CONJUGATION, TRANSFORMATION, TRANSDUCTION & COMPLEMENTATION TEST IN BACTERIOPHAGE

Introduction

The understanding of linkage relationships between genes in bacteria and viruses was made possible by the discovery of genetic recombination in these organisms. Gene recombination, coupled with the short generation time of these organisms, the numerous conveniently recognized mutants that they possessed and the ease with which recombinant colonies could be scored among very large parental populations gave bacteria and viruses decided advantages for recombination studies over those possessed by “higher organisms”. Collected together in this lecture are the discussions of three major mechanisms by which bacterial donor genes are transferred into bacterial recipients, along with brief reviews of how these mechanisms can be used to determine linkage relationships.

Conjugation: In this process, genetic material transfer occurs between two bacterial cells directly or by a bridge like connection, the sex pilus. The gene transfer, sometimes, also occurs by means of cell-to-cell contact and is promoted by a sex factor (also called a conjugative plasmid), that can become incorporated into the bacterial donor chromosome. The entire bacterial chromosome may then be mobilized for transfer into the recipient, although not all of it is necessarily transferred.

Transformation: A term used for the transfer of genetic information into recipient cells in the form of exogenous (extracellular) DNA extracted from donor cells.

Transduction: This mode involves the transfer of donor genes into recipient bacteria by viruses that act as intermediary carriers.

Complementation test: This test is used for finding out mutation location in the same or different gene, based on F1 progeny.

CONJUGATION

In the line of gene transfer, secondly conjugation was discovered by Lederberg and Tatum, in Escherichia coli, approximately 20 years after discovery of transformation. This discovery hinged upon the appearance of prototrophs, e.g., T+L+B1+B+Ph+C+, from a mixed culture of two kinds of autotrophs, i.e., T-L-B1-B+Ph+C+ X T+L+B1+B-Ph-C-. Since these prototrophs only arose through mutual cell contact between the strains and it was unlikely that mutation of all

three markers could occur simultaneously at the observed frequencies, transformation and mutation as possible explanations for the conjugation experiment could reasonably be excluded.

Hayes then demonstrated that the transfer of genes or genetic material occurred only from one parental auxotrophic strain to the other, but not reciprocally. For example, recombinant zygotes produced by mating strains A and B consisted of cells from only one of the two strains (e.g., A) with a genetic, but not cellular, contribution of the other (B). Only strain A cells appeared to be incorporated entirely into zygotes and their death, caused by a premating exposure to the antibiotic streptomycin, led to the loss of recombinant classes. Exposure of the complementary mating strain B to streptomycin, on the other hand, did not eliminate recombinants, showing that B cells could participate in sexual conjugation and then die without in anyway diminishing the frequency of recombinants. Thus strain A acted as the “recipient” of zygotic material, while the function of strain B seemed to be confined to the role of “donor”. Once classified as either donor or recipient, a strain appeared to remain fairly consistent in its sexual behavior; recipients never became donors spontaneously, although donors occasionally became recipients.

During conjugation, a copy of the F+ plasmid passes into the F- cell, presumably through the sex pilus (a bridge like connection), converting the F- cell into F+. A copy of the plasmid also remains in the donor bacterium so this cell remains F+ also. However, bacterial conjugation is important to geneticists not because the F plasmid is transferred but because chromosomal genes can also be passed from donor cell to recipient. How does this come about? There are several possible ways, the first being simple that a small random piece of the donor cell’s chromosome is transferred along with the F plasmid. This is thought to account for the gene transfer phenomenon originally discovered by Lederberg and Tatum, but probably occurs fairly infrequently. More important in genetics are the gene transfer properties of two additional types of donor cells, called Hfr and F’ (F-prime). In Hfr cells, the F plasmid has become integrated into the E. coli chromosome, by a recombination event analogous to that responsible for integration of the A genome into the E. coli DNA molecule. The integrated form of the F plasmid can still direct conjugal transfer, but in this case as well as transferring itself it will also carry into the F cell at least a portion of the E. coli chromosome to which it is attached. Consequently, a mating involving an Hfr cell will virtually always bring about transfer of some E. coli genes.

F’ cells, the second type of donor cell regularly associated with the transfer of chromosomal genes, occasionally arise from Hfr cells when the integrated F plasmid excises from the chromosomal DNA. Normally this event results in an F+ cell, but sometimes excision of the F plasmid is not entirely accurate and a small

segment of the adjacent chromosomal DNA is also snipped out. This will lead to an F' plasmid that carries a small piece of chromosomal DNA, possibly including few genes. Conjugation involving the F cell will always result in the transfer of the plasmid associated chromosomal genes.

Conjugative Transfer in Other Species: A large number of bacterial species are able to conjugate in the same way as E. coli and in all other species the process is controlled by a plasmid analogous to the F plasmid. Fertility plasmids of this type are called self-transmissible, which means that they can set up conjugation and mobilize themselves into the recipient cell. Conjugation and mobilization are two distinct characteristics and not all plasmids are able to direct both. A few can set up only the conjugation contact between cells and not mobilize on their own, whereas others can mobilize, but only if they are co-exisiting in the cell with a second plasmid that can set up the initial contact. Other plasmids are totally non-fertile and can neither conjugate nor mobilize.

TRANSFORMATION

The first evidence of genetic recombination or exchange of hereditary material in bacteria was noted by Griffith in the transformation of harmless streptococci (then called pneumococci) into virulent ones. As fully demonstrated by Avery, MacLeod and McCarty in 1944, the transforming agent responsible for the observed genetic change turned out to be pure DNA that could be extracted from donor strains. Recent studies have shown that during the process of extraction, the donor DNA is broken into smaller transforming molecules or fragments which are about 1/200 of the total DNA. These transforming molecules usually contains on an average about 20,000 nucleotide base pairs (20 Kbp). Smaller DNA pieces can also be absorbed by the recipient cell, but a minimum length of about 450 base pairs seems to be essential for transformation to occur.

There are three general stages in the transformation process. The first stage is that of *competence*, or the ability of cells to take up DNA through changes in the cell wall. This involves the formation or activation of special DNA receptor proteins, the "competence factors" that can be induced by polypeptide in some species, or by special growth conditions in others. In *Streptococcus* (formerly *diplococcus) pneumoniae* that have become competent, one or more of an estimated 30 to 80 cell wall receptor sites are capable of binding double-stranded DNA from almost any source. On the other hand, *Haemophilus influenzae* bacteria possess a more limited number of four to eight receptors which primarily recognize duplex DNA carrying special 11-base pair "uptake site" sequences (5'-

AAGTGCGGTCA-3'). The fact that such uptake sites are especially common in *Haemophilus* DNA (about 600 such sequences are found in the genome) and relatively rare in other DNAs, generally restricts the *Haemophilus* transformation process to donor bacteria of its own species.

The second stage, *DNA binding and uptake,* involves direct interaction between the cell wall receptors and donor DNA. This sequence is at first reversible, but as more cell membrane proteins become involved, DNA attachment to the cell wall is considerably increased. In *Streptococcus* and *Bacillus subtilis,* the bound DNA double helix is nicked by endonucleases, broken into smaller pieces that average about 14 Kbp and is then attacked by an exonuclease to yield single-stranded molecules. In *Haemophilus,* bound DNA is generally taken into the cell as intact double helices.

The last major step begins with intracellular transport of the transforming DNA to the recipient chromosome in some protected form, either complexed with a specific DNA-binding protein or in small vesicles derived from the recipient cell surface, or both. During the culmination of this last stage, *integration*, a single strand of donor DNA is incorporated into the recipient chromosome by displacing a homologous section of one of the recipient DNA strands, which is then excised and degraded. The integrated donor strand than replicates forming a double helix, while the remaining unpaired recipient strand is excised.

Transformation thus seems to arise from some form of recombination mechanism which produces gene exchange similar to that produced by sexual recombination. Atleast some complementary base pairing between single-stranded DNA sections appears necessary in transformation. Thus, if "foreign" donor DNA is used which bears little or no homology with the recipient, successful transformation will not take place. Only in cases of transfection by viruses or transformation by plasmids, extracted non-homologous nucleic acids successfully replicate themselves in a recipient cell.

Linkage: The chain of 20,000 nucleotide pairs in the average transforming DNA molecule is certainly sufficiently long to contain within it more than one gene. For example, a transforming molecule carrying gene A may also carry gene B. If these genes are closely linked, there is a good likelihood that transformation at the A locus produced by a single DNA molecule would also produce transformation at the B locus (double transformation). If A and B are not linked within one transforming DNA molecule, the frequency of double transformation will depend upon the presence of two different transforming DNA molecules. In the latter case, double transformation is caused by two independent events, i.e., its frequency is the product of both probabilities. Thus closely linked genes will produce a much higher frequency of double transformants than those that are not

linked or only distantly linked, especially if low concentrations of transforming DNA are used. The first example of linkage between two genes in transformation was shown by Hotchkiss and Marmur for streptomycin-resistant and mannitol-utilizing strains in *Streptococcus*. Since then linkage relationships have been analyzed in *Haemophilus* and in *Bacillus subtilis.*

TRANSDUCTION

Transduction is the transmission of DNA from a donor cell to a recipient by incorporation of a fragment of donor DNA into a viral particle. This fragmentation and incorporation of donor DNA is a consequence of viral growth and the mechanisms of viral growth can affect both the kind and extent of transduction that takes place.

From the time of their discovery, bacteriophages, the viruses that infect bacteria, were long assumed to bear a simple parasitic relationship to their hosts in which phages can only replicate through bacterial destruction (lysis). However, beginning in the 1920s and continuing since then, work by Bordet, the Wollmans, Lwoff and others has shown that, for some bacteria and for some phages, this relationship is partly symbiotic one, i.e., a phage may be carried within a bacterium without causing immediate lysis. Such bacterial strains are called *lysogenic*, since they are never capable of being lysed through occasional proliferation of the viruses they carry. This is in contrast to *non-lysogenic* bacterial strains, which do not carry viruses within them and will therefore not lyse unless newly infected by phage.

The phages involved in lysogenic relationships are called *temperate*, while phages that infect and destroy bacteria but cannot lysogenize them are called *virulent, lytic or non-temperate*. The “inducing” trigger that produces lysis in lysogenic strains involves a change in the activity of the phage from a quiescent *prophage* state to a proliferative *vegetative* stage. Induction of the vegetative state and consequent lysis may be spontaneous, or can be produced artificially by small doses of ultraviolet (UV) light, X rays and chemicals such as nitrogen mustards. If large doses of UV are used, the few surviving bacterial cells may be “cured” of their prophages, becoming non-lysogenic.

Lysogeny is an advantage to bacterial cells, since the presence of the prophage enables them to withstand infection and prevent vegetative growth by virus particles of the same variety. An *E. coli* cell lysogenized with λ prophage, for example, can be placed into the medium containing free λ particles and will not lyse; that is λ genetic material injected into such bacterial cells by a λ particle will not multiply because of the specific immunity against λ vegetative growth

conferred by the prophage. On the other hand, non-lysogenic *E. coli* placed in the same medium will soon be destroyed by the virus. Some cells may nevertheless escape destruction under such conditions, but these are often found to have become lysogenic, i.e., they now bear the λ prophage and produce lysogenic colonies that are immune to further lysis by new infections of λ.
In general, the effect of lysogeny is therefore a periodic release of temperate phages whose vegetative growth, as in some virulent phages, causes fragmentation of bacterial host DNA. Such fragmentation thus offers the opportunity for transduction through incorporation of host DNA into these viruses and their transmission to recipient cells.

Generalized Transduction: The first example of transduction was discovered by Zinder and Lederberg in 1952 when they found that a particular temperate phage may act as a carrier for bacterial genes from many different parts of the genome, a process called *generalized transduction.* Their experiments began as a test to discover whether the genetic exchange previously demonstrated in *E.coli* by Lederberg and Tatum also existed in the mouse typhoid bacterium *Salmonella typhimurium*. For this purpose, they combined various strains of auxotrophs on a minimal medium and then looked for new prototrophic combinations; eg. Strains unable to synthesize phenylalanine and tryptophan might combine with strains unable to synthesize methionine and histidine to form wild-type prototrophs. They found that such prototrophs were indeed formed. Moreover, since these new combinations occurred only among mixtures between strains and did not occur within the auxotrophic strains themselves, it seemed evident that genetic exchange was responsible for their formation. This exchange, however, did not occur with the same frequency for each strain examined and some gave very few recombinants. A combination of strains LA22 and LA2 appeared to be the most “fertile” and the capacity for genetic exchange between these two strains extended to numerous additional characteristics such as the utilization of galactose, xylose, mannitol, and maltose and streptomycin resistance.

Specialized (Restricted) Transduction: In 1956, Morse and Lederberg, looking for transducing viruses in *E. coli*, found that λ phage could also serve this function but that its transducing activity was restricted to the galactose locus. For example, wild-type prototrophs used as donors in λ-mediated transduction could affect mutant recipients only by changing gal^- to gal^+, but never transduced other loci, such as thr, trp, lac, etc. This phenomenon, known as specialized or restricted transduction, has been mostly investigated in *E. coli*, although it has also been found in other bacteria, such as *Salmonella*. The technique using λ is not dissimilar from other methods of specialized transduction except that a

special medium is used for the detection of gal^+ transductants. The λ phages are harvested from the donor and then used to infect recipient bacteria which are plated on an “indicator” agar to which galactose has been added. Although both gal^- and gal^+ bacteria can grow on this medium, the gal^+ transduced colonies form darkly pigmented colonies, while the gal^- colonies are lighter colored.

The isolation of transduced gal^+ colonies by these methods showed important novelties. First, in contrast to generalized transduction, the gal^+ colonies used as donors had to be lysogenic for the prophage, which was then induced by irradiation. If they were not carrying the λ prophage but were instead directly infected and lysed with λ particles, the λ phages harvested from these bacteria had no transducing power. Second, in contrast to normal gal^+ colonies, the transduced gal^+ colonies were unstable, producing one about one gal^- cell for each thousand bacterial divisions. Third, those of the transductants that remained gal^+ were all lysogenic for λ, either able to produce λ upon induction, or immune to lysis by new infective λ particles. On the other hand, about 90% of the gal^-segregants formed by unstable transductants were no longer lysogenic for λ but appeared to have lost the prophage. Fourth, when the transductant gal^+ colonies were induced and the λ phage harvested, about one-half of the phage particles were now able to transduce gal^- bacteria, in contrast to the initial transduction efficiency of one out of a million. This phage harvested, with its increased transducing efficiency, is called an*Hft* (high-frequency transduction) lysate.

COMPLEMENTATION TEST

Apart from the gene transfer, one most important goal is to find out the gene location and the mutation if it exists. Most commonly used techniques in genetics are recombination test and complementation test, in which first one is used for finding out the distance between the genes and later one is used to find out, whether two mutations are on the same or different gene. Bacteriophage is frequently used as model to understand these mentioned tests. Benzer demonstrated the experiment for gene fine structure and location of mutations as complementation test which is also known as **cis-trans test**.

Bacteriophages or phages are viruses which infect bacteria. They are of two kinds with respect to their infective life style; virulent and temperate. The former, as the name suggests, always kill and lyse the infected cells. Examples of this class are the T-odd phages (T1, T3, etc.) and T-even phages (T2, T4, etc.). Phages are identified by what are called ‘plaques’. This is called the wild type or

r+ morphology and mutation in this, forms r type morphology. Wild types of T4/λ phage infect *E. coli* strain B and K12, whereas mutant infects only B strain.
Benzer utilized the T4 rII mutants in two ways; the first was to carry out complementation test to see how many genes define the rII (mutation loci) function. For this, he co-infected E. coli K12 (λ) with two mutant, search of which does not develop in the host and observed whether the cultures lysed or not.
If they lysed, he examined the type of progeny phage (rII or r+). With some pairs of mutants, the infected cultures lysed and all the progeny phages were of the rII type, showing complementation between the mutants. With some other pairs there was no lysis and therefore, the mutants belonged to the same complementation group.
In this way, he was able to group all the rII muants (over 2000) in two groups: A and B. All mutants in group A mapped at one locus and those in group B mapped at a locus very close. Therefore, the rII locus consists of two functional units, rIIA and rIIB, which Benzer called cistrons. The rII gene region with many individual mutations was accurately mapped using complementation test.

- If there is just **one gene**, then no two mutations will complement each other. When **two mutant phages coinfect the same cell**, no rII gene product (an enzyme or other protein) is produced by either mutant. So the plaque formation that was observed showed **no complementation**.
- If there are **two genes**, then mutations in different genes will complement each other when **two mutant phages coinfect the same cell**. Each mutant is deficient with respect to one product, but when combined they will provide all the products necessary. So the plaque formation was observed. This phenomena found is **complementation** instead of the recombination, because the progeny phages are the original mutants.

Moreover, co-infection of same mutant discovery aids many hidden phenomena of the genetic fine structure and helps in developing various techniques.

SUMMARY

Gene transfer is most fascinating phenomena in the biological studies. Technological development particularly in the field of molecular biology reveals many hidden sides of the genetic composition and organization. Many processes of gene transfer were well studied which includes conjugation, transformation and transduction along with the most useful complementation test using bacteriophage and their host bacteria.

DNA, a genetic material in all living entity also exists as a naked DNA in the environment as well as plasmid DNA inside the cell. Transfer of all these forms of DNA from one microorganism to another can be possible by direct entry, physical contact or virus. Naked DNA can directly enter inside the nearest competent cells through process called transformation. Study on *Streptococcus pneumoniae* and *Haemophilus influenzae* showed the binding of foreign DNA (~450 bp) to receptor and integration of it with chromosomal DNA.

Moreover, few of the bacterial species like *E coli* possess plasmid which transfers by direct physical contact through sex pilli. This process is known as conjugation. Due to the effect of the many environmental factors, transfer process didn't complete successfully and led to the mutation. So it is very important to locate the specific region where mutation occurs.

Many times bacterial plasmid transfers by integration with viral genome. Especially this integrated viral genome were studied using *Salmonella typhi* and *E. coli* which showed conversion of prototrophic bacterium to auxotrophic bacterium via process called generalized transduction. In some case, this process is restricted to specific location of the host genome like gal gene and produce high frequency transductants through specialized transduction.

Complementation test is the method which focus on the gene based mutation study and finds whether the mutation is in the same or different gene using bacteriophage T4 or λ.