

Lecture #44 - Bacterial Genetics: Sexual Reproduction  
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Studies on bacterial mutation, particularly those concerning the effects of various chemical reagents on the production of new genetic forms in bacteria, have been of great value, and will continue to be in elucidating the structure of the genetic material. Nevertheless, there are serious limitations which are quite obvious in any genetic analysis which is confined to studying those changes which arise in the course of purely vegetative reproduction. If we are to conduct any experiments on the interaction of different genetic components, we must have some mechanism available to us for recombining genetic factors from more than one kind of cell.

Until about a dozen years ago this was not considered possible. Many investigators had looked for sexual processes in bacteria, using principally morphological analysis, searching through the microscope for evidence of unusual forms that might betray the fusion of dissimilar cells. The futility of these experiments is demonstrated, after the fact, by the calculation that in early experiments on recombination in *Escherichia coli* the interesting events occur so as to involve only about one per million of the bacteria in any given culture. In this situation there is an evident use for the principles of selective isolation of specific genotypes, as has already been applied for the measurement and demonstration of occurrence of genetic changes in the course of vegetative reproduction. Among the more useful markers for studies of this sort are the nutritional defects such as have been discovered and elaborated by Beadle and Tatum in *Neurospora*. Just as in *Neurospora*, genetic changes arising in *Escherichia coli*, either spontaneously or under the influence of various mutagens, are capable of resulting in defects in a biosynthetic sequence and thereby in establishing a requirement for a specific growth factor which must be furnished in the medium if the bacteria are to be capable of continued

growth. Using distinct blocks in different biosynthetic chains one can easily set up an advantageous system for the detection of genetic interaction, as I can illustrate in the following way. Let us suppose that starting from our wild-type strain, and, to speak the facts here we might refer to strain K12 of *Escherichia coli*, that we start out from the original wild-type, whose nutritional sufficiency can be indicated by the symbols  $M^+ T^+$ , and from this obtain two distinct mutants -- one of these a mutant requiring say methionine, which we will symbolize  $M^- T^+$ , another requiring an alternative amino acid, let us say threonine, we will symbolize  $M^+ T^-$ . These are two distinct auxotrophic, nutritionally dependent, organisms, and neither of them by itself will be capable of producing any colonies when cultures are plated on basal minimal media which contain the general carbon and nitrogen sources but are deprived of the specific supplements needed by these strains.

Therefore, if in a mixed population containing some  $M^- T^+$  cells and other  $M^+ T^-$  cells, there should be any recombinational events, these can be detected with great efficiency by the occurrence of a new form which will resemble the wild-type, and is therefore called a prototroph, and which is capable of growing on the synthetic medium. Since these will be the only cells capable of forming colonies on agar plates, even though they be innoculated with many millions of cells of either parent, we have indeed an efficient mechanism for the original detection of recombinational events. This was indeed the case when experiments were done with the K12 strain beginning about 1946.

The early experiments involved a mating type,  $F^+$ , to which I will refer a little bit later, and did give a very low frequency of recombination. Subsequently when work of this kind was continued, in my own and in many other laboratories, new strains dis-

playing very much higher rates of recombination have been discovered, and these strains which had a high frequency of recombination are therefore called Hfr strains. Continuing the analysis of sexual reproduction in bacteria by means of these strains, Wollman and Jacob have established in somewhat more detail some aspects of the system of fertilization. First, we consider that in microscopic examination of Hfr by F<sup>-</sup> crosses it has been possible to demonstrate the occurrence of conjugal pairs of cells where a male and a female cell having made a random contact with one another, a conjugation bridge is established between them. And I'll take the opportunity of displaying a photograph taken through the microscope of just such an event. In kinetic experiments where male and female cells were mixed and then subjected at various times to the great shearing forces which operate in a Waring blender, so as to separate what these Frenchmen call the "happy couples", Wollman and Jacob were able to show that there was a scission of the bridge separating the two cells, without disturbing their viability. And they showed furthermore that there was not an immediate transfer of a block of genetic material of the entire nucleus from one cell to another, but rather a progressive transfer of different genetic elements.

To signalize this progressive transfer which requires about 100 minutes for completion, we conclude that there is a substantial unwinding of the DNA so that it is represented no longer by a coherent blob of material as we see it in the usual stain preparations, and that one end of the DNA string is preferentially transmitted first, followed by the remainder of the DNA, until finally all, or almost all, of it is transferred. There is thus a linear progression of the various markers which are represented by the segments of DNA which are arranged in a linear sequence down this chromosome, from which Jacob and Wollman have succeeded in making the most meticulous maps of the genetic material that have been made available.

Expressed in somewhat different terms, the rate of transfer of the DNA corresponds to approximately one thousand nucleotide pairs, or to coin a new unit, n'its, per second, which is about half the extent of a typical gene -- a gene being here defined as one of the cistrons responsible for the production of a characteristic protein. By the way, Levinthal's analysis of the genetic control of the enzyme alkaline phosphatase, using procedures of this sort, and applying them to a variety of mutants in which this enzyme was defective, verified that there was approximately the four to one coding ratio, which is demanded by the elementary considerations of correspondence between nucleotides and amino acids, as other speakers have summarized.

After the genetic material has been transferred, and perhaps beginning before the transfer is complete, there must be some process of synapsis, of orientation of the newly incoming material with some of the DNA of a pre-existent nucleus. And following this, by mechanisms which are still quite imperfectly understood, an exchange between the two -- this resulting in a recombinant which may have some markers from the female parent which it has retained, some markers from the male parent, whose DNA has been transmitted into the female cell. It is by no means certain at the present time whether this recombination of the parts of the chromosome represents an actual physical breakage and reunion as envisaged in some of the canonical models of crossingover in higher organisms, or whether there is a mechanism of choice of alternative templates, that is a copy-choice, during the replication of daughter DNA, using as alternative models either the one or the other parental material.

As a consequence of all these processes, first the initial injection of the DNA during fertilization, its synapsis with the genetic material of the female parent, and the integration or crossingover involving information from the two DNA components,

we have the formation of the recombinants which are in the first instance detected by the selective procedure that was outlined before. However, this represents a cross in which only two markers are involved, and this would not give us a great deal of detailed information. Therefore, typically in crosses involving *Escherichia coli* we use six, eight, or even a dozen differential markers, scoring different biochemical, physiological, drug-resistance characteristics, and I have here on the light box a plate which illustrates for more or less typical recombinants, and how we score them. On the first line we have a lactose-negative clone, which on this medium scores as a colorless, or very lightly colored colony. This has been brushed across a line of a loopful of streptomycin which was placed on the plate before these streaks were made. And the streptomycin is in the vertical line, represented by this pencil. The first type, which might be one parental form, was a lactose-negative streptomycin-sensitive type. Another parental form might have been a lactose-positive streptomycin-resistant form, and then in addition to these two parents, two recombinants which might issue from such a cross would be a lactose-positive streptomycin-sensitive, and a lactose-negative streptomycin-resistant.

This type of marker can be extended by the use of other sugars for which we have fermentation mutants, by the use of other antibiotics for which resistance may develop by quite independent mutations, by the use of bacteriophages for which again we can develop resistant mutants, and in other instances by markers such as specific antigenic quality, ability to move in semi-viscose media, and so forth.

We now have to consider the initial basis of sexual differentiation in this species. I've referred loosely in the diagram to a male and a female type, and we might add that these designations are partly, although not entirely, arbitrary, and are based

on the fact that the male cell contributes a migratory gamete, whereas the female cell remains entirely sessile. Having contributed a migratory gamete, the male and female in the ordinary course of events, or in a blender experiment such as Jacob and Wollman have done, can be separated and will continue to propagate. The male cell, however, has suffered no genetic alterations, it has no new genetic information as a result of this experience, whereas the female cell will produce a clone which will be a mixture of unaltered markers that it originally had, representing those nuclei that had not been fertilized by this event, and will also segregate a number of recombinants resulting from the interaction of its newly received segment of DNA with the DNA of the existing form.

Now, what then determines the sexual distinction between these two types?

We first have to mention that the original wild-type strain was self-compatible, and for some time it was not realized that there was a differentiation into two mating types. However, in subsequent work mutant strains have been derived which have apparently lost the capacity to function as male, and these we call female strains, since this is the only function that they can entertain in the mating process. For reasons which will soon become apparent, we also call these female strains  $F^-$ . The typical male strain, in the sense that this was the type of the original culture, we call  $F^+$ , and these are our first examples of female and male strains, respectively.

Now in many organisms sexual differentiation is determined either by an entire chromosome, as is true, for example, in man, or by the balance between one chromosome and the rest, as is true in *Drosophila*, or, as is true in many fungi, by a single segregating gene. And we might expect, *ab initio*, that the same would pertain for this microorganism. It was therefore quite startling to discover that sex, or rather the determination of male sexuality in *Escherichia coli* was an infectious phenomenon.

That is to say, the introduction of just one male  $F^+$  cell into a population of  $F^-$  female cells, would rapidly result in the spread of the male character to many and eventually to all of the female cells in that culture. And this male character, newly acquired, was hereditarily stable, in the same sense as any other marker; all, or virtually all, of the progeny of the newly converted  $F^+$  cells would continue to be male.

We are dealing here then with the transmission of a factor which is highly contagious and which, from the dynamics of the spread through the population, must be capable of multiplying at a rate at least twice that of the typical cell. However, all attempts to isolate a cell-free virus particle, which one might have postulated in view of the high infectivity of the male character, have failed. And we are therefore constrained to conclude that while there is an extra chromosomal factor which distinguishes an  $F^-$  from an  $F^+$  cell, we can symbolize the genome of an  $F^-$  cell by a single unencumbered line. This represents one of the nuclei stretched out. Although not stretched out to its full extent, since a consideration of the number of n'its in a whole bacterium would tell you that there would be something like 22  $\mu$  in total length of the DNA, and that would be of the order of 5 to 10 times the length of the bacterium. So that we can see the important role of close packing of the DNA strand in maintaining a properly functioning organism.

However, we do represent the normal genome of an  $F^-$  cell as an unencumbered line, symbolically, so. Then the  $F^+$  cell must have something else, and by a variety of lines of evidence, chief among which is the high infectivity and the high reproductivity of this particle, we conclude that we are dealing with an extra chromosomal  $F$  particle. This particle is evidently transmitted from male to female cells when they are planted into the same culture medium by means of a transient mating similar to that which operates

in the transfer of a chromosome. However, these matings which involve the transfer of only an F particle are evidently much more unstable and do occur rather more frequently than those involving chromosome transfer, and therefore account for the rapid spread of the F factor among the female cells in a population, far exceeding the rate at which chromosomes as a whole are transmitted.

Another feature which distinguishes the F particle from the chromosomal factors is the possibility of eliminating it differentially by means of certain dyes, for example, acridine orange. The exposure of an  $F^+$  cell to these dyes results in its reversion, back to the  $F^-$  state, apparently owing to the differential inhibition of reproduction of the F particle. At the same time the other genes in the bacterium which have been exposed to the dye are apparently unaffected. The F particle confers several interesting attributes on the cell which it inhabits. In the first place, it must modify the cell wall of the male cell so that it is capable of recognizing, (with) and reacting with, female cells with which it may come into contact. It must then form a bridge between the two cells, and finally must confer motility on the chromosome so as to enable it to be transferred from the male to the female cell, as previously described. That it may do this by first attaching, at least temporarily, to the chromosome is indicated by the occurrence of a third mating type, designated Hfr. These mating types have been studied and the factor determining their maleness analyzed by the methods applicable to other markers. And it has been found that in these mating types we are dealing with a mating type determinant which is located on the chromosome itself. Quite characteristic of the Hfr types is the location of the F factor at the very end of the chromosome; consequently it is the very last marker to be transmitted in the course of a typical mating -- this, in distinction to the transfer of the extra chromo-



somal factors in an  $F^+$  mating type, which are easily transmitted even in the absence of any chromosomal transfer.

How do we know that we are dealing with an F factor, as the determinant of maleness in an Hfr strain? This assertion is based on two findings. First, that all Hfr strains so far described, and a great many of them have, are derived from  $F^+$  cultures. Well,  $F^-$  cultures have been stably female and never give rise to any male strains, unless they are first contaminated by exposure to male cells, permitting the transfer of an F particle.

Secondly, some, in fact many, Hfr cultures are relatively unstable, and will now and again give rise to a reversion from the chromosomal state of the F particle to an extra chromosomal state, at the same time losing the very high fertility which is characteristic of a male strain. And finally, while the male character of an Hfr strain is not susceptible to inhibition by the drug acridine orange, the character of an  $F^+$  strain, as already mentioned, is. The reversibility, then, of the position and manifestation of the F particle is indicated by the occurrence of Hfr mutants which are not disinfectable by acridine orange, which will not transmit F particles contagiously, but by their capacity to revert back to  $F^+$  strains which will.

To the cycle of facultative participation as an extra chromosomal or as a chromosomal element, the term episomal cycle has been given, and F itself can be called an episome. The episomic character of F which is revealed by the reversible alteration between  $F^+$  states and Hfr states may also play some role in the normal low residual fertility of  $F^+$  by  $F^-$  crosses. What may be proposed here is that in order for an  $F^+$  cell to be fertile there must first be a transient association of an F particle, originally from the cytoplasm, with the chromosome. This association will then confer upon the chro-

mosome of that cell the same capacity for movement into an F<sup>-</sup> cell, as is stably represented in an Hfr clone. However, owing to the infrequency of the fertile cells in populations of F<sup>+</sup> clones it is difficult to make categorical statements about the genetic basis of those rare events.

F is but one of a number of particles having episomic characteristics that have been identified in *Escherichia coli*, K12. Another of extraordinary interest for some aspects of genetic analysis is a bacterial virus called lambda. When originally isolated, strain K12 of *Escherichia coli* showed no overt evidence of carrying a bacteriophage, and it was handled in the laboratory by many investigators for many years before it was realized that this was so. A necessary condition for the detection of this symbiotic virus was the natural unexpected occurrence of a sensitive mutant strain which had, for no evident reason, lost the bacteriophage. Having once been established and recognized as such, these lambda-sensitive, lambda not carrying mutants, act as indicators for a bacteriophage, which is now known to be produced with some regularity by cells of *E. coli*, strain K12. This association between a bacterium and a virus, which it can potentially produce as an infectious particle, is called lysogenicity. Lysogenicity has been recognized for many years as a property of a number of bacteria. In fact, its recognition dates almost to the original discovery of bacteriophages by d'Herelle. Having once established the necessary cultures to determine whether or not a given clone was or was not carrying a particle of lambda, it became of interest to study the way in which this trait itself, the trait of lysogenicity as contrasted with sensitivity, was inherited. And accordingly, crosses were set up, again using the basic designs that have already been mentioned, to determine the inheritance of this particular quality. I might add that the probable expectation for the results of such experiments was that lambda would be found to be an

extra chromosomal factor, in the hope, or the expectation, that we would here have a system where we have living in the cytoplasm of the cell some unusual symbiont which was capable of occasionally being activated to kill the cell and then be released.

It was, therefore, somewhat surprising when the results of mating experiments between lysogenic and sensitive strains showed that this attribute also was inherited as a chromosomal marker. Not only could lambda be assigned a definite place on the chromosome, but it was found to be clearly linked to another marker, which is responsible for the fermentation of galactose. And to the locus for the positioning of lambda on the chromosome the symbol Lp was given. A definite demonstration of the chromosomal nature of lambda as it exists in the lysogenic cell comes from the isolation of certain clones which are non-disjunctive for this chromosome which are heterozygous for these traits.

These exceptional heterozygotes can be symbolized by the formula Gal<sup>+</sup> Lp<sup>+</sup> representing one chromosome, Gal<sup>-</sup> Lp<sup>S</sup> representing the other. These diploids are susceptible to fairly frequent segregation, in the course of which one can observe the close linkage between the Gal<sup>+</sup> marker and the capacity to produce bacteriophage which we call lysogenicity, and the Gal<sup>-</sup> marker and the sensitivity to the same bacteriophage as is produced by the heterozygote itself, and by the other class of segregants.

The completion of the episomic cycle for lambda is only manifest when this chromosomal factor, Lp, undergoes a stage of maturation to produce the intact virus, before which it goes through a cycle of vegetative multiplication in the cytoplasm. This event takes place quite frequently when cells carrying the Lp<sup>+</sup> gene are subject to ultraviolet light, as was shown by Lwoff. We do not know in any detail the events which transpire so as to result in the release of the genic factor into the cytoplasm, but, after an interval following ultraviolet treatment, there appear in the cytoplasm of lysogenic

cells a number of particles of the virus. This, however, is a lethal complex, and the cell must lyse and liberate intact free virus particles. As we will have occasion to analyze in somewhat further detail, these free particles are capable of entering other cells and there to follow one of two alternative pathways -- either to continue their multiplication as a parasite, or as a virus lambda, or to reenter the chromosome and thereby give again the lysogenic status. The fact that two of the most important elements of E. coli have proven to be episomic has naturally raised questions as to the generality of this phenomenon, of genic factors being capable under some circumstances of entering the cytoplasm and thereby mimicking cytoplasmic factors as are known in other organisms.