TRIANGLE

VOLUME V NO. 2 JULY 1961

BACTERIAL GENES

PROF. J. LEDERBERG

BACTERIAL GENES*

PROF. J. LEDERBERG
Department of Genetics, Stanford University School of Medicine,
Palo Alto, California

Fifteen years ago bacteria were already becoming recognised as experimental material very well suited for fundamental research in biochemistry and cell physiology. But these outstanding merits could only aggravate the expressed disappointments that bacteria were also too simple to have any mode of reproduction but a strictly asexual one of vegetative multiplication. It thus appeared that bacteria might be barred from the exciting race towards the solution of fundamental biological problems: the chemistry of heredity, and its converse, the genetics of biochemical individuality, and such derivative problems as differentiation, sexuality and cancer. In short, bacteria would remain a digression from the main sequence of biological evolution and from our theoretical synthesis of biology. Conversely, the special problems of microbiology would be isolated from the method and the theoretical insight of general biology.

Bacteria for physiological research

Today, no one will accuse bacteria of either poverty or simplicity in their genetic systems, and we may even voice some oversophisticated and unanswerable doubts as to their primitive character. Far from being incidental curiosities, bacteria are now salient in molecular and cell biology. The advantages of bacteria for physiological research include the following attributes and capabilities which are of course interrelated and must be classified rather arbitrarily:

1. the ease of their pure culture in the laboratory, under rigorous control of their environment, for an indefinite number of generations.

2. the density of their metabolic machinery, as reflected in a high concentration of nucleic acids and proteins, including specific enzymes and antigens.

3. the diversity of their metabolism: almost every known enzyme is represented in some bacterial species, sometimes uniquely. They also exhibit many other functions of great interest: motility, photosynthesis, antibiosis, virulence.

4. the very large populations, billions of organisms per ml., that can readily be achieved.

5. the speed of their multiplication, allowing many progeny to be assessed in a short period of time.

6. their amenability to procedures for artificial selection of specific cell types, however rare — for example in counting mutational events.

* The author's studies in bacterial genetics have been generously supported by research grants from the National Science Foundation and from the National Cancer Institute, U. S. Public Health Service (Grant No. C–4496).

7. the uniformity of the cells (barring mutation) that are produced in populations that have been grown under careful control.

8. the ease of counting populations by conventional plating methods of microbiology.

9. their liability to a series of specific viruses, the bacteriophages, and the force of the well-known methods for studying these viruses.

10. a variety of genetic mechanisms which allow particular insights into the chemistry of the gene, into the pathways of its action, and into problems of sexuality and recombination. To be sure, these mechanisms could not be so powerfully studied were it not for the technical advantages just listed. The main burden of this article will be to review these mechanisms.

These advantages are mainly technical but they have accelerated the pace of microbiological research, have reduced its initial costs *, and have permitted the design and execution of many experiments that would be too cumbersome even to think of with present techniques for larger organisms. We have therefore a capital accumulation of knowledge which has inestimable value for further progress. Needless to say, these advantages apply quite independently of the practical importance of bacteria in industry, in agriculture and in medicine — indeed, it has been repeatedly suggested that the urgency of the problems presented in these applied areas contributed to delaying a fundamental approach to microbial biology.

The author readily confesses to an unreasonable enthusiasm for the bacterium, but he must concede that some of their purported advantages are deterrents in other contexts. For example, the small size of the bacterium hinders our examination of genetic and environmental effects in single cells (though in part this may be attributed to an unwarranted phobia against the micromanipulator); and the bacterial nucleus and chromosomes still offer some of the most vexing problems in morphology and cytochemistry. As far as it applies, the uniformity of bacterial clones precludes embryology, and (with some forward exceptions) during the present century the neurobiologist has found little to interest him in bacterial behaviour.

* As marginal economics teaches, microbiological research does not remain cheap; its scale expands to the limits available funds, energy and ideas.

Genetic transfer, sexuality and transduction

In higher organisms genetic transfer is so far synonymous with sexual reproduction. The familiar process of fertilisation unites a complete set of genetic determinants, one gamete from each parent. In the production of gametes, the dual set of the parent is sampled at random. Each cycle of reproduction thus achieves a far-reaching reshuffling of the genes — except identical twins emerging from a single zygote, we can be sure that no two individuals of the human species have ever existed who were quite alike in their genetic endowment *. The biological importance of this variability, which helps insure the vitality of the species in a world with ever-changing demands, is shown by the evolution of the elaborate ritual of sexuality, with its hierarchy of biological, psychic and social supports. Equally persuasive is the obligatory connection of reproduction with sexuality in higher organisms: variation has become a *sine qua non* of survival.

* This fact can hardly be over-emphasised in its implications for medicine. Ample attention has been given to the importance of obvious hereditary abnormalities, but hardly enough to the biochemical and physical variation that falls within established criteria of the "normal".

Among bacteria, however, genetic recombination is connected only casually with reproduction, which is mainly vegetative. This may be a corollary of the immense size of bacterial populations, which ensures a trickle of variant organisms from even rare incidents of mutation and of genetic exchange. The size of these populations and the speed of their growth also compensate for an intensity of selection of apt variants — e.g., one survivor per billion organisms — that would soon extinguish larger, more complex and less numerous species.

In addition, sexuality involving cellular conjugation is only one of a number of mechanisms for genetic exchange that have been found to occur among various bacteria. Sexuality is likely to be visualised in the first place as an extrapolation of human experience; however, in more distant organisms, obviously less of the total ritual will be recognised and the definition of sexuality must become more abstract. We may define sexuality by its genetic effect, namely the union of two substantially complete gametes or sets of genes (genomes) from different individuals. Even this abstract definition has run into difficulties — for example, half the spermatozoa of man are already relatively deficient, with respect to the genes of the X chromosome — and the classification must be somewhat arbitrary. As the reader will have sensed from this line of argument, the other forms of genetic transfer found in bacteria differ from sexuality by embracing only a fragment of the genome. Such a fragment can be thought of as a chromosome fragment, or as a DNA molecule *, according to one's scientific standpoint. The transfer of a genetic fragment is called transduction; this may be done by a bacterial virus; alternatively, DNA itself, as a purified chemical entity, can sometimes be taken up by bacterial cells with much the same effect.

Experimentally, microscopy might be the most direct method for detecting the interactions of living bacteria just as morphological cytology has exposed the elements of fertilisation and gamete formation in higher organisms. However, this aim has been thwarted in the past by the smallness of bacteria and the delicacy of their nuclear structures, and the unpredictable rarity of pertinent events. Instead, our information comes mainly from genetic and chemical experiments and has only recently been corroborated by direct microscopy. Bizarre forms are not unusual in bacterial cultures and some morphologists have attributed them to sexual conjugation. However, by current evidence "cyclogeny" has little or nothing to do with bacterial sexuality and is more often a sign of a defect in the cell wall, as for example in the L forms of various bacteria.

Bacterial genetics

Research in bacterial genetics now relies on the recognition and manipulation of precise markers, simple hereditary differences which can be followed from one generation to another. A marker should be a clear-cut, easily symbolised alternative e.g., Lac^+ vs. Lac^-, which stands for the ability or inability to utilise lactose on the part of strains of *Escherichia coli.* The smallness and simplicity of bacteria give relatively little scope for the recognition of gross abnormalities in structure, as have played an important role in earlier genetic work with Drosophila, laboratory mammals and man. In-

* DNA = *d*eoxyribo*n*ucleic *a*cid. Modern genetic research is unintelligible without insight into the chemistry of this class of macromolecules; see references at the end of this article.

stead, we must rely for the most part on biochemical features, most often and by preference the presence or absence of a specific enzyme which can be detected in a sizeable clone of bacteria derived from a single cell. Once the necessary methods had been developed for the easy recognition of biochemical differences among bacteria, this necessity became an outstanding virtue in so far as biochemical defects represent the most immediate representation of the physiological action of genes. Anatomical defects generally are believed to have a similar basis in their development but most have eluded the detailed analysis needed to specify the primary effect of the mutant gene.

The conception that genes worked through the control of metabolic steps was foreshadowed in the work of GARROD on "Inborn Errors of Metabolism" in man. It remained for BEADLE and TATUM to reduce this conception to a precise experimental program with Neurospora, whereby genetic mutations, calculatedly produced in the laboratory, were detected by their effect on the nutritional requirements of strains of the mould. This work of course added new insight to the connection of nutrition and biochemistry, growth factors being just those metabolites for which the organism must depend on its diet, lacking the corresponding biosynthetic enzyme in its own repertoire. Accordingly we perceive that our own survival depends on the complementation of our genes by the genes of green plants whose enzymes outdo ours in the capacity to synthesise such compounds as thiamin, tryptophane, lysine and ascorbic acid, as well as in trapping the radiant energy of the sun.

Nutritional mutants are especially apt for specific selective procedures. For example, a few Try^+ (tryptophane-sufficient) cells are readily detected amongst a billion Try^- (tryptophane-dependent) cells, by plating the mixture on a minimal medium which lacks tryptophane and on which only Try^+ types can form colonies. This type of experimental selection with some elaboration has been the basis of most experiments on mutation and recombination in bacteria. Thus, sexual recombination in *E. coli* was first revealed by the occurrence of T^+M^+ cells (T = threonine, M = methionine) when T^-M^+ and T^+M^- cells had been grown together. Of course, it remained for further work to show that the evident recombination, $T^-M^+ \times T^+M^- \rightarrow T^+M^+$, was a consequence of cellular conjugation, and that it embraced a sufficient variety of markers to justify calling it sexual. The detailed exposition of this work would be beyond the scope of this article, but some features of sexuality in *E. coli* are too interesting to let pass without comment.

Sexuality in bacteria

Many, perhaps all, strains of *E. coli* are potentially fertile. But a necessary condition for fertility is that one strain carry a male-determinant particle, called F. This particle can be carried on the chromosome, behaving as a typical gene; alternatively, it can escape to the cytoplasm and multiply autonomously. In its cytoplasmic state, it is so readily transferred to other cells, probably by short-lived conjugations, that maleness spreads contagiously through a population of female cells. In this state, the F particle is, however, accessible to disinfection by acridine dyes. The ambivalence of F between chromosome and cytoplasm makes it an exemplar of the fundamental equivalence of viruses, plasmids and genes. F must determine two male-sexual attributes in the cell carrying it; the primary one of energising the

chromosome for its passage from the male to the female cell at conjugation, and the secondary one of modifying the cell surface so that female cells will be recognised, and conjugal bridges formed with them. The enzymes and substrates that must underly these reactions are still obscure; the sensitivity of the male surface to inactivation by periodic acid suggests the involvement of a carbohydrate component; a male surface antigen has also been detected by serological methods.

The only known genetic determinant of female receptivity is the absence of F; other determinants which are difficult to study by genetic methods presumably distinguish F- female-fertile strains from the many *E. coli* strains that have so far eluded crossing. Recently, F has been transferred to other species of enteric bacteria, so that *E. coli, Salmonellas, Shigellas* and even *Serratias* can be crossed with one another and among themselves. Only casual efforts have been made to hybridise *E. coli* with more distant species. A mating system analogous to that of *E. coli* has also been described in *Pseudomonas*. Other bacteria have been relatively little studied for sexuality by genetic methods: Actinomycete species have given the most promising results but their interpretation remains controversial.

The course of events during conjugation in *E. coli* has been clarified by the kinetic experiments of JACOB, WOLLMAN und HAYES. Within a few minutes after the cells are mixed, bridges are formed to make heterosexual pairs, $F^+ \cdot F^-$, and a male chromosome moves progressively into the female cell. This transfer starts at a definite point on the chromosome and proceeds over an interval of about 100 minutes until the entire chromosome, viz., all the genetic markers, have been transferred. The last marker to be transferred, when this is resident on the chromosome, is the male-determinant F itself *. Some contradictions of earlier genetic data were resolved when it became clear that the mating pairs (Fig.) might be prematurely broken, either accidentally, or deliberately for kinetic experiments, by intense agitation of the culture so that fertilization might be incomplete and proximal markers transferred more often than terminal ones. By preliminary labelling experiments, the chromosome transfer could also be translated into chemical units, that is, about 1,000 nucleotide pairs of DNA are trans-

Fig.: Photograph of mating E. coli (Giemsa stain for nuclei after HCL hydrolysis).

* It is difficult to think that the chromosome is pushed by the F particle. We might rather think that the F particle adheres to the wall of the male cell, perhaps near the point where the male-surface substance has been produced under its influence.

ferred per second; the total DNA content of the *E. coli* nucleus is about 5 million nucleotide pairs. Although in general it has not been possible to reintroduce chemically purified DNA into the genetic system of *E. coli* *, the process of progressive transfer allows some biological control over particular segments of special interest. This is reinforced by the opportunity for studying infrequent recombinants, which allows the resolution of adjacent nucleotide pairs. Studies along these lines have supported the now general conception of the gene as a linear ensemble of nucleotide units, gene specificity being the sequence of particular nucleotide (of adenine, thymine, guanine and cytosine respectively); they are also being exploited in several laboratories for the assault on the ultimate code, the exact correspondence between the array of nucleotides in the DNA and the amino acid units in the protein which is specified by that DNA.

Transduction by bacteriophage

A second type of genetic transfer is transduction by bacteriophage, first discovered in *Salmonella,* and subsequently found in other bacteria including the sexually fertile strains of *E. coli.* This new phenomenon can be epitomised as a mistake on the part of the maturing virus particle whereby it incorporates a small fragment of bacterial chromosome in place of the customary viral nucleus **. Very little is known of the circumstances of this error. Perhaps the saucier question is why transduction is not universal; what marks the viral from the bacterial DNA: "what is a virus"?

Transduction by bacterial viruses is a valuable technical tool, complementing genetic analysis by sexual conjugation; for markers within the fragment, the effect is much the same.

The possibility of transduction of markers among mammalian cells is of some current interest, particularly as it may relate to the biology of tumour viruses.

Genetic transfer by isolated DNA molecules

A third mechanism of genetic transfer, by isolated DNA molecules, has had enormous impact on contemporary biological thought. This experiment, the outgrowth of GRIFFITH's observations on the pneumococcus transformation in 1928, is the proof that genes are DNA, and our most direct avenue to the actual characterisation of nucleotide sequences in relation to specific protein structures. The isolation of functional DNA also allows its manipulation. The polynucleotide chain is susceptible not only to analysis of its existing structure, but also to artificial modifications whose effects can then be assessed after reintroduction of the molecule into the genetic system of a suitable acceptor cell.

The uptake of functional DNA by homologous bacteria is still accepted as a beneficent miracle by the biological investigators who gratefully exploit the event. Certainly it would hardly have been predicted, every alternative explanation of the early experiments was preferred, and we still lack a significant appreciation of the mechanism by which it is accomplished. The recipe for successful transfer smacks of alchemy: an empirically discovered

* An exciting exception, the uptake of DNA isolated from bacteria phage as reported by KAISER and HOGNESS is pregnant with new possibilities.

** The transducing particle is thus a lamb in wolf's clothing.

routine, which is different for each species, must be followed in order to "sensitise" the acceptor bacteria, and they remain competent for a relatively short time only. At present, three bacterial species dominate work in DNA transfer: the pneumococcus, *Hemophilus influenzae,* and a particular strain of *Bacillus subtilis.* In the author's opinion, the last is by far the most convenient of the three, the other two bacteria being rather finicky in culture and balky at yielding clear-cut nutritional markers. In *B. subtilis,* by dint of a few years more work in several laboratories, we may hope for an accumulation of background data approaching that available in *E. coli,* and studies on a broad front should advance accordingly.

While the preparation of the bacteria in a state of optimum competence requires a special routine, the other features of DNA transfer are experimentally as simple as are the corresponding experiments on genetic transfer by conjugation or by phage-mediated transduction. For example, DNA is prepared, more or less crudely, by the extraction of the wild-type Try^+ strain with lysozyme and deoxycholate. This DNA preparation is then added to a prepared culture of a marker strain, Try^-, and incubated with it for a few minutes. Unreacted DNA can then be destroyed by the addition of deoxyribonuclease. Genetic transfers, represented by new Try^+ descendants of the treated Try^- bacteria, are counted by plating the cultures on minimal medium.

In contrast to sexual conjugation in *E. coli,* only one or a few markers can be transferred in any one event, evidently because the chemical unit in the DNA is a molecule whose weight is only about 10 million*. However, by special search, small clusters of markers have been found to be linked on single fragments even in the transductional systems; e.g., the markers Try^+ and His^+ (His = histidine) are closely linked in *B. subtilis.* If we knew only these systems we might have had to conclude that there was no organised genome, and to deny the homology of bacterial genetics with that of other forms. But this is belied by our findings with *E. coli* and *Salmonella* where the conjugal process gives unmistakeable evidence of a single, well organised chromosome, while phage mediated transduction involves the substitution of small fragments into it. Thus, while the final convergence of chemistry and genetics may stem from studies on DNA transfer, the study of sexuality has furnished an indispensable bridge to the precedent traditions of genetic study coordinating our picture of terrestrial life into a gratifying unity**.

REFERENCES

LEDERBERG J. (1959): A view of genetics, Les Prix Nobel en 1958; also published in *Science* ***131,*** 269 (1960).

WOLLMANN E. and JACOB F. (1959): La Sexualité des Bactéries, Masson et Cie, Paris.

Symposium on Microbial Genetics, X, Society for General Microbiology, 1960, Cambridge University Press.

For the biochemistry of DNA, see:

KORNBERG A. (1960): Biologic synthesis of deoxyribonucleic acid, Les Prix Nobel en 1959; also published in *Science* ***131,*** 1503 (1960).

* Whether this molecule is a natural unit of organisation of the DNA *in vivo* or an artifact of its preparation is an important issue in current debate.

** In this brief review, it has not been feasible to do justice to the complexities of many of the issues, nor to the individual contributions of many workers in a field that has attracted high talents from several nations. More complete discussions of recent work and bibliographic references are available.