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Genetics of the bacterial cell

Nobel Lecture, December 11, 1965

If I find myself here today, sharing with Andre Lwoff and Jacques Monod this very great honor which is being bestowed upon us, it is undoubtedly because, when I entered research in 1950, I was fortunate enough to arrive at the right place at the right time. At the right place, because there, in the attics of the Pasteur Institute, a new discipline was emerging in an atmosphere of enthusiasm, lucid criticism, nonconformism, and friendship. At the right time, because then biology was bubbling with activity, changing its ways of thinking, discovering in microorganisms a new and simple material, and drawing closer to physics and chemistry. A rare moment, in which ignorance could become a virtue.

Lysogeny and Bacterial Conjugation

The laboratory of Andre Lwoff was traversed by a long corridor where everyone would meet for endless discussions of experiments and hypotheses. At one end of the corridor, Jacques Monod's group was adding β-galactosides to bacterial cultures to initiate the biosynthesis of β-galactosidase; at the other end, Andre Lwoff and his collaborators were dousing cultures of lysogenic bacteria with ultraviolet light, having just discovered that means of initiating the biosynthesis of bacteriophage. Each was therefore «inducing» in his own way, convinced that the two phenomena had nothing in common, save a word.

Having come to prepare a doctoral thesis with André Lwoff, I was assigned the study of lysogeny in *Pseudomonas pyocyanea*. Thus I conscientiously set out to irradiate this organism. However, it soon became apparent that the problem of lysogeny was primarily that of the relationship between the bacterium and bacteriophage, in other words, a matter of genetics.

The genetics of bacteria and bacteriophages was born just 10 years earlier, with a paper by Luria and Delbrück[1]. It had continued to grow with the investigations of Lederberg and Tatum[2], Delbriick and Bailey[3], and Hershey[4].

But this young science had already produced many surprises for biologists. The most important was the demonstration by Avery, MacLeod, and McCarty[5], and later by Hershey and Chase[6], that genetic specificity is carried by DNA. For the first time, it became possible to give some chemical and physical meaning to the old biological concepts of heredity, variation, and evolution. Such a molecular interpretation of genetic phenomena is exactly what was provided in the structure of DNA proposed by Watson and Crick[7].

Another surprise was the realization that their rapid growth rate, their ability to adapt to many different media, and the variety of their mechanisms of genetic transfer make bacteria and viruses objects of choice for studying the functions and reproduction of the cell. The work of Beadle and Tatum[8], Lederberg[9], and Benzer[10] had shown that with a little imagination one can exert on a population of microorganisms such a selective pressure as to isolate, almost at will, individuals in which a particular function has been altered by mutation. Indeed, one of the most effective ways of determining the normal mechanisms of the cell is to explore abnormalities in suitably selected monsters.

The first attempts to analyze lysogeny genetically, intended to determine the location of the prophage in the bacterial cell, were carried out in 1952 by E. and J. Lederberg[11] and by Wollman[12]. Certain crosses between lysogenic and nonlysogenic bacteria suggested some linkage between the lysogenic character-determined by the λ prophage of *Escherichia coli* and other characters controlled by bacterial genes. However, other crosses gave anomalous results. In fact, the answer obtained from these experiments could hardly be decisive, since the mechanism of conjugation was not understood at the time.

It was with the intention of continuing this study under somewhat different conditions that I began to work with Elie Wollman; very soon our collaboration became a particularly close and friendly one. We wanted in the first place to understand the anomalies observed in crosses between lysogenic and nonlysogenic bacteria, in particular the fact that the lysogenic character was not transmitted to recombinants except when carried by the female. To study this problem, we used a mutant male bacterium recently isolated by William Hayes and named *Hfr*, because, in crosses with females, it produced recombinants with high frequency[13]. Upon crossing such lysogenic *Hfr* males to nonlysogenic females, we were surprised to find out that the zygotes formed by more than half the males happened to lyse and produce phage[14]. This phenomenon, termed *zygotic induction,* showed that the equilibrium between the prophage and the bacterium is maintained by some regulatory system present

in the cytoplasm of a lysogenic bacterium but absent from a nonlysogenic one. Moreover, it showed that a genetic character transferred by the male can be expressed in the zygote without being integrated into the chromosome of the female bacterium. It thus became possible in bacterial conjugation to distinguish experimentally between transfer of genetic material and recombinational event.

In order to bring the analysis of conjugation down from the level of the population to the level of the individual bacterial pair, we had to understand how the genetic material of the male is transferred to the female. In particular, one could try to interrupt conjugation after various times in order to find out when the transfer takes place. Elie Wollman had the somewhat startling idea of interrupting conjugation by placing a mating mixture in one of those blenders which ordinarily find service in the kitchen. It turned out that the shearing forces generated in the blender separate males from females; the male chromosome is broken during transit, but the chromosomal fragment that has already penetrated the female can express its potentialities and undergo recombination. In this way, it could be shown that, after pairing, the male slowly injects its chromosome into the female. This injection follows a strict schedule and, with any particular strain, the injection always starts at the same point[15]. Marvellous organism, in which conjugal bliss can last for nearly three times the life-span of the individual!

With this system, it became relatively easy to analyze the genetic constitution of *E. coli,* to show that bacterial characters are arranged on a single linkage group, termed the bacterial chromosome, and to map them, not only by classical genetic methods, but also by physical and chemical measurements. Moreover, two new insights emerged from this study. First, the bacterial chromosome turned out to be a closed, or circular, structure. Second, it was not as fixed a unit as one might have believed: other genetic elements, termed *episomes* (for example, a phage chromosome or a sex factor), can be added to or subtracted from it[16]. These properties happened to be of great value in subsequent studies of the bacterial cell and of its functioning.

Expression of the Genetic Material: The Messenger

In addition to its interest for the analysis of strictly genetic phenomena, bacterial conjugation proved particularly well adapted for the analysis of cellular functions, since it provided a means of transferring to an entire population a

given gene at a given time. The phenotypic effects produced by the sudden appearance of a new gene in the recipient bacterium are then manifested without the accompanying complications that occur in higher organisms as a result of morphogenesis and cellular differentiation.

At his end of the corridor, Jacques Monod had reached the conclusion that further progress in the understanding of enzymatic induction required genetic analysis. Two types of mutations which altered the induced biosynthesis of β-galactosidase were known at that time. One type abolished the capacity to produce an active protein. The other changed the inducible character of enzyme synthesis so that it became *constitutive,* that is, able to proceed even in the absence of a β - galactoside inducer. How are these genes expressed? What is the relationship between the genetic determinants revealed. by these mutations? What do the "inducible" or "constitutive" characters result from? Many of these questions could be experimentally approached through bacterial conjugation. By using male and female bacteria of suitable genotypes, one could transfer the desired allele of a given gene into a bacterium, and then study the conditions of enzyme synthesis in the zygote.

Such experiments were carried out in collaboration with Arthur Pardee, who had come to spend a year at the Pasteur Instituter[17]. They led to two new concepts. The first was relevant to the mechanism of induction itself. Transfer into a constitutive bacterium of the genetic determinant for inducibility of the enzyme by β - galactosides resulted in formation of transitory diploids, heterozygous for the characters "inducible/constitutive". Obviously, the phenotype of such zygotes should permit a choice among the different hypotheses then proposed to explain induction. The experiments showed that the "inducible" allele can express itself independently of the gene controlling the synthesis of the enzyme; and that it is dominant over the "constitutive" allele. This result revealed the existence of a special gene which controls induction by forming a cytoplasmic product that inhibits synthesis of the enzyme in the absence of inducer. As we shall see later, this finding changed existing notions about the mechanism of induction and made possible a genetic analysis of the systems which regulate the rates of protein synthesis.

The second observation concerned the functioning of the genetic material. By transferring the gene that governs the structure of a protein into a bacterium which lacks it, one can determine the conditions under which this gene is expressed in the zygote. Here again, different predictions could be made, depending on the nature of the mechanisms postulated for information transfer in the formation of proteins. From kinetic analysis of protein synthesis,

one could expect information concerning the primary gene product, the time required for its synthesis, and its mode of action. The experiments showed that, once transferred into a bacterium, and before genetic recombination has occurred, the gene controlling the structure of a protein can begin to function without detectable delay, producing protein at the maximal rate.

This was quite a surprising observation, for it was inconsistent with the notions that were prevalent at the time. Gene expression was then usually believed to consist in the accumulation of stable structures in the cytoplasm, probably the RNA of ribosomes, which were assumed to serve as templates specifying protein structures (see ref. 18). Such a scheme, which can be summarized by the aphorism "one gene-one ribosome-one enzyme", was hardly compatible with an immediate protein synthesis at maximal rate.

Further study of this problem required the withdrawal of a gene from a bacterium in order to examine the consequences of this withdrawal on the synthesis of the corresponding protein: stable templates, if present, should permit residual synthesis. But, although conjugation made it easy to inject a particular gene, the extraction of a particular gene from a whole bacterial population appeared to be an impossible operation. What could be done, however, was to transfer a segment of chromosome heavily labeled with ^{32}P and then destroy the gene under study by ^{32}P decay. This delicate experiment was carried out by Monica Riley in the laboratory of Arthur Pardee; it showed unambiguously that capacity to produce the protein does not survive destruction of the genera.

The answer was clear: gene expression cannot proceed through formation of stable templates. About the same time, the genetic and kinetic analyses of induction further strengthened this belief. Induction appeared to take place almost instantaneously and to act on structures which often specified several proteins, not merely a single one. This finding was likewise inconsistent with the theories then current, since it did not fit with the observed homogeneity of the ribosomes.

By virtue of their stability, their homogeneity, and their base composition, the two known species of RNA did not fulfill the requirements for cytoplasmic templates. Since the notion that synthesis of proteins could occur directly on DNA was incompatible with the cytoplasmic localization of the ribosomes and their role in this synthesis, only one possible hypothesis remained: it was necessary to postulate the existence of a third species of RNA, the *messenger,* a short-lived molecule charged with transmission of genetic information to the cytoplasm[20]. According to this hypothesis, the ribosomes are non-

specific structures, which function as machines to translate the nucleic language, carried by the messenger, into the peptidic language, with the aid of the transfer RNA's. In other words, the synthesis of a protein must be a two-step process: the deoxyribonucleotide sequence of DNA is first *transcribed* into messenger, the primary gene product; this messenger binds to the ribosomes, bringing them a specific "program", and the nucleotide sequence of the messenger is then *translated* into the amino acid sequence. Despite the objections raised against it, this messenger hypothesis possessed two main virtues in our eyes: on the one hand, it allowed a coherent interpretation of a number of known facts which had, until then, remained isolated or incompatible; on the other hand, it led to some precise experimental predictions.

In fact, even before it had appeared in print, the messenger hypothesis received two experimental confirmations, Sydney Brenner and I had decided to spend the month of June 1960 with M. Meselson, hunting for the messenger in the laboratory of Max Delbrück at the California Institute of Technology. The best candidate for the role of messenger seemed to us to be the RNA detected by Hershey[21] and later by Volkin and Astrachan[22] in bacteria infected with T_2 phage. Thanks to the extraordinary intellectual and experimental agility of Sydney Brenner, we were able to show, within a few weeks, that the RNA formed by the phage associates with ribosomes synthesized wholly before infection, to produce on them phage proteins. The same ribosomes can thus make either phage or bacterial proteins, depending on the messenger with which they associate. Accordingly, it is the messenger which brings to the ribosomes a specific program for synthesis[23].

At this time, another member of our group at the Pasteur Institute, François Gros, had gone to spend several months at Harvard in the laboratory of J.D. Watson. With their collaborators, they rapidly succeeded in demonstrating the existence of a messenger fraction in the RNA of growing bacteria, and in establishing its principal properties[24]. The course of events which led to the recognition and isolation of the messenger has been described by J.D. Watson[25].

: *Activity and Its Regulation : The Operon*

Experiments on genetic transfer by conjugation not only led to a revision of the concepts on the mechanisms of information transfer which occur in protein synthesis; they also made it possible to analyze the regulation of this synthesis.

The most striking observation that emerged from the study of phage production by lysogenic bacteria and of induction of β -galactosidase synthesis was the extraordinary degree of analogy between the two systems. Despite the obvious differences between the production of a virus and that of an enzyme, the evidence showed that in both cases protein synthesis is subject to a double genetic determinism: on the one hand, by *structural genes,* which specify the configuration of the peptide chains; on the other hand, by *regulatory genes*, which control the expression of these structural genes. In both cases, the properties of mutants showed that the effect of a regulatory gene consists in inhibiting the expression of the structural genes, by forming a cytoplasmic product which was called the *repressor.* In both cases, the induction of synthesis (whether of phage or of enzyme) seemed to result from a similar process: an-inhibition of the inhibitor. Thus, to our surprise, these two phenomena, studied at opposite ends of the corridor, appeared to share a common fundamental mechanism. It should be emphasized that this analogy was invaluable to us. In biology, each material has its own virtues and is of particular value for a certain kind of experimental investigation. The combination of two systems significantly increased our means of analysis.

The existence of a specific inhibitor, the repressor, had an immediate corollary: the protein-forming apparatus must contain a site on which the repressor acts in order to block synthesis. The repressor itself could be regarded as a chemical signal emitted by the regulatory gene. The signal must have a receptor. The receptor had to be specific, hence genetically determined, and hence accessible to mutation. In a system that permits induced biosynthesis of an enzyme, any mutation damaging one element of the emitter-receptor system which inhibits the synthesis should result in constitutive enzyme production. Consequently it seemed difficult to distinguish mutations affecting the emitter from those affecting the receptor, until we realized that the distinction should be relatively easy to make in a diploid. This point can be illustrated by a simple analogy. Let us consider a house in which the opening of each of two doors is controlled by a little radio receiver. Let us suppose, furthermore, that somewhere in the vicinity there exist two transmitters, each sending out the same signal, which prevents the opening of the doors. If one of these transmitters is damaged, the other continues to send out signals and the doors remain closed: the damaged transmitter can be considered as "recessive" with respect to the normal one. On the other hand, if one of the receivers is damaged, it no longer responds to the inhibitory signal, and the door which it controls (but only that one) opens. The damaged receiver is thus "dominant" over the

normal one, but the lesion is manifested only by the door which it controls: the effect is *cis* and not *trans,* in genetic terminology[26].

Thus it should be possible in principle, by the use of diploid bacteria, to distinguish, among constitutive mutations, those due to the regulatory gene from those due to the receptor. In fact, phage mutants corresponding to one or the other of these types had been known for a long time, although their nature became clear only in the light of this scheme. The existence of such mutations in phages encouraged us to search for analogous bacterial mutations affecting the enzymes of the lactose system. For that purpose, however, diploid bacteria were required. Although conjugation allowed formation of transitory diploids, their production was tricky and their analysis complicated. However, certain observations which had recently been made by Edward Adelberg led to the idea that the sexual episome F, which governs conjugation in *E. coli,* might under certain conditions incorporate and subsequently replicate a small fragment of the bacterial chromosome[27]. By using a series of strains in which the sexual episome was inserted at various points along the bacterial chromosome, we succeeded in isolating episomes which had incorporated a neighboring chromosomal fragment. Bacteria harboring such an episome become stable diploids for a small genetic segment, so that it is easy to make all possible combinations of alleles in this segment[28].

Having thus constructed the requisite genetic tool for our analysis, we set out to isolate under different conditions a whole series of mutants constitutive for the lactose system, in order to subject them to functional analysis. These mutants proved to belong to two quite distinct groups, which possessed the predicted properties for the transmitter and the receiver, respectively.

Many of these mutations were found to be "recessive" with respect to the wild-type allele. They allowed a definition of the transmitter, that is, of the regulatory gene. Some of these mutations possessed characteristic properties which led to the indirect identification of the repressor, the product of the regulatory gene[29]. They are discussed in greater detail in Jacques Monod's lecture.

In the second group, the mutations turned out to be "dominant" over the wild-type allele, and only those genes which were located on the same chromosome, that is, in *cis* position, were expressed constitutively. With these mutations, it was possible to define the receptor of the repressor, termed the *operator*[30].

The study of these mutants led, furthermore, to the notion that in bacteria the genetic material is organized into units of activity called *operons,* which are

often more complex than the gene considered as the unit of function. In fact, the lactose system of *E. coli* contains three known proteins, and the three genes governing their structure are adjacent to one another on a small segment of the chromosome with the operator at one end (Fig. 1). Constitutive mutations, whether due to the alteration of the regulatory gene or of the operator, always display the remarkable property of being pleiotropic; that is, they affect simultaneously, and to the same extent, the production of the three proteins. The regulatory circuit therefore had to act on one integral structure containing the information which specifies the amino acid sequences of the three proteins. This structure could only be either the DNA itself or a messenger common to the three genes. This idea was further supported by the properties observed in mutations affecting the structural genes of the lactose system. Whereas some of these mutations obey Beadle and Tatum's "one gene-one enzyme" rule in the sense that they abolish only one of the three biochemical activities, others violate this rule by affecting the expression of several genes at a time[31,32].

The notion of the operon, a grouping of adjacent structural genes controlled by a common operator, explained why the genes controlling the enzymes of the same biochemical pathway tend to remain clustered in bacteria, as observed by Demereč and Hartman[33]. Similarly, it accounted for the coordinate production of enzymes already found in certain biochemical pathway@. Although at first the operon concept was based exclusively on genetic criteria, it now includes biochemical criteria as well. There are, in fact, a number of experimental arguments, both genetics[32,35] and biochemical[36], in support of the inference that an operon produces a single messenger, which binds to ribosomes to form the series of peptide chains determined by the different structural genes of the operon.

We can therefore envision the activity of the genome of *E. coli* as follows. The expression of the genetic material requires a continuous flow of unstable messengers which dictate to the ribosomal machinery the specificity of the proteins to be made. The genetic material consists of operons containing one or more genes, each operon giving rise to one messenger. The production of messenger by the operon is, in one way or another, inhibited by regulatory loops composed of three elements: regulatory gene, repressor, operator. Specific metabolites intervene at the level of these loops to play their role as signals: in inducible systems, to inactivate the repressor and hence allow production of messenger and ultimately of proteins; in repressible systems, to activate the repressor, and hence inhibit production of messenger and of proteins. Ac-

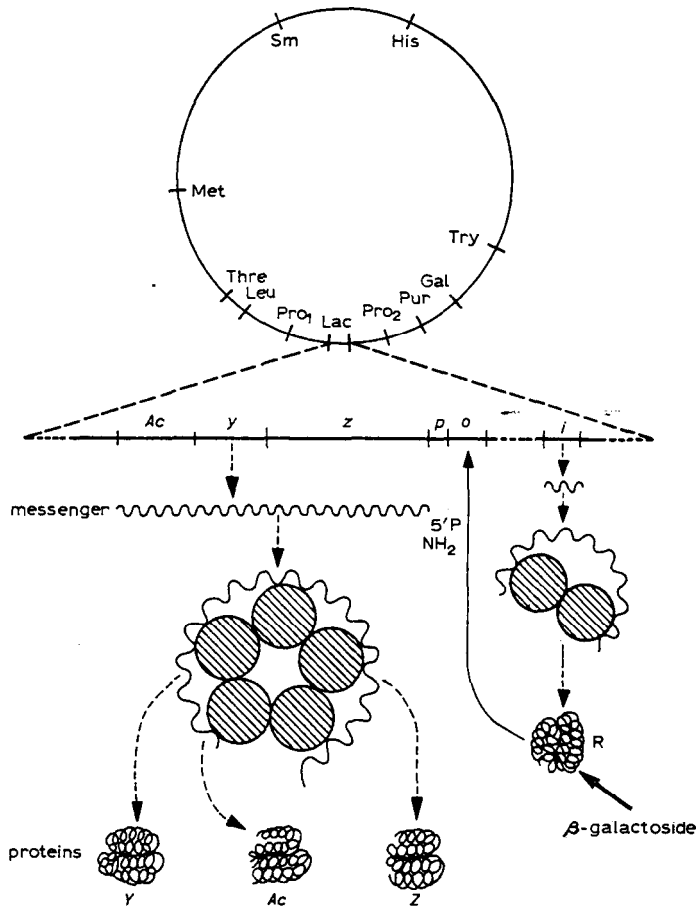

Fig. 1. The lactose region of *Escherichin coli.* The circle represents the *E. coli* chromosome and shows the position of the lactose region *(Lac)* among other markers. An enlargement of the lactose region is shown below. *i,* Regulatory gene; *o,* operator; *p,* promotor; *z,* structural gene for β-galactosidase; *y,* structural gene for β-galactoside permease; *Ac,* structural gene for β- galactoside transacetylase. The structural genes probably synthesize a single messenger (of which the 5′-phosphate end is most likely at the operator end) which associates with the ribosomes to form a polysome where the different peptide chains (of which the amino end probably corresponds to the operator end) are synthesized. The regulatory gene produces a specific repressor which, acting at the level of the operator, blocks the production of the messenger and hence of the proteins. The β-galactoside inducers act on the repressor to inactivate it, thus permitting the production of the messenger and consequently of the proteins determined by the operon.

cording to this scheme, only a fraction of the genes of the cell can be expressed at any moment, while the others remain repressed. The network of specific, genetically determined circuits selects at any given time the segments of DNA that are to be transcribed into messenger and consequently translated into proteins, as a function of the chemical signals coming from the cytoplasm and from the environment.

From the beginning, the conception of the genetic material as being formed of juxtaposed operons whose activity is regulated by a single operator site entailed a precise experimental prediction: a chromosomal rearrangement which would separate some structural genes from their operator and link them to a different operon controlled by its own operator should place the activity of these structural genes under a new regulatory control. But for a while, although some genes could be separated from their operators as a result of certain mutations, they became reattached to an unidentified region of the chromosome and consequently subject to an unknown system of regulation which remained beyond our experimental reach[37,38].

Only recently has it become possible to obtain a fusion of the lactose operon of *E. coli* with another known operon, by using bacteria which were diploid for the chosen region[39]. At present, we know only a limited number of genes on the bacterial chromosome and a still more limited number of genes whose activity can be modified by the action of external metabolites. Any deletion which fuses two of these regions is likely to be relatively large and therefore to include a gene whose product is required for growth or division; it would thus be lethal in a haploid bacterium. In diploid bacteria, on the other hand, it has been possible to isolate a series of deletions covering about 50 to 80 genes; at one end, these deletions terminate in the gene controlling the structure of β-galactosidase, and at the other, in different regions of the chromosome. Some terminate in one of the two cistrons belonging to a purine operon, while leaving the other cistron intact (Fig. 2).

In these mutants, synthesis of the two proteins of the lactose region determined by the two genes left intact by the deletion is no longer inducible by β-galactosides. Such a result could be predicted since the deletion has destroyed both of the elements (regulatory gene and operator) responsible for the specific regulation of the lactose system. *But this synthesis has become repressible by the addition of purines.* It is thus clear that, in the deletion, the fragment of the lactose operon and the fragment of the purine operon have been fused to form a new operon which, from all appearances, produces a single messenger containing the genetic information for the synthesis of the proteins

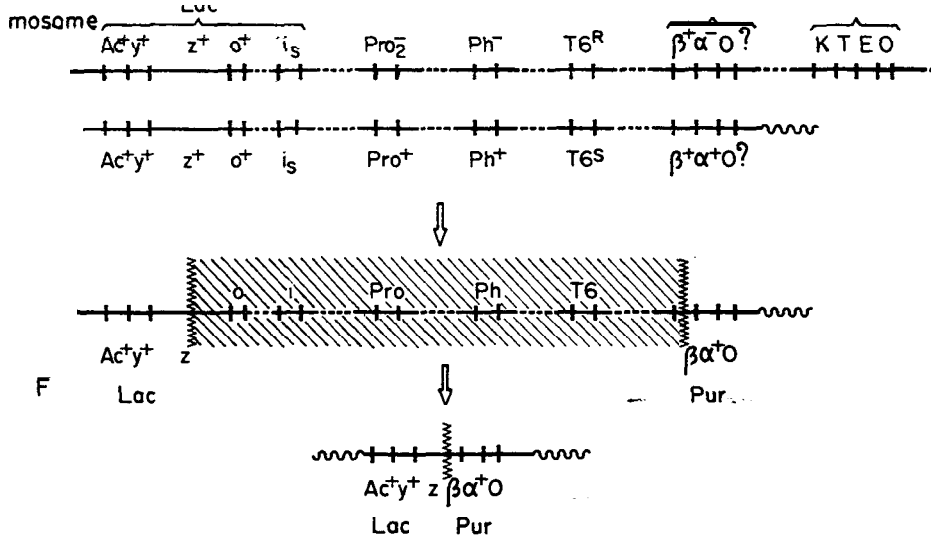

Fig. 2. Deletion fusing a fragment of the lactose operon and a fragment of a purine operon in *E.coli*. The upper part of the diagram represents the original heterozygous diploid structure of a bacterium containing a sexual episome which has incorporated an important chromosomal fragment by recombination. In the central part of the diagram, the hatched region shows the zone eliminated by a deletion which occurred in the episome. The lower part of the diagram shows the structure formed as a result of the deletion. The latter connected a terminal fragment of the *z* gene (determining β-galactosidase) with an initial fragment of the *Pur β* gene (determining an enzyme of purine biosynthesis). A new operon is thus formed from the *Pur α* gene (determining one of the proteins of purine biosynthesis), a structure formed by part of the *Pur β* gene and part of the *z* gene (probably producing a hybrid peptide chain consisting at the amino end of a *Pur β* sequence and at the carboxyl end of a sequence from *z*), the *y* gene (determining β-galactoside permease), and the *Ac* gene (determining β-galactoside transacetylase). The expression of the operon is repressed by purines, presumably at the level of a purine operator, which is itself sensitive to a repressor specifically activated by purines[39].

involved both in the biosynthesis of purines and in the utilization of lactose. But the system which determines the regulation of this messenger must be the operator of the purine region, sensitive to a repressor activated by purines.

In the same way, deletions fusing the lactose operon with the operon controlling tryptophan biosynthesis have recently been isolated[40]. The expression of the genes of the lactose operon which are still intact has consequently become repressible by tryptophan.

The type of regulation imposed on the expression of the genes belonging to

a given operon thus depends exclusively on the operator, that is, in some way, on the nucleotide sequence located at the proximal extremity of the operon. In this manner, both the nature of the metabolites on which regulation depends and the inducible or repressible character of the regulation are determined by the respective *positions* of the genes along the chromosome and, more particularly, by their *association* with a particular operator segment. Obviously, it is the associations most favorable to the organism that are selected.

The presence of units of activity and regulation constituted by polycistronic operons implies the existence of a double system ofpunctuation in the nucleic text. One type of punctuation must permit the "slicing" of the long DNA duplex into "sections of transcription" corresponding to operons: this must serve as the point of recognition for the RNA polymerase, to show it not only where to start and finish the transcription of an operon, but also which strand of DNA is to be transcribed. Under certain conditions, one can obtain transpositions of the lactose operon into a region of the chromosome different from the normal region, and these insertions can be oriented in either one direction or the other[40]. It should be noted that, in case of an inversion, the 3′, 5′ polarity of the DNA strands requires that the sequence to be transcribed into messenger must change with respect not only to direction, but also to strand (Fig. 3). For the insertions obtained, the lactose operon seems to be equally well expressed whether or not there has been an inversion. Conse-

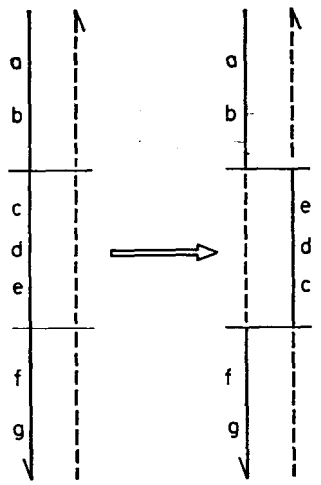

Fig. 3. Schematic diagram showing a change of polarity, and hence of strand, in an inversion.

quently, it must be assumed that *(i)* all the genetic information of *E. coli* is not necessarily contained in the same strand of DNA; *(ii)* a genetic signal ought to indicate the start of the operon, as well as the direction of transcription; and *(iii)* another signal ought to mark the end of the operon. If two operons of opposite orientations occur in juxtaposition, in the absence of a signal "end of transcription", the transcription of one operon could eventually proceed along the other, in the DNA strand which is not normally supposed to be transcribed.

The second punctuation mark of the nucleic text must, at the time of translation, allow the "slicing" of the messenger into the various peptide chains corresponding to the respective genes of the operon. This punctuation serves as a signal to the translation system (ribosomes, tRNA, and so on) to delimit the amino terminal and the carboxy terminal ends of each peptide chain.

In the lactose system of *E. coli,* the analysis of a series of deletions shows that the operator is situated outside the first known structural gene of the operon (refs.41,38), from which it appears to be separated by a region called the *promotor,* which is indispensable to the expression of the entire operon[37]. The promotor probably corresponds to one of the punctuation marks, either for transcription or for translation. There are reasons to believe that the operator itself is not translated into a peptide chain, but we still do not know whether it is transcribed into messenger and whether the repressor acts at the level of the messenger or of the DNA itself (Fig. 4). It is not possible to discuss in detail

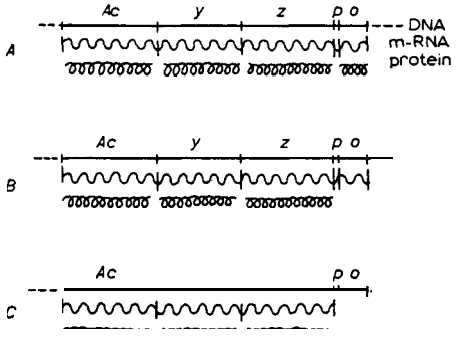

Fig. 4. The three possible models for the functioning of an operator. *A*, The operator (*o*) is transcribed and translated into protein. *B,* The operator is transcribed but not translated. C, The operator is neither transcribed nor translated. Obviously, depending on whether or not the operator is translated or is transcribed, the repressor could act at the level of the protein, of the messenger, or of the DNA itself.

here the experimental arguments or the hypotheses[20,32,42] concerning the site of action of the repressor. However, the ensemble of results recently obtained in various laboratories[43] indicates that synthesis of the messenger from its 5′-phosphate terminus, like that of the first peptide chain from its amino terminus, both begin at the operator end of the operon. The simplest hypothesis compatible with the results of genetic analysis, particularly with the study of deletions covering different segments of the operator region, is that the promotor represents the punctuation of transcription, providing the signal for RNA polymerase to start the synthesis of the messenger for this operon on one of the two DNA strands. If this is correct, the operator is not transcribed into messenger and repression can be exerted only at the level of DNA. This is the interpretation that now seems the most plausible to the geneticist; but it is clear that, as usual, the last word will belong to the chemist.

Organization of Genetic Material in the Bacterial Cell: The Replic

Genetic analysis had revealed the logic of the circuits involved in the regulation of protein synthesis by showing that these circuits are composed of elements which can be connected in different ways to respond to the needs of the cell. It seemed reasonable to assume that analogous circuits, constructed on similar principles and using similar elements, might participate in other aspects of cellular regulation, and in particular to direct the replication of DNA in coordination with cellular division.

The systems involved in the replication of DNA seem to function more subtly in the cell than when isolated in the test tube. There are a number of experimental arguments in favor of a semiconservative mechanism of replication, as predicted by Watson and Crick from their model. Thanks to the work of Kornberg[44] and his collaborators an enzyme is known which can polymerize deoxyribonucleotides in the order dictated by the sequence of a piece of DNA serving as template. However, if a fragment of bacterial DNA is transferred into a recipient bacterium by transformation or incomplete conjugation, this fragment is incapable of replicating by itself. It can replicate only when integrated by recombination with one of the genetic structures in the host bacterium.

In bacteria, the DNA is organized into much simpler units than those observed in the cells of higher organisms. The essential information for the growth and division of the bacterium is carried by a single element, the so-

called "bacterial chromosome". In addition, other nonessential elements, the "episomes", may be introduced into the bacterial cell[16].

Many different kinds of work have revealed that the best known of these genetic elements, the chromosome, behaves genetically, structurally, and biochemically as a single, integrated element. It seems to consist of one double-stranded chain of DNA, very probably closed or circular[45]. Replication appears to start at a fixed point on the molecule, and to continue regularly until the point of departure is reached again[45,46]. Under normal growth conditions, a new round of replication cannot begin until completion of the previous one[47].

Although other bacterial genetic elements are less well understood, their properties seem to be analogous. The genetic equipment of a bacterium can thus be considered to consist of distinct structures, each containing a "molecule" of DNA which is circular and of variable length.

Together with Sydney Brenner, we have tried to explain the regulation of DNA synthesis by means of circuits resembling those involved in the control ofprotein synthesis[4 8]. We have been led to postulate that each genetic element constitutes a unit of replication or *replicon,* which determines a circuit controlling its own replication in coordination with cell division. This hypothesis carries with it three distinct predictions.

(1) If each element contains some genetic determinants controlling its own replication, it should be possible to isolate mutants in which the regulatory circuit is impaired. In fact, for each of the three elements examined-bacterial chromosome, sexual episome, and phage-mutations can be obtained which abolish replication of the mutated element but not of others[49]. The nature and properties of these mutations suggest that they modify a diffusible product which normally acts on a punctuation mark of the replicon, that is, on a particular nucleotide sequence, to permit the start of replication. Once the reaction is initiated, the system replicates the entire sequence attached to this punctuation.

Here again, a genetically determined regulatory loop appears to operate. But, whereas in the synthesis of proteins the regulation seems to be *negative* or repressive, in the synthesis of DNA the available evidence indicates that the regulation involves a *positive* element, that is, one which acts on the DNA to trigger replication.

(2) The circumstances of bacterial conjugation can be best interpreted by assuming that the sexual episome is attached to the bacterial membrane near the zone through which the male chromosome passes during mating. Further-

more, to explain, during bacterial growth, the segregation of DNA after replication and the distribution of the two DNA copies in the two bacteria formed by cellular division, the simplest hypothesis consists in assuming that all cellular replicons are attached to the bacterial membrane. It is the synthesis of the membrane between the points of attachment of the two DNA copies that would insure their normal segregation.

The latter prediction appears to be confirmed by Antoinette Ryter's electron microscopic study of the "nuclear bodies" in *Bacillus subtilis*[50]. Each of these nuclear bodies seems to be attached to a "mesosome", a structure formed by an invagination of the membrane (Figs. 5 and 6). Furthermore, by staining the membrane with a tellurium salt, it can be shown that membrane synthesis does not take place uniformly over the bacterial surface, but rather in particular zones close to the attachment sites of the nuclear bodies. It is thus the growth of the membrane which seems to bring about the segregation of the DNA elements formed by replication. Finally, François Cuzin has succeeded in demonstrating that two independent replicons, such as the chromosome and the sex factor, do not segregate independently but remain associated during bacterial multiplication[51]; presumably, each of these structures is linked to the same element, possibly the same fragment of membrane. This fragment which might remain intact during growth and bacterial division would thus constitute the actual unit of segregation, analogous to a chromosome in a higher organism.

(3) To explain the coordination between the replication of DNA and the growth and subsequent division of the bacterial cell, it must be assumed that DNA replication and its regulation occur in the membrane. This seems to be indicated by certain phenomena observed during bacterial conjugation: presumably a surface reaction which occurs while the male and female come in contact triggers, in some way, a round of replication in the male. One of the structures thus synthesized remains in the male, while the other is progressively driven, during its formation, into the femalesa. Furthermore, the idea that DNA synthesis occurs in the membrane has recently received some biochemical support[53].

Thus one comes to envision the genetic equipment of bacteria as formed of circular DNA "molecules" constituting independent units of replication. These units would be associated with one membrane element, which would coordinate their replication with cell growth by means of regulatory circuits (Fig. 7). The basic genetic information would be contained in the longest of these units, but supplementary information could be added by the fixation of

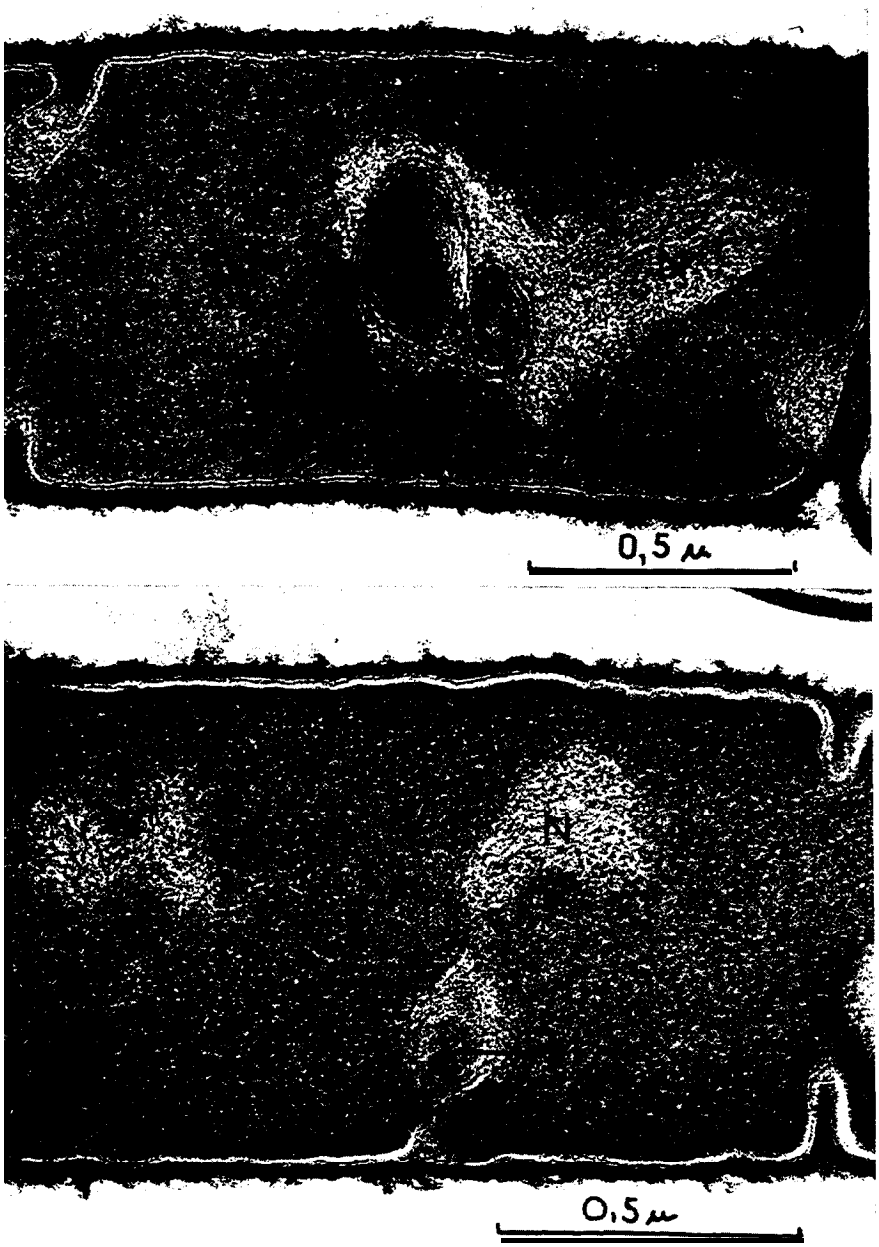

Fig. 5. Sections of *Bacillus subtilis.* The nuclear body (N) is bound to the membrane by means of a mesosome (M)[50].

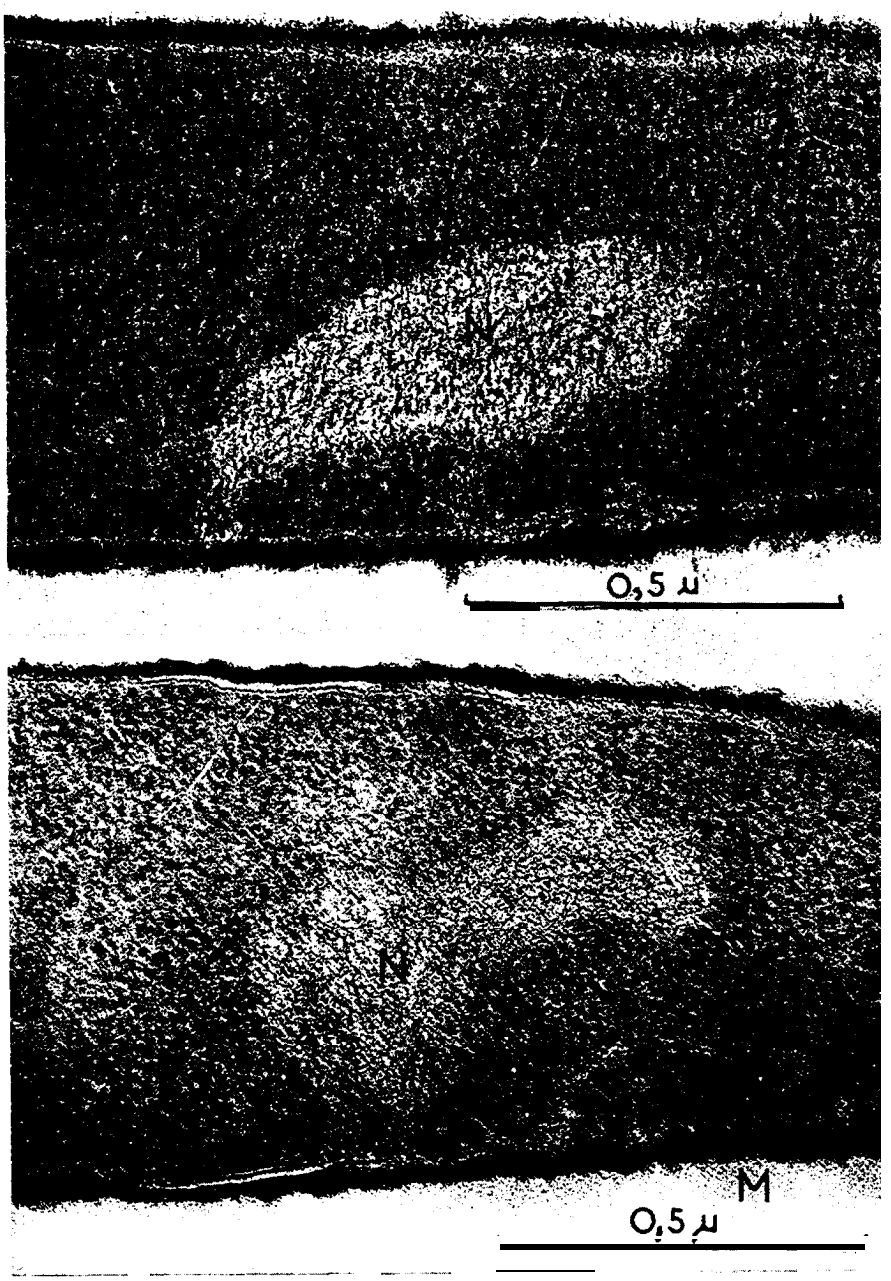

Fig. 6. Sections of *B. subtilis* placed for 30 min in 0.5 *M* sucrose. The mesosomes have been pushed out of the cytoplasm. During their retraction, they pull with them the nuclear bodies (N), which then appear bound directly to the membrane[50].

other replicons to the membrane. It seems that one of the important steps in the passage from the cellular organization of procaryotes to that of eucaryotes involves invaginations of membranous structures followed by differentiation into specialized organelles (mitochondria, genetic apparatus) whose functions were originally carried out by the bacterial membrane.

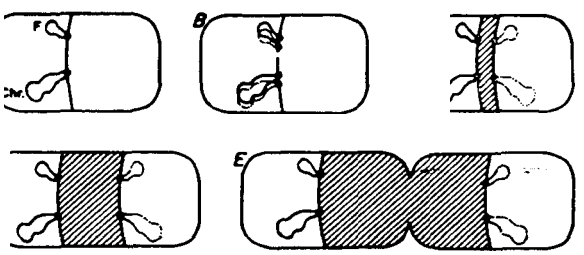

Fig. 7. Schematic diagram of DNA replication in bacteria. The bacterium represented contains two independent units: the "chromosome" and the sexual episome F. These two replicons are shown attached to the membrane at two distinct stites. At a given point in the division cycle, the membrane transmits to each replicon a signal permitting replication to start. Replication progresses linearly, each replicon turning slowly across the membrane in which the enzymatic complex responsible for replication is thought to occur. Two copies of each replicon are thus formed and are assumed to be attached to the membrane side by side. Membrane synthesis is considered to occur between these regions to which the two copies of each replicon are attached, thus drawing them to either side, with the septum forming in the median region. No new round of replication is allowed until the membrane, having returned to its original state following cell division transmits a new signal. The process is simplified in the sense that: *(i)* since the replication of DNA occurs one cycle ahead of division, bacteria generally have from two to four nuclear bodies (and not from one to two); and *(ii)* each step is considered to be completed before the start of the next one[48].

It is remarkable that the study of the bacterial cell leads us to attribute to the membrane such an important role in the coordination of growth and cell division, since a similar conclusion has been reached on the basis of the study of cells from higher organisms. In such cells, the process of morphogenesis and contact phenomena among cells indicate that the cell surface must also control cellular multiplication by means of signals transmitted, in one way or another, to the nucleus, Despite the evident complexity of such cells in comparison with bacteria, it must be assumed that evolution has conserved a system of molecular communication between cell surface and DNA.

Conclusions

The two chemical activities of DNA, transcription, the copying of a single strand into a ribonucleotide sequence, and replication, the copying of both strands into deoxyribonucleotide sequences, are controlled by a network of specific molecular interactions determined by the genes. The message inscribed in the genetic material thus contains not only the plans for the architecture of the cell, but also a program to coordinate the synthetic processes, as well as the means of insuring its execution.

Perhaps one of the most important contributions ofmicrobial genetics is the definitive answer given to the old problem of the interaction between genes and cytoplasm, between heredity and environment. Although the demonstration that acquired characters cannot be inherited had already been made by classical genetics, the explanation for this fact is now provided by the nature of the nucleic message and of the genetic code. It is clear, on the other hand, that the expression of the genetic material is subject to external influences. Ten years ago, it still seemed possible that, in certain processes such as the induced biosynthesis of enzymes or of antibodies, the presence of specific compounds could modify the synthesis of proteins, mold their configurations, and hence alter their properties. The environment seemed to exercise an *instructive* action on the genes, to use Lederberg's expression[54], and thus to modulate the sense of the genetic text. What the study of regulatory circuits has shown is that the compounds in question serve only as simple stimuli: they act as signals to initiate a synthesis whose mechanism and final product remain entirely determined by the nucleotide sequence of the DNA. If the nucleic message may be compared with the text of a book, the regulatory network determines which pages are to be read at any given time. In the expression of the nucleic message, as well as in its reproduction, adaptation results from an *elective* rather than an instructive effect of the environment.

Of course genetic analysis can do no more than indicate the existence of regulatory circuits. A chemical analysis, which should disclose the specific molecular interactions, remains to be made. No repressor has yet been isolated, and the nature of the complexes that it can form with an operator or a metabolite remains obscure. We do not know how molecules find each other, recognize each other, and combine to constitute the regulatory network or 'to form such cellular superstructures as a membrane, a mitochondrion, or a chromosome. We do not know how molecules transmit the signals which modify the activity of their neighbors. What is clear, however, is that the

problems to be solved by cellular biology and genetics in the years to come tend increasingly to merge with those in which biochemistry and physical chemistry are involved.

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1. S.E. Luria and M. Delbrück, *Genetics,* 28 (1943) 499.
2. J. Lederberg and E.L. Tatum, *Nature,* 158 (1946) 558.
3. M. Delbrück and W.T. Bailey Jr., *Cold Spring Harbor Symp.Quant.Biol.,* 11 (1946) 33.
4. A.D. Hershey, Cold Spring *Harbor Symp. Quant. Biol.,* 11 (1946) 67.
5. O.T. Avery, C.M. MacLeod, M. McCarty, *J. Exptl. Med.,* 79 (1944) 137.
6. A.D. Hershey and M. Chase, *J. Gen. Physiol.,* 36 (1952) 39.
7. J.D. Watson and F.H.C. Crick, *Nature,* 171 (1953) 737.
8. G.W. Beadle and E.L.Tatum, *Proc. Natl. Acad. Sci. (U.S.), 27* (1941) 499.
9. J. Lederberg, in J.H. Comroe Jr. (Ed.), *Methods in Medical Research,* Vol. 3, Year Book Publishers, Chicago, 1950, p. 5.
10. S. Benzer, in W.D. McElroy and B. Glass (Eds.), *The Chemical Basis of Heredity,* Johns Hopkins, Baltimore, 1957, p. 70.
11. E.M. Lederberg and J. Lederberg, *Genetics,* 38 (1953) 51.
12. E.L. Wollman, Ann. *Inst. Pasteur,* 84 (1953) 281.
13. W. Hayes, *Cold Spring Harbor Symp. Quant. Biol.,* 18 (1953) 75.
14. F. Jacob and E.L. Wollman, *Compt. Rend.,* 239 (1954) 455.
15. E.L. Wollman and F. Jacob, *Compt. Rend.,* 240 (1955) 2449.
16. F. Jacob and E.L. Wollman, *Sexuality and the Genetics of Bacteria,* Academic Press, New York, 1961.
17. A.B. Pardee, F. Jacob and J. Monod, *J. Mol. Biol.,* 1 (1959) 165.
18. T. Caspersson, *Chromosoma,* 1 (1940) 562; J. Brachet, *Enzymologia,* 10 (1941) 87; F.H.C. Crick, *Symp. Soc. Exptl. Biol.,* 12 (1958) 138; R.B. Roberts, *Microsonal Particles and Protein Synthesis,* Pergamon, Oxford, 1958; A. Tissières, J.D. Watson, D.

Schlesinger and B.R. Hollingworth, *J. Mol. Biol.,* 1 (1959) 221; C.I. Davern and M. Meselson, *J. Mol. Biol., 2* (1960) 153.
19. M. Riley, A.B. Pardee, F. Jacob and J. Monod, *J. Mol. Biol.,* 2 (1960) 216.
20. F. Jacob and J. Monod, *J. Mol. Biol.,* 3 (1961) 318.
21. A.D. Hershey, J. Dixon and M. Chase, *J. Gen. Physiol.,* 36 (1953) *777.*
22. E. Volkin and L. Astrachan, *Virology,* 2 (1956) 149.
23. S. Brenner, F. Jacob and M. Meselson, *Nature,* 190 (1961) 576.
24. F. Gros, W. Gilbert, H. Hiatt, C.G. Kurland, R.W. Risebrough and J.D. Watson, *Nature,* 190 (1961) 581.
25. J.D. Watson, The involvement of RNA in the synthesis of proteins, *in Nobel Lectures, Physiology* or *Medicine, 1942-1962,* Elsevier, Amsterdam, 1964, p. 785.
26. G. Pontecorvo, *Advan. Enzymol.,* 13 (1952) 121.
27. E.A. Adelberg and S.N. Burns, *J. Bacteriol.,* 79 (1960) 321.
28. F. Jacob and E.A. Adelberg, *Compt. Rend.,* 249 (1959) 189.
29. C. Willson, D. Perrin, M. Cohn, F. Jacob and J. Monod., *J. Mol. Biol.,* 8 (1964) 582; S. Bourgeois, M. Cohn and L. Orgel, *J. Mol. Biol.,* 14 (1965) 300.
30. F. Jacob, D. Perrin, C. Sanchez and J. Monod, *Compt. Rend.,* 250 (1960) 1727.
31. F. Jacob and J. Monod, *Cold Spring Harbor Symp. Quant. Biol.,* 26 (1961) 193; *N.* Franklin and S.E. Luria, *Virology,* 15 (1961) 299.
32. B.N. Ames and P. Hartman, *Cold Spring Harbor Symp.Quant.Biol.,* 28 (1963) 349.
33. M.Demerec̆ and P. Hartman, *Ann. Rev. Microbial.,* 13 (1959) 377.
34. B.N. Ames and B. Gary, *Proc. Natl. Acad. Sci. (U.S.),* 45 (1959) 1453.
35. J.R. Beckwith, *Structure and Function of the Genetic Material,* Akademie Verlag, Berlin, 1964, p.119.
36. G. Attardi, S. Naono, J. Rouviere, F. Jacob and F. Gros, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 363; B. Guttman and A. Novick, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 373; R.G. Martin, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 357; S. Spiegelman and M. Hayashi, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 161.
37. B.N. Ames, P. Hartman and F. Jacob, *J. Mol. Biol.,* 7 (1963) 23; A. Matsushiro, S. Kido, J. Ito, K. Sato and F. Imamoto,. *Biochem. Biophys. Res. Commun,* 9 (1962) 204.
38. F. Jacob, A. Ullmann and J. Monod, *Compt. Rend.,* 258 (1964) 3125.
39. F. Jacob, A. Ullmann and J. Monod, *J. Mol. Biol.,* 13 (1965) 704.
40. J.R. Beckwith, E. Signer and W. Epstein, *Cold Spring Harbor Symp. Quant. Biol.,* 23 (1966) 393.
41. J.R. Beckwith, *J. Mol. Biol.,* 8 (1964) 427.
42. L. Szilard, *Proc. Natl. Acad. Sci.(U.S.),* 46 (1960) 277; G.S. Stent, *Science,* 144 (1964) 816; W.K. Maas and E. McFall, *Ann. Rev. Microbiol.,* 18 (1964) 95.
43. R.L. Somerville and C. Yanofsky, *J. Mol. Biol.,* 8 (1964) 616; G. Streisinger, *Mendel Symposium on the Mutational* Process, Prague, 1965; H. Bremer, M.W. Konrad, K. Gaines and *G.S.* Stem, *J. Mol. Biol.,* 13 (1965) 540; F. Imamoto, N. Morikawa and K. Sato, *J. Mol. Biol.,* 13 (1965) 169; U. Maitra and J. Hurwitz, *Proc. Natl. Acad. Sci. (U.S.),* 54 (1965) 815; M. Salas, M.A. Smith, W.M. Stanley Jr., A.J. Wahba and S. Ochoa, *J. Biol. Chem.,* 240 (1965) 3988; R.E. Thach, M.A. Cecere, T.A. Sunarajan and P. Doty, *Proc. Natl. Acad. Sci. (U.S.),* 54 (1965) 1167.

44. A. Kornberg, The biologic synthesis of deoxyribonucleic acid, in *Nobel Lectures, Physiology or Medicine, 1942-1962,* Elsevier, Amsterdam, 1964, p. 665.
45. J. Cairns, *J. Mol. Biol.,* 6 (1963) 208.
46. N. Sueoka and H. Yoshikawa, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 47; F. Bonhoeffer and A. Gierer, *J. Mol. Biol.,* 7 (1963) 534.
47. O. Maaloe, *Cold Spring Harbor Symp. Quant. Biol.,* 26 (1961) 45; R.H. Pritchard and K.G. Lark, *J. Mol. Biol.,* 9 (1964) 288.
48. F. Jacob and S. Brenner, *Compt. Rend.,* 256 (1963) 298; F. Jacob, S. Brenner and F. Cuzin, *Cold Spring Harbor Symp. Quant. Biol.,* 28 (1963) 329.
49. M. Kohiyama, H. Lamfrom, S. Brenner and F. Jacob, *Compt. Rend.,* 257 (1963) 1979; F. Cuzin and F. Jacob, *Proc. Intern. Congr. Genet. 11th, The Hague 1962,* Vol. 1, 1963, p. 40; F. Jacob, C. Fuerst and E.L. Wollman, *Ann. Inst. Pasteur,* 93 (1957) 724.
50. A. Ryter and F. Jacob, *Ann. Inst. Pasteur,* 107 (1964) 384,
51. F. Cuzin and F. Jacob, *Compt. Rend.,* 260 *(1965)* 5411.
52. J.D. Gross and L.G. Caro, *Science,* 150 (1965) 1679; M. Ptashne, *J. Mol. Biol.,* 11 (1965) 829; A.A. Blinkova, S.E. Bresler and V.A. Lanzov, Z. *Vererbungsl.,* 96 (1965) 267.
53. A.T. Ganesan and J. Lederberg, *Biochem. Biophys. Res. Commun.,* 18 (1965) 824.
54. J. Lederberg, *J. Cellular Comp. Physiol.,* Suppl. 1, 52 (1958) 398.