Recombination

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 (Figure. 1). 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.

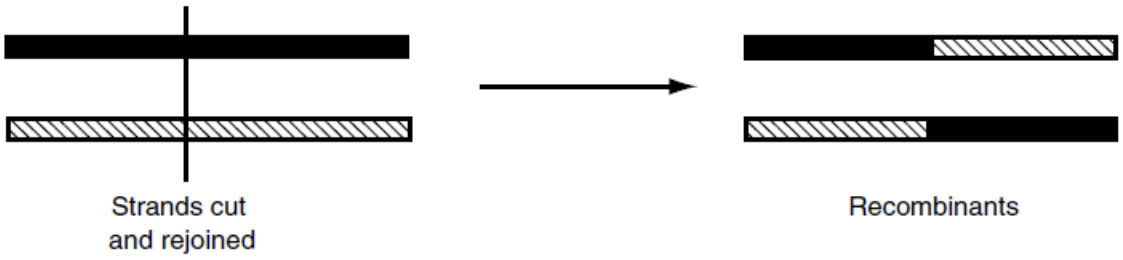

Figure .1 Recombination between two linear DNA molecules

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, for example in the integration and excision of bacteriophage and conjugative transposons.

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.

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 2). 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. 3, 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.

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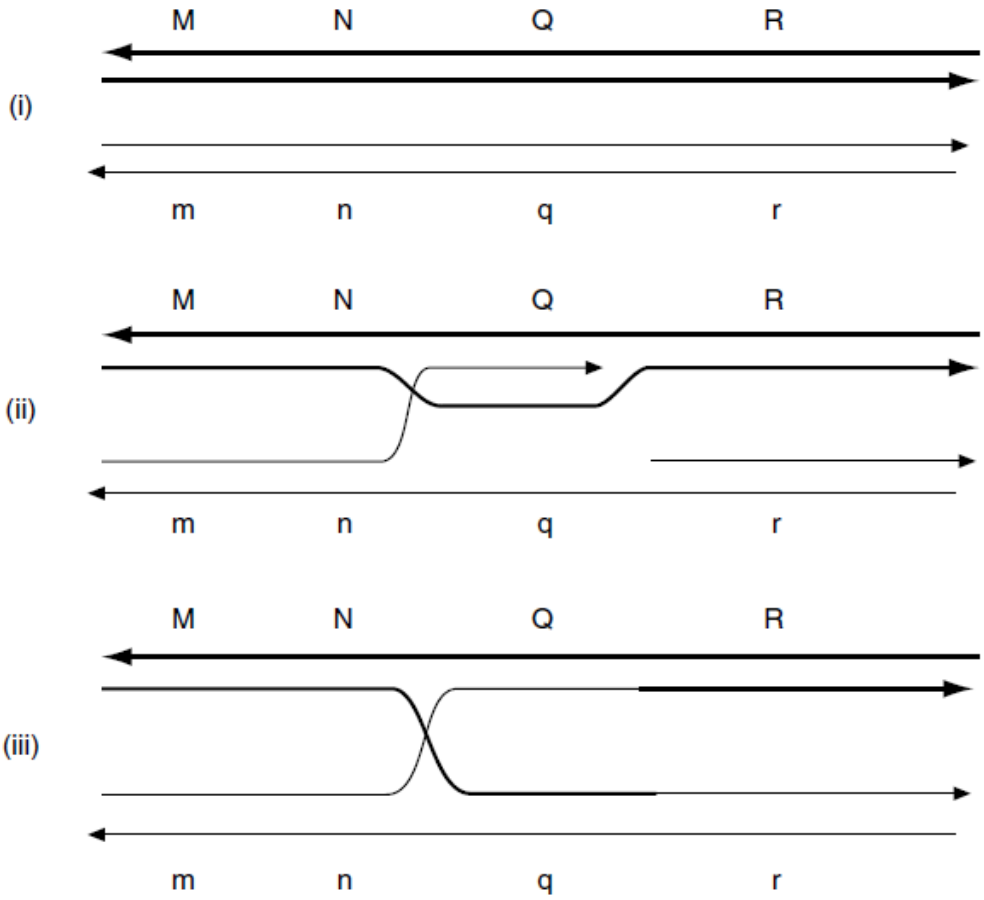

Figure .2 Initial stages of homologous recombination. (i) Pairing of the homologous regions. (ii) Nicked strand invades the opposite DNA molecule, displacing the corresponding strand. (iii) The displaced strand is nicked and the exchanged strands are re-joined

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. In the context of recombination however, its role is to stimulate the interaction between the recombining DNA molecules. 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).

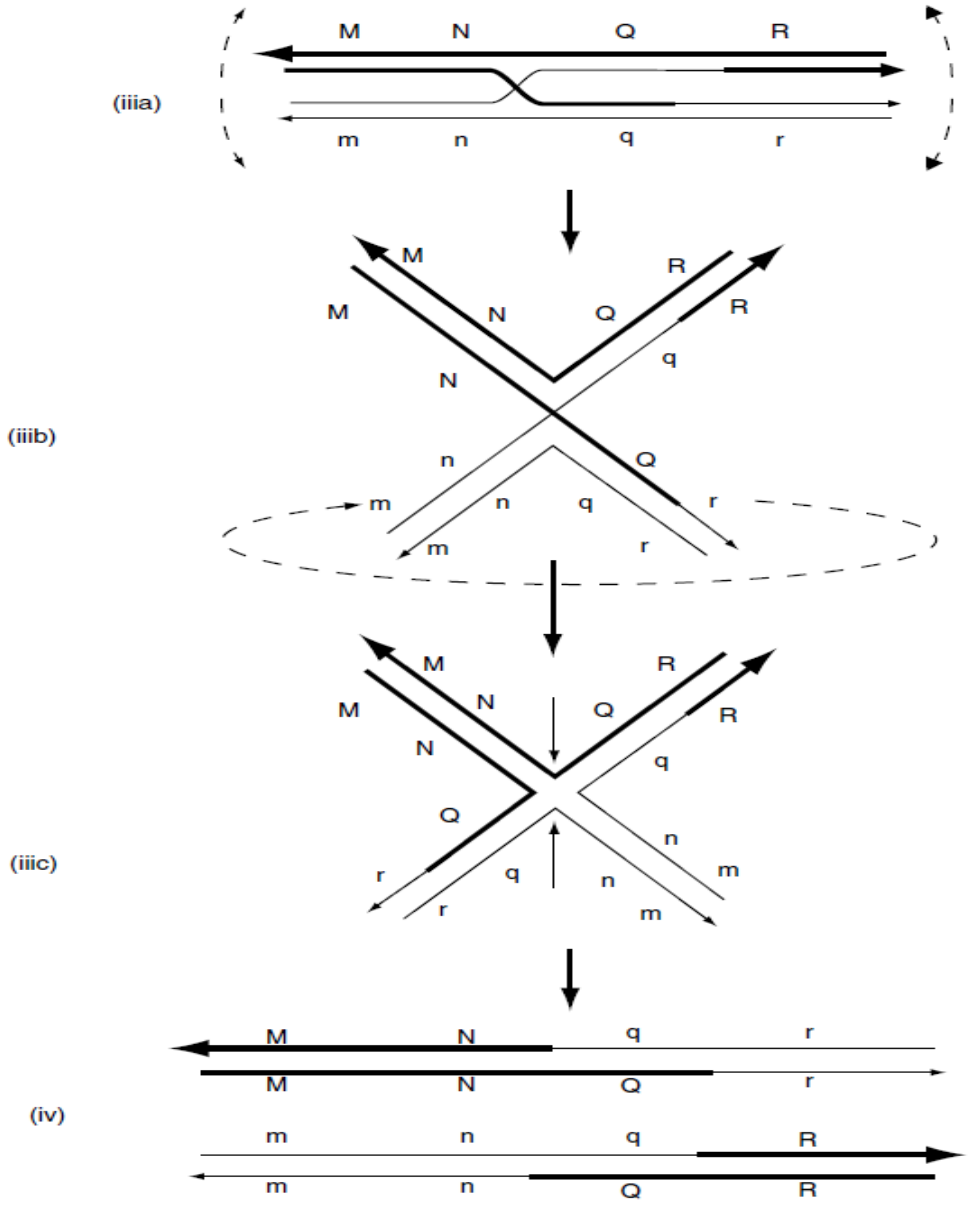

Figure 3 Homologous recombination: the Holliday junction. Structure iii from Figure 2 (iiia) is bent to an X shape (iiib) and the lower half is rotated to produce the Holliday junction (iiic). Resolution occurs by cutting the DNA at the arrowed points, producing the recombinant molecules shown (iv)

An eight-base 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.

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 which is needed for invasion of the sister strand.