THE *h* REGION OF BACTERIOPHAGE T2H, WITH SPECIAL REFERENCE TO AN ANOMALOUS MUTANT[1]

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IN bacteriophage T2 the host range (*h*) region controls the specific adsorption properties of the phage particles. The original isolate, T2h^+, adsorbs to *E. coli* strain B, but not to the mutant strain B/2. Spontaneous T2*h* mutants can be isolated from T2h^+ which now adsorb to both cell strains. STREISINGER and FRANKLIN, working with T2L, first investigated the genetic properties of this host range region (1956). They could detect no h^+ recombinants in crosses between different *h* isolates; however, crosses between different h^+ isolates derived from an *h* mutant usually yielded a small number of recombinants. From the mutational point of view the "wild type" of the host range region is thus its *h* configuration. This determines the formation of tail fibers which adsorb both to B and B/2 bacteria (FRANKLIN 1961). One particular class of alteration to this configuration leads to the formation of h^+ fibers, which adsorb now to B, but not to B/2 bacteria.

The results reported in the first part of this paper establish the same relationship between the *h* and h^+ mutants in T2H as exists between those mutants in T2L. Eleven independently isolated h^+ mutants were found to be closely linked. and to map at five distinguishable sites. These sites were then ordered in three-factor crosses which employed the outside marker *r*13 (rapid lysis, plaque morphology mutant), which in T2H lies close to the *h* region.

During these studies one of the h^+ isolates (h^+11) showed aberrant properties. behaving in some respects as if it were a double mutant. An intensive genetic analysis was therefore made of h^+11; its mutational pattern was compared to the patterns of the other mutants before and after treatment with several mutagens. To explain the experimental results we were led to consider the possibility that the mutant was formed by an acridine-type mutational event; that is. by the addition or deletion of a base pair in the *h* region. Although we have not been able to confirm this idea, we reconsidered the types of recombinational event which could be expected to occur in crosses with mutants of the acridine type. The investigation of the properties of this atypical mutant, with a discussion of their possible implications, is the basis for the second part of this paper.

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MATERIALS AND METHODS

Bacterial strains: B, B/2H, B/2L, B/2/h. (See BAYLOR, HURST, ALLEN and BERTANI 1957).

Phage stocks: All phages used were initially derived from T2 and T2*h* (from A. D. HERSHEY). The origin of the new *h* and *h*+ mutants is given in Table 1; the phenotypes of the mutants are characterized also.

Media: Nutrient broth: Bacto-peptone, 10 g, Bacto-beef extract, 3 g, NaCl, 5 g, glucose, 10 g, distilled H_2O, 1 liter. Tryptone broth: tryptone, 10 g, NaCl, 5 g, distilled H_2O, 1 liter. M9 synthetic medium: M9 salts: Na_2HPO_4, 60 g, KH_2PO_4, 30 g, NaCl, 5 g, NH_4Cl, 10 g, distilled H_2O, 1 liter. After autoclaving, salts are diluted 1:8 into distilled water along with 1 part 4% sterile glucose solution. Final medium is .001 M for $MgSO_4$; pH *is* 7.0. T2 phosphate buffer is described in BAYLOR *et al.* (1957).

Crosses: The host cell used was B(2×10^8/ml); phage input was 8 to 10 per bacterium. After adsorption in T2 buffer, the infected complexes were diluted into broth; 35 minutes later chloroform was added to break any residual infected complexes, and the growth tubes were assayed. In certain cases, infected complexes were irradiated with ultraviolt light (UV) to increase frequency of recombination. In other cases, the infecting phage were irradiated prior to use for infection. UV dose was equivalent to 20 to 50 hits per free phage. Nearly all crosses were three-factor and included the plaque morphology marker *r*13. All mutants described in this work proved by these crosses to be in the *h* region of HERSHEY. All stocks were tested exhaustively for mutant level because very low levels of recombination were determined. Only stocks giving unequivocal results were used in the crosses.

Scoring of recombinants: To detect mutants and recombinants of increased host range, we plated directly on mixed indicator cells (B and B/2) and also on B/2 after preadsorption on B to correct for phenotypic mixing (STREISINGER 1956). The B cells were treated with UV, which reduced the number of viable cells but did not affect ability of the cells to produce phage. The recombination frequency between two *h*+ mutants, estimated by occurrence of *h* recombinants, is the quotient of twice the number of plaques observed on B/2 divided by the number counted on mixed indicator. The plaques on B/2 were scored for *r* or *r*+, enabling us to locate them relative to the *r*13 markers, just outside the *h* region.

Genotypes capable of limited growth either on B/2 or B/2/*h* were also identified indirectly after velvet transfer from the B cell master plates to B/2 or B/2/*h* replica plates.

Heat inactivation procedure: Phage suspensions were diluted into pre-warmed broth (65°C) and assayed at the end of various time intervals. This distinguishes the *h* and *h*+ phenotypes because T2*h* has about 100-fold fewer survivors after this treatment than does T2*h*+. Sample mixtures of a mutant plus either the *h* or *h*+ standard of opposite *r* form were heat treated. Comparison of survivors with an unheated control mixture showed that mutants similar to T2*h*+ proved resistant (R); those similar to T2*h* were sensitive (S). No mutants of intermediate sensitivity were found.

Nitrous acid treatment: Experiments involving nitrous acid inactivation and mutagenesis (VIELMETTER and SCHUSTER 1960) were performed by diluting phage suspensions into 0.05 M KNO_2 in acetate-acetic acid buffer, pH 4.0, at room temperature. Samples were withdrawn and assayed for survivors and mutants at various times. The *h* mutants were detected by plating on B/2 after preadsorption on UV-treated B. In general, a standard multiplicity of infection of phage was employed to avoid cross reactivation of the nitrous acid inactivated phage (BAUTZ-FREESE and FREESE, 1961; BAYLOR and MAHLER 1962). In all mutagenesis experiments, stocks with a low mutation background were selected. This precaution was necessary so that the rise in newly formed mutants could be observed.

Proflavine mutagenesis: B cells (2×10^8/ml) infected with either *r*+*h*+0, *r*+*h*+1, or *r*+*h*+11 (multiplicity of infection with 4 phage per bacterium) were exposed to 4 μg/ml proflavine during an 8-minute adsorption period in M9 medium without glucose. There followed a tenfold dilution of the adsorption tube with tryptone broth plus 4 μg/ml proflavine for 16 minutes growth at 38°C. A 100-fold dilution into dye-free broth followed, and a 60-minute growth period was permitted for phage maturation. These growth tubes were then chloroformed and assayed

TABLE 1

Origins and characteristics of the mutants

	h mutants	*h*+ mutants	Heat sensitivity	Efficiency of transfer to B/2*
Originating from *h*+0 (Hershey): heat resistant, no transfer to B/2	*h*1	*h*+6	sensitive	none
	*h*2	*h*+7	resistant	turbid
	*h*3 through *h*10	*h*+8	sensitive	none
		h+11	sensitive	0†
Originating from *h*0 (Hershey): all *h*'s are heat sensitive; all *h*'s are clear on B/2		*h*+1	sensitive	very turbid
		h+2	resistant	very turbid
		h+3	resistant	very turbid
		h+4	sensitive	0
		h+5	resistant	very turbid
		h+9	sensitive	very turbid
		h+10	resistant	turbid

h+6, 7, 8, and 11 all originated from *h*3.
* All *h*+ produce *h* mutants, which are clear on B/2, heat sensitive.
† *h*+11 produces *hs*, which is clear on B/2, very turbid on B/2/*h*, heat resistant. *h*+11 produces *h* mutants as well.

on S cells to look for an increase in *r* mutants. Increase in *h* mutants was sought by preadsorbing the treated phages onto UV-treated B cells and plating these infected complexes on B/2L.

Hydroxylamine mutagenesis: The hydroxylamine solution (HA) contained 1 M NH_2OH, 1 M NaCl in phosphate buffer, pH 7.5 (see FREESE, BAUTZ-FREESE, and BAUTZ 1961). Stock phage were diluted 50 times in the HA and held at 38°C for 18 hours, after which they were assayed.

RESULTS

Origins and phenotypes: Table 1 shows the origin of the various mutants used in this study, and also indicates their phenotypes. It can be seen that all the *h* mutants isolated are equivalent in phenotype, as are all the *hs* mutants, but that the *h*+ mutants differ from one another.

Crosses among h *mutants:* The ten *h* mutants isolated from *h*+0 were each crossed with *h*0. Also the mutant *h*3 was crossed with *h*1, *h*2, and *h*6. Several thousand progeny phages from each cross were plated on mixed indicator. No turbid plaques were detected in the progeny of any of the crosses, indicating that no recombinants of lower host range had been produced.

To test whether recombinants with higher host range might have been produced, several thousand phages from a number of the crosses were plated on B/2 indicator, and the plaques transferred by velvet to B/2/*h*. No recombinants of higher host range were detected in this way.

Crosses among h+ *mutants:* Eleven different *h*+ mutants were crossed with *h*+8. The progeny were then plated on mixed indicator. Using this nonselective technique, it was already evident that a small fraction of *h* recombinants could be detected in the progeny of eight of the crosses, showing that some of the *h*+ mutants related to mutations at different, but closely linked, sites. Three-factor crosses were then carried out between pairs of the *h*+ mutants, the third factor in all cases being the *r*13 marker. Five distinct sites were located. Five of the *h*+

TABLE 2

Extent of recombination between h+ *mutants of T2H determined by 3-factor crosses*

Parental genotypes	No. of experiments	Percent recombination $2\times(h)$*	r^+/total on B/2†
$r13h^+0 \times h^+1$	2	1.30	105/839
h^+2	2	0.03	5/77
h^+3	1	<0.001	.....
h^+4	2	1.0	55/357
h^+5	1	<0.001	.....
h^+6	2	<0.002	.....
h^+7	2	0.02	2/49
h^+8	3	0.90	85/942
h^+9	2	1.20	145/1040
h^+11	4	0.24	978/1255
$h^+0 \times r13h^+11$	1	0.24	35/132
$h^+2 \times h^+5$	1	0.005	.....
$\times r13h^+11$	1	0.16	65/438
$r13h^+1 \times h^+2$	2	0.40	266/300
h^+3	1	1.0	200/226
h^+4	1	0.66	57/357
h^+5	1	0.90	544/650
h^+6	1	0.38	106/121
$r1h^+6$	1	0.34	.....
h^+7	1	0.08	.....
h^+8	3	0.40	101/751
h^+9	2	<0.002	.....
h^+11	2	1.20	319/351
$h^+1 \times r13h^+11$	1	1.20	56/420
$r1h^+11$	1	1.20	.....
$h^+4 \times r13h^+9$	1	0.4	50/259
$\times h^+8$	1	<0.001	.....

* The percent recombination was determined by doubling the number of plaques that plated on B/2L after preadsorption on UV-treated B, and dividing by the total progeny.
† The ratio of r^+ to the total on B/2L gives the order of the h^+ sites relative to the $r13$ locus.

mutants yielded less than 0.001% recombinant phages in crosses with h^+0, and two of the other sites also showed repeats. Figure 1, prepared from the data in Table 2, represents the map of the *h* region of T2H. The overall length of the region is approximately 2 recombination units. The closest sites to be distinguished, h^+0 and h^+2, are approximately 0.03 recombination units apart.

These results with T2H agree closely with those previously reported for T2L

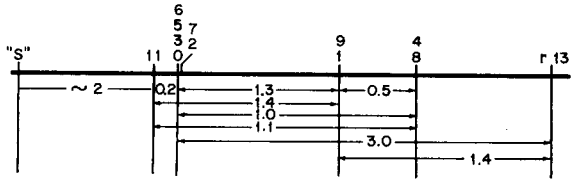

FIGURE 1.—Genetic map of the *h* region of T2H prepared from the data of Table 2.

(STREISINGER and FRANKLIN 1956). In both cases there appears to be a short region of the phage genome controlling the synthesis of the protein responsible for adsorption to B/2 bacteria—a protein which probably makes up the tail fibers of the phage particle. From the genetic analysis, the configuration restored by recobination between mutants is that which controls the synthesis of the protein conferring the *h* phenotype on phage particles. Changes in this unique configuration then produce phages with altered phenotypes, one class of which are the h^+ mutants which have lost their ability to adsorb to B/2 bacteria.

Crosses with ht *mutants:* The work of BAYLOR *et al.*, 1957, and BAYLOR, HESSLER, and BAIRD (1965) has demonstrated a number of mutations (*ht*), scattered throughout the genome, which modify the host range both of h^+ and *h* phages. These *ht* mutations in conjunction with the h^+0 allele, give turbid transfers from B to B/2H, and in conjunction with the *h* allele, give turbid transfers from B or B/2 to B/2/*h*. Differences exist among them; a "strong" *ht* in conjunction with h^+0 gives a less turbid transfer than a "weak" *ht*. These differences have been correlated quantitatively by BAYLOR and SILVER (1961) with differences in the abilities of the hth^+0 phages to adsorb to B/2H.

Combinations of various *ht*'s with different h^+ alleles at the *h* locus showed that the *ht* mutations acted nonspecifically on any of the h^+ mutations, any particular *ht* increasing the host range of all the h^+ mutants by a definite amount.

The strange case of h^+11 *crosses:* The mutant h^+11 showed unique genetic behavior and the rest of this paper is mainly concerned with an investigation of its properties.

When the progeny from the cross $h^+0 \times h^+11$ were plated on B/2H, and then transferred by velvet to B/2/*h*, two types of recombinants could be distinguished. The first gave no transfer, like the *h* recombinants found in crosses between other pairs of h^+ mutants. The second class, designated as *hs* recombinants ("super" *h*), gave a definite turbid transfer to B/2/*h*. The *h* and *hs* phages could also be distinguished by direct plating on B/2L, the former giving slightly turbid plaques, the latter producing completely clear plaques. The fraction of the progeny which yielded plaques on B/2H was 0.1%. Of these 12% were *hs* phages. The distribution of the marker *r*13 among both the *h* and *hs* progeny was predominantly that allele introduced by the h^+11 parent.

The mutant h^+11 was then crossed to other h^+ mutants (Table 3). In all cases the *hs* recombinant as well as the *h* recombinant was found. The ratio of *hs* to *h* phages in the progeny decreased as the distance between the two parent h^+ markers increased. As in the cross $h^+0 \times h11$, the *h* and *hs* recombinants showed the same distribution of the *r*13 marker.

The progeny from crosses of h^+11 with h^+0 and h^+1, which resulted from UV-irradiated infected complexes, showed an increased recombination frequency of five to ten times over the untreated control. The proportion of *h* and *hs* was the same in the control and the treated cultures. This suggests strongly that *hs* as well as *h* results from a recombinational event.

Crosses of h^+11 *with* h*:* Phages with the *hs* phenotype were found in the progeny of crosses between h^+11 and *h*, as shown in Table 3. Because the recombin-

TABLE 3

Recombination data of h+*11 with other genotypes*

Other parent*	No. of experiments	Percent recombination 2×(*h*+*hs*)†	*r*+/total on B/2	Percent *hs* of total on B/2
*r*13*h*+0	4	0.24	978/1255	12
h+0	1		35/132	
*r*13*h*+1	2	1.20	319/351	5.4
h+1	1		56/420	
*r*1*h*+1	1			
h+2	1	0.16	65/438	14
h+3	1	0.10	35/228	15.4
h+5	1	0.07	75/373	18.2
h+8	2	1.0	89/890	2.9
h+9	1	0.46	150/175	2.5
h	6	1.20	72/72	100

* The allele at the *r*13 locus of the *h*+11 parent was reversed depending on the cross. The *r*13 form of recombinant was that introduced by the *h* parent of the *h*×*h*+11 cross.
† The percent recombination was determined by doubling the number of plaques on B/2 divided by the total progeny.

ants cannot be selected by direct plating on B/2/*h*, this observation was confirmed at a higher level of resolution by a rescue experiment. A stock of *r*13*h* was treated with UV to a dose of 50 lethal hits per phage. A mixture of the treated *r*13*h* and unirradiated *h*+11 phages was then used to infect cells such that, on the average, each cell received 0.1 *r*13*h* and 8 *h*+11 phages. After adsorption the infected complexes were plated on B/2L and the plaques transferred by velvet to B/2/*h*. In the unirradiated control experiment, about one half of the large clear plaques on B/2 gave positive transfers to B/2/*h*. When the *r*13*h* parent was irradiated, most of the infected cells plated as mottled plaques, demonstrating that extensive recombination had occurred, and all of them transferred from B/2 to B/2/*h*. A similar result was found when *h*3 (the mutant from which *h*+11 arose) was used instead of *h*0. This result strongly suggests that the *hs* phages which result from *h* × *h*+11 crosses arose from a recombination event.

About 1% recombination occurs between *h* and *h*+11. To establish how many of the clear plaques on B and B/2 that transferred to B/2/*h* were true recombinants and not heterozygotes, plaques which transferred to B/2/*h* were sampled for homogeneity of genotype. Of 12 plaques tested, ten were homogeneous *hs*, one was mixed *h* and *hs*, and one was mixed *h* and a new genotype. This new genotype which has not been detected previously was turbid on B and B/2 but gave a clear transfer on B/2/*h*. Almost all the *hs* recombinants formed carry the *r*13 allele contributed by the *h* parent.

The recombination of the other *h*+ alleles with *h*0 was exhaustively tested by similar UV experiments. In no case did these crosses produce *hs* recombinants.

Mutational pattern of h+*11:* The mutants produced by *h*+11 which plate on B/2 are also of two types, *h* and *hs*. The number of mutants in the stocks varies according to the method of stock preparation. Stocks prepared by giving a small inoculum of *h*+11 to a culture of B in synthetic (M9) medium contain a high

proportion of mutants (1 in 10^4). Definite selection occurs for the higher host range mutants. Stocks prepared from confluent agar plates may contain fewer (1 in 10^7) mutants. In different stocks the proportion of *h* phages among the mutant class varies from 10 to 90%, although the *hs* generally predominates. All the *h* phenotypes tested were heat sensitive, while the *hs* mutants were all heat resistant. About 20 of each have been tested.

The most direct explanation of the genetic behavior of the h^+11 mutant, as described both by its mutational pattern and its behavior in genetic crosses, is that it is a double mutant, in which the two mutations are closely linked. The *h* mutants found in the h^+11 stocks would then be the phenotype resulting from a back mutation at one site, while the *hs* mutants would arise from a mutation at the other site.

If this were the case, then the *h* and *hs* mutants should be reciprocal, and should give h^+11 phages as recombinants when crossed with one another. To test if *h* and *hs* are reciprocal, three different *h* mutants arising from different h^+11 stocks were crossed with three different *hs* mutants. The progeny were plated on mixed indicator. No progeny phages yielding turbid plaques were produced to the level of 0.02%. It therefore seems that the *h* and *hs* mutants are not reciprocal.

Three *h* mutants which arose in h^+11 stock were crossed with h^+11, as was *h*0 as a control. In all four cases, about 1% *hs* recombinants were found in the progeny of unirradiated infected cells and up to 4% in the progeny of UV-irradiated cells. A further confirmation of the recombination of the parental h^+11 and its mutant *h* was obtained by using the *h* as a minority parent in a cross with h^+11 and plating the infected complexes on B/2L. In agreement with similar experiments with *h*0, about one half of the plaques transferred positively to B/2/*h* in the unirradiated control, and all transferred when the *h* parent had been irradiated. These results establish that *h* mutants recombine with the h^+11 from which they originated.

Crosses with hs *phage:* An *hs* mutant (isolated from a stock of h^+11), and an *hs* recombinant (from the cross $h^+11 \times h^+0$), were crossed with h^+0. About 0.5% of the progeny of each cross had the *h* phenotype. Because there was no selective method of detecting the *h* recombinant, the numbers were small. A cross was then performed in which the *hs* parent was irradiated to a dosage of 50 hits per phage and the infected cells plated on B/2 and transferred to B/2/*h*. In the irradiated sample, 31% of the plaques arising from the infected cells which had received the irradiated *hs* phages were unable to transfer to B/2/*h*, i.e., these cells no longer produced progeny phages with the *hs* phenotype. All plaques from the unirradiated sample transferred. The locus *r*13 also disappeared from the bursts receiving the irradiated parent. This result is consistent with the notion that the "*s*" mutation has a definite location (so that *h* recombinants would be expected in $h^+0 \times hs$ crosses), for upon irradiation the *h* locus would be rescued from the *r*13*hs* phages separately from the "super" locus (and also separately from the *r*13 locus).

Five stocks were prepared from *h*-like plaques arising in the previous experi-

ment. All five were heat sensitive, like *h*. Four gave normal recombinant frequencies with *r*13, one gave no recombination. It is therefore concluded that normal *h* recombinants can arise in crosses between *hs* and h^+0.

Crosses between *hs* and h^+11 were performed with and without irradiation of the parents. No *h* recombinants were detected among more than 1,000 progeny phages tested in each cross. In contrast to the *h* mutant, the *hs* mutant does not seem to recombine with parental h^+11.

Nitrous acid mutagenesis: Many host range mutants appear in increased numbers relative to the viable parent particles as nitrous acid treatment progresses. These mutants have all degrees of plaque turbidity on B/2, and their subdivision into defined groups of genotypes is difficult. The number of very turbid and small plaques increased in excess of all other types and eventually formed a background which obscured observations of the other types. One distinctive category, the *he* (host extended) mutants, plated on B/2 with large but turbid plaques and transferred positively to B/2/*h*, permitting us an extra phenotype to follow with some accuracy. Another distinctive category was the *h* mutant, which like *h*0, plated with large plaques on B/2 and did not transfer to B/2/*h*. About thirty of these *h* mutants from various experiments have been shown to have full efficiency of plating on B/2 and to be heat sensitive. Five from h^+0 were found by crosses to be within 2 to 4% of *r*13.

These five *h* mutants were made into stocks, mixed with parent h^+0, and tested for relative sensitivity to nitrous acid inactivation. They, and *h*0, were slightly less sensitive to nitrous acid inactivation than h^+0, but this difference was not sufficient to account for the large increase of mutant type.

With the important exception of h^+11 (and possibly h^+4), all other h^+ phages produced a significantly increased number of *h* mutants relative to surviving parent particles after treatment of mature phage by nitrous acid (Table 4). In general, at a dosage of about five hits the mutant level of *h* relative to the total

TABLE 4

Nitrous acid mutagenesis of h+ *mutants**

Genotype	Hits/Phage†	Increase in *h*‡	Increase in *he*‡	Increase in *hs*‡
h^+0	2.34	200	....	0
	6.2	500	4300	0
h^+1	4.7	300	~1000	0
h^+2	5.1	~2000	....	0
h^+4	5.1	100	3000	0
h^+6	5.8	660	....	0
h^+9	6.2	900	....	0
h^+11	7.1	100	3000	30
	4.35	8.4	1000	5.7
	4.2	2.1	~104	1.7

* Stocks of phage were diluted 100-fold into 0.05 M HNO_2 in acetate buffer pH 4.0. Samples were withdrawn and assayed for survivors on B and for mutants on B/2 after preadsorption on B (UV). Only the 10-minute period is presented above.

† Hits per phage calculated by e^{-x} where x = survivors.

‡ Increase in mutants determined by dividing the ratio of mutant to total in the treated by the ratio of mutant to total in the untreated sample.

number of viable particles had risen by 500 times above that in the untreated sample. Early samples frequently showed an actual increase in the number of *h* mutants over the initial number of *h* mutants. The *he* mutant type had increased to greater than a thousand times its frequency in the initial sample.

Stocks prepared of *he* mutants were all extremely unstable under a variety of methods of preparation and storage. Crosses of *r*13 with two of the *he* mutants showed that they were distantly linked to the *h* locus. The numbers of turbid plaques recovered after nitrous acid mutagenesis increased many more times than did *h* and *he* plaques. These turbids are probably *ht* mutants which occur over most of the genetic region determining external protein. Mutations from r^+ to *r* also increased.

The data in Table 4 show that the proportion of *h* and *hs* mutants to h^+11 increased slightly when h^+11 was treated with nitrous acid. On the other hand, in control experiments using artificial mixtures of h^+11 and its *h* or *hs* mutants, the treatment enriched for the mutant categories 25-fold. The selection for the mutants is sufficient to account for the increased proportion of the *h* and probably also of the *hs* mutants. The differential sensitivity of h^+11 and *hs* disappeared when the two were treated as 5-minute infected complexes (BAYLOR and MAHLER 1962), which indicates that a part of the sensitivity of h^+11 resides in its protein. We conclude that nitrous acid does not mutate h^+11.

Proflavine mutagenesis: Cells infected with either h^+0, h^+11, or h^+1, exposed to proflavine during the first 16 minutes of growth, produced no increase in the numbers of *h* or *hs* relative to the parent phage. Three different stocks of h^+11 were tested, and three replicas of the h^+1 experiment were made. A sufficient number of plaques (400 to 10,000) were inspected in each experiment and the method of screening for *h* mutants was efficient enough to establish that proflavine mutagenesis of h^+ stocks to *h* could not be accomplished under the conditions employed. In these same experiments, a significant increase in *r* mutants over background occurred (from 3 to 20-fold increase).

Hydroxylamine mutagenesis: The genotypes, h^+0 (two stocks), h^+1, h^+2, h^+4, h^+6, h^+7, h^+8, h^+9, and h^+11 (two stocks), were treated overnight in hydroxylamine and gave no increase in the numbers of *h* mutants, although the numbers of *r* and *r*-r^+ mottled plaques rose from 0.01 to 3.0%. Similar treatment of *h*0 resulted in an increase in h^+ mutants from a maximum of 0.15% in the untreated to about 3% in the treated.

Three of the hydroxylamine-induced h^+ mutants were tested for reversion with hydroxylamine and showed no increased number of *h* mutants. These same three stocks treated with nitrous acid showed a significant rise in *h* mutants. All the HA-induced h^+ mutants mapped within the *h* locus. If HA causes *h* to h^+ mutation, the base-pair transition CG to AT may be responsible. Reversion of h^+ mutants to *h* by HA would not be expected. Or the effect of HA on h^+ may not lead to a change which yields an *h* phenotype.

DISCUSSION

Apart from the anomalous results obtained with the mutant h^+11 which will

be discussed below, the properties of the host range region of T2H described in this paper are in good accord with those for T2L reported by STREISINGER and FRANKLIN (1956). The *h* configuration of the phage corresponds to the wild type, and all the h^+ mutants which have been mapped are closely linked and are contained probably in a single cistron with a map length of about two recombination units. Several lines of evidence support the notion that the *h* gene is an essential one, that is, that all alleles are functional. (1) The different h^+ mutants are phenotypically distinct; they have different heat sensitivities and make plaques of different turbidity on mixed indicator, and in fact, mutants of *E. coli* B have been isolated which will adsorb some of the h^+ mutants and not others. This suggests that each of the h^+ mutants contain a different altered *h* protein, rather than none at all. (2) Phenotypic mixing experiments demonstrate that both the *h* and the h^+ allele make products (STREISINGER 1956; BAYLOR and SILVER 1961). (3) The mutation rate *h* to h^+ and also the reversion rate of all the "well behaved" h^+ mutants can be increased by the base analogue mutagen nitrous acid, but the rates are unaffected by the acridine mutagen proflavine. This result (see also BRENNER, BARNETT, CRICK and ORGEL 1961) is to be expected for an essential gene, as nitrous acid can cause simple base transitions in DNA which could lead to the formation of protein with only slightly altered properties, while proflavine probably causes either additions or deletions to DNA and it is only in rare cases that the mutant gene could control the formation of sufficient protein for any function to be retained.

The discussion of the behavior of the mutant h^+11 can be facilitated by first summarizing its properties. These are as follows: (a) In crosses between h^+11 and *h*, about 1% of the progeny are recombinants with the phenotype *hs*, which is recognized by its ability to adsorb to B/2/*h* bacteria. (b) In crosses between h^+11 and other h^+ mutants, the *hs* recombinant occurs in addition to the *h* wild type. The percentage of the recombinants which are *hs* varies inversely with the distance between the two h^+ markers, the ratio *hs*/*h* taking on values between 0.02 and 0.2. (c) In crosses involving the closely linked outside marker *r*13 nearly all the *hs* recombinants formed between h^+11 and *h* carry the *r* allele in the form donated by the *h* parent; in crosses between h^+11 and other h^+ mutants, the *r* allele is in the form donated by the h^+11 parent. (d) The mutant *h* formed from h^+11 recombines with the h^+11 to produce *hs*. (e) Crosses between *hs* and h^+0 yield *h* recombinants which appear to be identical to the original *h*. (f) In stocks of h^+11 there occur spontaneously both *h* and *hs* phages with approximately equal frequencies. (g) The mutation rates of h^+11 to *h* and h^+11 to *hs* are affected neither by base analogue nor by acridine mutagens.

The simplest explanation of this behavior is that h^+11 is a double mutant, being compounded of one mutation which leads to the *hs* phenotype and another which leads to the *h* phenotype. This model requires that a cross between the *h* and *hs* phages arising in an h^+11 lysate should give h^+11 recombinants. Experimentally, however, no h^+11 recombinants were found. Furthermore, the *h* mutants arising spontaneously from h^+11 recombine with h^+11 to produce *hs*;

thus these *h* mutants would also have to be double mutants. A single event which produces two separable genetic changes is required to explain these data.

Because the idea that h^+11 is a double mutant cannot explain the experimental results, we considered the possibility that it was a single mutant, but that some nonclassical type of recombinational event occurred when it was crossed with its parent *h*, which yielded phenotypically recognizable recombinants. Let us represent the base sequence of the *h* by 1 2 3 4 5 6 7 8 9 — — —, and have the deletion in the h^+11 mutant missing a base pair at the position 8. Then during pairing between these two genotypes either the base pair at position 8 would loop out, allow homologous pairing to occur on either side of the deletion, and lead to classical results, or homologous pairing could occur only on one side of the deletion. Thus a short length of nonhomologous pairing might occur on the other side such that unequal crossing over could occur as follows:

1 2 3 4 5 6 7 8 9 10 11 12 ....
×
1 2 3 4 5 6 7 9 10 11 12 ....

The results of such a recombinational event would lead to the formation of two recombinants, one still with the normal complement of bases, and the other a deletion, with the missing base-pair now at position 4. Nonhomologous pairing with concomitant recombination is a mechanism for moving a deletion in either direction along the genome. No restriction as to the point at which the exchange occurs need be evoked, as only one or a few of the resulting sequences would be expected to produce an acceptable gene product. The deletion mechanism is very similar to that suggested by LERMAN (1963) to explain the origin of acridine mutagenesis, and by DEMEREC (1962) to explain the origin of "selfer" mutations in Salmonella.

All the crosses can be explained within the framework of the model of unequal crossing over if h^+11 is a deletion mutant. For example, the cross $h \times h^+11$ will yield recombinants in which the deletion has been moved to a new position in the genome. In these recombinants different lengths of the genome would be transcribed and in a number of cases this could lead to the production of phages with the *hs* phenotype. In crosses between h^+11 and other h^+ mutants, the *h* would arise from single crossovers, whereas the *hs* genotypes would need double crossovers to occur. Owing to negative interference the ratio of double to single crossovers would increase as the distance between the markers decreases, as indeed is found.

The spontaneous mutational pattern of h^+11 can also be interpreted in terms of the deletion theory. During growth of h^+11 the mutation h^+11 to *h* should occur spontaneously by the addition of a base. Then the *h* mutant arising from h^+11 can be backcrossed to the parent stock to give a unique recombinant. The origin of *hs* in stocks can be explained by the fact that *h* mutants arising during growth have an opportunity to cross with parental h^+11.

If h^+11 is a deletion and still produces a functional product, then up to the

point of the deletion most of the gene must have been transcribed. This assumes that the gene must be read from *r*13 towards the h^+11 mutation site, which is near the other end of the gene. At the moment, the data concerning the direction of reading of phage cistrons are very confusing (CRICK, BARNETT, BRENNER, and WATTS-TOBIN 1961; SARABHAI, STRETTON, BRENNER, and BOLLE 1964).

The deletion hypothesis led to the mutagenesis experiments, the results of which neither supported nor contradicted the hypothesis. On the one hand, nitrous acid increased the rate of reversion of all the *h* mutants tested except h^+11, which confirms the uniqueness of h^+11 and suggests it did not arise by a simple base substitution. On the other hand, in the critical test with proflavine, the reversion rate of h^+11 was also not increased. These results are not inconsistent with the notion that h^+11 is a deletion mutant, for it is known from the work of ORGEL and BRENNER (1961) that not all acridine-induced mutants can be reverted by acridine mutagenesis.

The suggestion that h^+11 is an acridine-type mutant, and that nonhomologous pairing which is followed by recombination can occur, is provisional and remains to be proved rigorously. The main point we would like to stress is that in considering recombination in systems where deletions are known to exist it is not certain that classical rules will always hold. In studying these problems the *h* locus has the advantage that it is potentially capable of producing many recognizable phenotypes by which the various recombinant classes can be identified. Serious consideration of the nature of enigmatic mutants, those unexplainable in terms of conventional phage genetics, is essential for a thorough understanding of the gene.

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SUMMARY

A genetic and mutagenic analysis has been carried out on 11 spontaneously occurring mutants at the *h* locus in phage T2H. All but one of these mutants have the behaviour expected for point mutations of the base analogue type. However, the exceptional mutant behaves anomalously both in crosses and with respect to its mutation pattern; its behaviour can be explained by assuming that it arose from an acridine-type mutational event.

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