

chapter 8

GENES AS DETERMINANTS OF PROTEIN STRUCTURE

Studies on the biochemical effects of mutations have given strong support to the notion that individual genes are concerned with the biosynthesis of individual proteins. In a large number of instances it has been possible to attribute the absence, or modification of an enzyme to a single gene mutation. The study of such biochemical lesions, not only in microorganisms like *Neurospora* and *E. coli* but in man and other higher organisms as well, has led to the concept of a "one gene-one enzyme" relationship, which we have already introduced in Chapter 2.

The term "gene" as used in this context has, until quite recently, been employed to convey the purely abstract concept of a unit of heredity. It represented a quantum of genetic information that in some way controlled the biosynthesis of a single protein or, in more cautious terms, of some "functional unit." The recent advances in the biochemistry of the chromosome and of DNA, and in the mapping of genetic "fine structure" of the sort we have discussed in relation to bacteriophage, now make it possible to speculate about gene action

in chemical terms instead of formal abstraction. The investigations of S. Benzer, G. Streisinger, M. Demerec and his collaborators, G. Pontecorvo, and many others have indicated that the idea of a one-dimensional array of "genes," divisible by genetic recombination, may very likely be extended down to molecular dimensions. Their results suggest that the word "pseudoallelism" needed to be invented only because of the difficulties of demonstrating extremely rare recombinations in unfavorable biological material.

If we accept the generalities of the "one gene-one enzyme" concept, and if we are willing to go along with the present trend of opinion on the role of DNA as the basic determinant of heredity, we must seriously consider the conclusion that the information which governs details of protein structure is present in the chemical structure of the DNA molecule. It is an undeniable temptation to suggest further that a point mutation is really just a very localized change in the sequence or the three-dimensional relationships within a polynucleotide chain and that such a localized change might reflect itself in the sequence and folding of the protein concerned. In spite of the fact that many investigators properly accept the generality as a working hypothesis, such speculations are, at present, mostly fancy with little fact. The pathway from gene structure to phenotypic protein may be a long and tortuous one, and we cannot rule out such possible complications as the combined action of several genes in the synthesis of a single protein or the involvement of cytoplasmic hereditary factors which might modify, or even initiate, steps in a biosynthetic pathway.

If this hypothesis is an approximately correct one, however, we should, as N. Horowitz has pointed out, be able to demonstrate mutations that lead to qualitative as well as quantitative changes in enzymes and other proteins. It should be possible, for example, to show that various mutations within a given protein-determining region of the genetic material of an organism can lead to "mutant" forms of a biologically active protein which exhibit varying degrees of functional adequacy. Mutations affecting portions of protein structure that are essential for function should be lethal ones, whereas those affecting less essential regions might either be undetected or "leaky," to use the genetic patois.

In spite of the fact that hundreds of examples have been found of gene-protein relationships, it has been possible to demonstrate a correlation between the mutation of a single gene and the chemical and physical properties of a *homogeneous* protein molecule in only a few instances. Many of these positive correlations have emerged from

studies on proteins of higher organisms for the simple reason that protein samples of sufficient purity are easier to come by with red cells, milk, and plasma than with microorganisms. However, the advantages offered by microorganisms in respect to genetic mapping has been a tremendous stimulus to gene-minded protein chemists, and it is likely that many of the major advances in this area will be made on material from such sources. If, for example, the protein whose biosynthesis is under the control of that region of genetic material in T4 bacteriophage so elegantly mapped by Benzer (see Chapter 4) could be identified and isolated in pure form, it is clear that a direct

TABLE 14
Alterations in Proteins Attributable to Mutations

Protein	Species	Demonstrated or Possible Effects of Mutation
Hemoglobin ^{1, 2}	Man	Composition and charge
	Sheep	Charge
	Mouse	Charge
β -Lactoglobulin ^{1, 2}	Cattle	Charge
Haptoglobin ²	Man	Charge
Pantothenate-synthesizing enzyme ¹	<i>E. coli</i>	Thermostability
Tyrosinase ¹	<i>Neurospora cr.</i>	Thermostability
Glutamic acid dehydrogenase ¹	<i>Neurospora cr.</i>	Reversible heat activation

For more detailed reference see:

1. N. Horowitz, *Federation Proc.*, **15**, 818 (1956).
2. D. Steinberg and E. Mihalyi, *Ann. Rev. Biochem.*, **26**, 373 (1957).

test, in enormous detail, could be made for the existence of a correspondence between "cistron" and protein. Such detail could never be achieved with human proteins because extensive gene mapping in man is limited by his lengthy generation time and his eugenic mores.

A partial list of those proteins for which gene-linked modification has been demonstrated is presented in Table 14. With one exception, human hemoglobin, the difference between the normal protein and that obtained from the mutant has been in electrophoretic mobility, heat stability, and serological behavior. The net charge, stability, and serology of a protein are, of course, quite distinctive characteristics, and the proteins in Table 14 which have been studied in respect to these parameters can almost certainly be assumed to exist

in forms whose differences are related to allelomorphous genes. Nevertheless, small organic molecules, tightly bound to proteins, can modify charge, and polysaccharides or other haptenic substances may influence antigenicity. For such reasons, the case of human hemoglobin is a particularly favorable one, since for this protein the electrophoretic and solubility differences between mutant forms are attributable to actual modifications in amino acid sequence.

In 1949, L. Pauling, H. A. Itano, S. J. Singer, and I. C. Wells¹ made the important observation that the hemoglobin of sickle-cell anemics is electrophoretically abnormal and that in individuals with sickle-cell trait (an asymptomatic condition) a mixture of the abnormal sickle-cell and the normal forms could be demonstrated. Extensive study of the familial relationships of sickle-cell anemia has indicated that this frequently fatal disease is inherited in a Mendelian fashion. By an analysis of the genetic relationships between sickle-cell anemia and sickle-cell trait, J. V. Neel established that the production of the abnormal hemoglobin was due to the presence of a single mutant gene. Genetically, the anemic may be characterized as homozygous for the sickling gene and the individual with the trait as heterozygous.

The studies of Pauling and Itano and their collaborators, together with the discovery by H. Hörlein and G. Weber² of a congenital methemoglobinemia involving an abnormal globin component, stimulated the search for other genetically linked aberrations in hemoglobin synthesis. At present writing a dozen or more types of abnormal hemoglobins which may be detected by their unusual physical properties are known. In addition, there are a number of clinical situations in which detection depends on hematologic examination but no changes in the physical properties of hemoglobin have been observed. Such abnormal individuals have microcytic red cells or cells showing some other deviation from the normal morphology of erythrocytes. These instances of inhibition of synthesis of normal hemoglobin are collectively named thalassemia, and Allison has proposed, on the basis of the observation that the locus controlling the thalassemia effect does not appear to be allelomorphous with the normal hemoglobin gene, Hb^A , that the locus for thalassemia be designated Th . The normal gene at this locus would then be termed Th^N and the thalassemia allele, Th^T .

Examples of the clinical nomenclature and genotypic designations for a number of abnormalities involving the hemoglobin molecule are given in Table 15. This compilation is taken from the excellent review by Itano to which the reader is referred for more detailed information. For our present purposes it is sufficient to recognize,

TABLE 15
The Human Hemoglobins*

Method of Detection		Method of Detection	
A	Normal adult	E	Electrophoresis
F	Foetal	G	Electrophoresis
X	Electrophoresis	H	Electrophoresis
S	Electrophoresis	I	Electrophoresis
	Solubility	J	Electrophoresis
	Tactoid formation	M	Spectrophotometry
C	Electrophoresis		
D	Electrophoresis and solubility		

Nomenclature of Syndromes Associated with Abnormalities in Hemoglobin Metabolism		
Condition	Genotype	
	Hb Locus	Th Locus
Homozygous		
Normal	$Hb^A Hb^A$	$Th^N Th^N$
Sickle-cell anemia	$Hb^S Hb^S$	
Hemoglobin C disease	$Hb^C Hb^C$	
Thalassemia major	$Hb^{t^h} Hb^{t^h}$	
Thalassemia major		$Th^T Th^T$
Heterozygous		
Sickle-cell trait	$Hb^A Hb^S$	
Hemoglobin C trait	$Hb^A Hb^C$	
Sickle-cell hemoglobin C disease	$Hb^S Hb^C$	
Thalassemia minor	$Hb^A Hb^{t^h}$	
Thalassemia minor		$Th^N Th^T$
Sickle-cell thalassemia disease	$Hb^S Hb^{t^h}$	
Hemoglobin C thalassemia disease	$Hb^C Hb^{t^h}$	
Doubly heterozygous		
Sickle-cell thalassemia disease	$Hb^A Hb^S$	$Th^N Th^T$
Hemoglobin C thalassemia disease	$Hb^A Hb^C$	$Th^N Th^T$

* From a review by H. Itano, *Advances in Protein Chemistry*, volume 12, (C. B. Anfinsen, M. L. Anson, K. Bailey, and J. T. Edsall, editors), Academic Press, p. 215, 1957.

first, that some of the various abnormal hemoglobins (Hb^c , Hb^s , etc.) are under the control of a series of genes which seem to be allelic (they are, perhaps, pseudoallelic) and, second, that certain other abnormalities, inclusively termed thalassemsias (Th^T , Th^T , etc.) involve genetic abnormalities for which no physical or chemical reflection in the structure of the hemoglobin molecule has been observed and which appear to be associated with genetic loci different from the Hb^A locus.

Let us now examine what chemical data we have. The differences observed by Pauling, Itano, and their colleagues in the electrophoretic mobility of normal and sickle-cell hemoglobin might be ascribed to modifications in the amino acid sequence leading to the introduction or deletion of charged side-chain groups. On the other hand, such charge differences might be apparent only and could reflect the manner of folding of the polypeptide chains of the protein to expose or to mask charged groups in response to configurational change. A direct test of these hypotheses has been made by V. Ingram,³ who has examined the details of sequence in the molecule (Figure 78) by means of the sensitive "fingerprinting" technique described in the previous chapter. His investigations have made it extremely likely that both sickle-cell hemoglobin and hemoglobin C differ from normal hemoglobin in only a single amino acid residue. The affected portion of the protein is shown in Figure 79. A glutamic acid residue in Hb^A has been replaced with valine and lysine, respectively, in Hb^s and Hb^c . The corresponding changes in net charge per mole (plus 2 for Hb^s and plus 4 for Hb^c , with respect to Hb^A) agree with that to be expected from the electrophoretic measurements, and no evidence has been obtained for other changes in sequence in the rest of the molecular structure of the protein. We have here, then, a direct test of the proposition that a mutation in a specific genetic locus causes a specific change in the covalent structure of the phenotypic protein related to this locus. Indeed, Ingram's experiments are a test with a vengeance. Not only do the allelic Mendelian genes Hb^A , Hb^s , and Hb^c have to do with a very restricted aspect of structure, but they all appear to be related to the *same* aspect, namely the sequence at one unique point. If the sequence of nucleotides in the polynucleotide chain of DNA determines polypeptide sequence, how can we explain the fact that these three genetically segregatable loci all influence the same position in the polypeptide?

A particularly intriguing possibility for explaining Ingram's results comes from a consideration of the theoretical model of Watson and Crick for DNA structure. The obligatory pairing of heterocyclic

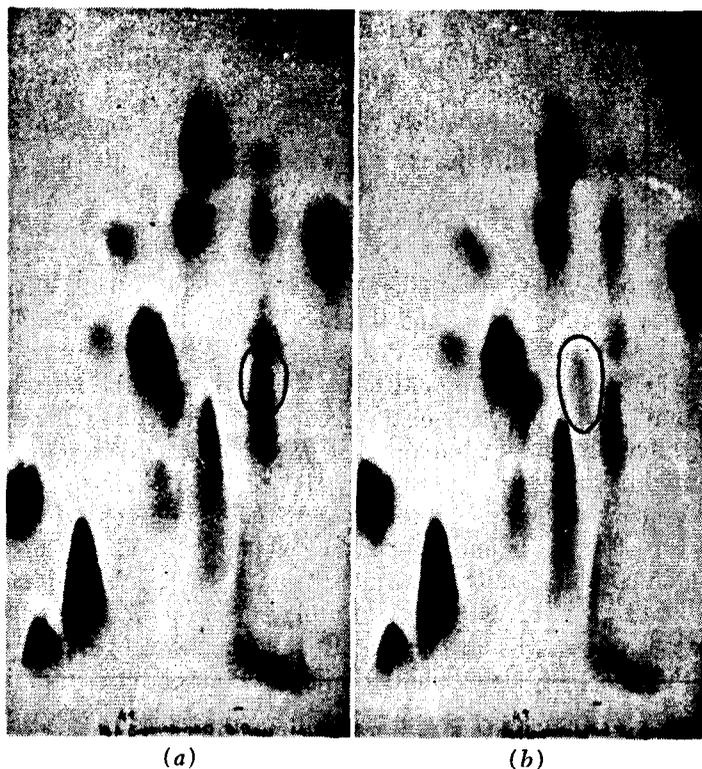


Figure 78. "Fingerprints" of the peptides produced by digestion of normal hemoglobin (a) and sickle-cell hemoglobin (b) with trypsin. The "fingerprints" were obtained by a combination of electrophoresis and chromatography, more or less as described in Figures 71 and 72. The encircled areas in the figure show where the fingerprints differ significantly. From V. M. Ingram, *Nature*, 180, 326 (1957).

bases in this structure has, as we have discussed earlier, been suggested as a basis for the accurate self-duplication of DNA strands. The specific sequences of the bases in the complementary strands of the double helix have also been viewed as a set of coded genetic information which might serve as the fundamental template for protein synthesis. The most popular code form has been one based on "triplets," in which various sets of three nucleotides correspond to a specific amino acid. Employing this idea, we may arbitrarily translate the sequence of amino acids in hemoglobin that differs in the three mutant forms into a corresponding nucleotide code as shown in Figure 80. The replacement of a single nucleotide with another within the critical trinucleotide sequence would give us the required

change in code. (The reader will obviously not take all this too seriously. The most improbable hypotheses in science have turned out to be true, however, and this one certainly deserves some serious consideration for its novelty and coherence.)

One very interesting question is raised by the existence of three mutant forms of hemoglobin differing from one another in respect to a single "locus." Why, with some 300 amino acid residues in a hemoglobin monomer to choose from, has the accident of mutation occurred, and been perpetuated, in the same place three times? The phenomenon is qualitatively reminiscent of the results obtained by Benzer in his analysis of mutants in the *rII* region of bacteriophage T4 where he observed that, out of many hundreds of mutant colonies selected, a disproportionately great number involved mutation in the *same* genetic locus, whereas others were modified only rarely. The nonrandom distribution of affected loci, both in the bacteriophage case for which we have a good deal of genetic information, and for human hemoglobin for which we unfortunately have very little, might mean that only certain mutations are "permissible" and that the degree of permissibility is slight in most of the genetic material. We might equally well suggest, however, that some unsuspected peculiarities of DNA structure favor the modification of some lengths of nucleotide sequence more than others. Most probably, the mutant hemoglobin genes have been preserved because of the selective advantage they have conferred on the affected individuals. (Sickle-cell anemia, for example, is correlated with decreased susceptibility to clinical malaria.)

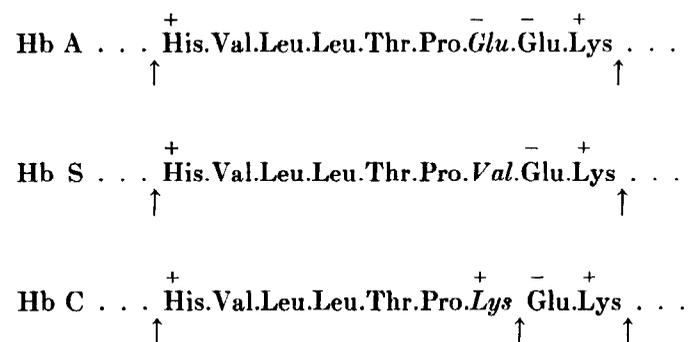


Figure 79. The differences in amino acid sequence between normal hemoglobin, sickle-cell hemoglobin and hemoglobin C. The arrows indicate the points of attack by trypsin which have led to the production of the peptide fragments shown in the figure.

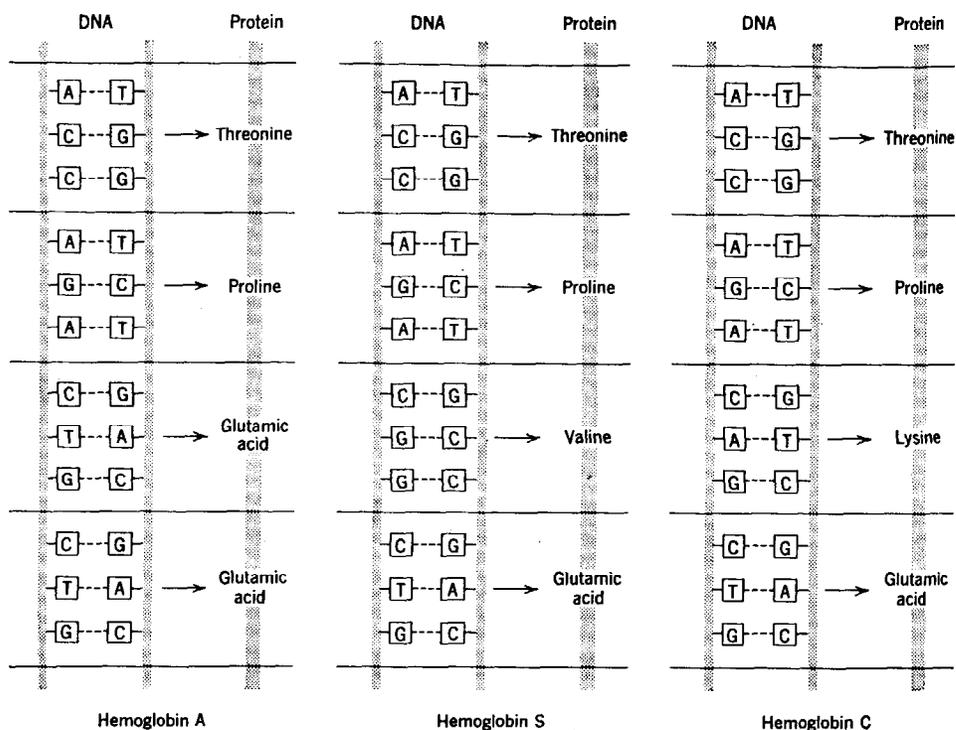


Figure 80. A hypothetical scheme showing how the structure of deoxyribonucleic acid might be related to the structures of hemoglobin A (normal hemoglobin), hemoglobin S (sickle-cell hemoglobin), and hemoglobin C. The diagram suggests a correspondence between triplets of purine and pyrimidine bases and individual amino acid residues. A change in the base sequence corresponding to the third amino acid from the top of the drawing could conceivably lead to the changes in code required for the modifications in sequence shown in Figure 79. The reader should be very much aware of the completely speculative nature of this diagram.

In the absence of further chemical data of the sort available for the human hemoglobins, it may be of value to examine in more detail some of the research now in progress which can be expected to settle some of the problems that we have posed. Many groups of investigators are busily engaged in attempts to isolate and characterize particular proteins from organisms that differ in genotype by a single mutation. We have already referred to the studies of Horowitz and Fling (Chapter 2) on the tyrosinases of *Neurospora* mutants in which differences in heat stability and activation energies of thermal inactivation were demonstrated. Rigid purification of these tyrosinases,

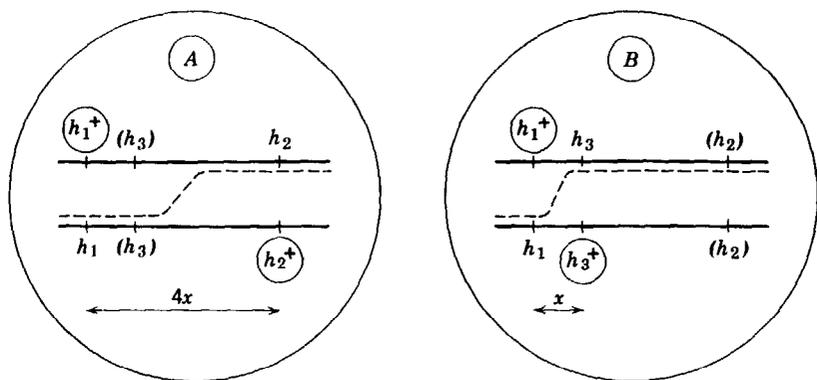
and subsequent study of their chemical structure, may well lead to another situation like that of the hemoglobins for which the direct chemical consequences of mutation can be shown. Others of the protein systems under investigation, listed in Table 14, also promise to be extremely informative, particularly those involving easily isolated proteins like the β -lactoglobulins of milk. Because of their flexibility as regards genetic analysis, however, the bacteria and bacteriophages are, at present, receiving the most concerted attention. For example, no less than three laboratories are in the midst of the particular problem of determining the effects of mutation in the *h* region of bacteriophage T2 on the chemical nature of the phage particle.

The host range (*h*) region of the genetic material of bacteriophage T2 determines whether or not a phage particle will adsorb to a specific bacterial cell host. Thus the wild-type phage, T2 h^+ , will adsorb to and infect *E. coli* of the B strain but not of the B/2 strain, whereas *h* mutants will attack both B and B/2. Thus wild-type particles (h^+), when grown on a Petri dish containing agar in which is uniformly distributed a mixture of B and B/2 *E. coli*, will lyse only the B cells and a turbid plaque will be formed. On the other hand, *h* mutants will attack both B and B/2 and a completely clear plaque will result. (It is convenient, in what follows, to think of an h^+ mutation as a "defect" in the "normal" *h* region of the genetic strand.) This difference in phenotypic behavior can be made the basis for a quantitative estimate of the proportion of *h* and h^+ particles and has been applied by Streisinger and Franklin⁴ for the location of various *h* mutants along the linear genetic map in a manner much like that employed by Benzer for the mapping of *r* mutants in bacteriophage T4.

Since use of the technique of fine-structure mapping in bacteriophage will become more and more common, it will be instructive to examine briefly the general approach to the mapping of the *h* region as an additional example.

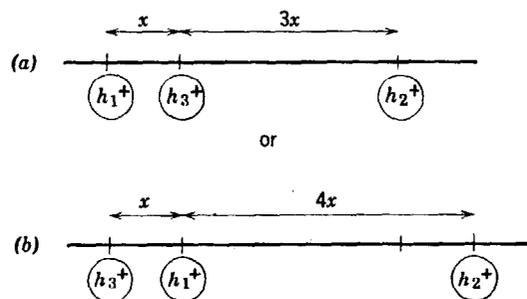
An *h*-type phage arbitrarily named h_0^0 , was plated on mixed B and B/2 cells as above described, and the turbid plaques were chosen as examples of reversions to the h^+ genotype. In this way there was obtained a series of mutants of the h^+ variety. Fourteen h^+ mutants having low reversion indices* were then crossed with each other as

* All the h^+ mutants were examined for their propensity to revert spontaneously to the *h* phenotype, and those having a high "reversion index" were discarded since such mutants would have introduced technical difficulties in subsequent studies of the ability of pairs of h^+ mutants to yield *h* phenotypes by genetic recombination.



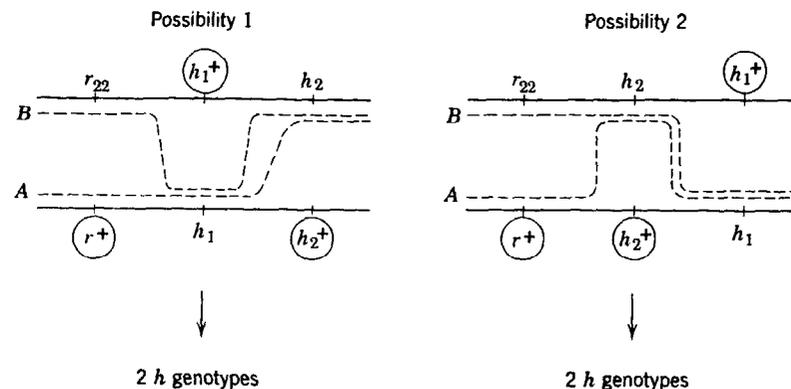
The “defective,” h^+ loci are encircled. During the formation of progeny a process analogous to crossing over takes place. The probability that recombination of h_1 and h_2 will occur in the doubly infected cell, A , is four times greater than the probability that h_1 and h_3 will recombine in B .

The relative positions of h_1^+ , h_2^+ , and h_3^+ might then be



The correct order, $h_1^+ - h_3^+ - h_2^+$, may be established by crossing mutant h_2^+ with h_3^+ . Recombination here will correspond to $3x$ rather than $5x$.

Figure 81. Establishing the relative separation and order of h^+ loci by two factor crosses.



Ⓐ $r_+ h_1 h_2$

Ⓑ $r_{22} h_1 h_2$

with many more r^+ since only one “crossover” is involved

Thus, order is $r_{22} - h_1 - h_2$ if r^+ are in excess,
or $r_{22} - h_2 - h_1$ if r_{22} are in excess.

Ⓐ $r_+ h_2 h_1$

Ⓑ $r_{22} h_2 h_1$

with many more r_{22}

Figure 82. Establishing the absolute order of h^+ loci by three-factor crosses ($r_{22} h_1^+ \times r^+ h_2^+$).

well as with the original h^+ strain by mixed infection of *E. coli* B. The progeny were examined (by the “turbid-or-clear” plaque test) for the relative proportion of h phenotypes that had formed through recombination (Figure 81). Each pair of h^+ mutants was found to yield h recombinants with a characteristic and reproducible frequency. All these frequencies were low, however (less than 1 per cent), indicating that the h^+ mutants examined all occurred within a region along the genetic map of less than two recombination units. (As discussed in Chapter 4, the total “length” of the genetic material in T2 phage may correspond to as much as 800 such units.)

Having established that all the various h^+ mutations occurred within a small region of the map, it was necessary to determine the order in which they were arranged with respect to one another. This was done through the use of three-factor crosses, a procedure with a forbidding name but one that is perfectly straightforward when thought of in terms of a simple model (Figure 82).

Crosses were made by mixed infection of strain B bacteria with bacteriophages containing, in addition to one of the h^+ loci, either the wild type, r^+ , or the mutant, r_{22} (which belong to the so-called

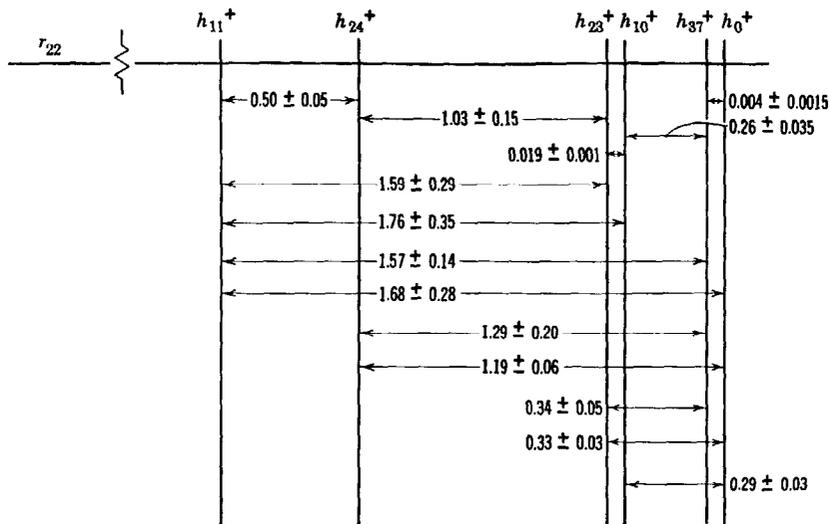


Figure 83. Genetic map of h^+ mutants. Taken from the studies of G. Streisinger and N. Franklin on the genetic determination of host range in bacteriophage T2, *Cold Spring Harbor Symposia Quant. Biology*, 21, 103 (1956).

plaque-type mutants that were mapped by Benzer). The preparation of these doubly marked mutants requires a considerable amount of technical manipulation involving repeated back-crossing and selection and we shall not attempt to describe the details of the chore. Suffice it to say that strains of bacteriophage were obtained which permitted crosses of the type $r_{22}h_1^+h_2 \times r^+h_1h_2^+$ to be made, where the r_{22} locus is situated 24 recombination units from the h region as determined by two-factor crosses.

The relative location of two h^+ loci, h_1^+ and h_2^+ , with respect to the r_{22} locus may be determined by the estimation of the proportion of the h mutants formed by recombination which is r^+ in character. The applicability of this test becomes clear upon inspection of the schematic representation shown in the figure. If the order of loci is $r_{22}-h_1-h_2$ rather than $r_{22}-h_2-h_1$, a far greater proportion of h -type recombinants will be r^+ since the incorporation of the segregated h alleles into one functional unit requires only one "crossover" in the first instance and two in the second. All of this deduction involves, of course, the assumptions we have mentioned earlier, including the reality of a linear arrangement of genetic loci in phage and the availability of a mechanism of crossover at least analogous to that generally invoked for recombination in the chromosomes of higher or-

ganisms. These assumptions are, operationally speaking, applicable in the present case. The order of h^+ loci shown on the map in Figure 83, which were determined from the three-factor cross data, are compatible with the distances between the loci which was indicated by the preliminary two-factor cross experiments.

Before considering these genetic observations in terms of the hereditary control of phage chemistry, one further observation needs to be reviewed. This concerns the demonstration of the functional unity of the h region. Do all the h^+ mutations in the "map" shown in Figure 83 belong to a single unit of function (a "cistron"), or is it possible that they are divided into more than one group and act cooperatively in the determination of host range specificity? A decision may be made by use of the *cis-trans* test which we have previously described in relation to the r mutants. Streisinger⁴ demonstrated that all the h mutants belong to a single functional unit by comparing the effectiveness of crosses of the *cis* type ($h \times h^+$) and of the *trans* type ($h_1^+ \times h_2^+$) in producing h phenotypes (i.e., phage which adsorbs to both B and B/2 bacteria). If, in Figure 84, each

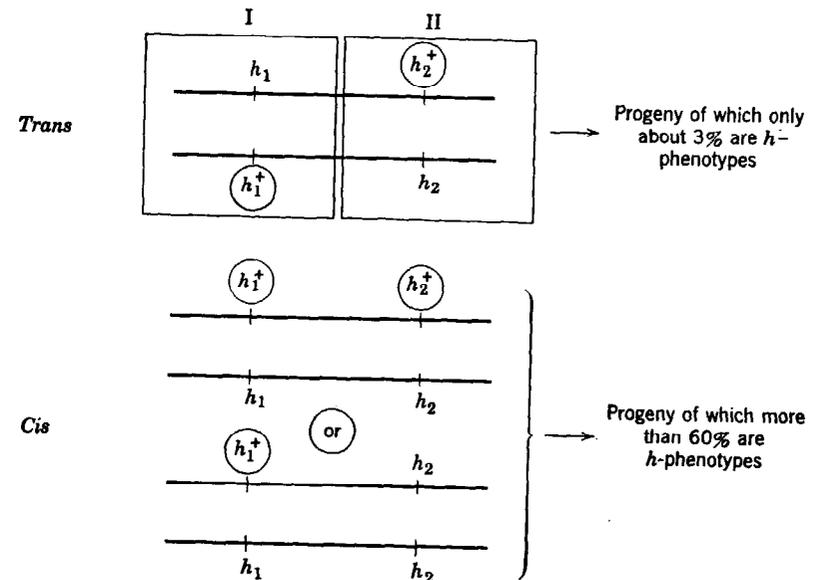


Figure 84. Application of the *cis-trans* test to determine the functional unity of the h region in the genetic material of bacteriophage T2. We may conclude that portions I and II of the h region of the hereditary material of bacteriophage T2 cannot act cooperatively to yield an "unblemished" h phenotype. The entire h region must be free of h^+ loci.

of the indicated portions of the genetic strand acts separately, and they cooperatively produce a normal *h* phenotype, all the progeny in such a mixed infection should have the *h* character. It was found, however, that only a very small proportion of the progeny were *h* in phenotype (about 3 per cent, of the order of that to be expected from crossover and other sequelae of recombination). In the case of the *cis* arrangement (Figure 84), a high percentage of *h* pheno-



Figure 85. Electron photomicrograph of bacteriophage T2 adsorbed on cell walls of *E. coli* B. Some of the adsorbed virus particles have lost their DNA, presumably by injection into the bacterial cell (see arrows). This photograph was obtained through the kindness of Dr. Thomas F. Anderson of the Institute for Cancer Research, Philadelphia, Pa.

types was observed (about 60 per cent). (Enough phenotypically *h* material is presumably made by the all-*h* strand to confer this character on some of the *h*⁺ genomes as well as the *h*. That is, genetically *h*⁺ phage may have, associated with their protein coats, some *h*-type host-range protein). It may be concluded, therefore, that only a functionally complete *h* region will suffice for the expression of the *h* character.

We are now in a position to consider the genetic map of the *h* region in terms of what it does for the bacteriophage particle. Our attention must, of course, be directed at that part of the chemistry (and morphology) of T2 which has to do with its adsorption to host cells. Phage particles attach to bacterial cells by the tips of their tails (Figure 85). The same sort of attachment occurs with phage "ghosts" prepared by suddenly exposing intact phage to an osmotic shock. Since the phage ghost is essentially all protein, except for traces of carbohydrate present in such small amounts that its functional importance is fairly unlikely, it may be concluded that the business of attachment involves a specific protein component. Further support for the protein nature of the adsorbing substance comes from the fact that the kinetics of inactivation of the adsorptive capacity by agents like urea are very similar to those of protein denaturation. It has also been observed that the blocking of amino groups in phage prevents attachment to bacteria.

We may approach the problem of isolating and characterizing the protein component responsible for host range specificity in two ways. First, we may proceed to isolate various fragments from disrupted phage particles. Such studies have been carried out by S. Brenner and his colleagues in the Cavendish Laboratory at the University of Cambridge. These investigators have concluded that the host-range function is carried in the slender fibers that are attached to, and wrapped around, the tail of the virus particle (Figure 86) and have prepared highly purified concentrates of free fibers for chemical study.

A second approach to the problem involves the fractionation of the total protein mixture making up phage ghosts in the same way that we would approach the isolation of an enzyme from a crude tissue extract. Phage ghosts may be solubilized in a number of ways that should not cause modification in the covalent structure of the component proteins, and solutions prepared with such agents as urea and guanidine appear to be amenable to study by chromatographic, electrophoretic, and ultracentrifugal techniques. (See Figures 87 and 88, for example.)



Figure 86. Electron photomicrograph of T2 bacteriophage, disrupted by treatment with N-ethyl maleimide. This photograph was obtained through the kindness of Mrs. E. R. Kaufman and Dr. A. M. Katz of the National Institutes of Health, Bethesda, Maryland.

Both the morphological and "chemical" attacks on the fractionation problem require a test for functional activity. Although not direct, a test has been devised based on the fact that the antigenicity of the T-even bacteriophages against rabbit antiphage antibody is controlled by the same genetic locus as that which determines the host range. Thus, Streisinger⁵ has shown that no measurable recombination occurs between the determinants of host range and the determinants of serotype. (The reader must be asked to assume the validity of this conclusion; he may, however, wish to read the elegant paper of Streisinger,⁵ in which the details and arguments are presented.) We may, then, hopefully assay any given protein fraction or morphological fraction for activity by estimating its ability to block the phage-neutralizing action of an inactivating antibody preparation.

The studies on the chemical consequences of mutation in the *h* region of bacteriophage have only just begun, and the problems of isolation must first be solved. It seems likely, however, that these investigations, as well as others concerned with other regions of the

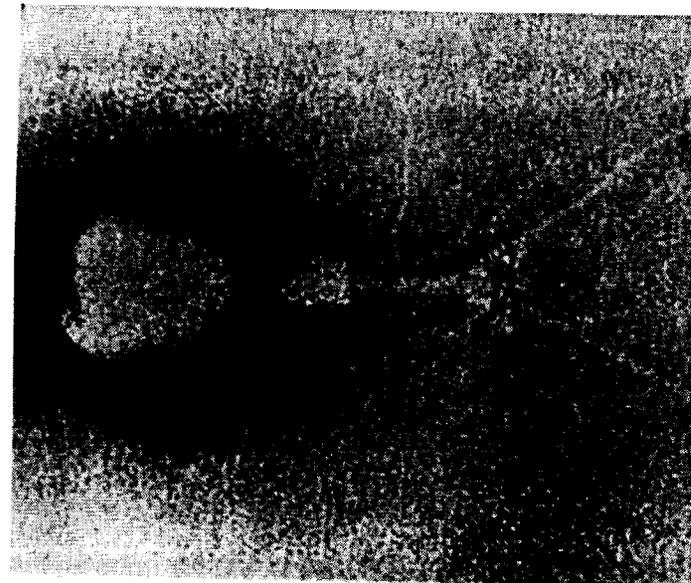
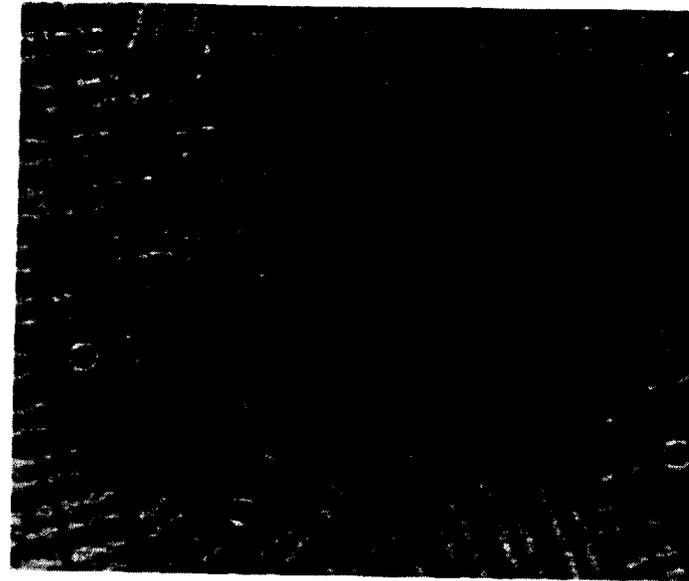


Figure 86 (continued). Left: Electronmicrograph of H₂O₂-treated bacteriophage T2 showing the head, core, contracted sheath, and tail fibers attached to the base of the core ($\times 300,000$). Right: Purified sheaths, some on end and some lying flat ($\times 300,000$). These photographs were obtained through the kindness of Dr. Sydney Brenner of the Cavendish Laboratory, University of Cambridge, England, who took these pictures together with his colleagues G. Streisinger, R. W. Horne, S. P. Champe, L. Barnett, and S. Benzer (*Journal of Molecular Biology*, 1959, in press). The preparations were made by the negative-staining technique described by S. Brenner and R. W. Horne (*Biochimica et Biophysica Acta*, 1959, in press).

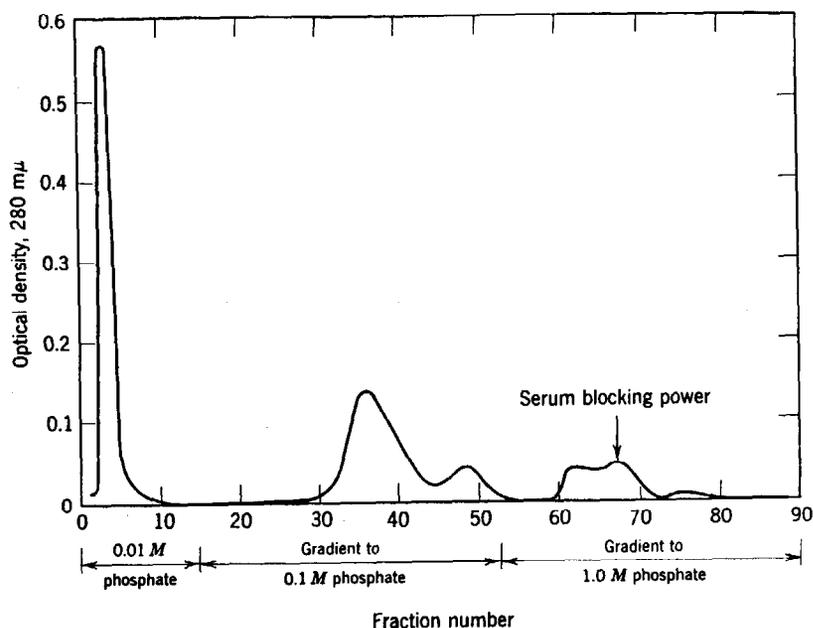


Figure 87. Partial purification of the protein component in ghosts of bacteriophage T2 which is responsible for serum blocking power (see text) and which presumably determines the host range of the phage. A preparation of "ghosts" was dissolved in ice-cold 5.2 M urea at pH 7.4 and chromatographed on a column of the cation exchanger XE-64. From W. J. Dreyer, A. Katz, and C. B. Anfinsen, *Federation Proc.*, 17, 214 (1958).

genetic map of phage, should ultimately enable us to make direct point-by-point comparisons of genetic changes and structural modifications in protein molecules. The particular power of the bacteriophage approach, and of similar studies on other microbial systems,* is the extreme discrimination of the genetic mapping for these "or-

* For example, C. Levinthal and A. Garen, of the Massachusetts Institute of Technology, have recently begun the mapping of the "cistron" which controls the synthesis of an alkaline phosphatase in *E. coli*. This enzyme is synthesized in large quantities when phosphate is limiting in the culture medium. Organisms are grown on plates, containing medium or low phosphate concentration, which are then sprayed with nitrophenylphosphate. Alkaline phosphatase-containing cells cleave the phosphate ester to yield the yellow-colored nitrophenol. Bacterial colonies containing the enzyme thus become yellow, some mutants remain white, and certain mutants, having enzyme of intermediate activity, are weakly colored. The enzymes, active or inactive, may be isolated from the various strains and are at present being subjected to structural analysis.

ganisms." If Benzer's calculation of the length of the "recon" in terms of nucleotide units in the DNA chain proves to be reasonably correct, we may expect to be able to distinguish, genetically, between loci as closely packed as those determining the three hemoglobins investigated by Ingram. In the *h* region, for example, the locus h_{31}^+ appears to be only 0.004 recombination units from h_0^+ . Translated into Benzer's nucleotide language, this distance would correspond to about the distance between one nucleotide pair. In spite of the wishful chemical thinking and genetic uncertainty involved in all these speculations, it must be very clear why the biochemist is willing to risk the gamble of time and effort required to test the gen-

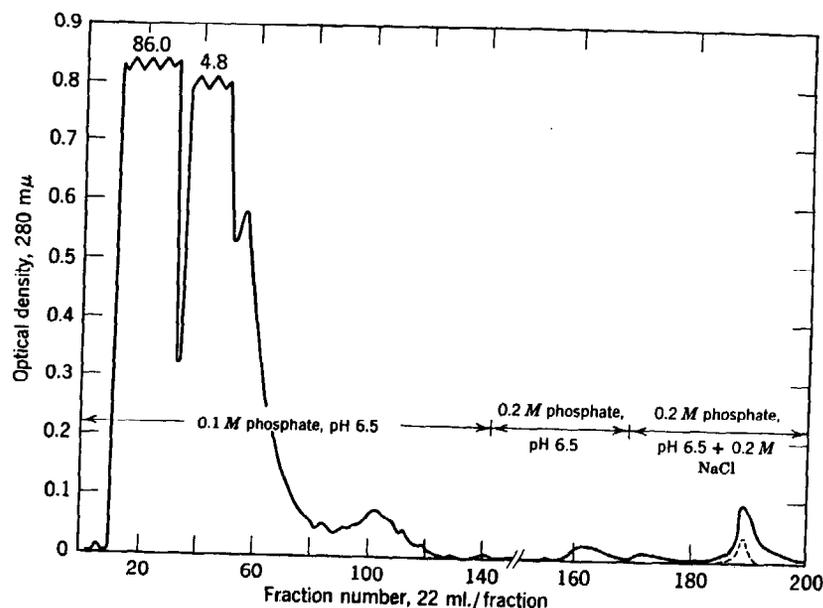


Figure 88. The purification of lysozyme from lysates of *E. coli* on a column of the cation exchanger, XE-64. The small chromatographic peak at the far right of the chromatogram contains the lysozyme activity (the dotted curve). Lysozyme may also be isolated from bacteriophage ghosts. The starting material of choice, however, is an *E. coli* lysate. Enough lysozyme is presumably synthesized following infection of *E. coli* cells with phage to more than satisfy the needs for the formation of progeny. The excess enzyme within the infected bacterial cells is then released into the surrounding culture medium upon lysis. The enzyme emerging from the ion exchange column is purified several thousandfold over its concentration in the crude lysate. From unpublished experiments of Dr. W. J. Dreyer, National Heart Institute, Bethesda, Maryland.

eral hypothesis. With luck, the answers might begin to clarify some of the most central problems of biology.

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