11,764

2941

Name: Margaret Sue Darsie Date of Degree: Aug. 8, 1964

Institution: Oklahoma State University Location: Stillwater, Okla.

Title of Study: Bacterial Transformations

Pages in Study: 28 Candidate for Master of Science Degree

Major Field: Natural Science

Summary: This report is a review of the literature on bacterial transformations. First, this paper discusses the historical development and discoveries from the time of Griffith in 1928 up through the years until 1963. Then examples of the known transformation reactions are given, followed by a discussion of competence. Also included is a chapter on the mechanism of transformation. All this leads to a final chapter on significance, which shows why research on transformation is important today.

Adviser's Approval Franklin R. Leach

BACTERIAL TRANSFORMATIONS

By

Margaret Sue Darsie

Bachelor of Science

Wheaton College

1963

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
Master of Science
August, 1964

BACTERIAL TRANSFORMATIONS

Report Approved:

Franklin R. Leach
Report Adviser

L. Hebert Bruneau

Robert MacIver
Dean of the Graduate School

TABLE OF CONTENTS

INTRODUCTION

Scientists have long desired to be able to induce predictable and specific mutations at will. For a long time those mutations which were spontaneous or induced were entirely undirected and unrelated to environmental conditions. Recently in bacteria specific inheritable changes have been induced in susceptible cells when grown in the presence of certain chemicals, dead cells, culture filtrates, or cell extracts from a genetically dissimilar member of the same species.

Since many of the reactions thus far induced involve rough and smooth strains, it will be necessary to first mention a few characteristics of these strains.

Smooth:
1. Smooth bacteria are virulent and have a capsule composed of polysaccharide.
2. They can be classified into many types according to the chemical composition and immunological properties of the capsular polysaccharide (I, II, etc.)
3. The serological type is inherited and maintained as long as cells are smooth (S).
4. S strains spontaneously mutate to R (rough) strains.

Rough:
1. These bacteria are nonencapsulated and avirulent.
2. They lack the serological type specificity of the parent.
3. They never revert spontaneously to any encapsulated S type.

HISTORY

In 1925 the first data was published regarding transformation. It was then discovered that one species of bacterium grown in the presence of a second, closely related species would acquire several properties of the second (Frobisher).

In 1928 Griffith conducted experiments in which avirulent, R strains of pneumococcus derived from one specific S type were inoculated subcutaneously into a mouse together with heat killed S cells of a heterologous type. Many of these mice died, and from them were obtained many virulent, encapsulated, living S cells of the heterologous type. These results may be represented by the following symbols:

$$\text{SII} \longrightarrow \text{R} \xrightarrow[\text{heat-killed SIII cells}]{\text{into mice}} \text{SIII}$$

Griffith's discovery was so novel and important that all alternative explanations had to be ruled out. In 1931 Dawson and Sia aided in eliminating the possibility that a few viable organisms had remained in Griffith's vaccine. They discovered this same transformation when small inocula of R forms were grown in media containing vaccines prepared from heterologous S cultures. This <u>in vitro</u> transformation was most readily effected when anti-R serum was employed in the culture medium.

Alloway in 1932 conducted <u>in vitro</u> transformations in the presence of cell-free extracts from S cells prepared by the dissolving action of sodium desoxycholate. Relatively small amounts of extract were effective when added to a broth

containing normal serum or serous fluid.

Since 1945 study has proceeded along two main lines. Chemical studies have confirmed that DNA is the transforming agent and have determined its chemical and physical properties.

The active transforming principle was isolated by Avery, MacLeod, and McCarty in 1943; and they found it to consist of a highly polymerized, viscous form of deoxyribonucleic acid. They determined this by the following tests:

1. Because of the determined C, N, and P ratios, protein impurities could not constitute a very significant portion of the transforming principle.
2. Biuret and Millon protein tests were negative.
3. The orcinol test for RNA was weakly positive, but no more so than the same test on calf thymus DNA.
4. No loss of transforming activity resulted after treatment with crystalline trypsin, chymotrypsin and ribonuclease.
5. The light absorption was maximum at 2600 A^{o} and minimum at 2300 A^{o}. This is similar to nucleic acids in general.
6. DNase enzymes rapidly denatured the transforming principle whereas other enzymes could not do this. When the DNase was denatured by heat, it could not inactivate the transforming principle. Also, the temperature at which DNase activity was lost closely resembled that at which the ability to destroy the transforming principle was lost.

Because of these and a few other tests Avery, MacLeod, and McCarty concluded that "within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid, or serologically reactive polysaccharide and consists principally, if not solely, of a highly polymerized, viscous form of deoxyribonucleic acid."

However, in 1944 the specific activity of DNA was unknown, but several of the following roles were postulated:

1. It is the genetic material capable of infecting cells.
2. It is capable of being replicated. This was known by the discovery that the active DNA capable of causing similar transformation isolated from transformed SIII cells and their progeny was in excess of the amount required for the initial alteration.
3. DNA is capable of controlling a specific metabolic function.

It was Mirsky in 1947 (Ravin) who said that DNA was essential for the activity of the transforming principle, but that it had not been proved to be the material responsible for the specificity transferred. He suggested that possibly a non-detectable protein, protected from denaturation and proteolytic agents by the DNA, could account for the specificity transferred.

Hotchkiss in 1948, 1952 (Ravin) showed by the following tests that the presence of such a genetically active impurity was highly improbable:

1. During purification the composition of the purine and pyrimidine bases became more nearly like that from calf thymus DNA.
2. N/P ratios of the transforming principle and calf thymus DNA were identical. If there were substantial contamination, this would not be so.
3. As the transforming principle was progressively purified, the α amino acids gradually disappeared. Glycine was the only α amino acid liberated, and the amount and rate of liberation resembled that at which glycine is liberated from the degradation of adenine rather than from a protein.

On the basis of this, Hotchkiss in 1952 (Ravin) estimated that any protein present could not constitute more than 0.02% of the purified transforming principle; and since these preparations were active when highly dilute, the chance of a contaminant was remote. Since this time further analyses with radiation and radioactive markers have shown that DNA alone is the genetically active transforming principle.

REACTIONS

Ravin gives a list of all the transformable bacteria and genetic characters as of 1961. Following is a partial duplication of that chart:

I. Capsular polysaccharide synthesis
II. Filamentous type of growth
III. Specific protein antigens (M proteins)
IV. Drug and antibiotic resistance (Penicillin, streptomycin, sulfanilamide, erythromycin, etc.)
V. Antibiotic dependence (streptomycin)
VI. Synthesis of specific enzymes
VII. Sporulation (Bacillus subtilis)
VIII. Other characters
    1. Ability to infect plants
    2. Intermediate encapsulated types
    3. Mixed or binary encapsulation
    4. Abnormal capsular type

It is impossible to elaborate on all of these reactions; therefore a few will suffice. The transformations related to specific somatic protein antigens and drug-resistance were helpful in ruling out accidental contamination as explanation of Griffith's early experiments.

Pneumococci contain a type-specific somatic protein, called M-protein, which is unalterable in the change from S to R. SII(M2) $\longrightarrow$ R(M2) + (TP from SIII(M3) $\longrightarrow$ SIII(M2) However, transformation of M-protein became possible when R

strains deficient in M proteins were used. Such R(m) strains resulted after a long period of growth in the presence of anti-M protein serum.

$$\text{SII(M2)} \longrightarrow \text{R(M2)} \longrightarrow \text{R(m2)} + (\text{DNA from SIII(M3)}) \begin{cases} \nearrow \text{SIII(m2)} \\ \longrightarrow \text{SIII(M3)} \end{cases}$$

In this reaction the SIII(M3) cells are the result of a double transformation and may have occurred in two successive reactions or simultaneously within one cell.

Double transformations have also occurred with drug sensitive strains as follows:

$$\underset{\substack{\uparrow \\ \text{penicillin} \\ \text{sensitive}}}{\text{SIIps}} \longrightarrow \text{Rps} + (\text{DNA from } \underset{\substack{\uparrow \\ \text{penicillin} \\ \text{resistant}}}{\text{SIIIpr}}) \begin{cases} \nearrow \text{Rpr} \\ \longrightarrow \text{SIIIps} \\ \searrow \text{SIIIpr (double)} \end{cases}$$

Nutritional transformations are also if importance. As an example of this, Escherichia coli (K12 strain) has successfully transformed to the ability to synthesize two enzymes necessary for the utilization of galactose.

A final type of transformation to be mentioned here involves mutants which differ from the fully encapsulated S types in quantity of capsular polysaccharide.

SIII-1 Synthesizes very little type III polysaccharide
SIII-2 Synthesizes a larger amount of polysaccharide
SIII-N Synthesizes a normal, fully encapsulated S type

The following interactions between the transforming principle of donor cells and the genetic determinants of the transformed cell are a bit unusual.

$$\text{SIII-1} + (\text{DNA from SIII-2}) \begin{cases} \nearrow \text{SIII-2 (many)} \\ \longrightarrow \text{SIII-N (few)} \end{cases}$$

SIII-2 formation has been termed "autogenic," whereas SIII-N has been termed "allogenic." Such allogenic transformations

suggest an interaction between subunits of the donor transforming principle and the DNA of the recipient cell.

Ravin says that transformation studies have been satisfactory on so few traits because it is necessary to separate the transformed from the untransformed bacteria especially if the frequency of transformation is low (less than 1% of the treated population). Thus, characteristics which can be readily screened on selective media have most often been studied. Examples of characters especially good for study are 1) drug or antibiotic resistance and 2) independence of specific growth substances in the medium.

COMPETENCE

Many of the types of transformations which have been known to occur have been mentioned above. It is surprising that only a small proportion of known bacteria and even of a specific population are competent, or capable of undergoing transformation. According to Ravin the most common competent bacteria as of 1961 were Pneumococcus, Hemophilus, Neisseria, and Streptococcus. Before 1949 there was an R strain of Escherichia coli which was easily transformed into an encapsulated S form, but it was lost shortly after the death of Boivin who was studying it. However, in 1960, Escherichia coli (K12 strain) underwent transformation, even though this DNA-mediated transformation was unusual in that a "helper" bacteriophage was necessary for the change to occur. Transformations have been reported in other species of bacteria such as Agrobacterium, Xanthomonas, and Rhizobium; but as yet not much work has been done on them. In general, among the enteric bacteria--Escherichia, Salmonella, and Shigella--transformations have never been reproduced regularly. Recently most of the work has been done on Bacillus subtilis.

The big question which arises is, what makes some bacteria competent and others unalterable? Is it the lack of potential to react or is it a lack of knowledge of the specific environmental conditions and techniques necessary for transformation? There has been a great deal of investigation along these lines. It is known that competence is influenced by the age of the

culture, the nature of the growth medium, and the phase of the division cycle.

In spite of all the research a single factor causing competence is relatively unknown. First of all, the cell's genetic constitution was suspected of having some effect. It was soon found that the nature of the cell coat was important in that thick, gummy, or mucoid capsules inhibit DNA penetration. Ravin found that in pneumococcal strains which differed quantitatively in the amount of polysaccharide capsule secreted, transformability was related inversely to the amount of capsule secreted. Along these same lines Braun tells of experiments where R strains of pneumococcus displaying different competence abilities also differ in bile solubility.

A second genetic factor preventing transformation is the exocellular secretion by certain strains of DNase which is capable of depolymerizing DNA before its entry into the cell. A question which now arises is what prevents endocellular DNase from destroying DNA which has penetrated. It has been suggested that this DNase is blocked from its action on DNA, and that this blocking may occur only during a certain part of the generation cycle of the bacterium (Ravin).

Studies by Young and Spizizen also suggested that there is some genetic control of the attainment of competence. These studies indicate that the sensitivity of Bacillus subtilis to transformation occurs at a stage of growth in which

presporulation events occur. Those strains of Bacillus subtilis which do not undergo sporulation (and thus not presporulation presumably) are not competent. It is suggested that associated with presporulation is an alteration in cell wall structure which may be the basis of competence (Anagnostopoulos and Spizizen).

When Rotheim was studying streptomycin-resistance markers, she reported that a genetic factor, a depressor, was linked to this marker. This factor apparently decreases the probability of integration of a streptomycin-resistance marker into pneumococcus. It was shown to be unlikely that this depressor existed before the mutation for streptomycin-resistance; therefore the two probably arose concomitantly.

In addition to these genetic factors it has been shown that certain environmental factors contribute to transformability. In early studies three factors--inorganic pyrophosphate, antibodies, and serum albumin--were found to be important ingredients of the media. The necessity of inorganic pyrophosphate depends on the nature of the medium, for it is successfully omitted today. Although its function is unknown, the chelating properties of polyphosphates suggest that pyrophosphate may complex ions which inhibit effective contact of the DNA with the recipient cells.

The antibodies were used in early research to agglutinate the recipient bacteria. More recently agar has been used to increase viscosity and thus hold the recipients. It was noted

by Hotchkiss (Ravin) that shaking to dispurse the cells after contact with the exogenous DNA inhibits transformation; therefore he suggested that the clumped state provides a suitable environment for the selective growth of transformants which are encapsulated.

Though the role of the serum albumin is unclear, Thomas (Ravin) showed that albumin in some way promotes and maintains the physiological state of competence, perhaps by making pneumococci more capable of making an effective contact with DNA. Recent research suggests that complexed reversibly to albumin are trace amounts of substances that stimulate the physiological development of competence. However, albumin is not essential, for pneumococci and Escherichia coli have been transformed without it. Shigella and Hemophilus influenza have been transformed in plain nutrient media. Thus, it seems that the conditions for many other transformations are far less complex.

There have been several successful attempts at increasing the number of competent cells among strains which normally undergo transformation. In 1959 Opara-Kubinska studied the effect of centrifuging on the competence of strain 36A of D. pneumoniae Type XII to be transformed to streptomycin resistance, and she noted that centrifuging results in a considerable rise in the number of transformed bacteria.

Anagnostopoulos and Spizizen studied the conditions required to develop optimal sensitivity of Bacillus subtilis

strain 168, which is indole-dependent, to transformation to prototrophy by wild-type strains with ^{32}P labeled DNA. Optimal sensitivity to transformation was achieved by growth in a glucose minimal medium which also contained an ammonium salt as C and N sources, indole or L-tryptophan, a chelator for cupric ions, and acid-hydrolyzed casein. If relatively large amounts of amino acid are present, sensitivity to transformation is reduced, possibly by permitting synthesis of wall components which prohibit DNA entrance.

It seems that allowing cells to multiply in the medium before DNA is added increases the number of competent cells. Braun mentions that a four hour growth of pneumococcus in a serum medium sensitizes cells. It was noted that this sensitization was lost if the cells were washed or if they were incubated two hours longer.

It has been obvious to investigators that there is a peak period of competence. Young and Spizizen find this competent period during the latter half of the logarithmic stage of growth, for it is during this time that the cells bind DNA irreversibly. Thus competence seems to be a physiological property that arises during a certain fraction of the generation period of the bacterium.

Ottolenghi and Hotchkiss noticed that when a population itself is most susceptible to transformation, the cells are producing the greatest quantity of material for the transformation of other cells. Accumulation of this extracellular DNA

has been reported by several authors in cultures of Brucella, Micrococcus, Alcoligenes, Pseudomonas, Flavobacterium, and Neisseria. This phenomenon indicates that transformations may not be restricted to laboratory conditions. Ottolenghi and Hotchkiss also have evidence that with growth there is released into the culture medium some factor which interferes with transformation by DNA, although this material does not behave like DNase.

This period of competence seems to be fairly short. Thomas (Ravin) calculated it to be fifteen minutes which is about one half the time required for the population to double in the medium. Experiments have shown that during this competent period, DNA needs to be in contact with the recipient cells only a short time. With Hemophilus influenzae three minutes of contact with DNA seems to be sufficient to initiate transformation. For pneumococcus fifteen minutes of exposure after a four hour sensitization period, seems to be sufficient.

Fox and Hotchkiss found that batches of competent bacteria could be preserved for transformation experiments by freezing at -20° in 10% glycerol. Following melting and incubation at 37° the cells lose their competence; however after twenty minutes in proper conditions (serum albumin, Ca^{++}, amino acids, and glucose) competence returns.

The physiological state of competence is related to DNA penetration through the cell. The question of how the DNA penetrates is one still not settled. There is evidence for

two different theories, and it has been suggested that both may be important. The "localized protoplast" theory proposes "naked" regions on the cell surface of competent bacteria. These may be where the division septum is forming and may temporarily be free of cell wall material. This would be an explanation of the periodicity of competence. The second theory termed "enzymatic receptor" holds that competent bacteria have synthesized on their surface enzymes which have the catalytic ability to bind DNA. Somewhere during the process of growth and reproduction this factor is synthesized; thus periodicity of competence is explained by this theory also. Research suggests that the walls of competent bacteria are temporarily permeable to large molecules including DNA. Fox and Hotchkiss in 1957 provided strong evidence that competence is related to the synthesis of enzymatically active receptor sites on the bacterium surface. They calculated that there were about 35-75 adsorption sites on the competent bacterium, and that the periodicity of competence was associated with the appearance and disappearance of such sites. It has already been mentioned that the presence of Ca^{++} and albumin are important for the development of competence. This plus the fact that conditions suitable for protein synthesis (amino acids and glucose) are required for competence to arise gives further evidence that DNA fixation is an enzymatically catalyzed process. Another good indication is that chloramphenicol, which is an inhibitor of protein synthesis, blocks the

development of competence. It has been suggested that possibly both of these theories may play a role in the entrance of DNA.

In 1963 Pakula and Walczak published their evidence along these lines. They reported that sterile, supernatant fluids from cultures of competent streptococci contain a factor which provokes competence of non-competent homologous and related cells. When this factor is added, transformations can occur in a medium containing neither serum nor albumin, i.e. in which conditions transformation of streptococci never or very infrequently occurs. They concluded that the presence of serum or albumin and probably an additional unknown substance is essential for production of this provoking factor and the subsequent development of competence in Streptococcus sanguis. This factor was never found in cultures which were not competent during a given period of growth. The factor which provokes competence seems to be an enzyme. The induction of competence is time and temperature dependent, and the factor, itself, is heat sensitive and does not pass through Visking dialysis tubing. Thus Pakula and Walczak concluded that "the conversion of non-competent cells into competent ones seems to be concomitant with an enzyme reaction."

Penetrance was also studied by "molecular shearing" or breaking the DNA molecule without separating the complementary strands. Spraying at high pressure through an atomizer or exposure of a DNA solution to ultrasonic oscillations will shear

the molecule. It was noted that solutions of these fragmented molecules still retained transforming activity, but the principal effect of shearing was to reduce the capacity to penetrate the bacterium. Ravin says that it is possible for less than entire molecules integrated into a cell to effect a transformation; however the molecule as a whole is necessary for effective contact with the recipient bacterium. According to him there seems to be no reason to invoke the notion that anything less than a native, high molecular weight DNA molecule enters the recipient bacterium. Also, only one unit of transforming activity, presumably one DNA molecule, is sufficient to make an effective contact with a bacterium and cause it to be transformed.

MECHANISM OF TRANSFORMATION

An important discovery was made when Marmur and Lane studied DNA denatured by heat. They noted that even when heated to temperatures where all the molecules are denatured (strands separated), some transforming activity is still retained. However, this single-stranded form is not readily taken up by the cells. Following fast cooling results single-stranded DNA with one half the molecular weight of the original DNA. During slow cooling strands recombine by complementary base pairing over most of their length. This form has as much as 50% of its original transforming activity and is called renatured. It appears that this reunion can take place between complementary strands or between strands which possess structures specifying each other over a large part of their respective lengths. Thus exists the possibility of forming heterozygous DNA molecules with genetic markers or chemical modifications in both strands. With the heterozygote as pictured here it would suggest that the information from both strands could be expressed. There is a discrepancy of results regarding transformations with DNA marked on both strands. The difference is between Hemophilus and pneumococcus. Lacks (1962) has described experiments which suggest that in pneumococcus one of the strands is degraded soon after uptake. With Hemophilus, Herriot (1963) found that the marker on each of the heterozygote strands appeared after transformations; therefore he concluded that both strands are utilized.

Recent studies of the transformation process in pneumococcus have shown that immediately after entry into the cells, the DNA loses its transforming ability, only to be gradually recovered within several minutes. The results of Lacks' observations indicate that very soon after or during its introduction into the cell, DNA is converted partly into low-molecular-weight fragments and partly into single-stranded polynucleotides, and that incorporation of a portion of the latter into the recipient DNA is the physical basis for the genetic transformation. Lacks suggests that this fragmentation process may be an essential step in the entry of DNA into the cell. This finding that single-stranded DNA is an intermediate in transformation explains why artificially produced single-stranded DNA is able to transform once it is incorporated into the cell, and also why the DNA is unable to express itself phenotypically before further integration. Wood and Berg in 1962 (Lacks) said that single-stranded DNA may be unable to transfer information in protein synthesis because it is unable to make messenger RNA. This single-stranded state is only transitory, for within ten minutes it has either been integrated or fragmented.

The next stage in the transformation process is synapsis, which is followed by recombination. Information about synapsis and recombination have been attained from studies of allelism and linkage in transforming agents. Synapsis occurs between exogenous and endogenous DNA. Herriot's (1961) experiments with Hemophilus showed that synapsis must take place between

two double helices in order for two linked markers of a hybrid molecule to be integrated into the recipient's genome. He said that recombination must involve the replacement of a segment of a double helix for a corresponding segment of the homologous double helix. In 1958 Schaffer (Ravin) assumed that the exactness of pairing depends on the extent of the structural homology between the donor and recipient DNA. The less exact the pairing, the less frequently is the exogenous DNA marker integrated into a genome. The homology is less between DNA of different species; this accounts for the difficulty of such transformations. In addition, it has been noted that the size of the marker affects the frequency of transformation. Two linked markers are incorporated much less frequently than either one separately. Thus it is assumed that the larger the segment to be integrated, the lower the probability of genetic integration. Ravin showed that markers more sensitive to ultraviolet light are integrated less frequently; hence they occupy a larger segment of DNA molecules bearing them. Also, if the regions of the molecule adjacent to the marker are homologous with the recipient DNA, integration will be more likely. Ravin also says that when a marker is integrated, it is removed from the major part of the molecule which bore it. Thus, any marker of heterospecific origin, if it succeeds in being integrated, is relieved of whatever part of it was dissimilar with the endogenous homolog.

Recombination has been defined as "any process in which

two parents differing by more than one genetic character give rise to progeny possessing a new combination of the parental characters" (Ravin). Taylor (Ravin) showed that transformation does not involve a mere addition of genetic material to the recipient's genome. The recipient cell contains a homolog of the exogenous DNA, and transformation comsists of a partial replacement of an endogenous with exogenous DNA.

Two classic schemes account for "crossing-over" between homologous chromosomes in higher organisms, but it is doubtful that either one can exactly account for the process in bacteria. The exact mechanism of crossing-over is unknown in bacteria. The two schemes in higher organisms are 1) breakage-reunion and 2) copy-choice. In breakage-reunion the homologous chromosomes break at homologous points along their lengths and two different chromosomes are formed by fusion of originally separate parts. During the copy-choice process the homologous chromosomes serve as templates, and the newly synthesized replicas are copied alternately from two templates. The breakage-reunion model would predict the recovery of reciprocal recombinant products, but these have not been observed.

For the most part the copy-choice mechanism seems excluded, for it has been shown that there is no replication of the DNA until after recombination. However, Ravin says that it is possible that "two distinct mechanisms of recombination occur: one resulting in reciprocal products and occurring over relatively

long distances of the chromosome, and the other capable of giving rise to nonreciprocal products occurring at the microchromosomal, perhaps molecular level." He also suggests a revised model of breakage-reunion where a portion of the exogenous molecule is transferred directly into the recipient molecule by substituting for the homologous segment which is eliminated.

It has been determined that for different characters there are separable agents which are transferred independently of each other. Evidence for this independent transfer indicates one of several mechanisms:

1. In a bacterium the genome is split up into separate DNA elements which are released independently when DNA is extracted from a cell.
2. The process of extracting DNA from a cell causes the break up of an organized organelle into separate DNA molecules.
3. The process of incorporation and integration into a recipient cell causes the DNA molecule to be broken into fragments which are then integrated at random, some being excluded.
4. Some combination of the above mechanism.

There is no cytological evidence for the first mechanism. The second is possible because there is physiochemical heterogeneity within a DNA preparation from one source. However, every DNA molecule carries several pieces of information, not

all of which are integrated into the recipient genome. On one hand there appear to be different DNA molecules of different genetic specificity obtainable from one source. The characters on each of these different molecules, of course, could be integrated separately. On the other hand, Ravin says that one molecule of DNA carries several different pieces of genetic information, and these pieces can be separated from each other during recombination. These determinants on a single molecule have been known to be transferred in a "linked" fashion. These linked factors are not necessarily related in any way biochemically and may affect quite different biochemical reactions. As of 1961 Ravin and Iyer (Ravin) had discovered five independent mutations, all on the same DNA molecule, which confer resistance to the antibiotic erythromycin in pneumococcus.

Following recombination whatever part of the molecule is not integrated into a genome is discarded, for reciprocal products do not occur. The next event to occur is the replication of the new genome at a rate identical to that of the resident genome. After several years of debate and such studies as Ephrussi-Taylor's (Ravin) with chloramphenicol inhibition, it has been determined that there is no copying of the introduced marker or DNA prior to genetic integration.

There are no current techniques to detect cells phenotypically active which are not replicating the marker, but it is thought that phenotypic expression of a genetic determinant does not require prior integration. This was a result of

Hotchkiss's experiments with a streptomycin-resistance marker. The marker was not always integrated, yet it was capable of altering the phenotype of the recipient cell. This later was termed pseudoresistance by Ephrussi-Taylor. These cells could survive but not reproduce in streptomycin. Ephrussi-Taylor (Ravin) noted that only twelve minutes after uptake of the DNA this resistance appears; however the ability to reproduce does not appear till three hours after uptake.

In spite of this phenomenon of pseudoresistance, experiments suggest that DNA must not only be incorporated but must be permanently integrated into the cell in order to elicit enzyme synthesis (Lacks and Hotchkiss). In addition to integration it seems that replication of the newly introduced DNA must also occur before protein synthesis. More significance can be attached to this fact when it is remembered that the function of the infecting gene is the ordering of the synthesis of a specific protein.

The three events--penetration into the cell, recombination, and replication all seem to occur within a brief period. In Hemophilus influenzae studied by Voll and Goodgal the half-maximum amount of recombination is attained within fifteen minutes after exposure to DNA. Lacks and Hotchkiss's studies of streptomycin-resistance in pneumococcus have shown that this character manifests itself sixty minutes after DNA incorporation. Among some bacteria the number of replications by the second generation depends on the environmental conditions and the

physiological state of the cells. In some clones replication appears later than this; however by applying proper environmental conditions it is possible to cause replication in the majority of infected clones by the second generation.

In cases where streptomycin-resistance is being transferred to sensitive cells and the resistance does not appear for several generations, it may be that there is a substance produced by the sensitive cells which must be diluted out of the cells by repeated divisions before any cells can become resistant.

SIGNIFICANCE

Any study such as this on the process of transformation would not be complete without mention of its applicability to current and future practical problems. These phenomena are of biological significance in that they aid in elucidating the mode of action of genetic determinants and thus attempt to solve some of the problems of inheritance in all forms of life. Thus far studies related to transformation have shown that directed changes in the hereditary properties of bacteria are possible under certain conditions. Secondly, they have confirmed that it is the DNA compound which is genetically active. Thirdly, they have revealed that DNA can vary in its biological specificity, and that one DNA preparation may affect several hereditary properties. In addition to these, transformation reactions are being used to discover the relation between molecular size or weight of DNA and its activity, and also the relation between a change in DNA structure and the mutation resulting. Transformation is used for these studies because, so far, this is a good way to measure DNA activity.

Transformations have great significance for the field of medicine, also. Many transformations of cells have been induced here, among which are transformations of human lymphocytes from the peripheral blood into larger cells capable of division. In another study strands of mouse subcutaneous

connective tissue--one which forms tumors and one which does not form them--were transformed in mixed cultures into a new cell line similar in chromosomal, biological, and morphological characteristics to the parent lines; however these cells were highly malignant. Also in the field of medicine, allogenic transformations can be of great epidemiologic significance. Cells of moderate virulence or relatively avirulent when coexisting within a host under conditions favoring transformation can result in highly virulent strains.

Genetic transformations are also suspected to have played a role in the evolution of bacteria since they can give rise to unusual combinations of characteristics. Since there are so many combinations formed, it would not be long before adaptations for certain environmental conditions and ecological niches would arise. It has already been mentioned in this paper that pneumococci, when most susceptible to transformation, release DNA into the medium. This suggests that transformations may not be restricted to laboratory conditions and transformation may provide a natural mechanism for genetic recombination in an organism in which no such mechanism has been noted thus far. Takahasi suggests that this type of transformation could be a prevalent mode of genetic exchange in such favorable habitats as the root surface and the rhizosphere.

By a knowledge of the internal and external conditions required for transformation it is hoped that transformations can be extended to other species of microorganisms and to the

cells of higher plants and animals. It has been reported that attempted genetic transformation in rabbits by artificial insemination with semen incubated with DNA have resulted in no transformations at the albino or sex loci.

Through this survey of the field of transformation it is seen that there remains a lot to be uncovered, and that every new discovery is of value.

SELECTED BIBLIOGRAPHY

Alloway, J. Lionel. J. Exptl. Med., 57, 265 (1932).

Anagnostopoulos, C., and Crawford, I.P. Proc. Natl. Acad. Sci. U.S., 47, 378 (1961).

Anagnostopoulos, C., and Spizizen, John. J. Bact., 81, 741 (1961).

Avery, Oswald T., and MacLeod, Collin M., and McCarty, Maclyn. J. Exptl. Med., 79, 137 (1943).

Braun, Werner. Bacterial Genetics (Philadelphia: W.B. Saunders Company, 1957).

Dawson, Martin H., and Sia, Richard. J. Exptl. Med., 54, 681 (193]

Doty, P., and Marmur, J., and Eigner, J., and Schildkraut, C. Proc. Natl. Acad. Sci. U.S., 46, 461 (1960).

Dove, William F., and Yamane, Tetsuo. Biochem. Biophys. Res. Commun., 3, 608 (1960).

Fox, Maurice S., and Hotchkiss, Rollin D. Nature, 187, 1002 (1960)

Frobisher, Martin. Fundamentals of Microbiology. (Philadelphia: W.B. Saunders Co., 1958).

Griffith, Fred. J. Hyg., 27, 113 (1928).

Herriott, Roger M. Proc. Natl. Acad. Sci. U.S., 47, 146 (1961).

Herriott, Roger M. Biochem. Zeit., 338, 179 (1963).

Lacks. Sanford, and Hotchkiss, Rollin D. Biochim. Biophys. Acta. 45, 155 (1960).

Lacks, Sanford. J. Molec. Biol., 5, 119 (1962).

Lerman, L. S., and Tolmack, L. J. Biochim. Biophys. Acta. 26, 68 (1957).

Marmur, J., and Lane, D. Proc. Natl. Acad. Sci. U. S. 46, 453 (1960).

Nester, Eugene W. and Lederberg, Joshua. Proc. Natl. Acad. Sci. U. S., 47, 52 (1961).

Opara-Kubinska. Bull. Acad. Polonaise Sci. Ser. Sci. Biol. 7, 433 (1959). (Biol. Abst.)

Ottolenghi, Elena, and Hotchkiss, Rollin D. Science., 187, 1257 (1960).

Pakula, R., and Walczak, W. J. Gen. Microbiol., 31, 125 (1963).

Ravin, Arnold W. Advances in Genetics 10, 61 (1961).

Rotheim, Minna B. Genetics 47, 599 (1962).

Ryan, F. J. Biochem. Biophys. Res. Commun. 12, 445 (1963).

Tabor, H. Fed. Proc. 20, 351(1961).

Takahasi, I. Biochem. Biophys. Res. Commun. 7, 467 (1962).

Voll, Mary Jane and Goodgal, Sol H. Proc. Natl. Acad. Sci. 47, 505 (1961).

Young, F.E. and Spizizen, John. J. Bacteriol. 81, 823 (1961).

Young, F.E. and Spizizen, John. Fed. Proc. 20, 254 (1961).

Zamenhof, Stephen and Alexander, Hottie and Leidy, Grace. J. Exptl. Med. 98, 373 (1953).

Zinder, Norton and Lederberg, Joshua. J. Bacteriol. 64, 679 (1952

VITA

Margaret Sue Darsie

Candidate for the Degree of

Master of Science

Report: Bacterial Transformations

Major Field: Natural Science

Biographical:

Personal Data: Born in Pittsburgh, Pennsylvania, November 2, 1941, the daughter of Leigh and Margaret Darsie.

Education: Attended grade school in Munhall, Pennsylvania; junior high school in Munhall; and graduated from Munhall High School in 1959; attended Wheaton College, Wheaton, Illinois, and received the Bachelor of Science degree from Wheaton College, with a major in biology in June, 1963; did graduate work at Oklahoma State University during the summer of 1963 and the academic year of 1963-1964; completed requirements for the Master of Science degree in July 1964.