Transduction

- Transduction is the phage-mediated transfer of genetic material. The key step in transduction is the packaging of DNA into the phage heads during lytic growth of the phage.
- 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 (see Figure 1).
- These transducing particles are capable 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 that will not 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 (as opposed to specialized 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 percent 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 F0 plasmids referred to earlier. As with the F' plasmids, it is now much easier to add genes to l DNA by creating recombinants *in vitro* .
- Another phage that has been employed in a similar way is the phage 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

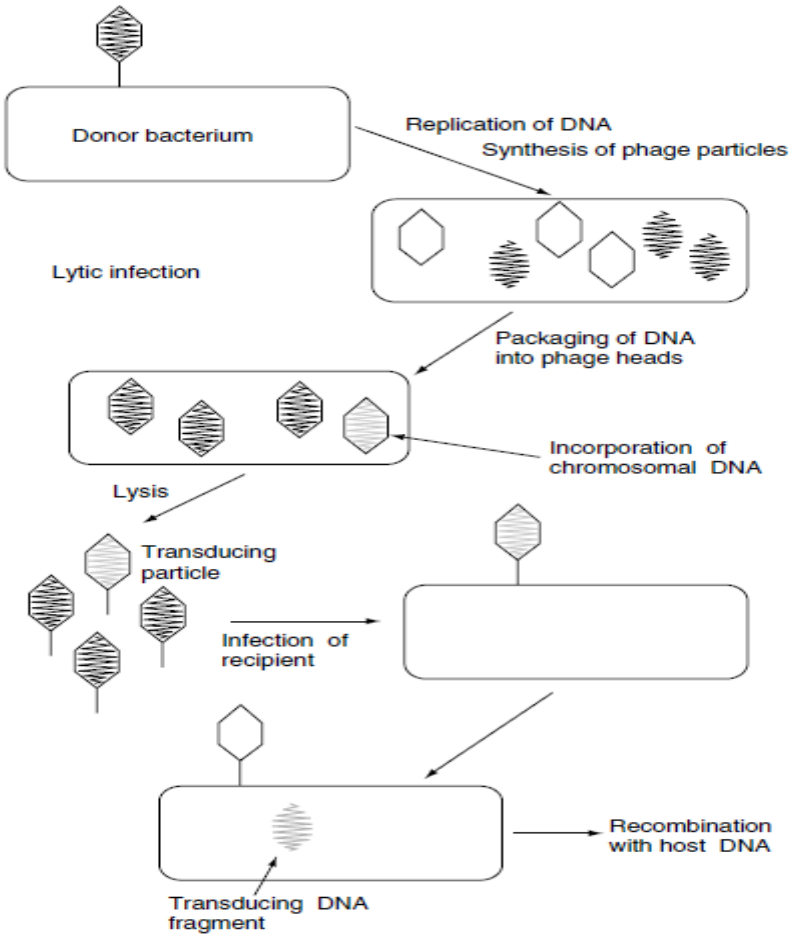

Figure 1. Generalized transduction

- wide range of specialized transducing phages with Mu which can be used both in genetic mapping and in mutagenesis.

Recombination

- **Recombination**, in the sense of re- assorting the observable characteristics in the progeny of a cross, has been a fundamental feature of genetics since long before its inception as a formal discipline. The **term 'recombination'** can be used in an analogous fashion in bacterial genetics, but is also used to refer to the physical breaking and joining of DNA molecules.
- At the simplest level, we can consider two linear DNA molecules: breaking both molecules at a single point, crossing them over and rejoining them will produce two recombinant DNA molecules, both of which have a part of each of the parental molecules.
- This general concept applies to a variety of recombinational mechanisms, of which the principal one is known as **general or homologous recombination**; this requires a substantial degree of homology between the sequences to be recombined but will work with any two pieces of homologous DNA. In contrast, site-specific recombinational mechanisms require little or no homology, but (as the name implies) operate only within specific sequences.
- The RecA protein is required for homologous recombination, but not for site-specific processes. Site-specific recombination is particularly important, for example in the integration and excision of bacteriophage and conjugative transposons.

A model of the recombination process

One model of the process of homologous recombination envisages firstly a pairing of the two DNA molecules in the homologous region (Figure 3). This is followed (ii) by a nick in one of the strands, which leads to that strand displacing part of the corresponding strand from the second molecule. The displaced strand is in turn nicked (iii) to produce an intermediate form with partially exchanged strands and the nicks are sealed to produce a structure with interlinked strands.

In Figure. 2, structure iii is redrawn in alternative forms, first by bending the arms to produce the X-shaped structure iiib and then by rotating the lower half by 180^{o} yielding the structure iiic, which is **known as a Holliday junction**, after Robin Holliday who first suggested the model from which this scheme is derived. This structure can be resolved by cutting the DNA strands in structure iiic at the positions marked with arrows. Ligation of the ends will then produce the recombinant structures shown in iv. (One of the simplifications used in this representation is the omission of other pathways that lead to alternative products. These structures contain some genetic markers from each parent and

are therefore recombinant in both the genetic and molecular senses. Note that in this diagram a short region, containing the marker Q/q, is a heteroduplex, i.e. one strand is from one parent and the second strand is from the other parent. This heteroduplex will either be repaired (i.e. q will be converted to Q, or vice versa), or if replication occurs first, the progeny will be mixed for this character.

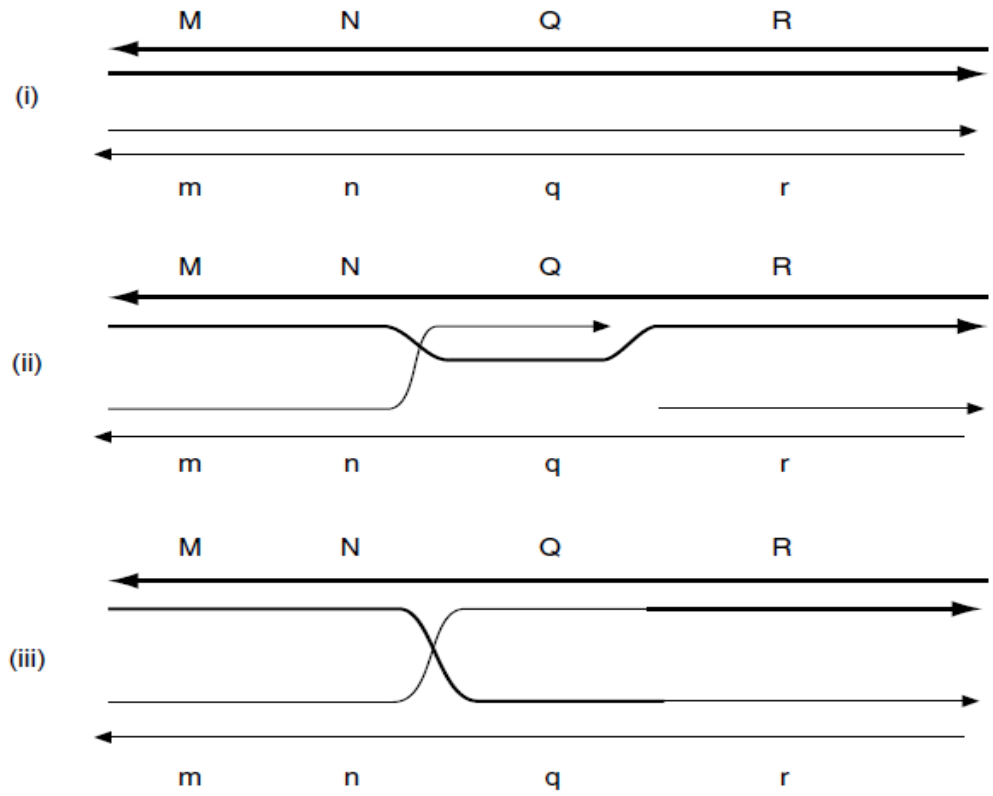

Figure 2. Initial stages of homologous recombination. (i) Pairing of the homologous regions. (ii) Nicked strand invades the opposite DNA molecule, displacing the corresponding strand. (iii) The displaced strand is nicked and the exchanged strands are re-joined

Enzymes involved in recombination

- One of the key enzymes in this process is the RecA protein as playing a key role in the induction of the SOS response. RecA protein can polymerize on DNA strands forming regular helical filaments in which the DNA helix is in a stretched conformation, thus facilitating an interaction with another DNA molecule.
- A second protein which is involved is an **endonuclease** with three subunits coded for by the *recB, recC and recD* genes (and hence known as the **RecBCD endonuclease**). This is a multifunctional enzyme with both endonuclease and exonuclease activity and is also able to unwind DNA molecules to provide the necessary single-stranded regions. As it

unwinds the DNA, one strand is degraded, until the enzyme reaches a specific **sequence known as a chi (χ) site**. Further nuclease degradation is then inhibited, leaving a single-stranded tail that is able to participate in strand invasion (with the assistance of RecA). These χ sites are therefore **hot-spots for recombination**. In *E. coli,* this sequence is 5^0GCTGGTGG3^0 (but may be different in other bacteria). An eight-base s adjacent DNA, enabling the junction to migrate along the DNA (thus increasing the extent of the heteroduplex).

- RuvC is the nuclease responsible for cutting the DNA strands as required for resolution of the Holliday junction. A different pathway (although still RecA-dependent) is required for repair of single-stranded gaps in the DNA.
- RecBCD is not able to participate in this system which uses instead RecF and several other proteins to prepare the single-stranded DNA for the loading of RecA that is needed for invasion of the sister strand. Sequence would be expected to occur within 65 kb on average if randomly distributed and most of the fragments generated during chromosome transfer by conjugation will be large enough to be likely to contain a χ site. However, when smaller fragments are involved the absence of a χ site in the DNA may limit the amount of recombination observed. This may be the case during transduction for example, and even more so during genetic manipulation experiments such as gene replacement.
- Three proteins, RuvA, RuvB and RuvC, are responsible for events at the Holliday junction. RuvA binds to the Holliday junction and stabilizes the structure needed for the subsequent events, while RuvB is a helicase that unwinds the adjacent DNA, enabling the junction to migrate along the DNA (thus increasing the extent of the heteroduplex). RuvC is the nuclease responsible for cutting the DNA strands as required for resolution of the Holliday junction.
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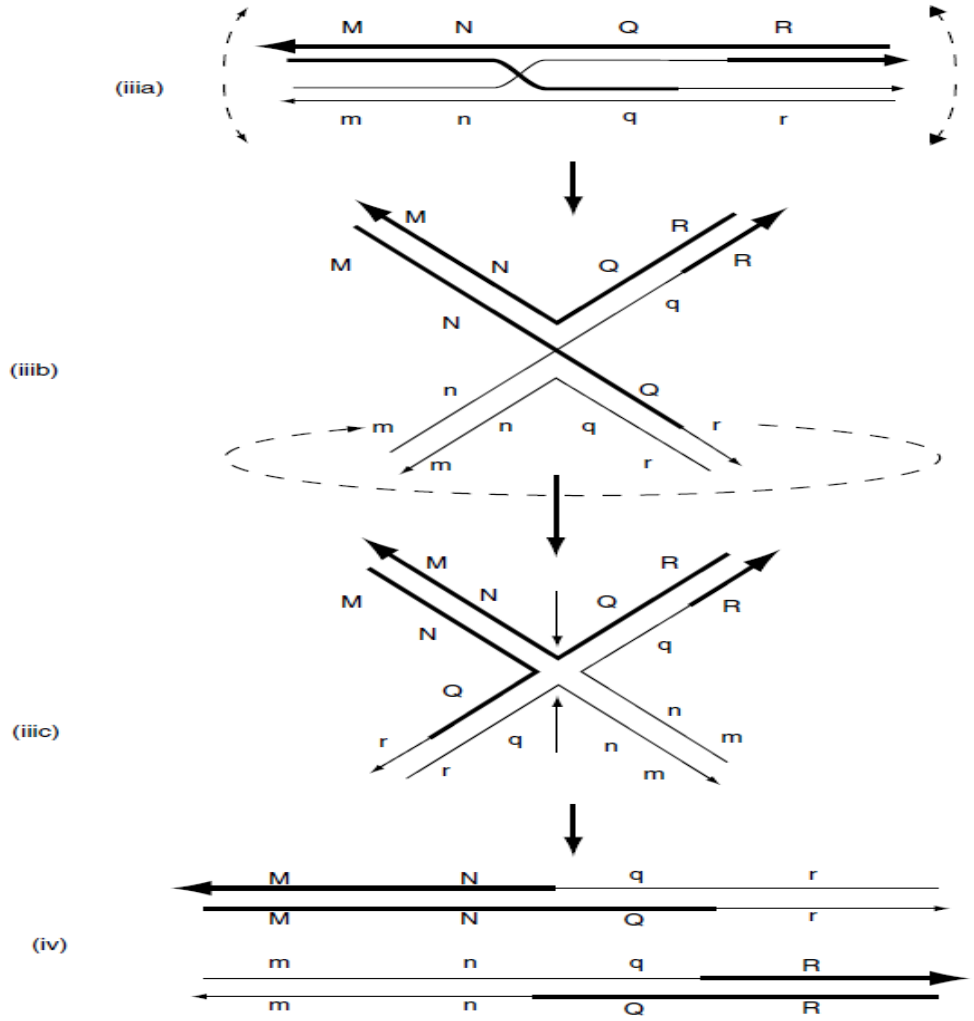

Figure 3. Homologous recombination: the Holliday junction. Structure iii from Figure 2 (iiia) is bent to an X shape (iiib) and the lower half is rotated to produce the Holliday junction (iiic). Resolution occurs by cutting the DNA at the arrowed points, producing the recombinant molecules shown (iv)

Site-specific and non-homologous (illegitimate) recombination

Recombination between DNA molecules can occur in a variety of other ways which are not dependent on the presence of extensive regions of homology nor on the action of RecA. Examples of these, include the integration of bacteriophage λ DNA into the chromosome, which involves a site-specific recombination between a defined sequence on the phage DNA and a specific chromosomal site and the transposition of mobile elements (insertion sequences and transposons) .