Gene Expression

Volume 3

Plasmids and Phages

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PREFACE

Gene Expression is intended to provide a critical analysis of the organization and expression of the genetic apparatus at the molecular level. In this series I hope to demonstrate the design that becomes apparent when the genetic operations of procaryotic and eucaryotic cells are analyzed from the perspective of the gene. The first volume, *Bacterial Genomes*, considers procaryotic nucleic acid and protein synthesis; the second volume, *Eucaryotic Chromosomes*, discusses the structure and function of the eucaryotic genetic apparatus. The present volume, *Plasmids and Phages*, attempts to define the events involved in the expression and reproduction of these small genetic elements. This volume is therefore closely related to the first: there I have considered replication, transcription and translation, and the systems responsible for their control in bacteria; here I consider the uses to which these systems are put, what may be thought of as the genetic strategies displayed by plasmids and phages.

Two themes constitute this book: the nature of the systems by which genetic information is transferred between bacterial cells; and the means by which the small elements that constitute the plasmids and phages are perpetuated. The transfer of genetic information between bacterial genomes is accomplished by transformation with DNA as well as through the conjugation promoted by plasmids and the transduction sponsored by some phages. Although not a plasmid or phage function, transformation is included so that discussion of genetic transfer is not limited to the achievements of plasmid and phage vectors. As a vector, the sex factor is the best characterized plasmid; and its counterpart among the lysogenic phages is, of course, phage lambda. Discussion of genetic transfer by vectors therefore focuses on conjugation and sexduction and on specialized transduction. As self-perpetuating elements, the sex factor and the drug factors related to it, and the smaller colicinogenic factors, are the best characterized; these are the focus of this discussion. Reproductive mechanisms are well characterized for many phages and this book therefore is able to provide fairly detailed accounts of their life cycles. This includes the lytic cycle of lambda as well as the virulent infection

that is the only option for survival open to nonlysogenic phages. Obviously it is scarcely possible to consider in a single volume all the systems that now have been investigated and discussion therefore concerns the best characterized: the large phage T4, the smaller phages T3 and T7; the single stranded DNA phages; and the RNA phages.

In this book as in the earlier volumes, I have been concerned not simply to summarize what is thought to be the status quo, but to consider critically the original experiments and to establish the limits of the conclusions that can be drawn from them. Sometimes, of course, my conclusions may differ from those of the authors reporting the data. And obviously it is as important to ask questions as to provide answers: any attempt to define the conclusions supported by current data can hardly be divorced from consideration of their further implications. I have tried therefore to point to the questions that may now be asked and to consider the lines along which their resolution may be possible.

I have followed my usual policy on references; I have attempted to provide a complete citation of those papers that comprise the mainstream of research (some 2000 in this volume) but without necessarily referring to every confirmatory report. Virtually all the references cited represent papers published in the major research journals; only in those rare instances where no other source is to be found have I relied upon contributions to symposia and other such volumes.

Finally it is a pleasure to thank colleagues who have provided material included in this book and especially Drs. E. P. Geiduschek and W. Szybalski, who read some of the chapters and made many helpful comments. Although any expression of gratitude here can only be inadequate, I should like to conclude by thanking my wife, Ann, without whose encouragement this book would never have been started nor completed.

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CONTENTS

CHAPTER 1

Transformation

Genetic Transfer by DNA Molecules

Discovery of Transformation and the Transforming Principle

Transformation was the first type of transfer of genetic information to be discovered in bacteria; and it was the subsequent identification of the "transforming principle" with deoxyribonucleic acid that led to the development of the concept that genetic information is stored in the form of nucleotide sequences. Transformation was first observed in the study of pneumococcal infection of mice reported by Griffith in 1928. Pneumococcus causes pneumonia in man, but the bacterium is pathogenic for mice and so on infection may prove fatal. Virulence in pneumococci depends upon a component of the surface of the bacterium, the capsular polysaccharide, which confers protection against phagocytosis in the animal and gives colonies of virulent bacteria a smooth (S) appearance. Several "types" of pneumococcus differ from each other in the constitution of their capsular polysaccharide and thus in the antigenic response which can be provoked against them. Mutants may be derived (from any type) which fail to synthesize the capsular polysaccharide; these R bacteria produce colonies with a rough surface and are avirulent when injected into mice.

An appeal of this system for experimental analysis is that it is not difficult to distinguish R and S bacteria and to type the S strains according to their antigenic properties. As Griffith described the system, "virulence and type characters are closely related in the pneumococcus. When pneumococci are grown in homologous immune serum, some descendents become attentuated in virulence and these can be recognized by their formation on solid media of a distinctive variety of colony known as the R form of pneumococcus. The virulent or S form of pneumococcus produces in fluid media, and still more abundantly in the peritoneal cavity of the mouse, a soluble substance which, although not itself antigenic, gives a copious precipitate with the appropriate antiserum. Each type of pneumococcus forms a special soluble substance which has no affinity for

an antiserum prepared against any other pneumococcal type and it is to this property that the remarkably clear definition of the serological races of pneumococcus is due. . . . As a result of its change to the R form, the pneumococcus generally loses this power of producing soluble substance."

The critical observation which Griffith made in this system was that injection into mice of either avirulent mutant R bacteria or of heat killed virulent S bacteria did not harm the animal; but injection of a mixture of the two bacterial preparations killed some animals and from them he could recover live virulent S bacteria. When live R bacteria derived from type II were injected together with dead S bacteria of type I, the live virulent (S) bacteria recovered post mortem were of type I; thus some property of the dead type I bacteria allows them to confer upon living R bacteria the ability to make the capsular polysaccharide characteristic of type I. This change induced in the properties of the R bacteria was called *transformation*.

These experiments were confirmed and extended to other types of pneumococcus by Dawson at the Rockefeller laboratories in 1930, but, like Griffith, he was at first unable to demonstrate transformation in vitro. In 1931, however, Dawson and Sia converted R bacteria incubated in vitro with heat killed S bacteria to give live S bacteria; and Alloway (1932, 1933) was then able to use cell free and filtered extracts of dead S bacteria to transform R bacteria into the specific type of S bacteria from which the cell free preparations were derived.

The significance of Griffith's original observations was not realized at the time and the idea that the changes induced by transformation are hereditary was proposed only later. One early idea, for example, was that some autocatalytic utilization by the live R bacteria of the capsular polysaccharide from dead S bacteria might be implicated. The nature of the system was seen clearly, however, by Avery and his colleagues in their studies at the Rockefeller laboratories of the chemical nature of the transforming principle. In describing the pneumococcal system, McCarty, Taylor and Avery (1946) wrote: "The phenomenon of transformation of pneumococcal types provides an outstanding example of the induction of specific and heritable modifications in microorganisms. Basically the phenomenon represents the transformation of a nonencapsulated (R) variant derived from one specific type of pneumococcus into encapsulated (S) cells of heterologous specific type. By the techniques employed at present, this is accomplished by growing the nonencapsulated cells in a special serum broth to which has been added the active fraction extracted from encapsulated pneumococci of a heterologous type. The production of a new polysaccharide capsule is induced in the R cells so that they

acquire the type specificity of the organisms from which the extract was obtained. The property of forming the new capsule is transmitted indefinitely to subsequent generations, and, in addition the substance responsible for the induction of transformation is itself reduplicated in the transformed cells. It is thus apparent that one is dealing with hereditary bacterial modifications, which are predictable and subject to direct experimental control."

The importance of identifying the *transforming principle*, the name given to the active component of the cell free system, was obvious, and in introducing their classic results, Avery, Macleod and McCarty (1944) noted that "biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microorganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of pneumococci. . . . The present paper is concerned with a more detailed analysis of the phenomenon of transformation of specific types of pneumococcus. The major interest has centered in attempts to isolate the active principle from crude bacterial extracts and to identify if possible its chemical nature or at least to characterize it sufficiently to place it in a general group of known chemical substances."

Chemical analysis of the transforming principle suggested that it comprised deoxyribonucleic acid. That it might be protein or ribonucleic acid was excluded by the failure of trypsin, chymotrypsin or ribonuclease to impair its activity. Confirmation that the transforming principle was DNA was provided by the experiments of McCarty and Avery (1946a), in which deoxyribonuclease was purified and it was shown that it irreversibly destroyed the transforming principle. Demonstration of the general nature of this conclusion came from the improved purification of transforming DNA which McCarty and Avery (1946b) used to achieve transformation of pneumococcus types II and VI as well as the type III previously used.

The significance of the identification of genetic material with DNA was immediately realized by Avery, who in 1943 described the isolation of the transforming principle in a letter (quoted by Hotchkiss, 1966). "When alcohol reaches a concentration of about 9/10 volume there separates out a fibrous substance which on stirring the mixture wraps itself about the glass rod like thread on a spool and the other impurities stay behind as a granular precipitate. The fibrous material is redissolved and the process repeated several times. In short, this substance is highly reactive and on elementary analysis conforms *very* closely to the theoretical values of

pure desoxyribose nucleic acid (thymus) type (who could have guessed it). This type of nucleic acid has not to my knowledge been recognised in pneumococcus before, though it has been found in other bacteria. . . . If we are right, and of course that is not yet proven, then it means that nucleic acids are not merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells and that by means of a known chemical substance it is possible to induce predictable and hereditary changes in cells."

The implications of the conclusion that the transforming principle and the cellular component which it influences possess different chemical natures were well expressed by Avery, Macleod and McCarty. "The inducing substance, on the basis of its chemical and physical properties, appears to be a highly polymerized and viscous form of sodium desoxyribonucleate. On the other hand, the type III capsular substance, the synthesis of which is evoked by this transforming agent, consists chiefly of a nonnitrogenous polysaccharide constituted of glucose-glucuronic acid units linked in glycosidic union. The presence of the newly formed capsule containing this type specific polysaccharide confers on the transformed cells all the distinguishing characteristics of pneumococcus type III. Thus, it is evident that the inducing substance and the substance produced in turn are chemically distinct and biologically specific in their action and that both are requisite in determining the type specificity of the cell of which they form a part." By the early fifties, when studies had started on the nature of the cellular events involved in transformation, the distinction between genetic material and the products of its expression was implicit.

The implication of DNA in some genetic capacity was clear to the participants in these and subsequent experiments, although how such functions might be exercised remained a matter for imagination. In concluding their classic paper, Avery, Macleod and McCarty thus expressed the caution that "if the results of the present study on the nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined." Extensive scepticism of the idea that DNA is the transforming principle prevailed among others, however, largely due to misapprehension of its structure. Because DNA was thought to have a simple, tetrameric repeating structure, objections were raised to the idea that it might have any biological specificity—its role was thought to be solely structural. Indeed, although its presence was recognized in eucaryotic chromosomes, it had not even been established as a component of all procaryotic cells. One frequent criticism was that a small amount of

contaminating protein might be present to provide the active component in transforming DNA preparations, but this, of course, was inconsistent with the responses of transforming principle to deoxyribonucleases and proteinases. And increased purification of transforming DNA by Hotchkiss reduced to ridiculously small proportions the amount of contaminating protein that might be present (see Hotchkiss, 1966). Two important advances helped to establish the role of DNA as genetic material. One, of course, was the demonstration by Hershey and Chase in 1952 that DNA carries the genetic information of a bacteriophage (see Chapter 4). And another was the growing realization that the structure of DNA was more complex than hitherto thought, this leading into the elucidation of the double helix structure and culminating in the breaking of the genetic code.

In implicating DNA as the active agent in the transforming principle, it was of obvious importance to demonstrate that bacteria other than pneumococcus can exchange genetic information by transformation and to show that markers other than the capsular polysaccharide can be carried by transforming DNA. In 1951, Alexander and Leidy used R and S forms of Hemophilus influenzae to show that DNA preparations from type specific S cells can change nontypeable R cells into the specific type from which the DNA was derived. And working with pneumococcus, Ephrussi-Taylor (1951) showed that other capsular phenotypes in addition to the original R and S forms can be transferred by DNA preparations derived from appropriate bacteria. Also in 1951, Hotchkiss reported that resistance to penicillin can be transferred between pneumococci. The use of medium containing penicillin provided an easy selective technique to isolate mutants resistant to different levels of the antibiotic and then to identify transformants of sensitive cells (compared with the more cumbersome agglutination technique used to identify transformants of antigenic type). These experiments clearly excluded any interpretation that the role of DNA might be connected in some physiological (rather than hereditary) manner with the synthesis of capsular polysaccharide.

Identification of the genetic material with DNA was therefore by this time clear, as Hotchkiss noted. "The sudden appearance of penicillin resistant pneumococci after transformation so far removed in time from any exposure to penicillin, is a strong indication that the genetic mechanism which has preserved the resistance property in the donor strain has been transferred to the receptor strain. It is therefore of considerable interest that this property is apparently carried in the desoxyribonucleate fraction prepared from these cells." And in another early paper on transformation, noting that several characters in addition to the original R and S phenotypes could be transformed, Ephrussi-Taylor (1951) summarized

the situation. "Transformation consists of the replacement of the nucleic acid of an autoreproducing cell element by a nucleic acid of an extraneous origin having a closely related structure and function."

Kinetics of Transformation

Transformation has now been achieved in many species of bacteria, although it remains best characterized in pneumococcus and H.influenzae, with which the first experiments were performed. With these and other bacteria, transformation can be demonstrated for any characteristic whose acquisition be measured in the recipient bacteria. Quantitative studies to measure the frequency of transformation require markers which can be easily selected and counted in the recipient population, a condition not fulfilled by the original mutants of capsular polysaccharides. Resistance to antibiotics was used to provide such markers in early studies of transformation of pneumococcus and H.influenzae; B.subtilis later came into use as a system for studying transformation and the many auxotrophic mutants available have extended the range of markers that can be utilized. We shall concentrate here largely on these three bacterial species.

Transformation involves two successive types of event: the transfer of genetic material from donor to recipient; and the utilization of donor genetic material within the recipient. Because transformation is achieved by the addition of donor DNA to recipient bacteria, this method of genetic transfer differs from others in making it possible to establish by direct experiment the structural parameters that are demanded for genetic activity in transforming DNA. Questions such as whether the DNA must be duplex or single stranded, whether there are any necessary minimum or maximum lengths, can be answered by subjecting the donor DNA molecules to appropriate treatments before transformation. By using preparations of radioactively labeled transforming DNA, it is possible also to follow the fate of the nucleic acid after its entry into a recipient cell. Such experiments show that transforming DNA enters recipient cells most effectively when provided in the duplex state, but that only one of the two donor strands is utilized for providing genetic information after uptake; recombination with the host genome is necessary to establish donor DNA in the recipient cell as a source of genetic information, and following such integration the donor sequence is replicated as part of the recipient and its descendent genomes.

A characteristic of all transformation systems is that an increase in the concentration of the transforming DNA preparation causes a proportional increase in the number of transformants, up to a plateau level. Figure 1.1