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Lecture #45 - Bacterial Genetics: Genetic Transduction
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In the previous lecture we discussed some aspects of the sexual mechanisms in Escherichia coli. Although necessarily any extrapolation of the concept of sexuality to simpler microorganisms is bound to be somewhat arbitrary, we must admit that in its main features the process of genetic recombination that was described involving the mediation of the F factor does bear at least reminiscent resemblances to the fertilization processes in higher organisms. The main feature that we would stress here is the participation of intact cells as the agents which come together in the process of recombination and also the integrity of all, or almost all, of an entire genome as the unit of transfer.

Only a few bacteria have been extensively studied for the detection of recombination processes, and, so far, Escherichia coli and its relatives among the enteric bacteria and some filamentous bacteria, the actinomycetes, are the only ones in which we have strong evidence for the existence of sexual processes. This is however more testimony of the limited extent to which bacteria have been subjected to experimental analysis than to the likely restriction of interesting genetic phenomena to a few forms. Further studies have been made, some of them in an attempt to widen the range of organisms in which sexuality could be demonstrated, and, in the course of some of these, other phenomena have been described which are still aspects of genetic recombination, but owing to their greater simplicity, have to be given a different category. We are concerned here now with processes of fragmentary genetic exchange which are subsumed under the heading of "Genetic Transductions".

One of the systems which has given rise to a large volume of experimentation is one in which bacteriophage is intimately involved, and which concerns a reasonably close relative of Escherichia coli, the species Salmonella typhimurium. This is the mouse typhoid organism, and is an important agent in food poisoning disease in man. The group Salmonella is moderately closely related to Escherichia coli in its general features, which is one characteristic that recommends the group for experimental physiological and genetic study, since, like Escherichia coli, these bacteria can be grown in simple media, and are subject to many of the same physiological tests which have already been developed for E. coli.

In the course of attempts to determine whether or not strains of Salmonella typhimurium were subject to sexual recombination along the lines described previously, a rather erratic pattern of genetic exchange was found. It was discovered by Zinder and myself that certain combinations of cultures when mixed together in nutrient medium would generate new recombinant forms, somewhat along the lines previously discussed. However, this was true only of certain combinations of strains, and it was then eventually discovered that in contrast to the necessary participation of entire cells, which characterizes the sexual process in E. coli, that, in Salmonella, from mixed cultures it was possible to obtain cell-free filtrates that had the same activity. That is to say, from a mixture of strains, one of them by analogy with our previous demonstration, $M^+ T^-$, and another $M^- T^+$, it was possible to obtain what we call a filtrable agent that had M^+ activity. That is to say, this cell-free filtrate, from which the cells of the original culture had been freed by sedimenting them in the centrifuge and then filtering the culture, and was also subject to such additional precautions as heating the filtrates until no bacteria could have survived, these cell-free preparations, nevertheless, had in

them a factor which was capable of reacting with a suitable indicator strain to transfer the M^+ activity. And as was given in the previous design, this is demonstrated experimentally by plating a mixture of the filtrate, which of course has no viable elements in it, and of the viable cells which are methionine-less, which would be unable to grow in minimal medium, and from that mixture obtaining a yield of these recombinant prototrophs.

The chief feature that distinguishes this system from sexuality is the participation of an element, which owing to its capacity to pass through filters which will retain bacteria, must be smaller than the bacteria in size. And it then became necessary to spend some effort to determine the nature of this filtrable agent. It was rather peculiar that attempts to isolate this agent from cultures of M^+ T^- alone were unsuccessful, although this culture must have been involved in donating the necessary genetic element, the M^+ factor, to the filtrate. It was eventually found that the filtrable activity itself could be used to propagate additional M^+ activity. That is to say, a drop of a filtrate once made from this mixture could be added to fresh M^+ cells and give rise, again, to a new crop with great increase of M^+ activity. And this could be passed in series.

So it is now apparent that in fact we have two agents present, or at least one agent with two manifestations. First, that agent which was capable of transferring the M^+ activity, and second the agent that was capable of evoking the first agent from the M^+ bacteria. Eventually it was discovered that this second agent, the provocative agent, was a bacteriophage, and this bacteriophage was one for which the second strain was lysogenic. That is to say, cells of the M^- strain were carrying a bacteriophage which we have since labeled P22, phage number 22, and phage 22 when grown on an M^+ strain yields particles that have the capacity to transmit M^+ activity to a suitable indicator. Now it turns out that in our original system we used as an indicator the same

strain from which the P22 had been derived. However, once it was recognized that phage 22 was the important element in provoking the transfer of genetic activity, the routine procedure for conducting these experiments was simply to add one or a few particles of a stock suspension of P22, add those to any of a variety of genetic types, and then from those filtrates to obtain new preparations that would have a genetic activity characteristic of the type of bacteria on which that phage had grown.

It would then be possible to use those filtrates having these activities on a variety of different indicator strains, including this original M^- strain, but also including any of a large number of other Salmonella types. And in fact, it was subsequently shown to be possible to transfer genetic factors almost universally to even other species in the Salmonella group.

Well, what does a bacteriophage now do to the cells of a suitable susceptible strain in order to provoke the development of this activity? The answer to this question depended on further studies of the factors that were transmitting the genes themselves. That is to say, although at first we knew that it required the bacteriophage to evoke the genetic activity in these filtrates, we did not until further studies, know the nature of the agent that was present in those filtrates. However, the simplest hypothesis that one could formulate was that the bacteriophage itself had acquired the capacity to transmit genetic factors from one strain to another. That is to say, we could make as a preliminary proposal that the bacteriophage had acquired fragments of genetic material from the host on which it had been grown. This would imply that in the filtrates that had genetic activity we should find that activity associated with the bacteriophage itself.

This association has been verified by means of a number of tests. First the agent shows the same quantitative susceptibility to inactivation by heat as does the bacteriophage. That temperature and time treatment which will inactivate 90% of the bacteriophage particles as measured by their activity as a virus, also inactivates 90% of the transductional, that is to say, the genetic transferring capacity of the bacteriophage suspension. Second, the genetic particles and the virus particles have the same susceptibility to specific antiserum, since one can obtain, by injecting the bacteriophage into rabbits, antibodies which specifically block the capacity of the bacteriophage to adsorb and at tach itself to susceptible cells. Thirdly, the attachment to susceptible cells occurs at the same rate for the genetic activity and for the virus activity of these suspensions. This is measured by adding known aliquots of the filtrates to an excess of bacteria, allowing this mixture to remain together for a period of time, and then removing the bacteria together with that fraction of the virus which had adsorbed to the bacteria. Then we would conclude from filtration experiments and from sedimentation experiments that the size and mass of the genetic activity was approximately the same as the bacteriophage activity.

From these measures we could conclude, with a fair degree of certainty, that the transductional activity in a filtrate was indeed associated with particles which had essentially the same exterior properties in every respect, chemical and physical, as the bacteriophage itself. However, owing to the very low frequency with which any given phage particle is likely to include a particular marker, we could not be absolutely sure from these experiments that the same particle which included a genetic fragment also included a virus genome. In fact, there is a strong suspicion, which has been much better substantiated in some other systems of transduction, that this is not

the case — that is to say, that those particles which are carrying a genetic fragment of the bacterium are at least defective in respect to the virus which is included in the same particle, so that to some extent we should consider that there has been an accidental replacement of a bacterial fragment in place of the virus genome which should occupy the interior of that phage particle.

We may now recapitulate the way in which a transduction experiment is done in Salmonella. We must first start with a suspension of bacteria which already contain those genes in which we are interested. And it is an important experimental fact, for the interpretation to which we give this phenomenon, that the range of activity displayed by a given bacteriophage filtrate is precisely the same as the genetic factors carried by strain on which the bacteriophage is grown, and independent of the ultimate origin of the bacteriophage. This can be shown by first growing a crop of phage 22 on a bacterium that might have the genotype for example $M^+ T^+ X^+ Y^- Z^-$. This crop of bacteriophage when tested on suitable indicators one at a time, these indicators being strains that lack M, lack T, lack X^+, lack Y^+, lack Z^+, on these indicators one can show that that filtrate will have activity for the factors which were present in the donor, in the host, and not for those factors which were absent. If this crop of phage, which I might call the first crop, is then put onto a new bacterial strain, let's say one which is $M^+ T^- X^+ Y^+ Z^-$, we then find that that crop of phage has a new genetic activity, namely it has now lost the activity for the T factor and has gained the activity for the Y factor, precisely in correspondence with the new genetic factors present in those bacteria used as the host. The bacteriophage is therefore an essentially passive element in respect to the genetic content of the fragments that it carries.

Having obtained this crop of bacteriophage from the bacterial culture that we wish to use as donor in a recombination experiment, we then remove the excess cells which may have survived the initial attack of the phage. And this is done most simply by a quick sedimentation in the centrifuge, and then heating the supernates which are almost free of bacteria to a temperature of 60° for 20 or 30 minutes, which will kill rapidly any of the remaining bacteria in the supernate and leave the phage intact. These supernates containing a fairly high titre of bacteriophage are then simply mixed with the susceptible indicator bacteria to which one wishes to demonstrate the transfer of the genetic markers in question. After a short period of incubation the bacteriophage particles attach to these recipient bacteria, as we will discuss in a later lecture, the DNA content of the bacteriophage is injected into the recipient bacterium, and, then provided the bacteria happens to survive these particular attacks, they then have a certain definite probability, in the present case however a rather low one, of acquiring one of the specific markers of the strains used as a donor. This is demonstrated, as was true in the original test experiments, by simply plating the treated bacteria on a selected medium which will suppress all of the original genotypes and permit the expression of bacteria which have been altered in respect to the gene that we are studying. For example, we study the transmission of the M^+ factor, by mixing phage grown on an M^+ strain with indicator bacteria of the M^+ type, and then plating that mixture on medium deficient in methionine which will suppress all of the M minuses and allow only the M^+ to come up. Now we can symbolize this general operation, since we wish to distinguish the transductive phenomenon from those of sexuality, in the following way. We simply write M^+, instead of cross or X, we write dash X M^-, and we will in this case have demonstrated getting out an M^+, and, since the vector in this case is phage P22, we may

write the reaction in this way.

We now have to consider the scope of genetic exchange, since although we have now demonstrated that the material element of transfer is something much less than the intact cell, in fact is incorporated in an element whose DNA content is about one one hundredth of that of the bacterium, we still have not yet said anything concerning the genetic scope of the activity in each phage particle. This can be done by pursuit of experiments similar to the one just described, in which we examine the coincidence of transfer of different markers. And for example, if we conduct an experiment and, now using the abbreviated notation, $M^+ T^+ X^+$ where these are three factors, for examples, methionine, threonine independence, and xylose fermentation, prepare the phage filtrate, put the filtrate on an indicator strain, which will be lacking the competence for each of the three functions indicated here, and then plating these mixtures on a variety of medium, first a medium that selects for M^+, then a medium that selects for T^+, and then a medium that selects for X^+. And then we examine those progeny which are M^+, after having purified them by picking the colonies that developed on the selected medium, and then restreaking them a few times to reestablish definitely pure clones, and we type them in respect to the other markers. And the typical result of such an experiment is that those bacteria that have received the M^+ factor and are now manifesting it are still of the original recipient type in respect to the other two markers. And similarly, those that are T^+ are still M^- and X^-, and those that are X^+ are still M^- and T^-.

From these results we conclude that, in general, each phage particle carries but a single marker from the donor, whose markers it is transducing. This is in contrast to the genetic consequences of sexual recombination, where we find large blocks of genes being transmitted in community from the Hfr cell into the F^- cell. However, more detailed studies have uncovered a number of specific examples where markers are

transduced together. From these results we conclude that there is a possibility of linked transduction and from a comparison of transduction by a bacteriophage P1, which is active in Escherichia coli, with the results of sexual recombination in Escherichia coli, we conclude that the average genetic content of each bacteriophage particle is approximately 1% of the total genotype of the donor strain. This is at least roughly concordant with the proportion of DNA which can be contained in a phage particle, with the DNA in a bacterial nucleus. As it happens, the linkage that can be demonstrated by co-transduction gives us a very powerful tool for studying the close linkage of biochemical markers which are physiologically related. Demerec and his co-workers at Cold Spring Harbor have extensively studied the relationships between markers involved in related steps of a biosynthetic sequence, for example, the synthesis of tryptophan which proceeds via anthranilic acid and indol as precursors of tryptophan. They have found that not only are those distinct mutants which are involved in various ways in introducing defects into the formation of one particular enzyme closely linked, in a cluster at a particular point on the bacterial chromosome, but also those mutants which are involved in the sequential steps in the biosynthesis of tryptophan are also closely linked to one another. And the same pattern has been found with respect to the biosynthetic patterns of histidine as well as of other substances. In fact, we may take it that as a general rule, to which there are however some exceptions, there will be this correspondence between biosynthetic and genetic association, to the point where we can assign a definite segment of the chromosome to a cluster of genes concerned with related functions.

Since this situation appears not to pertain to some higher organisms and does not apply in general for example even to Neurospora, we are not in position to assess the

exact significance of this correlation. However, it has been suggested that the adaptive value of this linkage of physiologically related genes must be in the regulatory functions which should function so as to turn an entire set of enzymes on and off in accord with the physiological requirements of the organism in different situations.

The study of transduction by bacteriophage has been greatly helped by the appearance of another type of transduction in which the frequency with which markers are carried is several orders of magnitude higher than it is in Salmonella. We find that the villain of the piece occurs again; we are dealing here once more with the bacteriophage, lambda. Now you may recall that we had evidence from bacterial crosses in which the markers lysogenicity and Gal were in coupling and both segregating, that these markers are closely linked on the bacterial chromosome. A second testimonial to this linkage is the fact that when bacteriophage lambda is harvested by the ultraviolet induction of the prophage in a galactose-positive lysogenic cell, that that lambda is found to have transducing activity for the Gal genes to which it is linked. Unlike the Salmonella situation where we find that any marker of the entire genome is susceptible of transudction by phage P22, so far we have not found any markers other than Gal, the ones which are closely linked to the prophage, to be capable of being transduced by this particular element.

The rate of transduction by lambda, originally grown from typical haploid lysogenic cells, is again quite small, and of the order of one per hundred thousand of the phage particles per marker. However, the progeny of the transduction from galactose-positive lysogenic to a galactose-negative strain had been found to exhibit an unusual structure, which is similar to heterozygosis but which involves only the Gal and perhaps the lambda segment. Because of the restricted scope of this duplication of genetic material we have called these unusual types heterogenotes. And the

simplest way to display the genetic behavior of these heterogenotes is to suppose that in addition to the unchanged chromosome of the recipient type, which we might indicate as having a Gal⁻ marker and some kind of prophage site, that there is an additional fragment which remains outside the chromosome although perhaps attached to it by some still not very well specified mode of attachment, and this fragment has the gene that had been carried over in the lambda segment. Now we know that the heterogenotes are capable of undergoing further changes equivalent to reduction as we see it in diploid forms, which permit the exchange of the galactose factor into the galactose minus form.

Of some importance is the fact that the lambda isolated from a heterogenote has a very high activity, to the point where nearly every lambda particle will transmit Gal activity. This is indicated by a simple experiment which I've diagrammed here, if one cross-brushes a suspension of lambda, (with) containing a relatively small number of particles, that lambda having come from a galactose positive heterogenote, against a suspension of galactose-negative bacteria, one can almost count the lambda particles by the number of galactose-positive colonies that appear in the zone of intersection. This is displayed further in the accompanying slide.

We have accordingly discussed the major mechanisms of genetic recombination as displayed in bacteria: heterozygosis and the sexual mechanisms of E. coli K12, a more complex form which is preceded by the concurrence of nuclei in common cytoplasm in filamentous fungi, previous lectures have discussed the direct role of DNA in the pneumococcus transformation, and we have just referred in the present lecture to the role of bacteriophage as a vector.