Name: Jerry Lee Cushman Date of Degree: May, 1967

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: GENETICS OF MICROORGANISMS

Pages in Study: 50 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope and Method of Study: This report has been designed to present the contributions that microorganisms have made to the field of genetics since the early 1940's. Primary emphasis is placed on the fungus Neurospora crassa, the bacteria Escherichia coli, and the bacteriophages emphasizing the T_4 strain. Classic experiments involving these organisms are presented in an attempt to further define their role in modern genetic studies.

Findings and Conclusions: The techniques and refinements that countless workers have contributed have made these organisms the most intensely studied organisms on earth outside of man at the present time. The employment of microorganisms has made possible a great refinement and method of experimentation leading ultimately to the study of genetics on a molecular level. Present work has sharply defined the concept of the gene and has led to the predicting of the amino acid sequences on a molecular basis.

Indications are that work with these microorganisms will become even more intense in coming years. For investigations involving the gene-protein relationships, work with these microorganisms has become indispensable.

ADVISER'S APPROVAL L. Herbert Bruneau

GENETICS OF MICROORGANISMS

By

JERRY LEE CUSHMAN

Bachelor of Science
Michigan State University
East Lansing, Michigan
1957

Master of Arts
Michigan State University
East Lansing, Michigan
1961

Submitted to the faculty of the Graduate College of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1967

GENETICS OF MICROORGANISMS

Report Approved:

L. Herbert Bunnell
Report Adviser

Elizabeth T. Gundy

Norman N. Durham
Dean of the Graduate College

Name: Jerry Lee Cushman Date of Degree: May, 1967

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: GENETICS OF MICROORGANISMS

Pages in Study: 50 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope and Method of Study: This report has been designed to present the contributions that microorganisms have made to the field of genetics since the early 1940's. Primary emphasis is placed on the fungus Neurospora crassa, the bacteria Escherichia coli, and the bacteriophages emphasizing the T_4 strain. Classic experiments involving these organisms are presented in an attempt to further define their role in modern genetic studies.

Findings and Conclusions: The techniques and refinements that countless workers have contributed have made these organisms the most intensely studied organisms on earth outside of man at the present time. The employment of microorganisms has made possible a great refinement and method of experimentation leading ultimately to the study of genetics on a molecular level. Present work has sharply defined the concept of the gene and has led to the predicting of the amino acid sequences on a molecular basis.

Indications are that work with these microorganisms will become even more intense in coming years. For investigations involving the gene-protein relationships, work with these microorganisms has become indispensable.

ADVISER'S APPROVAL L. Herbert Bruncau

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

CHAPTER I

INTRODUCTION

For many years the structure of genes and the chemical way in which they control cellular characteristics were a mystery. After a large number of spontaneous mutations had been described in a number of organisms, it became obvious that a one genome character relationship does not exist, but that all complex characters are under the control of many genes. The most sensible idea, postulated clearly by Garrod as early as 1909, was that genes affect the synthesis of enzymes. However, in general, the tools of the Mendelian geneticists, organisms such as the corn plant, the mouse, and even the fruit fly, Drosophila, were not suitable for chemical investigations of gene-protein relations. For this type of analysis, work with much simpler microorganisms became indispensable.

Before developments in microbial genetics, the only way of carrying out genetic analysis was through experiments making use of appropriately designed matings, that is, from results of sexual reproduction. Experiments of this type have serious limitations in that generation times are frequently very long and the number of progeny is generally quite small. Several other factors have contributed to the success of microorganisms in genetic experiments. Microorganisms are small requiring little space and they multiply very rapidly. Also, the basic principles of genetics can be

conveniently studied one at a time because of their relatively simple developmental cycle. Nevertheless, when considered collectively, microorganisms provide sufficient diversity to permit step-by-step experimentation of more complicated systems.

The employment of microorganisms has made possible a great refinement in concept and method of experimentation from an organismal approach toward the description of genetics from a cellular viewpoint leading ultimately to the molecular level. In fact many feel that the period of "molecular biology" is now in full flower.

It is conceivable that the modern age of molecular genetics was conceived by the recognition of DNA as the genetic material of the transforming principle in pneumococcus and this is where this paper begins.

It would be impossible to even dent the surface of the multitude of research that is available in the area of genetics of microorganisms. Therefore, this paper will attempt to describe the contributions of primarily three extremely important microorganisms, Neurospora crassa or common bread mold, Escherichia coli as a bacterium, and bacteriophage or viruses.

It is unfortunate that mention of many other outstanding findings using these three organisms cannot be made. Therefore it is impossible for this paper to be a complete description of microbial genetics.

The primary function of this paper is to convey to the reader an awareness of the contributions of microbial genetics in recent years and some of the impact thay have made on science today.

CHAPTER II

NEUROSPORA CRASSA

The rediscovery of Mendel's classic work at the turn of the century was followed by a great deal of effort on the part of geneticists in verification of the chromosomal theory of heredity and the mechanisms of Mendelian genetics.

One of the problems that geneticists encountered was the difficulty of finding an organism that could be used to test the concept that chemical reactions are controlled in some manner by genes. Without a clear concept of the nature of the gene and its product little progress could be made.

The chromosomal basis of Mendelian inheritance was established in the decade 1910-1920, primarily by Morgan and his students working on Drosophila, at Columbia University (Sager and Ryan, 1961). But a more direct system of demonstrating the relationship between genetic and cytological behavior was needed.

The breakthrough came with the introduction of microorganisms into genetic research. Beadle and Tatum (1941) began their work on the bread mold Neurospora in the early 1940's. Because of the importance of Neurospora crassa as an organism of genetic research it should be worthwhile to look at the basic characteristics of this organism.

Life Cycle of Neurospora Crassa

Beadle (1946) described Neurospora crassa as follows:

> It produces great masses of brilliant orange-red asexual spores. It is a heterothallic fungus, although molds of the two sexes are not visibly different. One can tell the sex of an individual only by putting it with another individual of a known sex for observation. If nothing happens, they are of the same sex. If fusion and formation of fruiting bodies takes place, they are of opposite sex. The bread mold differs from man in having in each of its cell nuclei only one set of chromosomes and genes instead of two. In this respect it corresponds to our gametes- eggs and sperms. The double condition, corresponding to the fertilized egg in man in having a set of chromosomes from each parent, is limited in Neurospora to a single cell generation. Because the stage of the organism ordinarily observed has nuclei with single sets of chromosomes, Neurospora is relatively simple genetically. There is no need to worry about dominance and recessiveness- there is only one representative of each gene in each nucleus. The Mendelian ratio usually observed is, therefore, the basic one-to-one ratio so often observed in higher organisms (Beadle, 1946).

A further advantage of the Neurospora as stated by Beadle (1946) is the fact that it also carries out an asexual reproductive cycle producing millions of microscopic orange spores called conidia. The conidia are capable of multiplying without genetic change and this is a big advantage in biochemical work, since it enables the growth of an unlimited amount of any one individual.

Tatum (1941) determined that the only growth factor required, other than the usual inorganic salts and sugar, was the then recently discovered vitamin biotin. From these basic materials it makes everything that it requires for normal development and functioning.

The life cycle of Neurospora is illustrated in Figure 1 on the following page. According to the figure by Beadle (1946) Neurospora is not only able to multiply by means of the particular asexual cycle

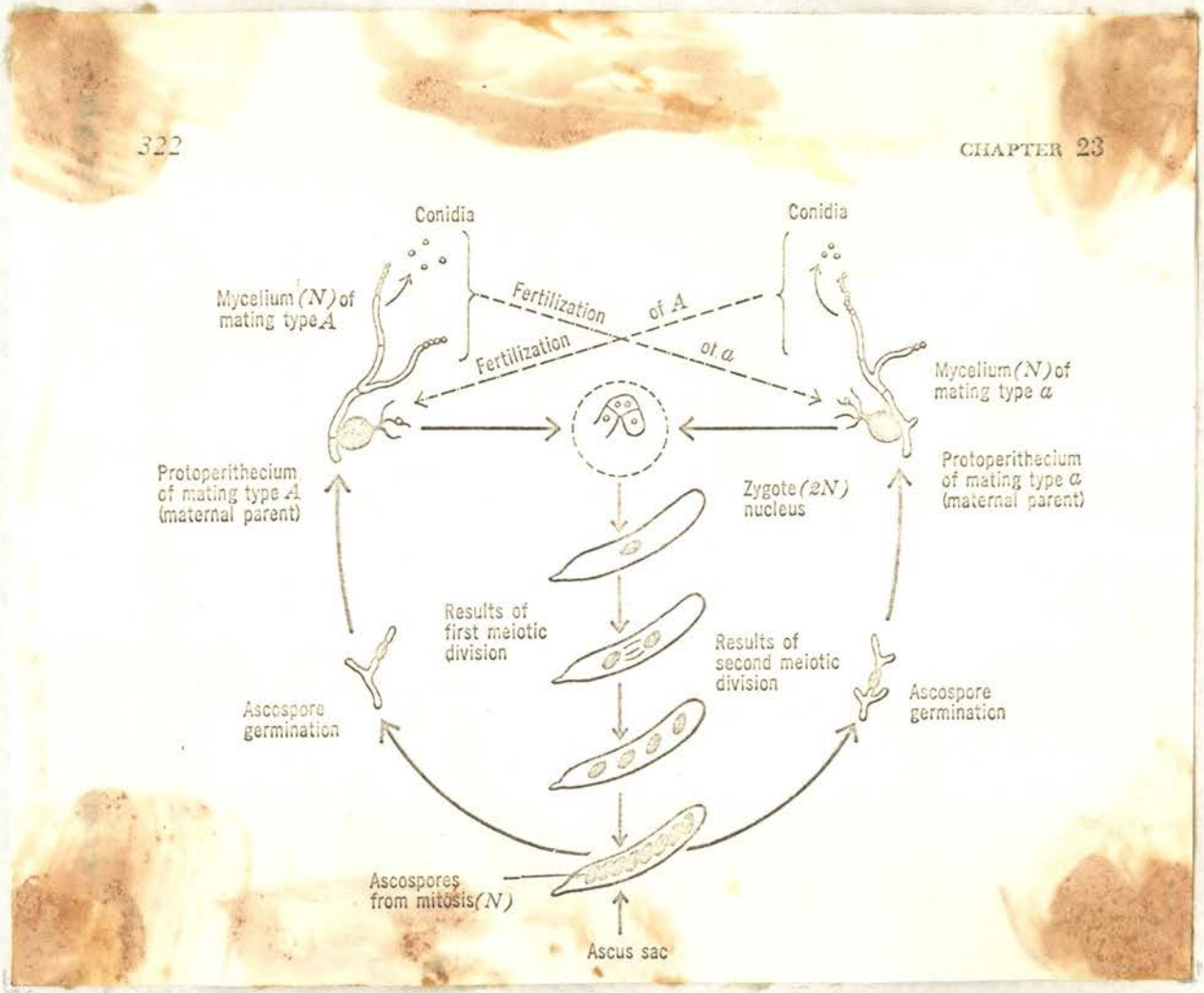

Figure 1. The Life Cycle of Bread Mold, Neurospora

shown, but the mold is able to multiply asexually through structures referred to as microconidia and by fragments of the mycelium.

One-Gene, One-Enzyme Theory

Beadle and Tatum (1941) were interested in testing the unproved assumption that a given gene has a single primary action. They further assumed that the primary gene action was the result of enzyme production.

With the use of Neurospora crassa, Beadle and Tatum (1941) set out to determine if and how genes control known biochemical reactions. Their procedure was based on the assumption that x-ray treatment will induce mutations in genes concerned with the control of known specific chemical reactions. If the organism must be able to carry out a certain chemical reaction to survive on a given medium, a mutation unable to do this will be lethal on the same medium. However, if this mutant can be grown on a medium to which the essential product of the genetically blocked reaction has been added the mutant can be maintained and studied.

The experimental procedure by which biochemical mutants are produced and detected in Neurospora is illustrated in figure 2 on the following page, of Beadle and Tatum (1941).

Three initial mutants were established by Beadle and Tatum (1941).

> In one of these the ability to synthesize vitamin B has been wholly or largely lost. In a second the ability to synthesize the thiazole half of the vitamin B_1 molecule is absent, and in the third para-aminobenzoic acid is not synthesized. It is therefore clear that all of these substances are essential growth factors for Neurospora.
>
> Growth of the pyridoxinless mutant (a mutant unable to synthesize vitamin B_6) is a function of the B_6 content

> of the medium on which it is grown. ---Inability to synthesize vitamin B_6 is apparently differentiated by a single gene from the ability of the organism to elaborate this essential growth substance (Beadle and Tatum, 1941).

Carlson (1966) commented that by 1945 Beadle and Tatum had detected numerous biochemical mutants affecting large numbers of products, establishing the generality of their technique for the study of biochemical pathways.

A more recent technique of detecting biochemical mutants in Neurospora was explained by Herskowitz (1965). This technique can eliminate nonmutant strains selectively. The spores are given an opportunity to grow for a short time on a minimal medium and then can be subjected to filtration, which separates the larger, growing nonmutant cultures from the smaller, nongrowing mutant ones, or to an antibiotic which kills actively-growing cultures but has less or no effect on nongrowing ones. Herskowitz (1965) further states it is even possible to find mutants for unknown growth factors by supplementing the culture medium with extracts of normal strains of Neurospora containing various substances, both known and unknown, needed by the mold. The same mutants requiring unknown growth factors can then be used in the specific assays needed for the isolation and identification of such substances.

Such improvements in the techniques for detecting biochemical mutants have expedited additional tests of the postulated enzyme-gene relationship. As a result of Beadle and Tatum (1941) the one-gene, one-enzyme theory was developed and they received the Nobel prize for their work in 1958.

Heterocaryosis and the Complementation Test

Fincham (1966) states that in Neurospora crassa as well as other filamentous fungi heterokaryons are formed as the result of fusions between hyphae of different genotypes. He further states that stable heterokaryons can only be formed when the component strains are of the same mating type. Stable heterokaryosis requires that the two participating nuclei be similar with respect to at least two other genes.

Emerson (1952) stated two ways in which a mixture of different kinds of nuclei within a single Neurospora cell can come about. In the growth resulting from a sexually produced ascospore, or from a uninucleate asexual microconidium, all nuclei are directly descended from a single haploid nucleus. Barring mutation, they should all have the same genetic constitution. Emerson (1952) further states that after the growth has become multinucleate, if a mutation should occur in one nucleus, the descendants of that nucleus would then have a different genetic constitution from the remaining nuclei in the common cytoplasm, and a condition of heterocaryosis would exist.

The second way in which heterocaryons arise according to Emerson (1952) is from the direct fusion of branches or hyphae of different strains, with the subsequent intermingling of their nuclei. By this method, heterocaryons of predetermined genetic constitution can be made at will. An illustration of heterocaryon formation resulting from hyphae fusion of two strains of Neurospora is shown in Figure 3 on the following page.

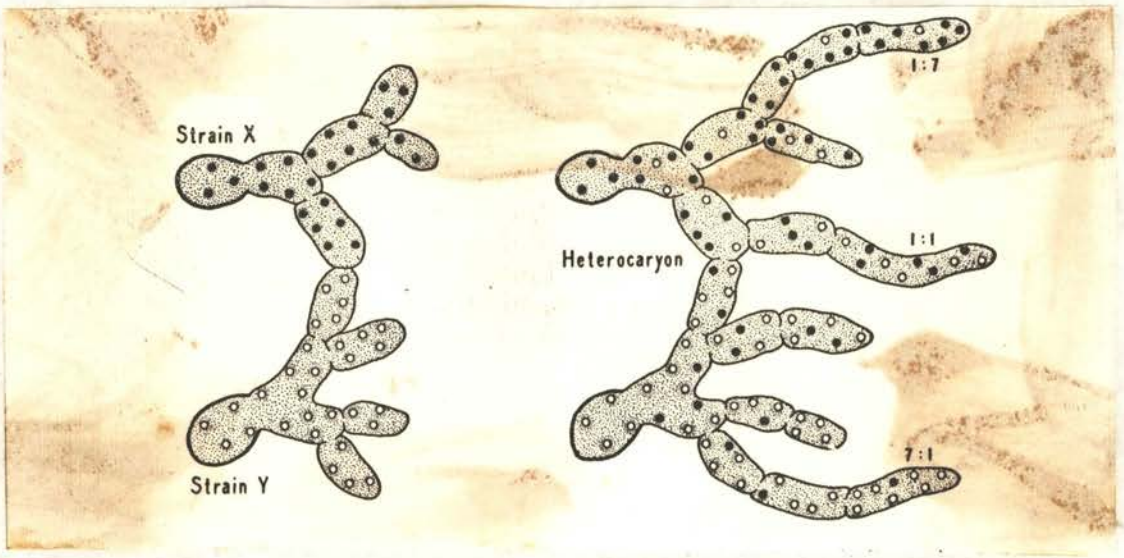

Figure 3. Heterocaryon Formation Resulting From Hypha Fusion of two Strain of Neurospora

In the above illustration strain X is represented as having black nuclei to distinguish them from the nuclei of strain Y, which are pictured as being white. After fusion between hyphae, nuclei of strain Y may migrate into cells of strain X, and those of X into Y. It is possible that different hyphal tips, growing from this common mass of cells will have different relative numbers of the two sorts of nuclei, as shown by the ratios of 1:7, 1:1, and 7:1, in three of the illustrated tips (Emerson, 1952).

When heterokaryosis occurs in Neurospora it can be used as an important tool in genetics. This tool useful in genetic analysis is called the complementation test.

Giles and Case (1960) made intensive studies on the pan-2 gene of Neurospora crassa. Mutations of this gene result in the inability of the mutant to convert keto-valine into keto-pantoic acid. In order to grow, the mutants require pantothenic acid, which is a vitamin and

the end product of this particular pathway of synthesis. Srb, Owen and Edgar (1965) states that some 75 mutants have been isolated and mapped in one small region of chromosome 6. The missing step is believed to be controlled by a single enzyme, which is defective or lacking in the pan-2 mutants.

Srb, Owen and Edgar (1965) described the Giles and Case experiment by noting that heterokaryons are established that contain two different kinds of nuclei to be tested with respect to mutations at the pan-2 locus. Since heterokaryons between any pan-2 mutants and wild types are able to grow in the absence of pantothenic acid, the pan-2 mutants can be considered recessive to wild type. The mutants, therefore, fall into two classes with regard to their growth requirements in heterokaryons with other mutants.

Members of the larger group according to Srb, Owen and Edgar (1965) show no growth on unsupplemented medium when in combination with other mutants. These mutants are designated noncomplementing mutants, because they do not complement the growth defect of any of the other mutants. Mutants of the second class, termed complementing, are able to grow in combination with particular other mutants of this class.

The complementing pan-2 mutants can be arranged into a linear "complementation map", where the mutations can be represented by numbers. In the following illustration the map of the pan-2 locus in Neurospora is plotted. The genetic map is shown at the bottom by the double line and the mutations indicated below the double line are noncomplementing while those above the double line are complementing (Srb, Owen and Edgar 1965).

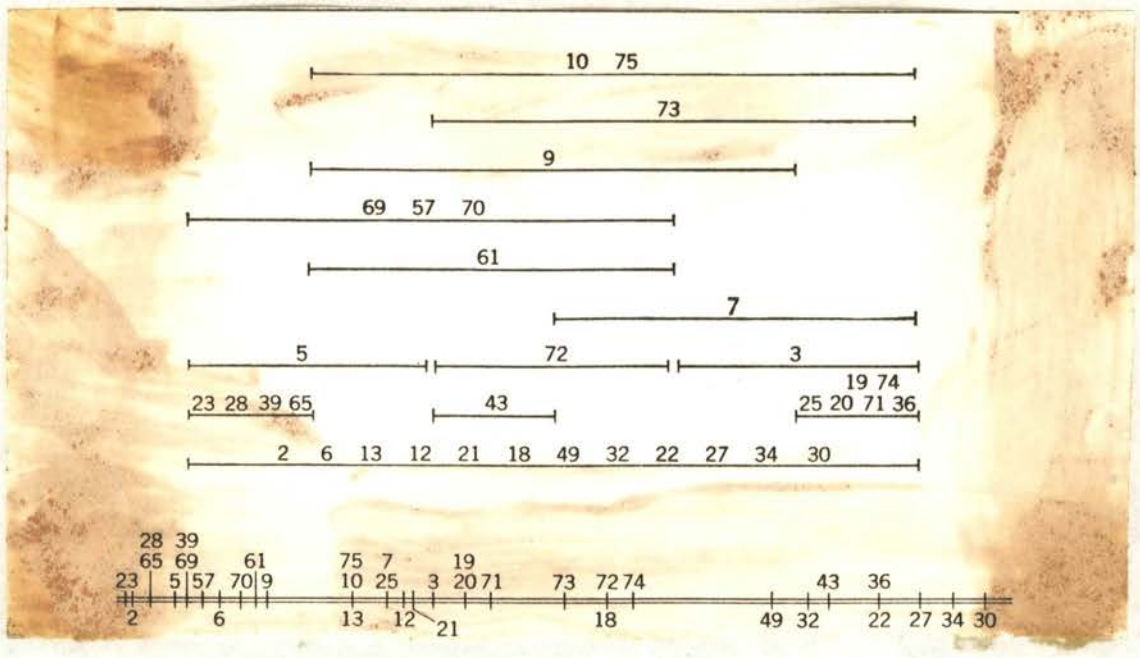

Figure 4. Map of the Pan-2 Locus in Neurospora

Complementation tests have been carried out more extensively in Neurospora crassa than in any other organism, almost always with auxotrophic mutants according to Fincham (1966). The usual method is simply to superimpose small inocula of conidia of the two strains under test at a marked point on a plate of minimal agar medium, with the usual surcrose or glucose partly replaced by sorbose to bring about a tight colonial type of growth conducive to easy scoring. The auxotrophic conidia in a moderately dense mixed inoculum can always grow enough on the basis of their own reserves to form a heterokaryotic mycelium, after which growth usually stops, if the mutants are non-complementary, or continue indefinitely at more or less wild-type rate if they are complementary. Fully complementary and compatible strains will usually be seen to be growing strongly after one day at 25 degrees centigrade, but imperfectly complementary allelic mutants may have to

be incubated for as long as five or six days before showing a positive result (Fincham, 1966).

The contributions of Neurospora have been many and varied. Since the well known work of Beadle and Tatum (1941) an extremely important consequence of this work has been the uniting of genetics with the area of biochemistry.

Beadle (1945) emphasized the co-operation of genetics and biochemistry when he stated:

> It is perhaps unnecessary to state the obvious conclusion that if one is to understand the metabolism of the organism in the most complete way possible, genes must be taken into account. Too often in the past these units have been regarded as the exclusive property of the geneticist. The biochemist cannot understand what goes on chemically in the organism without considering genes anymore than a geneticist can fully appreciate the gene without taking into account what it is and what it does. It is a most unfortunate consequence of human limitations and the inflexible organization of our institutions of higher learning that investigators tend to be forced into laboratories with such labels as "biochemistry" or "genetics". The gene does not recognize the distinction-- we should at least minimize it (Beadle, 1945).

Thus, the union of genetics and biochemistry was made and there is little doubt that the success of the direct study of biochemical genetics through Neurospora was a major achievement.

Within a few years Joshua Lederberg would provide the essential tools for applying biochemical genetics to bacteria, bringing in the microbiologists. Bacterial genetics, in turn, would draw in the physicists who, through Delbruck's initiative, were using bacteriophage as genetic systems in the hope of establishing molecular models of genetics (Carlson, 1966).

CHAPTER III

BACTERIA

The discovery of bacterial transformation by Griffith (1928) began the use of bacteria in transformation experiments. Griffith observed in Diplococcus pneumoniae that the change from avirulent, rough colony type (R) to virulent, smooth colony type (S) occurred when a very large mass of R cells was placed under the skin of a mouse.

Pneumococci used in Griffiths (1928) experiment, exist as a variety of strains, distinguished by the type-specific capsular polysaccharide which they produce. The type S produces a colony whose smooth surface is directly related to the capsule of polysaccharide material each bacterium possesses. The other type of colony, R, has a rough surface because its bacteria lack this polysaccharide capsule. Griffith (1928) inoculated one group of mice with avirulent cells, a second group with heat-killed virulent cells, and a third group with both avirulent and heat-killed virulent cells. The first two groups of animals were unaffected by the inoculations. Mice in the last group, however, developed severe septicemia. The interaction within the mice between viable avirulent cells and heat-killed virulent cells led to the appearance of viable virulent cells (Griffith, 1928).

In 1928, however, the concept of bacterial mutation and selection was not fully established, and Griffith (1928) formulated instead the hypothesis that the R cells contained traces of S "antigen" which,

liberated from the large mass of disintegrating cells, was used by a surviving cell to build up its own S "antigen". To test this hypothesis, he injected into a mouse a small number of R cells together with a large mass of heat-killed S cells. As his theory predicted, live virulent S cells were recovered from the animal (Adelberg, 1966).

Later Dawson and Sia (1931) succeeded in inducing transformation in vitro. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system.

One of the major problems of the biologist was the determination of the chemical substances which carry the information that determines the characteristics of the cell and is also capable of transferring this information to each of the daughter cells. This set the stage for the important contribution of three men that determined the chemical nature of the specific substance concerned with the transmission of genetic information.

Avery, MacLeod, and MacCarty (1944) using Pneumococcus presented the results of their attempt to determine the chemical nature of the substance inducing specific transformation of pneumococcal types. A desoxyribonucleic acid fraction was isolated from Type III pneumococci which is capable of transforming unencapsulated R variants derived from Pneumococcus Type II into fully encapsulated Type III cells.

These investigators demonstrated that transformation of pneumococcus types is accomplished by DNA. Probably the most exciting period in biological research followed this important finding. Figure 5 on the

following page illustrates the procedure by which Avery, MacLeod, and McCarty (1944) isolated pure DNA with high transforming activity from the pneumococcus cells.

Genetic Recombination

The modern era of bacterial biology began with the demonstration of genetic recombination in Escherichia coli by Joshua Lederberg and E. L. Tatum (1946). These workers developed techniques for detecting recombinational events which might occur only very infrequently. They established a series of strains which differed from each other in a number of metabolic capacities. One strain required biotin and methionine; another required threonine, leucine and thiamine (vitamin B_1). These two strains may be represented as B^- M^- T^+ L^+ B_1^+ and B^+ M^+ T^- L^- B_1^-. When the two strains are mixed and plated on a culture medium lacking all of the five compounds required by one or the other of the parents, a small fraction of the cells, about 1 in 10^7, gave rise to colonies; they had the genetic formula of B^+ M^+ T^+ L^+ B_1^+.

Initially several explanations for the appearance of these "wild types" or "prototrophic" colonies could be advanced. Stern and Nanney (1965) commented that mutation for example, of B^- to B^+ and of M^- to M^+ in the same cell of one of the strains, would yield a prototroph. And the simultaneous mutation of three genes in the other strain would also give the results observed. However, probability considerations were against this interpretation. Still other interpretations were considered and subjected to test, but all were systematically excluded except the interpretation that cell to cell contact, followed by genetic

recombination, occurred in this bacterial species. This was the beginning of formal bacterial genetics (Stern and Nanney, 1965).

Bacterial Mating

Subsequent studies, namely by Hayes (1953) demonstrated that three different classes of E. coli strains can be distinguished, and that genetic recombination occurs only with certain combinations. More specifically, some strains, designated as F^- strains, behave as recipient or "female" cells. When in contact with appropriate donors they receive genetic material, but are incapable of contributing genes to their mates. In contrast, other strains designated as F^+ strains when mixed with F^- strains donate but do not receive genes. A more thorough study of the F^+ strains indicates that they contain a small proportion of distinctly different cells which mate avidly and are in fact responsible for the donor capacity of the strains. Pure lines of these distinctive cells have been established and they are designated as Hfr lines (for high frequency recombination); when they are mixed with F^- strains the frequency of mating increases from the one in 10^7 observed with $F^+ \times F^-$ crosses to nearly 100% and mating pairs can be readily observed (Hayes, 1953).

The methods of approach to Hfr x F^- crosses are described by Wollman, Jacob, and Hayes (1957). For comparative quantitative studies, crosses are made under the following basic conditions:

> Exponentially growing cultures of the Hfr and F^- strains are mixed in liquid medium and aerated, the population density of one of the strains, usually the F^- recipient, generally being in 20-fold excess in order to increase the efficiency of specific collisions. The frequency of the effect to be measured is then expressed as a percentage of the number of minority parental cells initially present.

> Samples of the mixture are removed at suitable times, diluted to prevent further contacts, and plated either on a selective minimal median if recombination is being studied or, in the case of zygotic induction, with a phage-sensitive indicator strain on nutrient agar (Wollman, Jacob, and Hayes, 1957).

Once bacterial mating could be controlled, formal genetic analysis could be conducted in much the same way as that in higher forms except that special techniques are required to isolate and score the recombinants. In addition, the bacteria have provided another means of genetic mapping which has demonstrated an unusual organization of the genetic material.

Jacob and Wollman (1955) had earlier demonstrated that conjugation in bacteria can be interrupted at will by subjecting a suspension of conjugating cells to the shear forces created by rapid stirring. For any conjugating pair, the interruption of conjugation leads to the failure of some markers to be transferred; apparently the chromosome is broken along with the conjugation tube. They determined that in the transfer of a chromosomal segment of the Hfr donor into the F^- recipient that the Hfr chromosome segment penetrated in a predetermined order with the same extremity, O (for origin) always first. This technique provides an unambiguous ordering of the genes from a single injection point. The genome of the Hfr bacterium is slowly pulled into the F^- cell through the narrow bridge connecting the two cells. Evidently no explanation is given for the force that drives the male chromosome into the female cell.

The experiments using the Waring blendor permit a representation of the distances between genetic characters in time units by comparing their relative time of penetration into the recipient. The

validity of this representation depends upon whether or not the transferred segement proceeds at a constant rate. Comparison between experiments done at different temperatures (Fisher, 1957) and even more the comparison between blendor and 32_p disintegration experiments indicate that this is the case for characters which represent about one-third of the total chromosome length (Jacob and Wollman 1956). The rate of penetration appears to decrease for characters located farther from the point of origin. Jacob and Wollman estimated the time for penetration of the whole chromosome at a constant rate would take about a hundred minutes. Taylor and Thoman (1964) state that the complete transfer can take place in approximately 89 minutes.

Nevertheless, the Hfr cell after mating contains a greater or smaller fragment of the genome, as well as one or more complete genomes, since E. coli cells are commonly multinucleate. The fate of the fragment in the Hfr cell has not been followed; persumably it is destroyed or diluted out at subsequent cell divisions (Stern and Nanney, 1965). The fragment which enters the F- cell, however, can be studied further since it may contain genes not present in the original F-genome. By some process not yet fully understood, but perhaps similar to crossing-over in higher forms, the Hfr genes may be incorporated into a genome of the F- cell, replacing the genes originally present. Since the F- cell is also multinucleate, the genes may be incorporated into one F- genome and not into another and an assortment of genes occurs for several cell divisions after mating.

Genetic Mapping

The time intervening between the transfer of two markers is a

measure of the distance between them; hence a genetic map based on time units can be constructed by this method.

No single Hfr strain can be used to map the entire chromosome because the speed of transfer, which is constant during injection of the first one-third to one-half of the chromosome, apparently decreases later on (Jacob and Wollman, 1958). This limitation is offset, however, by the fact that Hfr strains may have different "points-of-origin"; that is, they may transfer different chromosomal regions initially during conjugation (Jacob and Wollman 1957). Taylor and Adelberg (1961) made it possible to extend genetic mapping over the full length of the chromosome by using the unique points-of-origin of three very high frequency (Vhf) donor strains of E. coli. Further work by Taylor and Thoman (1964) have brought greater accuracy to the basic mapping method and consequently much of the E. coli mapping data found in earlier references has been refined. The genetic map of E. coli as determined by Taylor and Thoman (1964) is shown in Figure 6 on the following page.

One interesting point exists in the arrangement of the bacterial genes on its chromosome as compared to the chromosome in higher organisms. Cairns (1963) determined the genetic map of E. coli is a circle. The male chromosome as stated previously must break at a certain point before a free end can move into a female cell. Jacob and Wollman (1956) discovered the closed nature ("cirularity") of the genetic map of F^+ E. coli; Hfr cells, however, were believed to have an open ("linear") map. Furthermore, it was often pointed out that a circular map does not necessarily imply a circular physical structure.

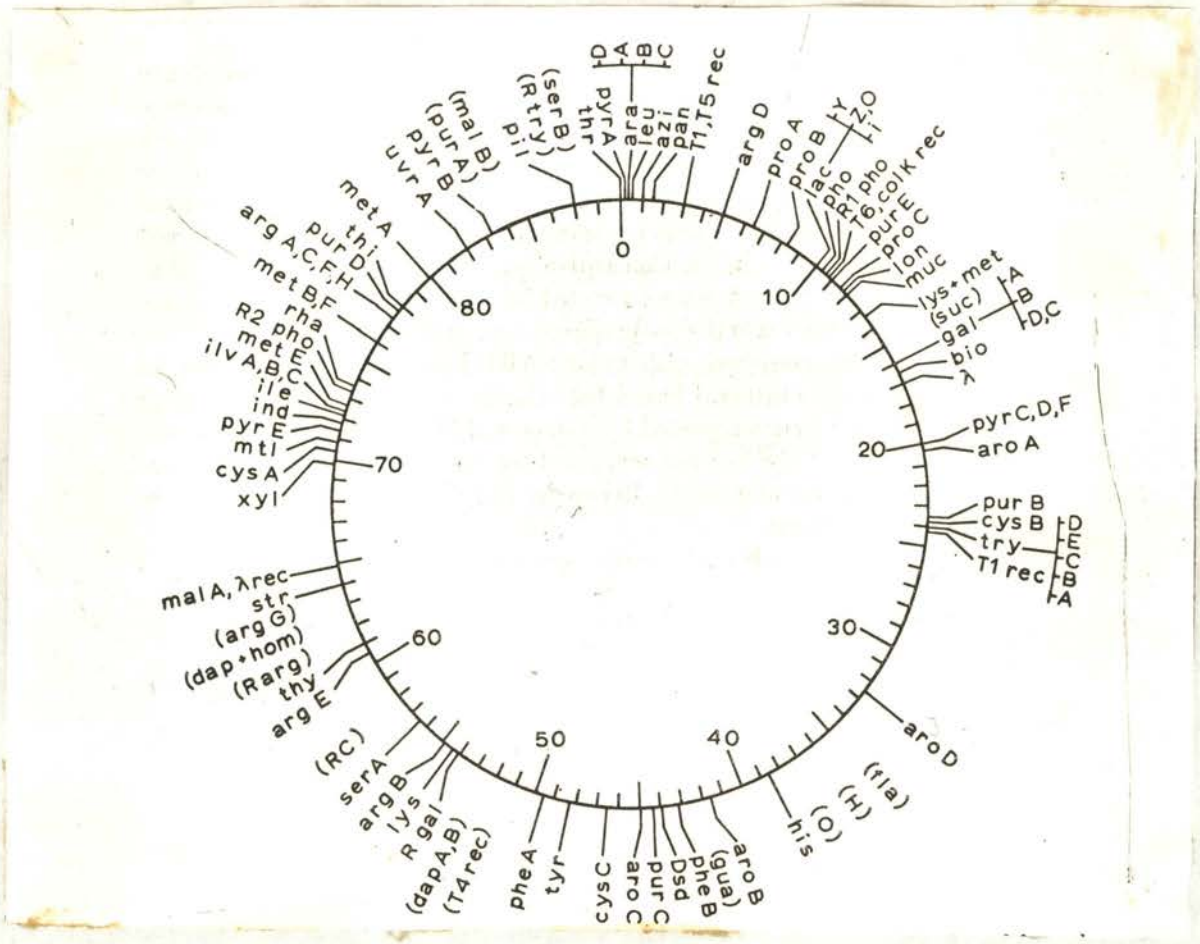

Figure 6. Genetic Map of E. Coli

Taylor and Adelberg (1961) showed that Hfr cells which are not acting as donors also possess a circular linkage group (Adelberg, 1966).

Cairns (1963) by the use of autoradiography of E. coli, labeled with tritiated thymidine and lysed with duponol, has shown that the bacterial chromosome comprises a single piece of DNA which is probably duplicated at a single growing point. Other experiments unrelated to Cairns also show that the bacterial chromosome is duplicated at a single growing point (Bonhoeffer and Gierer, 1963) which, at least in E. coli Hfr and in certain strains of Bacillus subtilis, always starts at the same place and moves in the same direction (Nagata, 1963; Yoshikawa and Sueoka, 1963).

Cairns comments on replication of the circular chromosome:

> At first sight it seemed surprising to find that the chromosome is physically in the form of a circle even while it is being replicated, for this arrangement demands that somewhere in the circle there must be something that acts as a swivel. However, in view of the apparent importance of the structure that unites the ends of the chromosome and so completes the circle, one must now consider the possibility that the structure actively drives DNA replication by rotating one end of the chromosome relative to the other; in this way, single stranded DNA might be continually produced at the replicating fork to act as primer for the polymerase. In short, it now seems conceivable that rapid DNA synthesis is possible only for circles (Cairns, 1963).

Figure 7 which appears on the following page illustrates the swivel idea of Cairns (1963).

The replication process is, in the terminology of Delbruck and Stent (1957), semiconservative, and continues until the entire chromosome has been duplicated. The point at which replication begins called the "replicator" (Jacob and Brenner, 1963) can be inferred to provide at least two functions essential to the replication process.

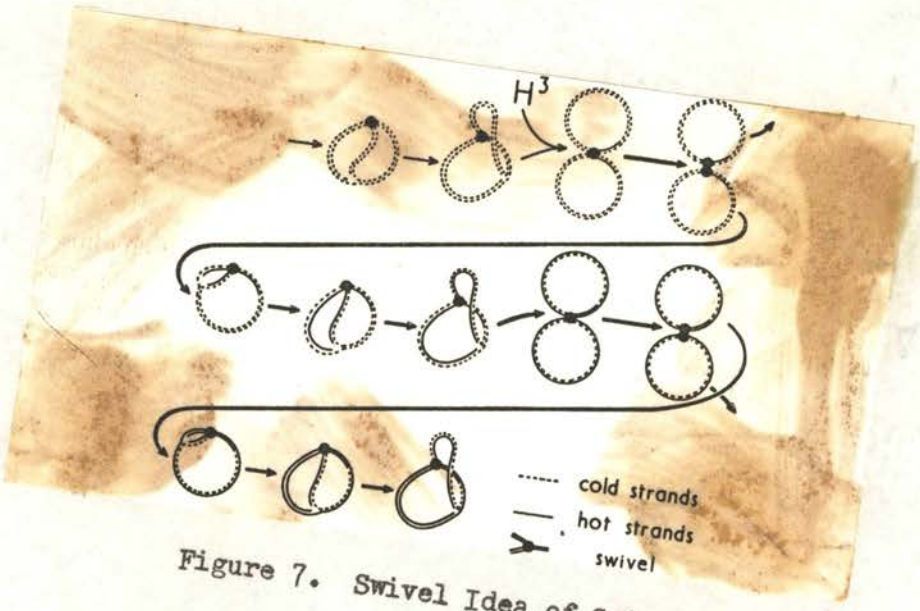

Figure 7. Swivel Idea of Cairn

It could allow the double helix to open, providing single strands to act as templates for the DNA replicating enzyme, and it could also function as a swivel. The need for a swivel is a consequence of the circularity of the chromosome; the unduplicated region must rotate in order to unwind, in a sense opposite to the rotation of one of the two arms. The mechanism by which the replicator acts as a swivel is still not known (Adelberg, 1966).

The following illustration, Figure 8, is a model of genetic transfer by replication during mating. In the donor (F^+ or Hfr) the F system of replication is assumed to be attached to the bacterial membrane at the vicinity of the point where the intercellular bridge is formed by contact with an F^- recipient. The DNA is assumed to move through the system of replication, one of the daughter replicas being driven through the bridge into the F^- cell, the other remaining in the donor bacterium (Jacob, Brenner and Cuzin, 1963).

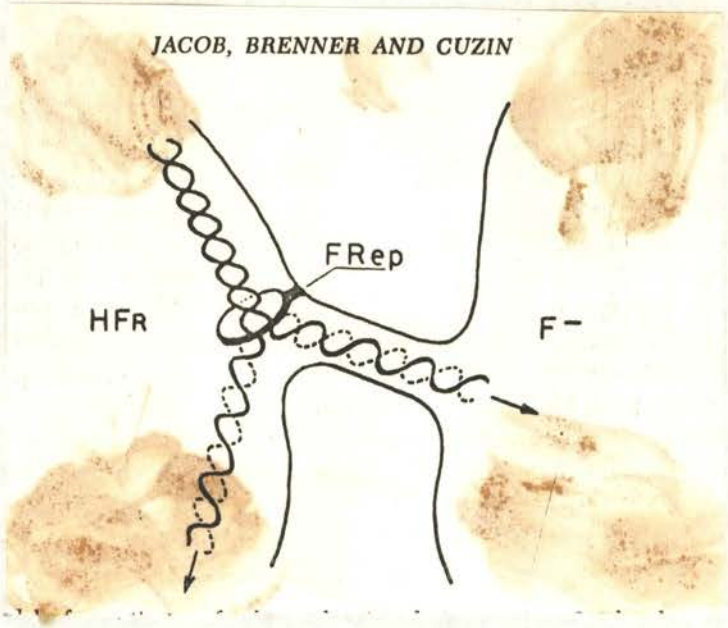

Figure 8. Model of Genetic Transfer by Replication

CHAPTER IV

VIRUSES

Bacterial viruses are commonly referred to as bacteriophage which literally means "eater of bacteria." Phages were discovered by Twort (1915) and d'Herelle (1917) when they noted that something was dissolving or lysing their bacterial cultures. D'Herelle (1917) believed that lysis is the process by which the phage, which has grown within the bacterium, is liberated from the cell and dispersed in solution. Delbrück (1940) in later research working with a sensitive strain of Bacillus coli found that there were two entirely different types of lysis, which were designated as "lysis from within" and "lysis from without."

> "Lysis from within" is caused by infection of a bacterium by a single phage particle and multiplication of this particle up to a threshold value. The cell contents are then liberated into solution without deformation of the cell wall.
>
> "Lysis from without" is caused by adsorption of phage above a threshold value. The cell contents are liberated by a distension and destruction of the cell wall. The adsorbed phage is not retrieved upon lysis. No new phage is formed (Delbrück, 1940).

Not all strains of bacteriophages are fully destructive to their host cells. The temperate (as opposed to the virulent) strains of phages have two distinct modes of parasitic reproduction according to Stahl (1964). Early in the course of infection of a bacterium a temperate phage chooses between two mutually exclusive paths of development. In some of the infected cells phage development proceeds

lytically; a period of DNA duplication is followed by the appearance in the cell of mature phages with lysis occurring soon after. In others of the infected cells one (or sometimes more) of the phage chromosomes becomes added to the bacterial chromosome (Stahl, 1964).

The first evidence for virus recombination came from studies in which viruses with different mutations were allowed to enter the same bacterial cells. A mixed infection is achieved when suspensions of the two viruses are added to a bacterial culture simultaneously in concentrations high enough so that most of the bacteria receive at least one of each of the virus particles (Watson, 1965).

Hershey and Chase (1951) demonstrated that when a phage infects a bacterium its DNA enters the host cell, but over 90 per cent of its protein remains attached to the outside surface of the cell.

In the Hershey and Chase (1951) experiment two batches of phage particles were prepared. One batch was prepared by growing phages in bacteria containing radioactive sulfur (S^{35}); the other batch was prepared in bacteria containing radioactive phosphorus (P^{32}). Since several amino acids contain sulfur but DNA does not, the first batch of phage particles was "labeled" in its protein moiety; since phosphorus is a component of DNA but not of proteins, the second batch was labeled in its DNA. Each batch of phages was permitted to attack bacteria. The infected bacteria were subjected to shear forces in a blender, and the amount of radioactivity removed from the cells was measured. The S^{35} was easily removed, but the P^{32} was not. The number of infected bacteria that produced a crop of phage particles was diminished by the treatment only slightly, if at all (Stahl, 1964).

The Hershey and Chase (1951) experiments along with other evidence indicated that the phage particles attach to the bacterium by means of a tail. The phage tail fibers penetrates the bacterial cell surface and injects the DNA into the bacterium. Figure 9 shows the basic phage structure.

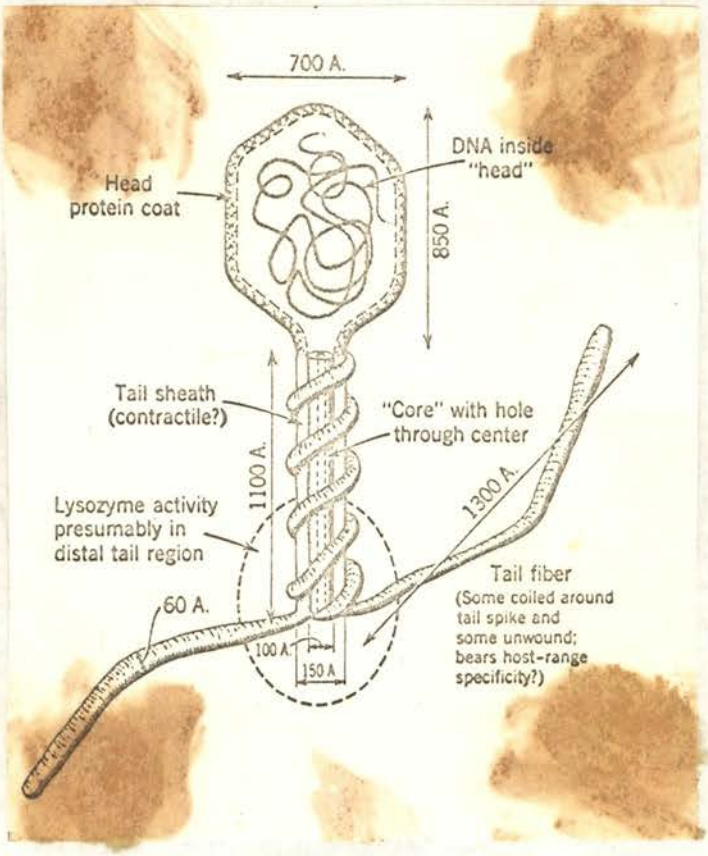

Figure 9. Basic Phage Structure

Viral Recombination

Delbrück, Bailey, and Hershey (1946) working independently made a most important discovery when examining the genetic character of the phage yield issuing from bacterial cells infected with two related parent viruses differing from each other in two mutant factors. It was found that among the progeny of such mixed infection there appear virus

offspring carrying DNA of the two parents, demonstrating that bacterial viruses can undergo genetic recombination (Stent, 1965).

Almost all the work has concentrated on several particular phages, arbitrarily given names like T1, T2, P1, F2, or λ. The best known are the closely related strains T2, T4, and T6. These similar strains reproduce in essentially the same way according to Watson (1965). The growth cycle starts when a phage particle collides with a sensitive bacterium and the phage tail specifically attaches to the bacterial wall. An enzyme in the phage tail then breaks down a small portion of the cell wall, creating a small hole through which the viral chromosomes enters the cell. The viral chromosome duplicates, and the daughter chromosomes continue to duplicate, to form eventually 100 to 1,000 new chromosomes, which become encapsulated with newly synthesized protective coats, to form a large number of newbacteriophage particles. The growth cycle is complete when the bacterial cell wall breaks open (lyses) and releases the progeny particles into the surrounding medium (Watson, 1965).

Adelberg (1965) states that about 20 to 40 minutes after infection, the infected bacteria produce enzymes called endolysins which rupture the bacterial cell wall and liberate infective phage into the medium.

Stern and Nanney (1965) described the technique of phage study. When phages are plated on a solid sheet of bacteria in a petri dish, each virus enters a bacterial cell, and after a characteristic latent period lyses the cell releasing the progeny. Eventually, a sufficiently large number of bacteria are destroyed to leave a clear area (or plaque) in the otherwise opaque sheet of bacteria. The number of initial particles can be determined by counting the plaques produced in appro-

priate dilutions. Also, occasional mutant viruses produce plaques of distinctive appearance. Their distinctiveness and constancy make them useful as genetic markers. Another set of markers is concerned with host specificity. Phages of a particular strain may attack some bacterial strains and not others, but mutants arise with modified host ranges (Stern and Nanney, 1965).

The important idea is the fact that when the lysate of the infected bacterial culture is assayed, recombinants appear in frequencies characteristic for the particular mutants employed and provide a means for mapping the virus "chromosome".

The classical conception of a gene assumed it to be a unitary particle by criteria involving each of the three kinds of observation:

1. A gene is a unit of chromosomal structure not subdivisible by chromosomal breakage or crossing over.
2. A gene is a unit of physiological function or expression.
3. A gene is a unit of mutation (Srb, Owen, and Edgar, 1965).

Studies on virus recombination have demonstrated the inadequacy of this concept.

Benzer and the rII Region

The work of Benzer (1955) involving the rII region of the phage T4 has clarified the concept of the gene.

There are a number of closely linked genes in the r region of the T4 bacteriophage of E. coli. The r mutants of T4 occur in three distinct regions designated as rI, rII, and rIII. The r mutants in all three regions produce plaques when E. coli strain B is used as host. However, mutants in the rII region are unique in that they cannot form

plaques when their host is strain K12 of E. coli, whereas the rI and rIII mutants can. Thus, among r mutants, only those in region II have this restriction in host range. The primary work involving the rII mutant was performed by Benzer (1955).

The work of Benzer (1955 and 1957) is an example of how bacterial viruses have been employed in determining the fine structure of the gene itself. As stated previously a bacterium can be infected simultaneously with two mutant phages and recombination between both DNA molecules can take place. With this type of fine analysis it has been possible to make genetic maps that approach the molecular level. Figure 10 on the following page shows the genetic map of the rII region, (Champe and Benzer, 1962).

Watson (1965) described the work of Benzer as follows:

> Up to now, the most striking results on the genetic structure of the gene itself have some from the work with the rIIA and rIIB genes of the bacterial virus T4. These are two adjacent genes which influence the length of the T4 life cycle; mutations in both thereby affect the size of plaques produced in a bacterial layer growing on an agar plate. The presence of either an rIIA or an rIIB mutation can cause a shorter life cycle of T4 phage within an E. coli cell. T4 infected cells on an agar plate normally do not break open and release new progeny phage until several hours after they have been infected. Cells infected with rII mutants, however, always break open more rapidly, hence the designation r (II stands for the fact that there do exist other genes which cause rapid cell lysis). Thus rII mutants produce larger plaques than wild-type phage. The rII mutants were chosen to work with because of the possibility of detecting a very large number of mutants. Although the wild-type and the rII mutants grow equally well on E. coli strain B, there is another strain, E. coli K(λ), on which only the wild-type can multiply. Thus when the progeny of a genetic cross between two different rII mutants are added to K12 (λ), only the wild-type recombinant per 10^6 progeny is easily detected.
>
> Over two thousand independent mutations in the rIIA and rIIB genes have been isolated and used in breeding experiments. In a typical cross, E. coli strain B bacteria

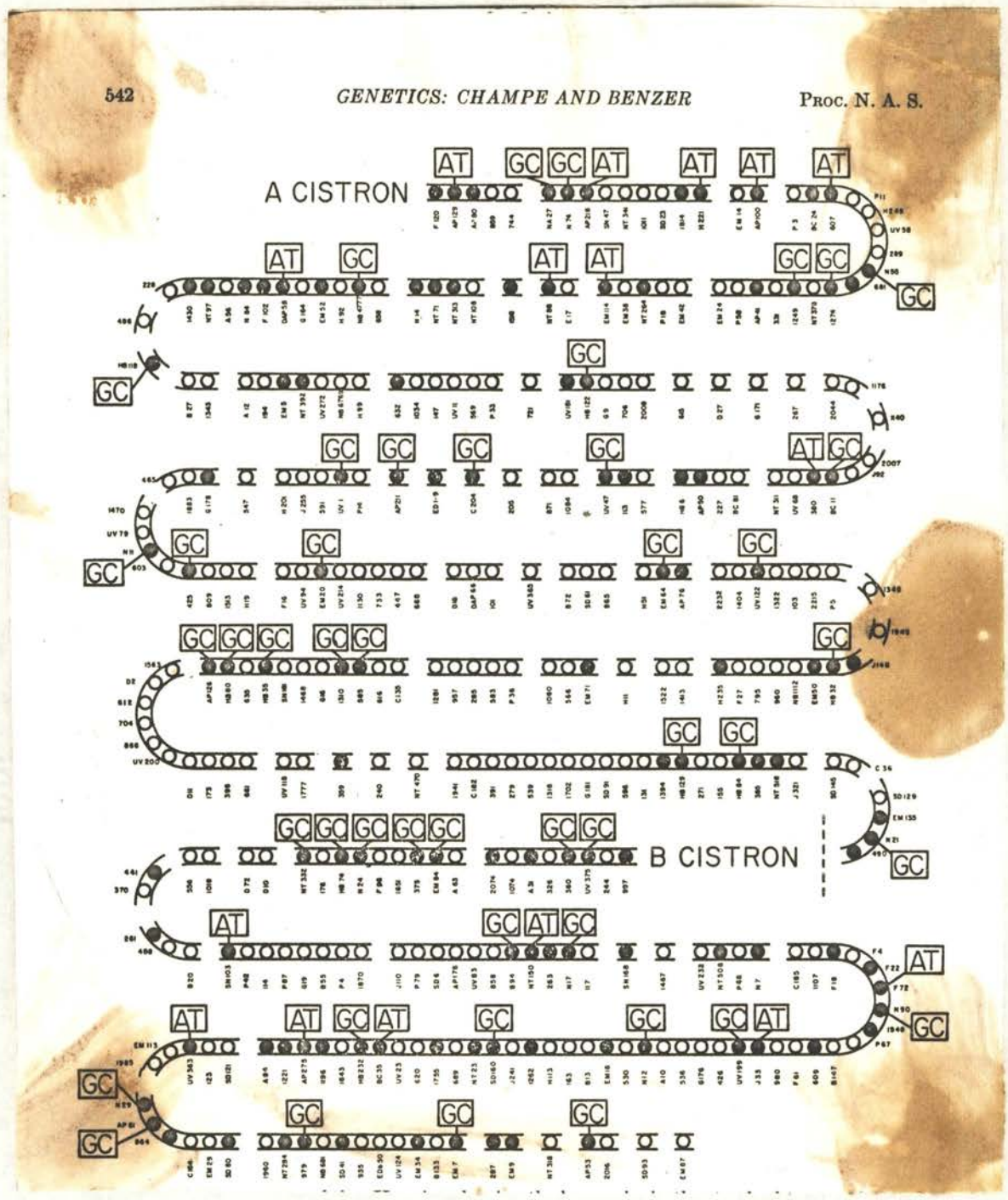

Figure 10. The Genetic Map of the rII Region

> were infected with two phage particles, each bearing an independently isolated rIIA (or rIIB) mutation. As the virus particles multiplied, genetic recombination occurred. The progeny were then grown on E. coli K(λ) to test for the wild type. Normal particles were found in a very large fraction of the crosses, indicating recombination within the gene, (Watson, 1965).

A very elegant test was used by Benzer (1957) to determine a functional unit he referred to as the "cistron." This test because of the part it has played in determining the fine structure of the gene requires explanation.

Stern and Nanney (1965) described the use of the cis-trans test:

> The rII region of phage T4 may be represented as (A) (B) and an rII mutant defective in the A section as (x) ( ); similarly, s mutant defective in the B section would be ( ) (x). A doubly infected K cell will be similar to a heterozygote, and if it is infected with mutants of the two kinds it can be represented as (x) ( ). If, however, it is infected with two mutants, both defective in the A section, it will appear as (x) ( ). In each of these "heterozygotes" the defects are placed in different chromosomes, across from each other in what may be called the trans configuration. By recombination, however, both defects can be placed in the same strand and the double heterozygotes will appear as (x) (x) and (x x) ( ). The defects are now ( ) ( ) ( ) ( ) in the same strands, in the cis position. The change from a trans to a cis arrangement does not change the composition of the elements but only their positions relative to each other. The change does, however, have consequences for the functioning of the material. In both cis arrangements a normal functional A region and a normal B region are available; but one of the trans arrangements is normal and the other defective. When two mutants are defective in the same function, a difference is detected between the cis and the trans configurations, but if the two mutants are defective in different functions, no such differences are seen in the cis-trans test (Stern and Nanney, 1965).

As a result of Benzer (1957) several new terms have come into common use. According to Benzer: (1) a recon is the unit of recombination, and in its minimal expression corresponds to the distance between

adjacent nucleotides in the DNA chain. (2) A muton is the unit of mutation, and corresponds, in its smallest expression, to a change in a pair of nucleotides. (3) A cistron is a unit of function within a gene.

The estimates of the molecular weights of these three units depended on the same basic information as the two previous attempts to give nucleotide dimensions to the conceptual aspects of the gene. On the basis of a view later shown to be in error, Carlson (1966) stated that Benzer assumed that only 40 per cent of the total phage DNA was genetically active.

Benzer (1957) estimated that the level of genetic fine structure which he had reached in his experiments with the rII mutants was not far removed from that of the individual nucleotides. He further stated that if a cross between two (single) T4 mutants does not give at least 0.01 per cent recombination, the locations of the two mutations probably are not separated by even one nucleotide pair.

The molecular sizes of the genetic units proposed by Benzer (1957) as a result of his experiments were listed as:

> Recon: The smallest non-zero recombination value so far observed among the rII mutants of T4 is around 0.02 per cent recombination. If the estimate of 0.01 per cent recombination per nucleotide pair should prove to be correct, the size of the recon would be limited to no more than two nucleotide pairs.
>
> Muton: The muton is defined as the smallest element, alteration of which can be effective in causing a mutation. In the case of reverting mutants, it has not been possible, so far, to demonstrate any appreciable mutation size greater than around 0.05 per cent recombination. This would indicate that alteration of very few nucleotide (no more than five, according to the present estimate) is capable of causing a visible mutation.

> Cistron: ....it seems safe to conclude that in the A cistron alone there are over a hundred "sensitive" points, i.e., locations at which a mutational event leads to an observable phenotypic effect. Just as in the case of the entire genetic map of an organism, the portrait of a cistron is weighted by considerations of which alterations are effectual (Benzer, 1957).

Watson (1965) states that in place of the slogan "one gene-one protein (enzyme), we now realize that a more correct statement is "one gene-one polypeptide chain."

In discussing this statement Peacocke and Drysdale (1965) noted that all gene mutations result in differences in amount of the specific protein of a mutant organism. Numerous cases are now known in which a specific mutation has resulted in a structural change in a particular protein. These are the cases which have revealed the relationship between genes and proteins and shown that one function of genes is to control the sequence of amino acids in proteins (Peacocke and Drysdale, 1965).

CHAPTER V

GENETIC CONTROL OF PROTEINS AND REGULATION

Perhaps the most extensive genetic and biochemical information in this area has been reported for the tryptophan synthetase system of E. coli studied by Yanofsky (1963) and his colleagues.

Relationship Between Genes and Proteins

The Escherichia coli tryptophan synthetase consists of two separable protein subunite, designated as A and B. According to Yanofsky (1963) these proteins catalyze three reactions:

(1) indole + L-serine $\longrightarrow$ L-tryptophan
(2) indoleglycerol phosphate $\rightleftharpoons$ indole + 3-phosphoglyceraldehyde
(3) indoleglycerol phosphate + L-serine $\longrightarrow$ L-tryptophan + 3-phosphoglyceraldehyde (Yanofsky, 1963).

Reaction (3) is believed to be the physiologically essential reaction in tryptophan biosynthesis.

Peacocke and Drysdale (1965) commented that Yanofsky's studies with E. coli have resulted in a correlation of gene mutation with specific amino acids at specific sites within the A protein of the tryptophan synthetase system.

The A mutants and suppressed A mutants according to Yanofsky (1963) were produced by ultraviolet irradiation of the K-12 strain. A large number of these mutants unable to synthesize tryptophan have been isolated and because they lack what is called a functional A chain they are enzymatically inactive.

Mutants were classified as to their production of different types of altered A protein first by Maling and Yanofsky (1961). Two main types of A mutants have been found on the basis of enzymic and serological tests. One type forms an altered A protein, the other does not form any protein detectable by these methods. The mutant A proteins differ from wild-type A protein and among themselves in regard to heat stability and stability at low pH. Yanofsky (1963) stated that such differences appear to be characteristic of the mutant proteins themselves and are not due to other substances present in the preparations. Figure 11 on the following page shows the relative order of mutational alterations in the A gene.

Yanofsky's studies on the relationships between genes and protein structure in the tryptophan synthetase system are very simply explained by Peacocke and Drysdale (1965):

> To obtain information about the precise chemical nature of the modification induced in the protein as a result of mutation, an analysis of the amino acid composition and sequence in the protein, or peptides derived from it, was necessary. Fortunately the A protein has a fairly low molecular weight of 29,500 and has proved amenable to more detailed analysis using the methods developed originally by Sanger and Smith for insulin and Ingram for haemoglobin
>
> Tryptophan synthetase A protein from wild-type strains gave twenty-five major peptides. The 'finger-prints' of the altered A proteins from a number of mutants differed in a number of cases from that of the wild type. It should be pointed out that not all amino acid substitutions were expected to result in altered peptide patterns since not all amino acid substitutions caused differences in the mobility of peptides under the conditions used.
>
> The A protein of two mutants, A23 and A46, which mapped at the same site on the genetic map were found to differ from normal A protein in the composition of a particular peptide. In the protein from A23 an arginine residue replaced a glycine present in wild-type A protein while in A46 this same glycine residue was replaced by glutamic acid. Several revertants, that is, strains which have regained the ability to grow on minimal medium as a result of mutation, have been recovered from each of the strains and where these map at the same

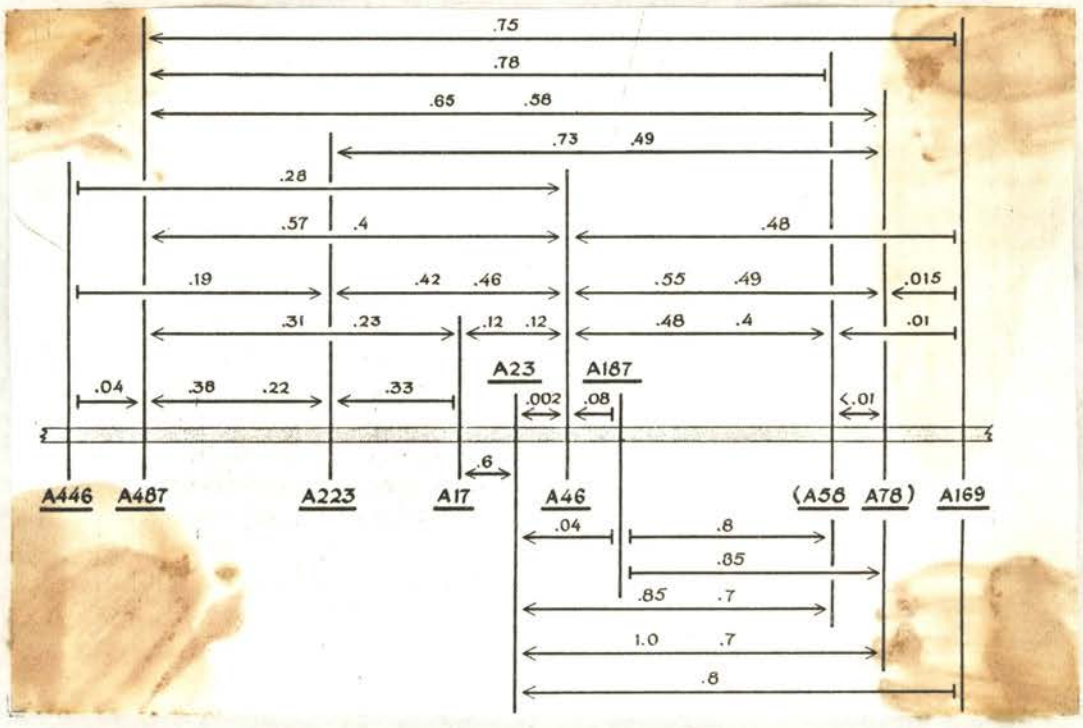

Figure 11. The Order of Mutationally Altered Sites in the A Gene

> genetic site it has been shown that the amino acids substituted as a result of mutation occupy the same position in the corresponding protein. The results provide evidence that eight independent mutations, mapping at the same site, have resulted in the substitution of six different amino acids at the same position in the polypeptide chain of tryptophan synthetase A protein (Peacocke and Drysdale, 1965).

According to Herskowitz (1965) the primary effect of at least some genes is to specify completely the amino acid content of a polypeptide. If the one polypeptide-one gene hypothesis is correct, we expect every polypeptide chain in every protein- including proteins that are not enzymes- to be completely specified by the primary and solitary action of a single gene.

The work of Yanofsky (1963 and 1964) has established in most detail the colinearity of the genetic map and the protein sequence. Figure 12 on the following page demonstrates the colinearity of gene structure and protein structure. Yanofsky has localized the sites of the A region of tryptophan synthetase. The figure shows the mutations causing amino acid replacements which result in a loss of enzyme activity. The tryptic peptides of the A protein show that closely linked sites are usually present in the same peptide. Furthermore, the sequence of tryptic peptides in the A protein is colinear with the sequence of sites in the genetic map. The colinearity is a consequence of the coding relation between the nucleotide sequence of the gene and the amino sequence of the protein specified by the gene (Carlson, 1966).

The function of the tryptophan synthetase A cistron has been worked out more thoroughly than that of any other cistron in any organism according to Fincham (1966). Most of the amino acid sequence of the A polypeptide chain is now known, and the effects on this primary structure of some dozen different mutations have been established.

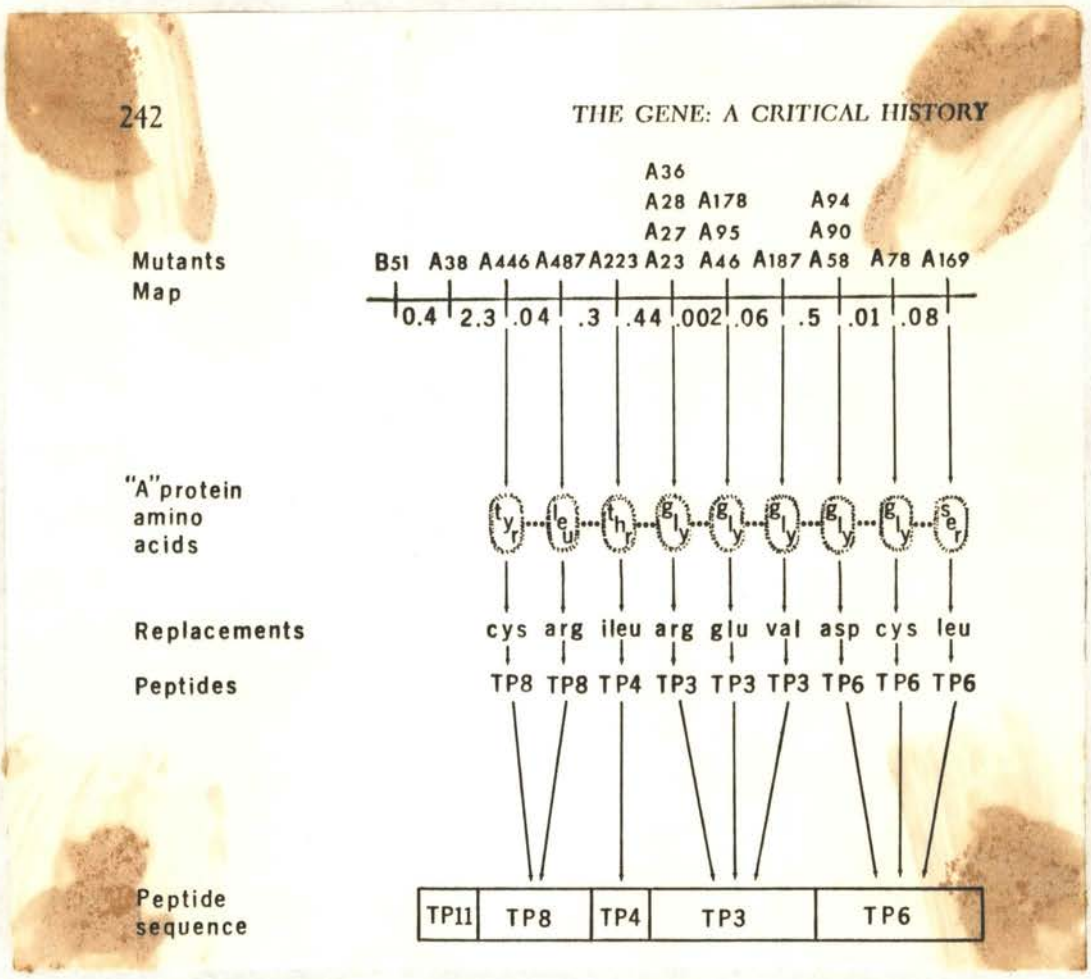

Figure 12. Colinearity of Gene and Protein

Fincham (1966) further states that this cistron forms a linear code for the amino acid sequence of a specific polypeptide chain. It appears overwhelmingly likely that this case, though the only one so far worked out in such detail, can stand as a general model of cistron action.

The Operon Theory

Although we are beginning to understand the way in which genes control proteins we are not as well grounded in the problem of regulation. This is probably one of the most rapidly developing areas of molecular biology where exciting developments are taking place. It would be impossible to discuss all the developments that are occurring but the evidence available in bacteria concerning the genetic regulatory mechanism by Jacob and Monod (1961) is of special importance.

The model, which was proposed in detail by F. Jacob and J. Monod (1961), was analyzed genetically in the B-galactosidase system of E. coli. As a result of their experimentation the evidence indicates that, at least in bacteria, genes with regulating or controlling functions exist in two types: (a) regulator genes in which mutations affect the conditions and rate of sythesis of the corresponding protein and (b) operator genes which control the rate of transcription of one or more structural genes adjacent to them in the chromosome (Peacocke and Drysdale, 1965).

Jacob and Monod (1961) summarized their model as follows:

> The molecular structure of proteins is determined by specific elements, the structural genes. These act by forming a cytoplasmic "transcript" of themselves, the structural messenger, which in turn synthesizes the protein. The synthesis of the messenger by the structural gene is a sequential replicative process, which can be initiated only at certain points on the DNA strand, and the cytoplasmic tran-

> scription of several, linked, structrual genes may depend upon a single initiating point or operator. The genes whose activity is thus co-ordinated form an operon.
>
> The operator tends to combine (by virtue of possessing a particular base sequence) specifically and reversibly with a certain (RNA) fraction possessing the proper (complementary) sequence. This combination blocks the initiation of cytoplasmic transcription and therefore the formation of the messenger by the structural genes in the whole operon. The specific "repressor" (RNA), acting with a given operator, is synthesized by a regulator gene, (Jacob and Monod, 1961).

By 1961 the operon theory according to Carlson (1966) dissolved the impasse between the cytoplasmic and nuclear outlooks. The operon became an extreme example of a mechanistic system of circuits, feedbacks, and blueprints.

Carlson (1966) further described the operon theory. In inducible systems the repressor would ordinarily seal off the operator and thus prevent the transcription of its structural genes. When inducer (such as galactoside bearing molecule) was introduced in the B-galactosidase system, the repressor and the inducer would form a complex which would be incapable of turning off the operator, and thus the enzyme for the degradation of the inducer would be transcribed and decoded from its structural gene. In the case of a repressible system, the repressor was assumed to be incomplete. Thus the structural genes would be transcribed and their enzymes would synthesize a metabolite. This metabolite would form a complex that would complete the repressor. The combination of repressor and co-repressor in this complex would seal off the operator and thus prevent the further formation of enzymes for the synthesis of the metabolite (or co-repressor) according to the description of Carlson (1966). The model proposed by Jacob and Monod (1961) is illustrated on the following page in figure 13.

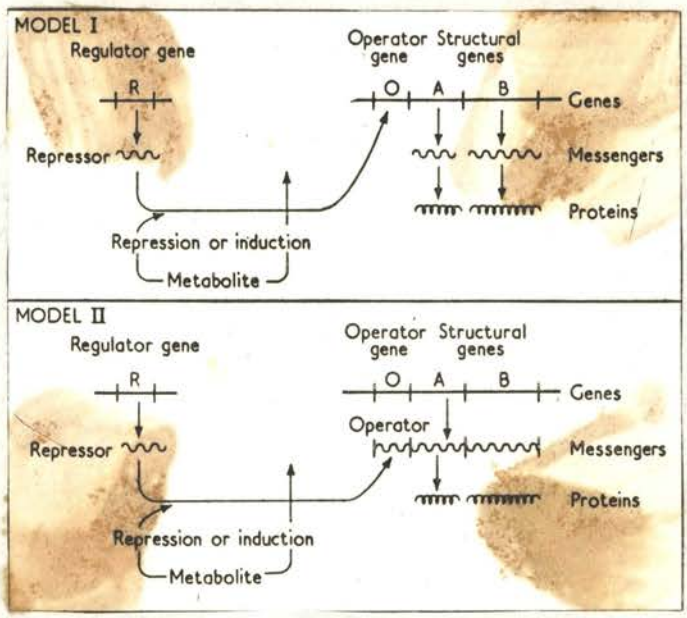

Figure 13. Models of the Regulation of Protein Synthesis

Concerning the position of the genes DeRobertis, Nowinski, and Saez (1965) commented that the regulator genes need not be near the structural genes on which they act and in fact they may even be in another chromosome since they act by way of the repressor. The operator gene in order to function however, requires a close linkage and a cis configuration with the structural gene. They further stated that both regulator and operator genes are carried in the chromosomes, can recombine and are composed of DNA.

Peacocke and Drysdale (1965) stated that according to the operon theory, if a structural gene is removed from the operon and incorporated elsewhere in the genome as a result of a chromosomal rearrangement then the structural gene should escape from the control of the operon. Using the Salmonella histidine system this prediction has been tested and found to be correct (Peacocke and Drysdale, 1965).

The application of the operon theory to a variety of inducible and repressible enzyme systems in bacteria has evidently been quite successful. Its application to higher levels of organization still needs to be achieved. Probably the biggest hindrance at this level is the lack of detailed experimental procedures similar to those that have been used in bacteria. Nevertheless, it is quite evident that this theory has stimulated a renewed hope that regulatory systems at higher levels will be uncovered in the very near future.

CHAPTER VI

SUMMARY

This paper has been an attempt to discuss the contributions that microorganisms have made to genetics with primary emphasis on Neurospora crassa, Escherichia coli, and the T bacteriophages.

The rapid development of modern biochemical genetics dates from the work of Beadle and Tatum in 1941. Their work with the Neurospora crassa was discussed.

In 1928 Griffith demonstrated that a hereditary determinant was involved in the transformation experiment he performed with Pneumococcus. Avery, MacLeod and McCarty then demonstrated in 1944 that this hereditary material was deoxyribonucleic acid (DNA).

The modern era of bacterial biology began with the work of Lederberg and Tatum in 1946, working with Escherichia coli. Further work with this organism primarily by Wollman, Jacob, and Hayes demonstrated that bacterial mating could be controlled. Other research led to the identification of the circular chromosome and also the technique of mapping the chromosome primarily by bacterial conjugation.

Bacteriophages are viruses which attack bacteria. The most intensively studied group are the T bacteriophages which infect Escherichia coli cells. The role of the phage nucleic acid in viral reproduction was established initially by the work of Hershey and Chase in the early fifties.

The work of Benzer involving the rII region of the phage T4 has made a big contribution in the clarification of the gene concept. As a result of his work in the middle fifties several new terms are now in common use.

During the past 20 years, mainly through developments in microbial genetics, it has become firmly established that the function of genes is to control the structure or synthesis of proteins. The work of Yanofsky in the early sixties has provided us with evidence that demonstrates a correlation of gene mutation with specific amino acids at specific sites within the A protein of the tryptophan synthetase system of E. coli.

To demonstrate the new concept of the regulation of gene activity the outstanding work of Jacob and Monod was presented. This is a very intensively studied area today. A very likely outgrowth of this valuable research will be that of the regulation and control of gene action during differentiation and development.

These and many other interesting areas such as the evolutionary aspects and implications will undoubtedly receive great attention in due course of time.

The continued study of microorganisms during the latter decades of the twentieth century should provide even newer and more exciting phases of genetics.

A SELECTED BIBLIOGRAPHY

Adelberg, Edward A. Papers on Bacterial Genetics, 2nd ed. Boston: Little, Brown and Company, 1965.

Avery, O. T., C. M. MacLeod, and M. McCarty. "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III." Journal of Experimental Medicine. 79: 137-158. 1944.

Beadle, G. W. "The Genetic Control of Biochemical Reactions". Harvey Lectures, Series 40: 179-194. 1945.

Beadle, G. W. and E. L. Tatum. "Genetic Control of Biochemical Reactions in Neurospora." Proc. of the Nat'l. Acad. of Sci. 27: 499-506. 1941.

Benzer, S. "Fine Structure of a Genetic Region in Bacteriophage." Proceedings Natl. Acad. Sci. U.S. 41: 344-354. 1955.

Benzer, S. "The Elementary Units of Heredity," in The Chemical Basis of Heredity, W. D. McElroy and B. Glass, editors, pp. 70-93. The John Hopkins Press, Baltimore. 1957.

Bonhoeffer, F., and A. Gierer. "On the Growth Mechanism of the Bacterial Chromosome". Journal of Molecular Biology. 7: 534-540. 1963.

Cairns, J. "The Bacterial Chromosome and its Manner of Replication as Seen by Radioautography." Journal of Molecular Biology. 6: 208-213. 1963.

Cairns, J. "The Chromosome of Escherichia Coli". Cold Spring Harbor Symposium on Quantitative Biology. 28: 43-46. 1963.

Carlson, Elof A. The Gene. Philadelphia: W. B. Saunders Co., 1966.

Champe, S. P. and S. Benzer. "Reversal of Mutant Phenotypes by 5-Fluorouracil: An Approach to Nucleotide Sequences in Messenger RNA." Proc. Natl. Acad. Sci., Washington. 48: 532-546. 1962.

Dawson, M. H., and R. H. P. Sia. "In vitro Transformation of Pneumococcal Types. A Technique for Inducing Transformation of Pneumococcal Types in Vitro." Journal of Experimental Medicine. 54: 681-710. 1931.

Delbruck, M. "Adsorption of Bacteriophages Under Various Physiological Conditions of the Host". Journal of General Physiology. 23: 631-642. 1940.

Delbruck, M. and W. T. Bailey. "Induced Mutations in Bacterial Viruses." Cold Spring Harbor Symposium on Quantitative Biology. 11: 33. 1946.

Delbruck, M. and G. Stent. On the Mechanism of DNA Replication. In "The Chemical Basis of Heredity", W. D. McElroy and B. Glass, Eds., Baltimore: Johns Hopkins Press. p. 699. 1957.

d'Herelle, F. "The Bacteriophage and its Behavior". (Reprinted in Papers on Bacterial Viruses). Baltimore: Williams and Wilkins. p. 135. 1926.

DeRobertis, E. D. P., Wiktor W. Nowinski and Francisco A. Saez. Cell Biology. Philadelphia: W. B. Saunders Company, 1965.

Emerson, Sterling. "Biochemical Models of Heterosis in Neurospora." In Heterosis edited by John W.Gowen. Iowa State College Press Ames-Iowa. 1952.

Fincham, J. R. S. Genetic Complementation. New York: W. A. Benjamin, Inc., 1966.

Fisher, K. "The Role of the Krebs Cycle in Conjugation in Escherichia coli K-12. Journal General Microbiology. 16: 120-135. 1957.

Giles, N. and M. Case. "Comparative Complementation and Genetic Map of the Pan-2 Locus in Neurospora crassa." Proc. of the Natl. Acad. of Sci. 46: 659-676. 1960.

Griffith, F. "The Significance of Pneumococcal Types". Journal of Hygiene. 27: 113-159. 1928.

Hayes, W. "The Mechanism of Genetic Recombination of Escherichia coli." Cold Spring Harbor Symposium on Quantitative Biology. 18: 74-93. 1953.

Hershey, A. D. and M. Chase. "Genetic Recombination and Heterozgosis in Bacteriophage." Cold Spring Harbor Symposium on Quantitative Biology. 16: 471-479. 1951.

Herskowitz, Irwin J. Genetics. Boston: Little, Brown and Company, 1965.

Jacob, F. and S. Brenner. Sur la Regulation de la Synthese du DNA Chez des Bacteries: l'hypothese du replicon. Compt. Rend. Acad. Sci., (Reprinted in E. A. Adelberg, Papers on Bacterial Genetics)., Paris. 248-321. 1963.

Jacob, F., S. Brenner, and F. Cuzin. "On the Regulation of DNA Replication in Bacteria." Cold Spring Harbor Symposium on Quantitative Biology. 28: 329-348. 1963

Jacob, F. and J. Monod. "On the Regulation of gene Activity." Cold Spring Harbor Symposium on Quantitative Biology. 26: 193-211. 1961.

Jacob, F., and E. L. Wollman. "Recombination Genetique et mutants de Fertilite Chez E. coli K-12." Compt. Rend. Acad, Scil. Paris, 242: 303-306. 1956.

Jacob, F., and E. L. Wollman. "Genetic and Physical Determinations of Chromosomal Segments in Escherichia coli." Symp. Soc. for Exptl. Biol., 12: 75-92. 1958.

Lederberg, J., and E. L. Tatum. "Novel Genotypes in Mixed Cultures of Biochemical Mutants of Bacteria." Cold Spring Harbor Symposium on Quantitative Biology. 11: 113-114. 1946.

Lwoff, A. Lysogeny. Bacteriol. Rev., 17: 269-337. 1953.

McElroy, W. D., and Bentley Glass. The Chemical Basis of Heredity. Baltimore: The Johns Hopkins Press, 1957.

Maling, B., and C. Yanofsky. "Suppressor Gene Alteration of Protein Primary Structure". Selected Papers on Molecular Genetics. 47: 551-641. 1961.

Nagata, T. "The Molecular Synchrony and Sequential Replication of DNA in Escherichia coli. Proc. Natl. Acad. Scil., Washington. 49: 551-559. 1963.

Peacocke, A. R., and R. B. Drysdale. The Molecular Basis of Heredity. Washington: Butterworth & Co., 1965.

Sager, Ruth and Francis J. Rayan. Cell Heredity. New York: John Wiley and Sons, Inc., 1961.

Srb, Adrian M., Ray D. Owen, and Robert S. Edgar. General Genetics. San Francisco: W. H. Freeman and Company, 1965.

Stahl, Franklin W. The Mechanics of Inheritance. Englewood Cliffs, New Jersey: Prentice-Hall, Inc., 1964.

Stern, Herbert, and David L. Nanney, The Biology of Cells. New York: John Wiley & Sons, Inc., 1965.

Stent, Gunther S. Papers on Bacterial Viruses, 2nd ed. Boston: Little Brown and Company, 1965.

Taylor, A. L., and E. A. Adelberg. "Evidence for a Closed Linkage Group in Hfr Males of Escherichia coli k-12." Biochemical and Biophysical Res. Com. 5: 400-404. 1961.

Taylor, J. Herbert. Selected Papers on Molecular Genetics. New York: Academic Press, 1965.

Taylor, A. L., and M. S. Thoman. "The Genetic Map of Escherichia Coli K-12." Genetics. 50: 659-677. 1964.

Twort, F. W. "An Investigation on the Nature of the Ultramicroscopic Viruses." (Reprinted in Papers on Bacterial Viruses). II, 1241. 1915.

Watson, J. D. Molecular Biology of the Gene. New York: W. A. Benjamin Inc., 1965.

Wollman, E. L., and F. Jacob. "Sur le Mecanisme du Transfert de Material Genetique au cours de la Recombinaison chez Escherichia Coli K-12." Compt. Rend. Acad. Sci., Paris: 240: 2449-2451. (Reprinted in E. A. Adelbert, Papers on Bacterial Genetics). Little, Brown and Company. Boston. 1955.

Wollman, E. L., F. Jacob, and W. Hayes. "Conjugation and Genetic Recombination in Escherichia Coli, K-12." Cold Spring Harbor Symposium on Quantitative Biology. 21: 141-161.

Yanofsky, C. "Amino Acid Replacements Associated with Mutation and Recombination in the A Gene and Their Relationship to in vitro coding Data." Cold Spring Symposium on Quantitative Biology. 28: 581-588. 1963.

Yanofsky, C., B. C. Carlton, J. R. Guest, D. R. Helinski, and U. Henning. "On the Colinearity of Gene Structure and Protein Structure." Proc. Natl. Acad. Sci. 51: 266-272. 1964.

Yoshikawa, H., and N. Sueoka. "Sequential Replication of Bacillus subtilis Chromosome II. Isotopic Transfer Experiments." Proc. Natl. Acad. Sci., Wash., 49: 806-813. 1963.

VITA

Jerry Lee Cushman

Candidate for the Degree of

Master of Science

Report: GENETICS OF MICROORGANISMS

Major Field: Natural Science

Biographical:

Personal Data: Born in Lansing, Michigan, April 30, 1935, the son of Seward R. and Julia E. Cushman.

Education: Attended grade school in Lansing, Michigan; graduated from Lansing Eastern High School in 1952; received the Bachelor of Science degree from Michigan State University in 1957; completed the requirements for the Master of Arts degree in August, 1961; attended Oklahoma State University on a NSF-AYI during the year 1966-67; completed the requirements for the Master of Science degree from Oklahoma State University, with a major in Natural Science, in May, 1957.

Professional experience: Served as a member of the Michigan National Guard from 1954 to 1960 attaining the M.O.S. of Sergeant First Class and the title of Chief of Survey in the Field Artillery. Since 1957 has been teaching biology and mathematics at Lansing Everett High School in Michigan.