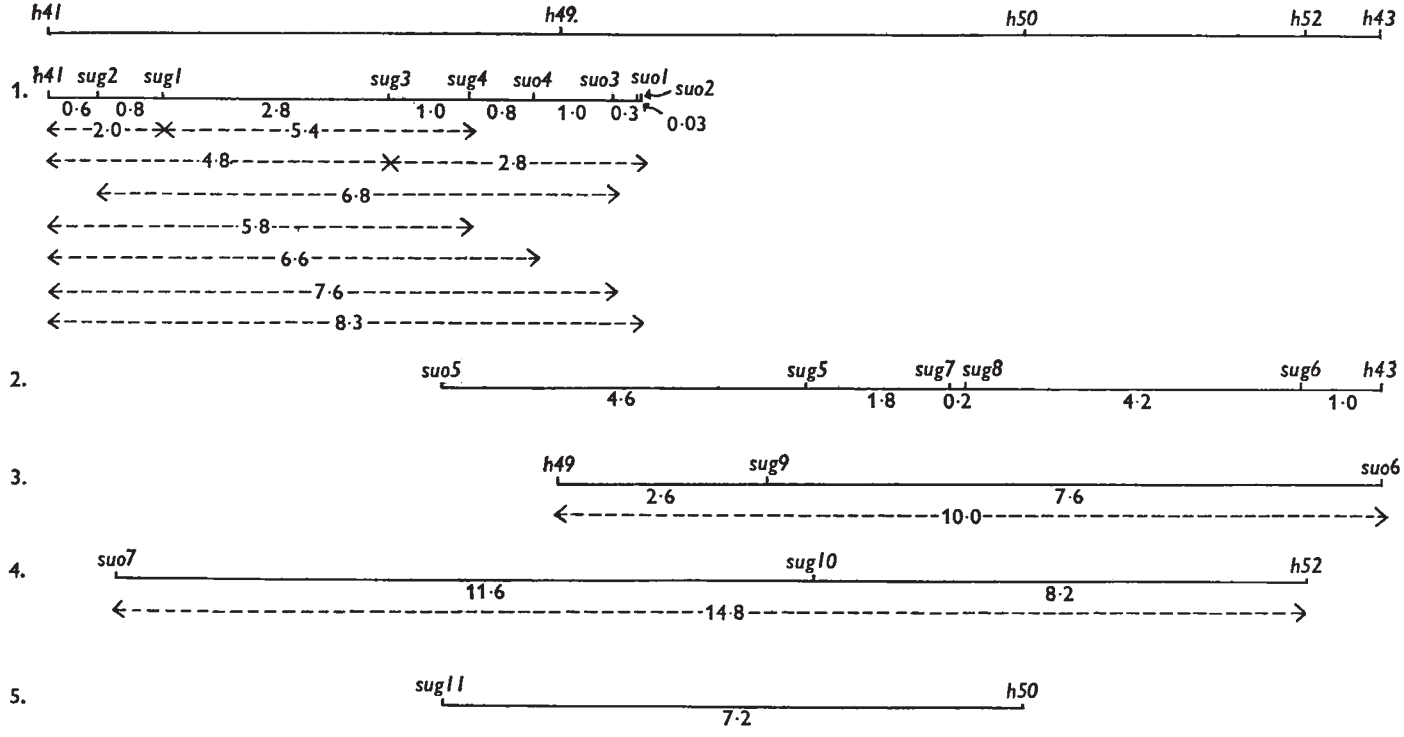

FIG. 1.—Linkage maps 1 to 5 show the positions of the suppressors of the h_{III} mutants mapped relative to the *h* mutant against which they were first isolated and to one another. The extent of the confirmatory evidence of the order of the suppressors revealed by crosses between them is only illustrated in Linkage map 1 for the suppressors of *h41*. The known length of the h_{III} region and the position of the h_{III} mutants within it are shown at the top of the figure for comparison (see Jinks, 1961 for fuller details). As reference to the text shows the relative order of the suppressors has also been confirmed by crosses to *tu45* mutants. Two types of suppressors are indicated, *sug* and *suo* which lead to wild type and non-wild type behaviour on KS/4 respectively.

and *suo1* had h_{III} phenotypes then approximately half the backcrosses would have been between these suppressors and *h41*. Hence, these backcross progenies would have included a small proportion of suppressed *h41* recombinants with their wild type plating morphology.

The absence of the latter in all 20 backcrosses suggests that these suppressor mutants do not have h_{III} phenotypes. For one of these revertants, *h41 suo1*, the more difficult task of testing whether the suppressor had a wild phenotype was undertaken. The wild type plaques obtained from crossing *h41 suo1* by wild type contain both parents and possibly the suppressor mutant, together accounting for some 96 per cent. of the progeny. Of the latter only 4 per cent. are prospectively the suppressor mutant. Ultra-violet irradiation of the parents before crossing increases recombination in phage (Epstein, 1958). Thus 4 minutes of irradiation before crossing *h41 suo1* by wild type raised the frequency of *h41* recombinants in the progeny to 17·3 per cent. and hence the expected frequency of *suo1* recombinants among the wild type progeny to 21 per cent. With this higher expectation the further analysis of only 20 wild type plaques in the progeny is sufficient to determine whether or not the suppressor mutant has a wild phenotype. Twenty wild type plaques, therefore, were isolated, built into stocks in the usual way and each backcrossed to both *h41 suo1* and the wild type parent. The suppressor mutant *suo1* will give no h_{III} plaques in the progeny of either backcross. None of the 20 isolates behaved in this way. Ten gave h_{III} plaques in the backcross to wild type and hence were *h41 suo1* in genotype while the remaining 10 gave h_{III} plaques in the backcross to *h41 suo1* and were therefore wild type. We must conclude, therefore, that *suo1* is neither h_{III} in phenotype nor wild type, in which case it must be lethal on the indicator strains used.

Clearly this technique for establishing the probable phenotype of a suppressor mutant is not readily applicable to large scale analyses and no similar analysis of the other suppressor mutants has been made.

(iv) *Phenotype of the suppressed* h_{III} *mutants*

The plaque morphology of the suppressed h_{III} mutants on the mixed indicator KS/4 and S/6 is similar to wild type and forms the basis of their recognition. On the basis of their plating properties on KS/4 alone at 37° C. the suppressed h_{III} mutants can be classified into two qualitative groups.

(1) Those which like wild type give a plating efficiency of about 10 per cent., all the plaques being turbid "ghosts". The suppressors responsible for this class will be referred to as *sug*.

(2) Those which give no plaques at all on KS/4 even at plating densities as heavy as 10^6 per plate. The suppressors responsible for this class will be referred to as *suo*.

The relative frequencies of these two classes of suppressors obtained

with the various h_{III} mutants are given in table 1. The frequencies of the two classes are those in random samples of 40 revertants isolated by the selective technique described earlier.

The adsorption curve of one of the revertants *h41 sug1* to KS/4 at 35° C. was followed in detail using wild type and *h41* as a control (see Jinks, 1961 for graphs). Under these conditions the total adsorption of the suppressed mutant was less than 5 per cent. and only 0·2 per cent. of the bacterial host cells released viable phage. These figures compare with 8 and 5 per cent. respectively for wild type.

In so far as the revertants are concentrated selectively in an h_{III} stock by heat inactivation at 45° C. they clearly have a heat stability

TABLE 1

The relative frequencies of sug *and* suo *suppressors among revertants of the various* h_{III} *mutants*

Type of suppressor / h_{III} mutant	*h41*	*h49*	*h52*	*h50*	*h43*	Total
sug	16	1	1	1	10	29
suo	8	1	1	0	1	11

comparable with that of wild type. Fourteen of these revertants equally of the *sug* and *suo* types have been compared with wild type for heat stability over a period of four hours of heat inactivation at 45° C. In no case could any difference between the revertants and wild type be detected.

(v) *Phenotype of the suppressed* tu45 *mutants*

All the reverted *tu45* mutants are less turbid on S/6 although some are still sufficiently turbid to be separable from true wild type when jointly plated on S/6. No classification of the revertants on this basis has, however, been attempted. Two types have been recognised on their behaviour on crossing to wild type and on their heat stabilities.

(1) Two of the suppressed *tu45* mutants had heat sensitivities which were identical with those of the *tu45* mutants from which they arose. Furthermore on crossing to wild type both *tu45* recombinants and a novel phenotype occurred with equal frequencies in the progenies. The latter gave plaques which were even clearer than those of wild type on S/6. These were in fact the suppressor mutant. They do not belong to the general system of suppressors under discussion in the paper, therefore they will not be considered further here.

(2) The remaining suppressed *tu45* mutants had the heat stability of wild type. On crossing to wild type no novel phenotype which might have been the suppressor mutant was present in the progeny.

(vi) *Joint suppressors of* h_{III} *and* tu45 *mutants*

Revertants of both the h_{III} and *tu45* mutants are present in the double mutant h_{III} *tu45* stocks with about the same frequency with which they occur in the single mutant stocks and they may be accumulated by selection during heat inactivation. This provides a direct comparison of the reversion rates of h_{III} and *tu45* mutants. At the same time any double reversions affecting h_{III} and *tu45* simultaneously will be recognised.

Twenty independent single cycle stocks of *h43 tu45a* and *h41 tu45a* made from four hour plaques were heat inactivated for five hours at 45° C. and the number of stocks containing h_{III}, *tu45* and joint reversions were scored by plating the survivors on the mixed indicator and on S/6.

TABLE 2

The frequencies of h_{III}, tu45 *and joint reversions in 20 independent stocks of* h41 tu45a *and* h43 tu45a

Type of suppressor / Stock	*h41 tu45a*	*h43 tu45a*	Total
h_{III} *sug* . . .	11	8	19
h_{III} *suo* . . .	6	1	7
tu45	3	4	7
h_{III} and *tu45* . . .	0	1	1

The h_{III} reversions which were recognised on the mixed indicator were tested for their turbidity on S/6. Similarly, the *tu45* reversions which were recognised on S/6 were test for h_{III} characteristics on the mixed indicator. The frequency of the three types of reversions are summarised in table 2. The h_{III} reversions are subdivided into *sug* and *suo* types.

These results confirm the greater frequency of the *sug* type revertants (see table 1) and the higher reversion rate of h_{III} mutants compared with the *tu45* mutants. One joint reversion out of 34 was recovered for *h43* and *tu45a*. Two explanations of the origin of this joint reversion are possible. Firstly, there were simultaneous mutations at a suppressor locus of *h43* and of *tu45a* and secondly, there is a class of suppressors which can suppress both h_{III} and *tu45* mutants. These alternatives are pursued later.

The heat sensitivities of the revertants confirm the classification based on plaque morphology. Thus, those in which only the h_{III} mutant had reverted had the heat sensitivities of the *tu45* mutant while those in which the *tu45a* mutant had reverted had the heat sensitivities of the h_{III} mutant they carried. The joint reversion of *h43* and *tu45a*, on the other hand, had a wild type heat stability.

(vii) *Mapping the suppressors*

In all 21 of the 129 suppressors have been ordered relative to the h_{III} or *tu45* mutants they suppress and also to one another by means of the following crossing programme.

1. All 129 of the revertants were crossed to wild type and in every cross a small proportion of the original h_{III}, or *tu45*, mutant segregated in the progeny—this is our principal evidence that the revertants are indeed due in all cases to suppressor mutations. The probable phenotype of the suppressor mutant has been established in only one case, where it was lethal. In order to determine the recombination frequency between an h_{III} or a *tu45* mutant and its suppressor all the latter mutations have been assumed to be lethal.

In practice the bias introduced by this assumption if incorrect is extremely small unless the suppressor mutant has in fact the phenotype of the mutant it suppresses, in which case the recombination frequencies will be too large by a factor almost of two.

2. Fourteen h_{III} revertants, involving the same h_{III} mutant, were crossed together to determine whether the suppressors lay on the same or opposite sides of the h_{III} mutant they suppress. Thus if they are on the same side the frequency of h_{III} recombinants in the progeny of such crosses will be the difference between the recombination frequencies of the suppressors and the h_{III} mutant. If they are on opposite sides it will be the sum of these frequencies.

3. To determine whether the suppressors of *h41* lay on the *tu45* side of *h41*, revertants of the latter were crossed to *tu45a*. If the suppressor lies between *h41* and *tu45a* then most of the h_{III} progeny will also be turbid; on the other hand if the *h41* locus lies between the suppressor and *tu45a* most of the h_{III} recombinants in the progeny will be non-turbid. The former is true of all the suppressors of *h41* and an example of the segregation observed in such a cross is included in table 3.

4. Six of the *h41* and *h43* revertants were isolated in *h41 tu45a* and *h43 tu45a* stocks and on crossing these to wild type the relative frequency of h_{III} to h_{III} *tu45* segregants in the progenies can be used to establish the order of the h_{III}, *tu45* and suppressor mutations. Thus if the suppressor lies between the h_{III} and *tu45* mutants most of the h_{III} recombinants will be non-turbid while if the h_{III} mutant lies between the other two most of the h_{III} recombinants in the progeny will be turbid. On this test all the suppressors of *h41* fall between it and *tu45a*, while those of *h43* lie on the opposite side to the *tu45* locus. Examples of the segregations observed in some of the crosses are included in table 3.

On the combined basis of these tests the maps shown in fig. 1 have been constructed for the suppressors of the h_{III} mutants.

All the suppressors of the h_{III} mutants map within the h_{III} region delimited by *h41* and *h43*. Furthermore, apart from the possible joint suppressor of *h43* and *tu45a*, all the suppressors of the *tu45* mutants

map on the *tu45* side of the h_{III} region. It is also clear that the two types of suppressors (*sug* and *suo*) of the h_{III} mutants do not map at random to the mutants against which they were isolated. Thus the *sug* suppressors invariably map closer than the *suo* suppressors to the mutant they are suppressing, that is the suppressors which produce the nearest reversion to a wild phenotype map the closest.

If we consider only the suppressors isolated against *h41* and *h43* it appears that *h49* marks the dividing line between the positions of *sug* and *suo* suppressors. Thus, all the *sug* suppressors of *h41* lie between *h41* and *h49*, similarly all the *sug* suppressors of *h43* lie between the latter and *h49*. The same relationship also holds for the two suppressors of *h52*. It breaks down, however, for the suppressors

TABLE 3

The ordering of the h_{III}, tu45 *and suppresssor loci on the relative frequency of turbid and non-turbid* h *recombinants*

Cross	Per cent. recombinants in progeny		Inferred order of loci
	h_{III} *tu45*	h_{III}	
h41 suo1 × *tu45a* . .	3·62	1·32	*h-su-tu*
h43 suo5 tu45a × + . .	4·77	1·18	*su-h-tu*
h43 sug5 tu45a × + . .	3·31	0·21	*su-h-tu*
h43 sug6 tu45a × + . .	0·35	0·12	*su-h-tu*
h43 sug7 tu45a × + . .	2·17	0·64	*su-h-tu*
h41 sug1 tu45a × + . .	0·74	3·12	*h-su-tu*
h41 sug2 tu45a × + . .	0·40	3·00	*h-su-tu*

isolated against *h49* itself, both the *sug* and *suo* suppressors falling on the same side between it and *h43*. Nevertheless, the *sug* suppressor is still the closer of the two.

(viii) *Specificity of the suppressors*

While there is a correlation between the type of suppression and the distance of the suppressor from the h_{III} mutant it is suppressing there is no association between an h_{III} mutant and the suppressors isolated against it. Thus the suppressors of *h41* and *h43* overlap in their distribution along the segment between them as do also the suppressors of *h49*, *h50* and *h52* (fig. 1). It seems unlikely, therefore, that the suppressors will be specific for a particular h_{III} mutant. Apart from one possible joint suppressor, however, there is no overlap in the distribution of h_{III} and *tu45* suppressors. It seems probable, therefore, that the suppressors of h_{III} will not suppress the *tu45* mutants and vice-versa.

Since the suppressors have not been successfully isolated alone, and indeed are probably lethal, all the evidence relating to their

specificity is necessarily indirect. The clearest evidence for non-specificity of a suppressor and the mutant against which it is isolated is obtained where two suppressors isolated against different mutants prove to be allelic in the sense that there is no recombination between them. While a number of probable examples were encountered during the initial screening of the revertants in only one case namely the *sug5* of *h43* and *sug10* of *h52* were sufficient progeny scored to leave no doubt that they were allelic in the sense defined above.

Various double h_{III} mutant stocks were available from earlier experiments (Jinks, 1961). The frequency of revertants in the stocks was compared with that of their component single mutants. From each of the single and double mutant stocks 100 independent single cycle stocks were made from four hour plaques. The reversion rates were then calculated from the observed frequency of stocks containing revertants after first selectively concentrating the latter to a known extent by heating at 45° C. In comparisons of stocks of *h41*, *h49*, *h43*, *h50*, *h41 h49*, *h41 h43* and *h50 h43* no difference in reversion frequency could be detected. The conclusion is, therefore, that the majority of the suppressors which arose in the single mutant stocks must have been capable of suppressing both mutants in the double mutant stocks.

Suppressed stocks of different h_{III} mutants can be crossed together and the frequency of h_{III} recombinants in the progeny determined by plating on the mixed indicator. From the recombination frequency between each h_{III} mutant and its suppressor and between the two h_{III} mutants the expected frequency of h_{III} recombinants in the progeny of such crosses may be estimated assuming specificity and non-specificity of the suppressors. For example, in the cross $h_A\, su_A \times h_B\, su_B$ where the known recombination frequencies a, c and d suggest the following order of the loci and our estimate of b is $d-a-c$,

$$\begin{array}{ccccccc} h_A & a & su_A & b & su_B & c & h_B \\ \longleftarrow & & & d & & & \longrightarrow \end{array}$$

the expected frequency of h recombinants is

$$\tfrac{1}{2}[a(1-b)(1-c)+c(1-a)(1-b)+ab(1-c)+bc(1-a)+abc]+ac(1-b)$$

if the suppressors are specific, and

$$\tfrac{1}{2}[ab(1-c)+bc(1-a)+abc]$$

if they are non-specific. That is, non-specificity reduces the frequency of h_{III} recombinants in the progeny by

$$\tfrac{1}{2}[(1-b)(a+c)].$$

This test of specificity reaches its maximum efficiency when the recombination frequency between the two suppressors (b) becomes very small compared with the recombination frequency between the two h_{III} mutants (d). These expectations, of course, differ if the

order of the loci is different from that in the example. However, expectations can be derived in a similar way for any order.

Table 4 contains six illustrations of this test for specificity chosen because they involve *sug* type suppressors which from other evidence are more likely to show some degree of specificity. Since this class of suppressor maps relatively close to the h_{III} mutant it is suppressing the method is not particularly efficient, that is, the two expectations in columns 2 and 3 of table 4 are not always sufficiently different to allow discrimination between the alternatives.

Evidence has already been presented that the two suppressors in the first cross in table 4 are probably allelic. The results in table 4 shows that whether or not they are allelic they are certainly non-specific; each will suppress both *h43* and *h52*. Similarly, the second

TABLE 4

The observed frequency of h_{III} *recombinants in crosses of the type* $h_A su_A \times h_B su_B$ *compared with that of the suppressors are specific and non-specific*

Cross	Observed frequency of h_{III} recombinants	Expected frequency if specific	Expected frequency if non-specific
h43 sug5 × *h52 sug10* . . .	0·0	7·6	0·0
h43 sug5 × *h50 sug11* . . .	1·9±0·2 *	7·1	1·9
h43 sug8 × *h41 suo1* . . .	3·4±0·3 *	7·0	1·8
h43 sug5 × *h49 sug9* . . .	4·6±0·5 *	5·1	3·6
h41 suo1 × *h49 sug9* . . .	3·9±0·3 *	5·8	1·1
h41 sug2 × *h49 suo6* . . .	5·9±0·4 *	5·7	5·3

* In addition to these sampling errors there may be an inherent error, which can be as high as 20 per cent., between recombination values from independent crosses. This must be borne in mind in assessing the outcome of these tests.

cross in table 4 shows that each suppressor will suppress both *h43* and *h50*. The results of the third cross, while not so clear cut, agree better with the expectation based on non-specificity of the suppressors. For the fourth and sixth cross in table 4 the difference between the two expectations is too small to discriminate between them and the observed values agree equally with either. In the fifth cross the two expectations are quite different, the observed value, however, is intermediate and significantly different from both. This anomalous result could have a number of causes. For example, where, as in this case, the recombination frequency between the suppressors is apparently small its estimation as a difference between too larger recombination frequencies is unreliable and could lead to the incorrect ordering of the loci. Unfortunately, no independent ordering of the loci can be made without assumptions about the specificity of the suppressors. On the other hand one of the suppressors may be specific and the other non-specific. The latter possibility can be tested.

Thus if the *sug* suppressor is specific the expected frequency of h_{III} recombinants in the progeny is 5·4 per cent. while if only the *suo* suppressor is specific the expectation is 2·2 per cent. While both are somewhat closer to the observed frequency the latter is still significantly different from both. We can only assume that the alternative explanation of the anomaly is the more likely and hence no conclusion regarding specificity can be made. No evidence in favour of specificity of the suppressors of the h_{III} mutants has, therefore, emerged from any of the indirect tests.

This lack of specificity among the suppressors of the h_{III} mutants contrasts with the specificity they show in their inability to suppress the related *tu45* mutants and the similar inability of suppressors of the *tu45* mutants to affect the h_{III} mutants. Thus in a random sample of 34 suppressors of h_{III} and *tu45* mutants only 1 could suppress the morphology and heat sensitivity of both. It is possible, however, that this apparent single non-specific suppressor is in fact two simultaneous mutations of a specific h_{III} and a specific *tu45* suppressing locus. Observations have been made to distinguish between these alternatives. In mapping experiments the suppressor of the h_{III} mutant (*h43*) is 0·9 per cent. recombination units from *h43* on the side away from *tu45a*. Similarly the suppressor of the *tu45* mutant (*tu45a*) is 6·1 per cent. recombination units away from *tu45a* on the far side of *h43*. Since the distance between *h43* and *tu45a* is 6 per cent. in the same units (Jinks, 1961) the suppressors of *h43* and of *tu45a* map in the same position. Thus if they are independent they must be very closely linked. It is virtually impossible to distinguish between this explanation and one based on a single non-specific suppressor. Two characteristics, however, make an explanation based on a single suppressor more attractive. Firstly, no other suppressors of *tu45* mutants map on the opposite side of *h43*. Secondly, no other suppressors of h_{III} mutants map closer to the *tu45* region. Thus our supposed double suppressor occupies a special position relative to the h_{III} and *tu45* regions, a position one would expect from the relationship between position and function shown by the specific suppressors of the h_{III} and *tu45* mutants.

3. DISCUSSION

The functional relationships of the genes in the h_{III}-*tu45* system can be portrayed in a linear complementation map the essential features of which, apart from its non-correspondence with the linkage map, is that the *tu45* mutants are functionally inseparable from *h49*, *h50* and *h52* but functionally different from *h41* and *h43* (Jinks, 1961). The suppressors show no corresponding groupings in their specificities. Indeed the only consistent specificity among the suppressors is between those which suppress the h_{III} mutants and those which suppress the *tu45* mutants. Thus the suppressors detect a functional difference

between the h_{III} and *tu45* mutants which is not revealed by the complementation tests. On the other hand there appear to be suppressors which do not detect functional differences recognised by the earlier complementation tests. For example, the evidence suggests that most, if not all, the suppressors of *h41* will suppress the other functionally independent h_{III} mutants. A further, though less well established example, is the probable joint suppressor of the functionally independent *h43* and *tu45* mutants.

Although there is no basis for the grouping of the suppressors into functional units or cistrons similar to the complementation test used to classify members of the h_{III}-*tu45* system, they can be grouped into three classes: those which suppress only the h_{III} mutants and map internal to the h_{III} region, those which suppress only the *tu45* mutants and the one suppressor which probably suppresses both. These three classes show no physical overlap on the linkage map; the only mutant which appears to suppress both lying between the other two classes. Thus on this classification there is a correspondence between the spatial and functional relationship of the suppressors.

The class which suppresses the h_{III} mutants has been further subdivided into two types, designated as *sug* and *suo*, on the basis of their plating efficiency on bacterial strain KS/4. There is a complete correspondence between the type of suppressor they lead to and the spatial relations between the suppressor and the h_{III} mutant against which they were both isolated and tested, the *sug* type, in all cases, resulting from the closer suppressors. Although deliberately looked for, but of necessity by indirect tests, no evidence of a different specificity between the two types of suppressors has been found. Indeed, what evidence exists suggests that most, or all, the suppressors of one h_{III} mutant will suppress all others. If this is true then it follows that there is only one type of suppressor of the h_{III} mutants but two types of suppression, the latter depending on the map distance between the suppressor and the h_{III} mutant it is suppressing. For example, if, as seems likely, *sug2* of *h41* (fig. 1) can suppress *h43* and since suppressors of *h43* as distant as *sug2* give a *suo* type of suppression (*e.g. suo5* of *h43*) then *sug2* of *h41* should give a *suo* type of suppression in combination with *h43*. A test of this and similar predictions has yet to be made.

Linked suppressors in microorganisms are rare, the only example in the literature being a single suppressor of leucineless in *Salmonella typhimurium* (Smith-Keary, 1960). "Internal suppressors" of one mutant in the r_{II} system have, however, been found in phage T4 (Feynman, personal communication). Unlinked suppressors are somewhat more common possibly because of the relative ease with which they can be distinguished from true back mutations (see Smith-Keary for review). It is, therefore, remarkable that not only has no example of a true back mutation been picked up in the h_{III}-*tu45* system but that only three unlinked suppressors have

been recovered for the h_{III} mutants, that is, some 2 per cent. of the total.

This study of the suppressors of the h_{III}-*tu45* system has clearly not simplified the rather complex picture which emerged from the earlier complementation tests. On the contrary, the specificities of the suppressors lead to subdivisions of the members of this system into different classes from those delineated by the earlier test. However, perhaps it is naive to expect that a classification of genetic material based on functional relationships should be independent of the functional test applied.

4. SUMMARY

All of the h_{III} and functionally related *tu45* mutants are inactivated at 45° C. with rates ranging from twice to a hundred times that of wild type. Heat stable reversions have been obtained from all of those mutants with frequencies from 10^{-6} to 10^{-7} by strongly inactivating stocks of the mutants.

Of 129 independent reversions examined all are due to suppressor mutations rather than back-mutations. None of the suppressors have been isolated alone. In two cases it has been determined that the suppressors do not have an h_{III} phenotype, while in one of these cases the suppressor has been shown to be lethal. All are closely linked.

The suppressors may be classified as follows.

1. Those which suppress only h_{III} mutants and map within the h_{III} region.
2. Those which suppress only *tu45* mutants and map outside the h_{III} region at the *tu45* end.
3. One example which suppresses both h_{III} and *tu45* mutants and maps between the other two classes.

These three classes thus show a correspondence between spatial relationships and specificity.

The first class may be subdivided into two. Those designated *sug* which plate on KS/4 with the same efficiency as wild type and those designated *suo* which are less leaky on KS/4 than wild type. The former map closer to the h_{III} mutant against which they were isolated than do the *suo* type. There is no evidence of differences in specificity between these two types, most or all appear to suppress all h_{III} mutants.

The functional relationships within the h_{III}-*tu45* system previously established by complementation tests are re-examined in the light of the specificities of the suppressors.

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