**Transduction**

Transduction is the process by which cellular genes can be transferred from a donor to a recipient cell by a virus particle (phage-mediated transfer), the recipient being known as a transductant following transfer .

This process is normally highly specific for phage DNA. However, with some phages, errors can be made and fragments of bacterial DNA (produced by phage-mediated degradation of the host chromosome) are occasionally packaged by mistake leading to phage-like particles that contain a segment of bacterial genome . These transducing particles arecapable of infecting a recipient cell, since the information necessary for attachment and injection of DNA is carried by the proteins of the phage particle, irrespective of the nucleic acid it contains. The transduced segment of DNA will therefore be injected into the new host cell.

Not all bacteriophages are capable of carrying out transduction. The basic requirements of an effective transducing phage are that infection should result in an appropriate level of degradation of the chromosomal DNA to form suitably sized fragments at the right time for packaging and that the specificity of the packaging process should be comparatively low.

In some cases, the transduced DNA is a bacterial plasmid, in which case the injected DNA molecule is capable of being replicated and inherited. More commonly the DNA incorporated into the transducing particle is a fragment of chromosomal DNA which will be unable to replicate in the recipient cell. For it to be replicated and inherited, it must be incorporated into the recipient chromosome (by homologous recombination), as is the case with other mechanisms of gene transfer.

This process is known **as generalized transduction** since essentially any gene has an equal chance of being transduced.



**Specialized transduction**

some phages (temperate phages) are able to establish a state known as lysogeny, in which expression of phage genes and replication of the phage is repressed. In many cases the prophage is inserted into the bacterial DNA and replicates as part of the chromosome. When lysogeny breaks down and the phage enters the lytic cycle, it is excised from the chromosome by recombination between sequences at each end of the integrated prophage. If this recombination event happens in the wrong place, an adjacent region of bacterial DNA is incorporated into the phage DNA. All the progeny of this phage will then contain this bacterial gene which will therefore be transduced at a very high frequency (effectively 100 per cent per phage particle) once the transducing phage has been isolated. Since the DNA transferred is limited to a very small region of the chromosome, the phenomenon is known as specialized (or restricted) transduction.

This is very similar to the formation of F- plasmids referred to earlier . As with the F- plasmids, it is now much easier to add genes to DNA by creating recombinants *in vitro* .

Another phage that has been employed in a similar way is the phage Mu which has the advantage of inserting at multiple sites in the chromosome by a transposon-like mechanism. It is therefore much easier to create a wide range of specialized transducing phages with Mu which can be used both in genetic mapping and in mutagenesis.

When a normal temperate phage (that is, a nondefective phage) lysogenizes a cell and its DNA is converted to the prophage state, the cell is immune to further infection by the same type of phage. This acquisition of immunity can be considered a change in phenotype. However, other phenotypic alterations can often be detected in the lysogenized cell that are unrelated to phage immunity. Such a change, which is brought about through lysogenization by a normal temperate phage, is called phage conversion.

Two cases of phage conversion have been especially well studied. One involves a change in structure of a polysaccharide on the cell surface of *Salmonella anatum* on lysogenization with bacteriophage 815.The second involves the conversion of non toxin-producing strains of *Corynebacterium diphtheriae* (the bacterium that causes the disease diphtheria) to toxin-producing (pathogenic) strainsupon lysogenization with phage *f3* . In both of these situations, the genes encoding the necessary molecules are an integral part of the phage genome and hence are automatically (and exclusively) transferred upon infection by the phage and lysogenization.

Lysogeny probably carries a strong selective value for the host cell because it confers resistance to infection by viruses of the same type. Phage conversion may also be of considerable evolutionary significance because it results in efficient genetic alteration of host cells. Many bacteria isolated from nature are natural lysogens. It seems reasonable to conclude, therefore, that lysogeny is a common condition and may often be essential for survival

of the host in nature.



**Recombination**

**General (homologous) recombination**

A common feature of all the forms of gene transfer between bacteria, except for the transfer of plasmids (which can replicate independently), is the requirement for the transferred piece of DNA to be inserted into the recipient chromosome by breaking both DNA molecules, crossing them over and rejoining them. This process, known as recombination. There are several different forms of recombination, but the mechanisms that require the presence of homologous regions of DNA which must be highly similar but do not have to be identical are of specific interest in this context. It is therefore known as homologous recombination. Of the alternative forms of recombination, site-specific recombination is particularly important.

It should be noted that recombination mechanisms have other roles within the cell apart from the incorporation of foreign DNA. In particular, recombination mechanisms are involved with some types of DNA repair . These may actually be of more fundamental importance to the cell and may be the real reason why bacteria have evolved to contain several mechanisms for recombining DNA molecules.

Homologous recombination is divided into four stages: synapsis, strand transfer, repaira nd resolution.

(1) In synapsis, homologous duplexes are aligned.

(2) During strand transfer , a single DNA strand is transferred from one duplex to the other. The first strand transfer marks the initiation of recombination as it invades the homologous duplex and (if the recipient duplex is intact) displaces a resident strand. This process may generate a short region of hetero duplex DNA duplex DNA comprising strands from different parental molecules which may contain base mismatches reflecting sequence differences (different alleles) in the parental duplexes. If the recipient duplex is intact, the displaced resident strand is able to pair with the free strand of the initiating duplex. The two transferred strands cross each other, forming a structure termed a cross bridge, cross branch or Holliday junction.The site of the Holliday junction may move in relation to its original position by progressive strand exchange between duplexes. This is branch migration, and may increase or decrease the amount of hetero duplex DNA.

(3) Repair and resolution do not occur in a fixed order as this depends upon the recombining partners and the availability of appropriate enzymes. Repair

refers to three different processes. In the simplest case, the recombining duplexes are intact (i.e. there is no genetic information missing from either duplex) and repair involves religation of the broken strands*.* This is conservative recombination .The Holliday junction can then be resolved in either of two planes to generate one of two products a patch of heteroduplex

DNA in a non recombinant background, or a splice of heteroduplex DNA with recombination of flanking markers.

However, if genetic information is missing from either duplex( i.e. if there is a single-strand gap, or a break) DNA repair synthesis replaces the missing informationusing information from the homologous duplex as a template . Recombination including the synthesis of new DNA is non conservative recombination. In the extreme case where an entire chromosome segment is missing, resolution of the Holliday junction yields a replication fork which can duplicate the missing segment .

Molecular basis of homologous recombination***.*** In *E.* coli, proteins responsible for all the major stages of recombination - synapsis, strand exchange, branch migration and Holliday junction resolution have been identified . The remaining requirement or recombination, single stranded DNA, appears to be provided by a number of overlapping pathways.

The RecBCD pathwayis the major source of recombinogenic substrates in *E.* coli. The products of the genes recB, recC and recD combine to form a large complex, the RecBCD enzyme or exonuclease V,which has three activities: (1) ATP-dependent exonuclease activity; (2) ATP-enhanced endonuclease activity; (3) ATP-dependent helicase activity.

Recombination is stimulated by cis-acting sites termed chi (GCTGGTGG), which occur approximately one every **5** kbp in the *E.* coli genome and represent preferential cleavage sites for the endonuclease. RecBCD cuts the strand containing the chi sequence approximately f ive bases to the 3' side in an orientation dependent manner. Chi sites are thus recombination hotspots(q.v.). Blunt ended linear duplex DNA is the substrate for the helicase activity. The enzyme binds to the DNA and progressively unwinds it, producing paired single-stranded loops in its wake. These loops are occasionally cleaved by the endonuclease activity of the enzyme, and following cleavage further unwinding generates the single-stranded tail necessary for homologous recombination.

Although the RecBCD pathway is important for recombination, *rec*BC mutants retain up to 10% homologous recombination activity in, dicating the presence of other pathways for producing recombinogenic DNA. Additionally, almost full recombination proficiency can be restored to *rec*BdC mutants by mutations at other loci, notably *sbc*A, *sbc*B and *sbc*C. The analysis of mutant strains with the genotype *rec*BC *sbc*BC has facilitated the identification of further recombination genes. These have overlapping functions, and collectively define the RecF pathway.Another system, revealed b y studying *rec*BC *sbc*A mutants, involves upregulation of the ***recE*** gene

**Site-specific and non-homologous (illegitimate) recombination**

Does not require homology between recombining partners. The proteins mediating this process (site-specific recombinases) recognize short, specific DNA sequences in the donor and recipient molecules, and interaction between the proteins facilitates recombination. Homology often exists between the donor and recipient sites because the same recombinase protein binds to both recognition sites.