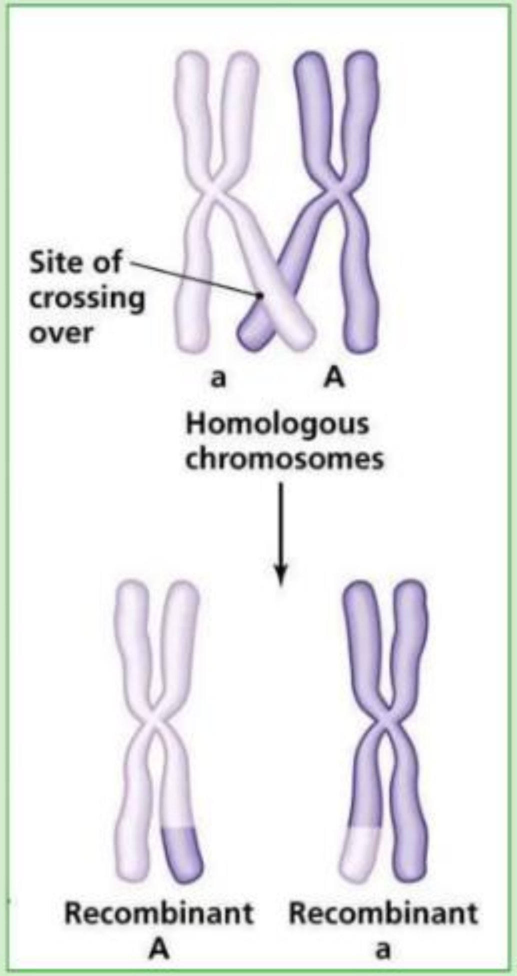
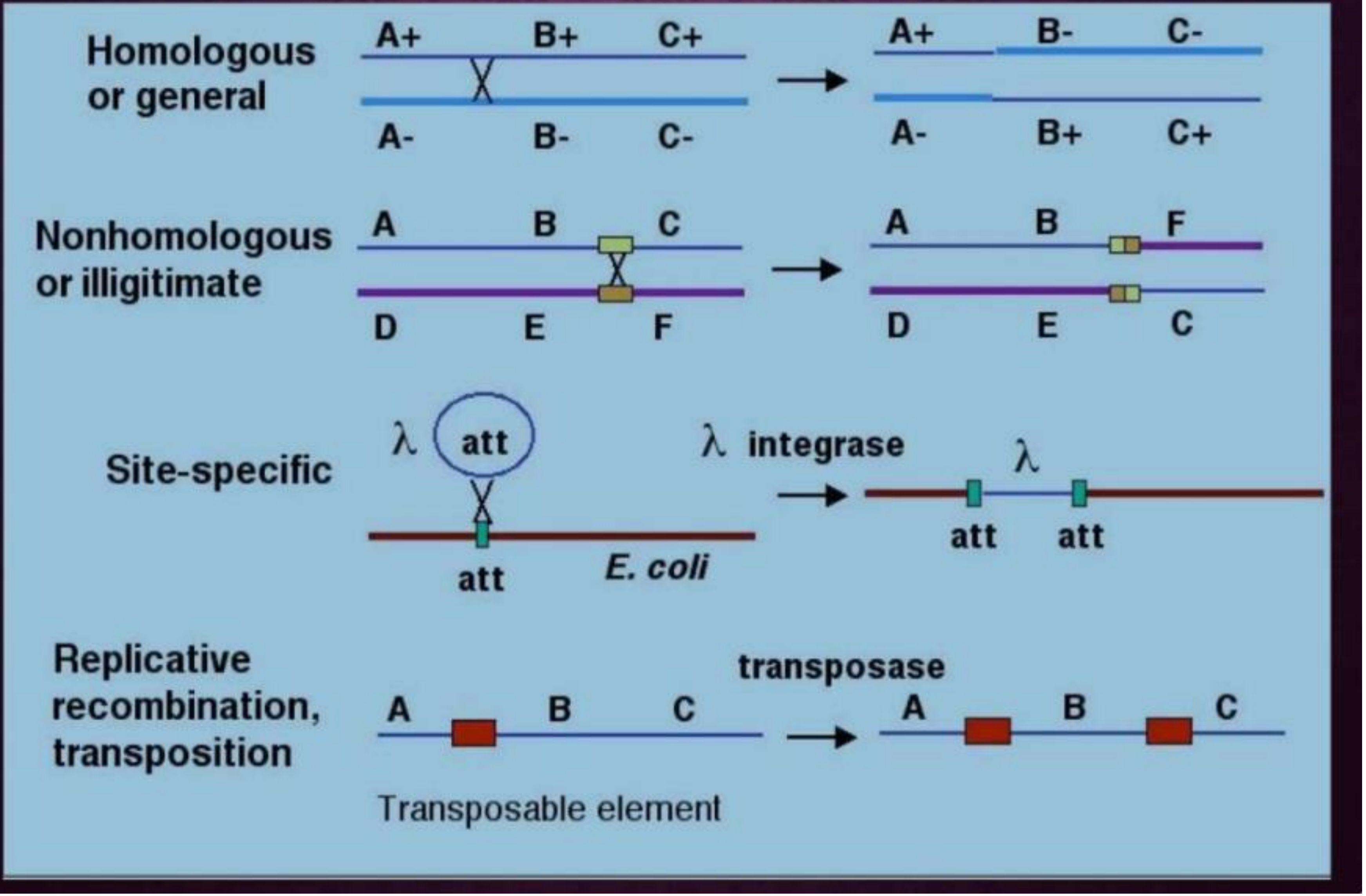
RECOMBINATION

RECOMBINATION

- Recombination is the rearrangement of DNA molecule or formation of new combination of genes.
- Recombination by crossing over is the process most molecular biologists often associate with the term recombination.
- But crossing over is not only the mechanism for recombination.



TYPES OF RECOMBINATION



Recombination is the production of new DNA molecule(s) from two parental DNA molecules or different segments of the same DNA molecule.

Types of recombination:

- **General or homologous recombination occurs between DNA molecules** of very similar sequence, such as homologous chromosomes in diploid organisms (meiotic recombination).
- **Illegitimate or nonhomologous recombination occurs in regions where no large scale** sequence similarity is apparent,
  - e.g. translocations between different chromosomes or deletions that remove several genes along a chromosome.

- **Site-specific recombination** systems mediate DNA rearrangements by breaking and joining DNA molecules at two specific sites, termed recombination targets (RTs).
- Site-specific recombination requires a special enzymatic machinery, basically one enzyme or enzyme system for each particular site.
- Good examples are the systems for integration of some bacteriophage, such as λ, into a bacterial chromosome and the rearrangement of immunoglobulin genes in vertebrate animals.
- **Replicative recombination** generates a new copy of a segment of DNA.
- Many transposable elements use a process of replicative recombination to generate a new copy of the transposable element at a new location.

HOMOLOGOUS RECOMBINATION

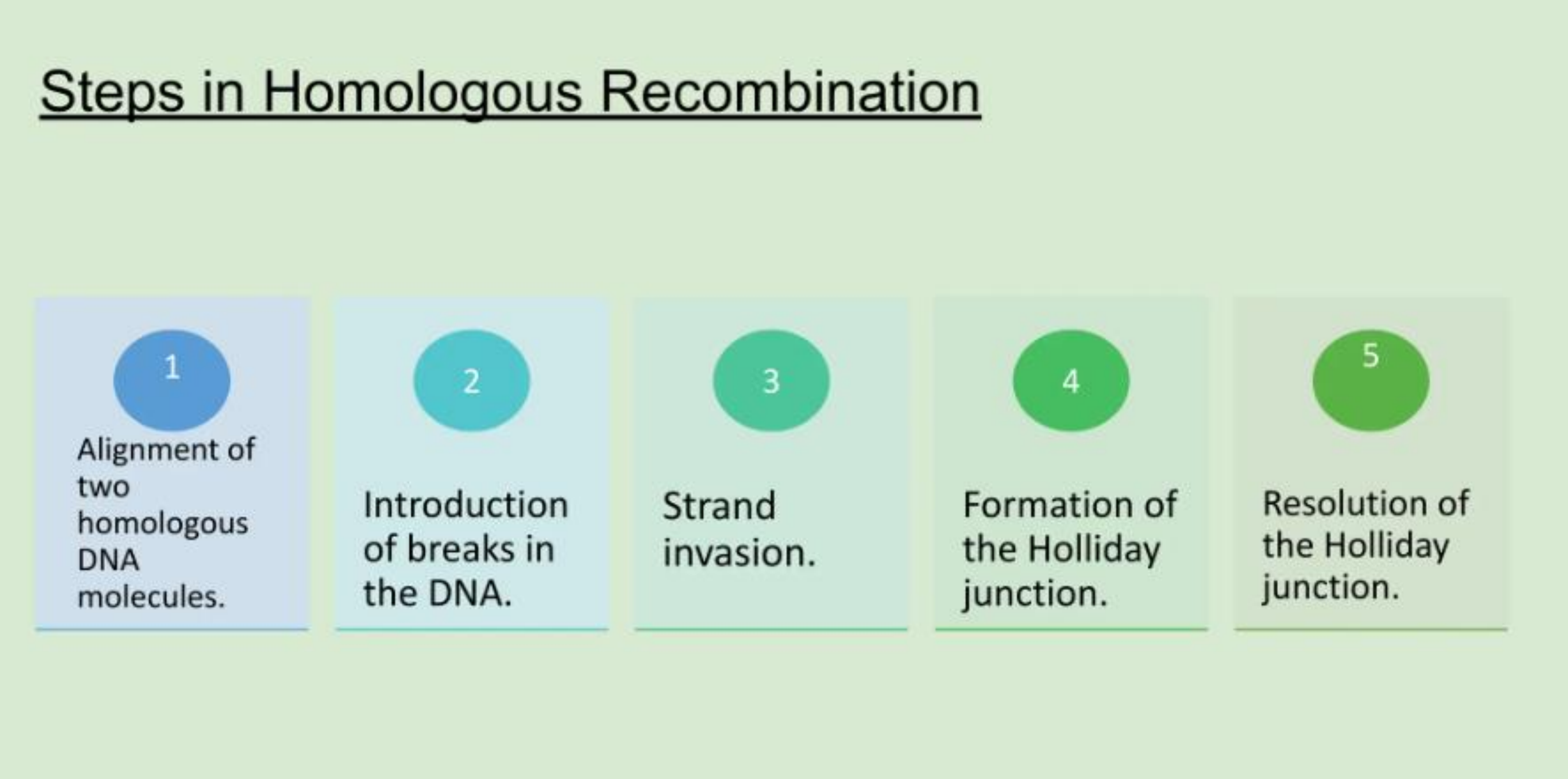
- Functions served:
  - Meiotic crossing over leading to reassortment of alleles on a chromosome
  - DNA repair
  - Resuming a stalled replication fork

Models Explaining Homologous Recombination

1. Holliday Model
2. Meselson- Radding Model
3. Double- strand Break Model

1.HOLLIDAY MODEL

- Robin Holliday proposed a model in 1964 to explain the molecular steps that occur during homologous recombination.
- This model describes a molecular mechanism of the recombination process.

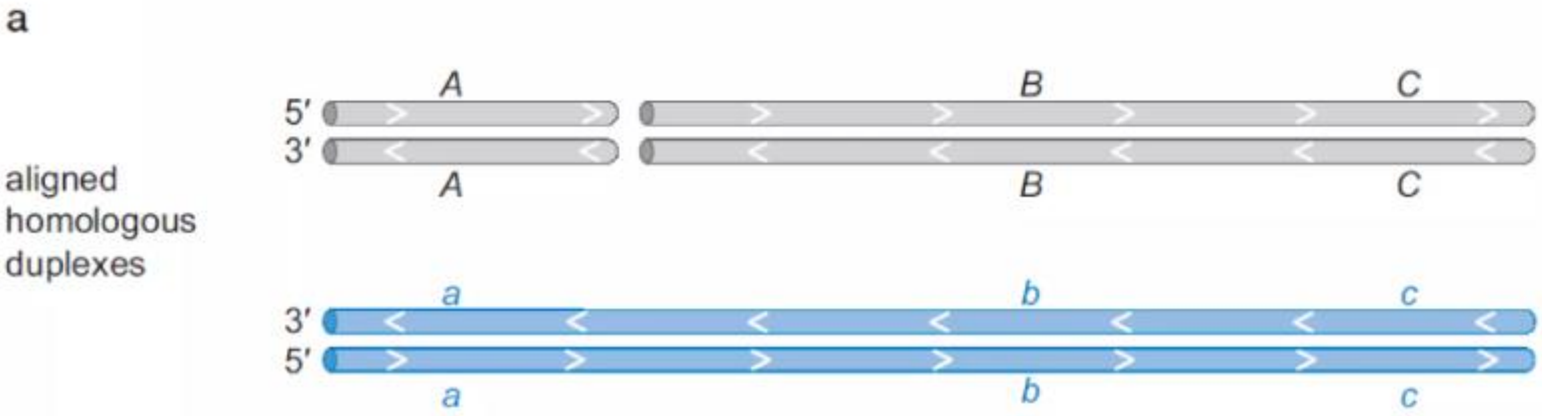
Steps in Homologous Recombination

1. Alignment of two homologous DNA molecules.
2. Introduction of breaks in the DNA.
3. Strand invasion.
4. Formation of the Holliday junction.
5. Resolution of the Holliday junction.

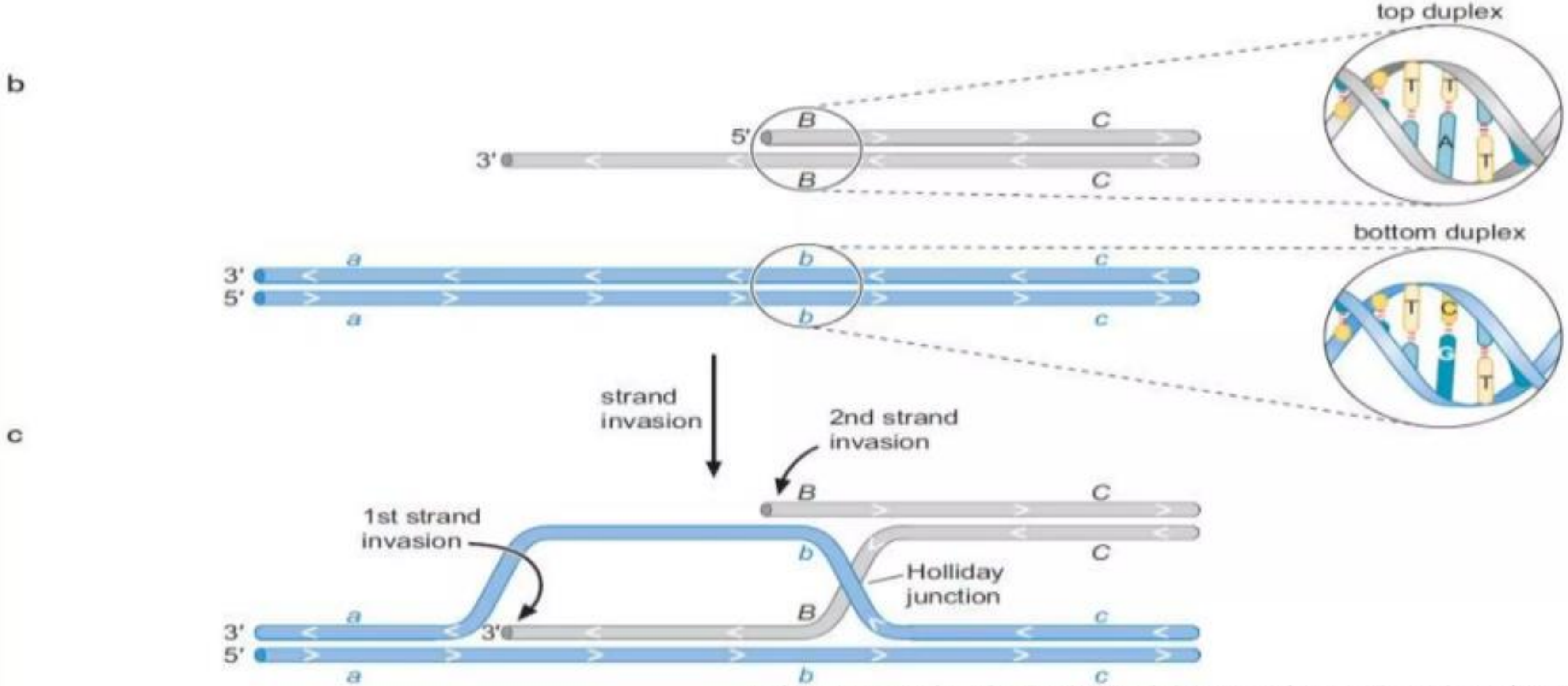
MODELS FOR HOMOLOGOUS RECOMBINATION

- ***THE HOLIDAY MODEL*** **:**

1. Alignment of two homologous DNA molecules. By homologous we mean that the DNA sequences are identical or nearly identical for a reason of at least 100 base pairs or so. Despite this high degree of similarity, DNA molecules can have small region of sequence difference and, may for example, carry different sequence variants known as **alleles**, of the same gene.

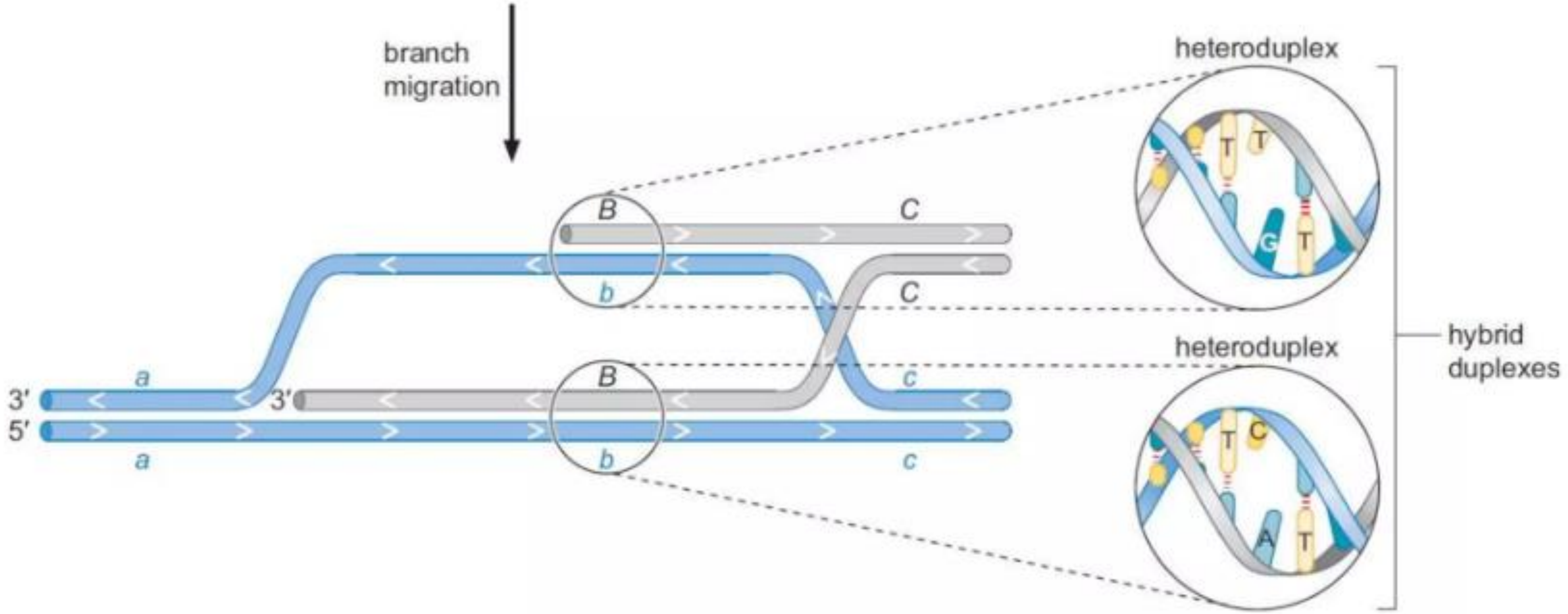


2. Introduction of breaks into the DNA. The break occur in one DNA strand or involve both DNA strands.
3. Formation of initial short regions of base pairing between the two recombinant molecules. This pairing occur when a single stranded region of DNA originating from one parental molecule pairs with its complementary stand in homologous duplex duplex DNA molecule. This step is known as **strand invasion** . As a result of strand invasion, the two DNA molecules become connected by crossing DNA strands. This structure is called a **Holliday Junction .**



Reference: Molecular biology of the gene (seventh addition) 2012

5. Cleavage of holiday junction. Cutting the DNA strands within the holiday junction regenerates two separate duplex DNA molecules, and therefore finishes genetic exchange. This process is called **resolution**.
6. If the two DNA molecules are not identical but, for example, carry a few small sequence differences, as is true often between two alleles of the same gene- branch migration through these regions of sequence difference generates DNA duplex carrying one or a few sequence mismatches (see *B* and *b* alleles) . Such regions are called **heteroduplex DNA** .



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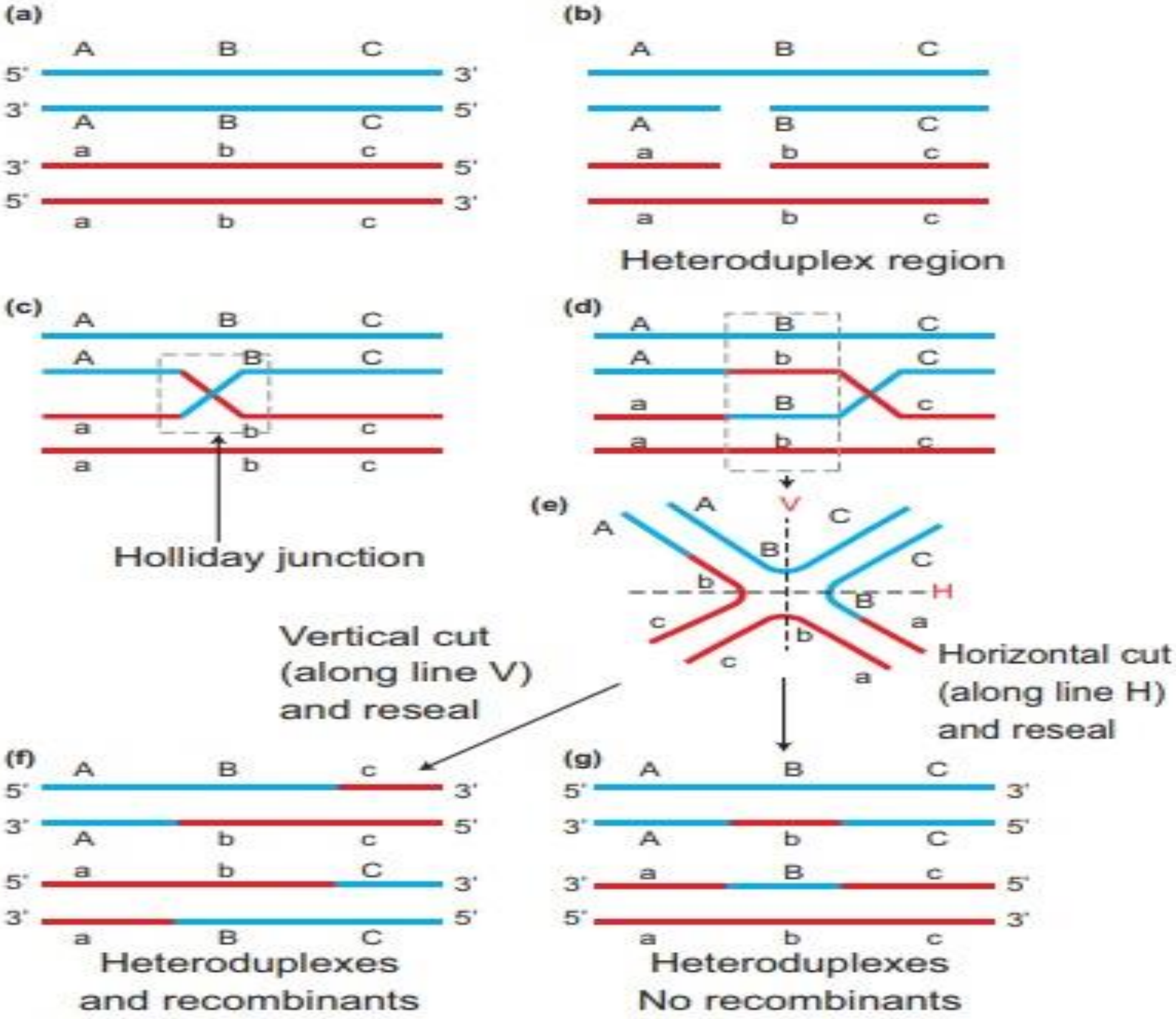


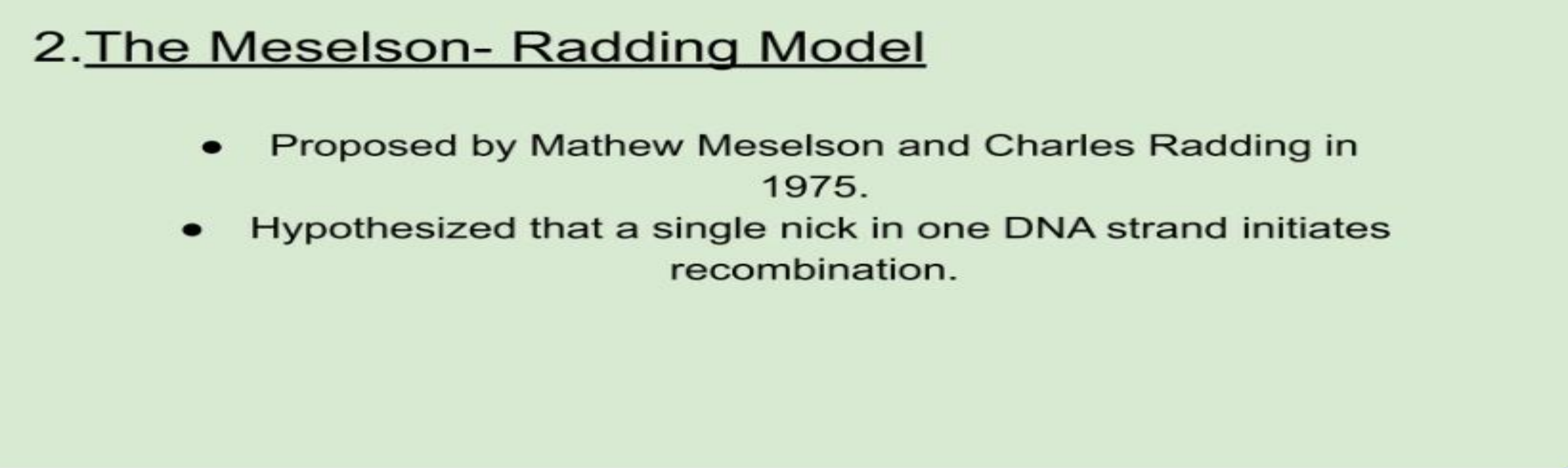
Figure 3.13: Holliday model showing Recombination

MODEL 2

MESELSON RADDING MODEL

- It is a second recombination model that is based on one single-strand nick for initiation
- The model retains some key features of the holliday model namely,

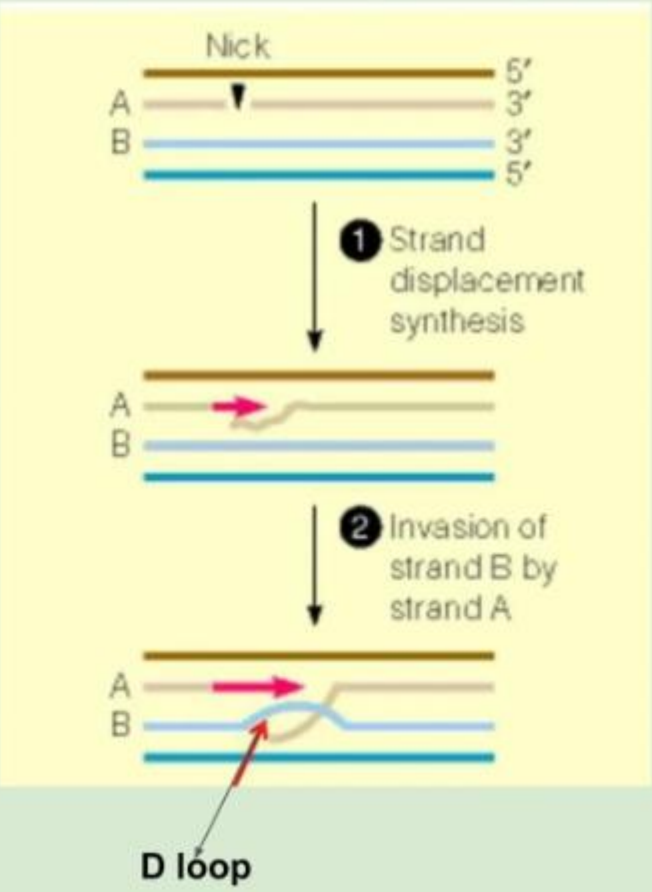
1. Formation of heteroduplex DNA
2. Mismatch repair of the heteroduplex DNA to give 3:1 and 1:3 gene conversion events
3. The holliday junction intermediate that could be resolved as non crossover or cross over



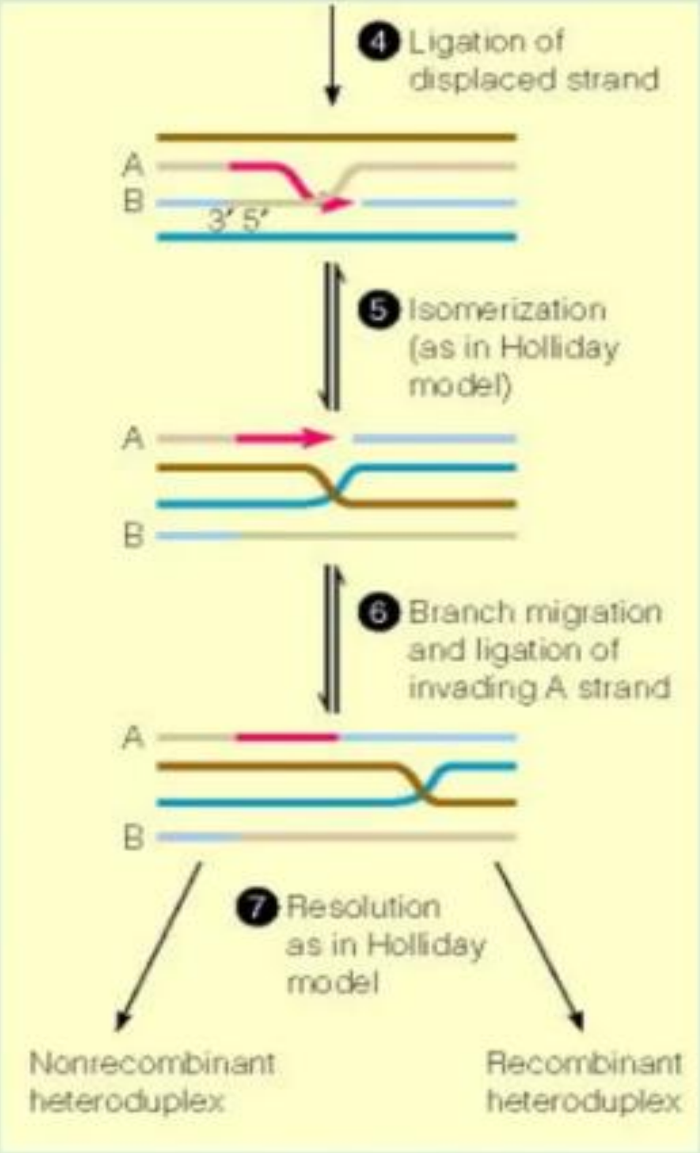
2.The Meselson- Radding Model

- Proposed by Mathew Meselson and Charles Radding in 1975.
- Hypothesized that a single nick in one DNA strand initiates recombination.

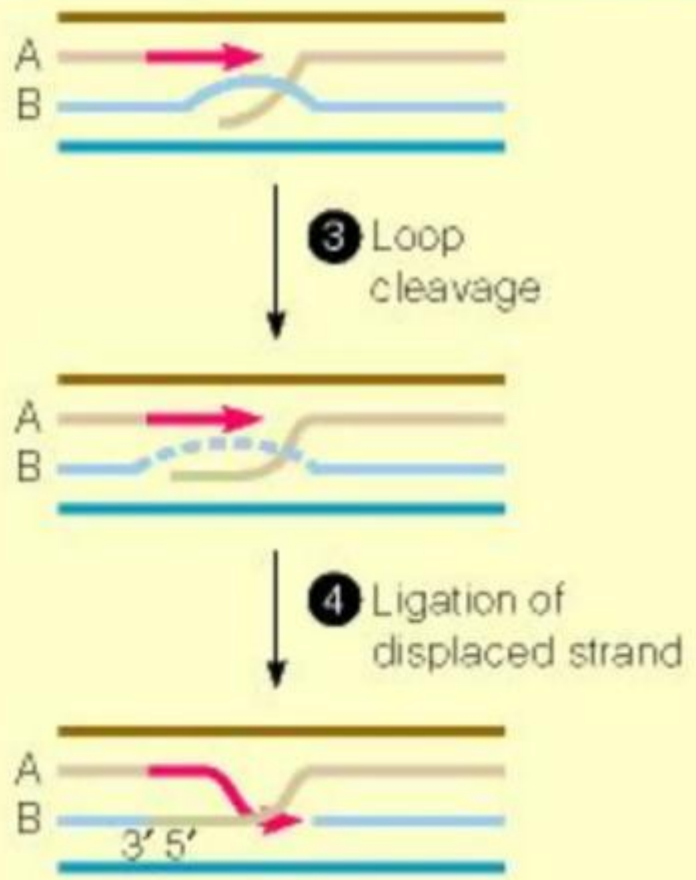
- This model suggests, Single-strand nick occurs in one of the double helices, one of the free end invades the homologous double helix(unbroken), displacing one of its strands forming a D-loop (Displacement loop)



- The Final steps(branch migration and resolution) is as same as in Holliday model.



- Eventually second nick occurs at the D-loop, creating the Holliday structure.

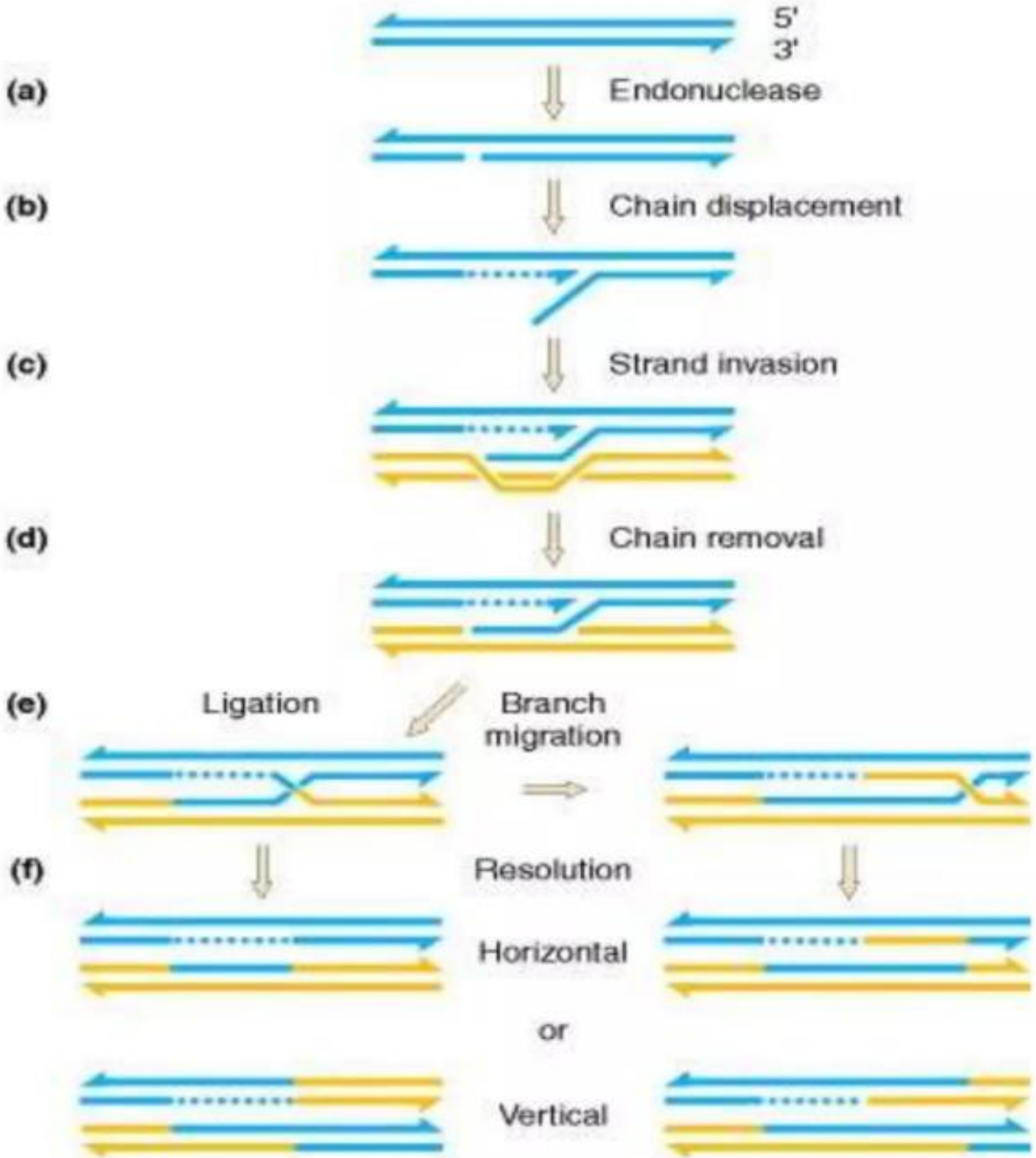


MECHANISM

- A single strand nick is introduced into a strand of one of the duplexes
- DNA polymerase extends the newly created 3’ end causing displacement of strand on the other side of the nick
- With the assistance of Rec A, the displaced strand with a 5’P tail invades a homologous region in the second duplex to form a D loop
- The D loop is cleaved forming a single strand links between the two duplexes

MECHANISM (ctd)

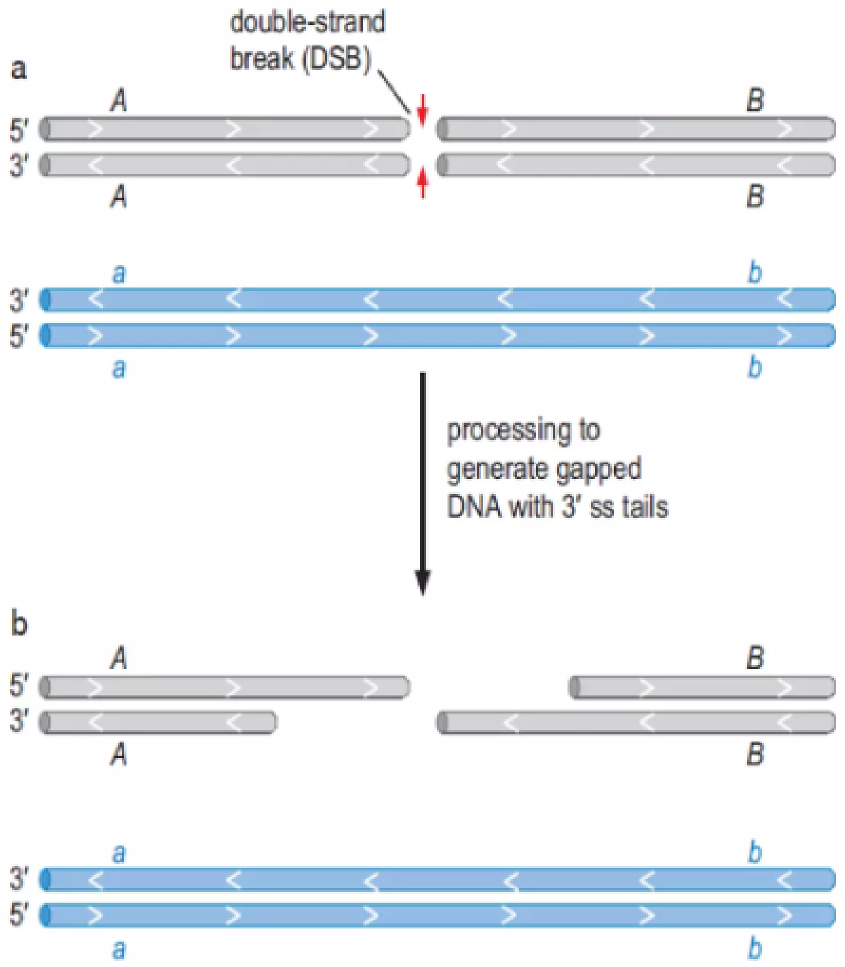
- The strands are isomerised or rotated around the crossed strand point to allow the free ends at the exchange site to be ligated, forming a holliday junction
- The holliday junction can branch migrate.
- Branch migration form the initial site of strand invasion forms symmetric heteroduplex DNA


5'
3'
(a)
Endonuclease
(b)
Chain displacement
(c)
Strand invasion
(d)
Chain removal
(e)
Ligation
Branch migration
(f)
Resolution
Horizontal
or
Vertical

MODEL 3

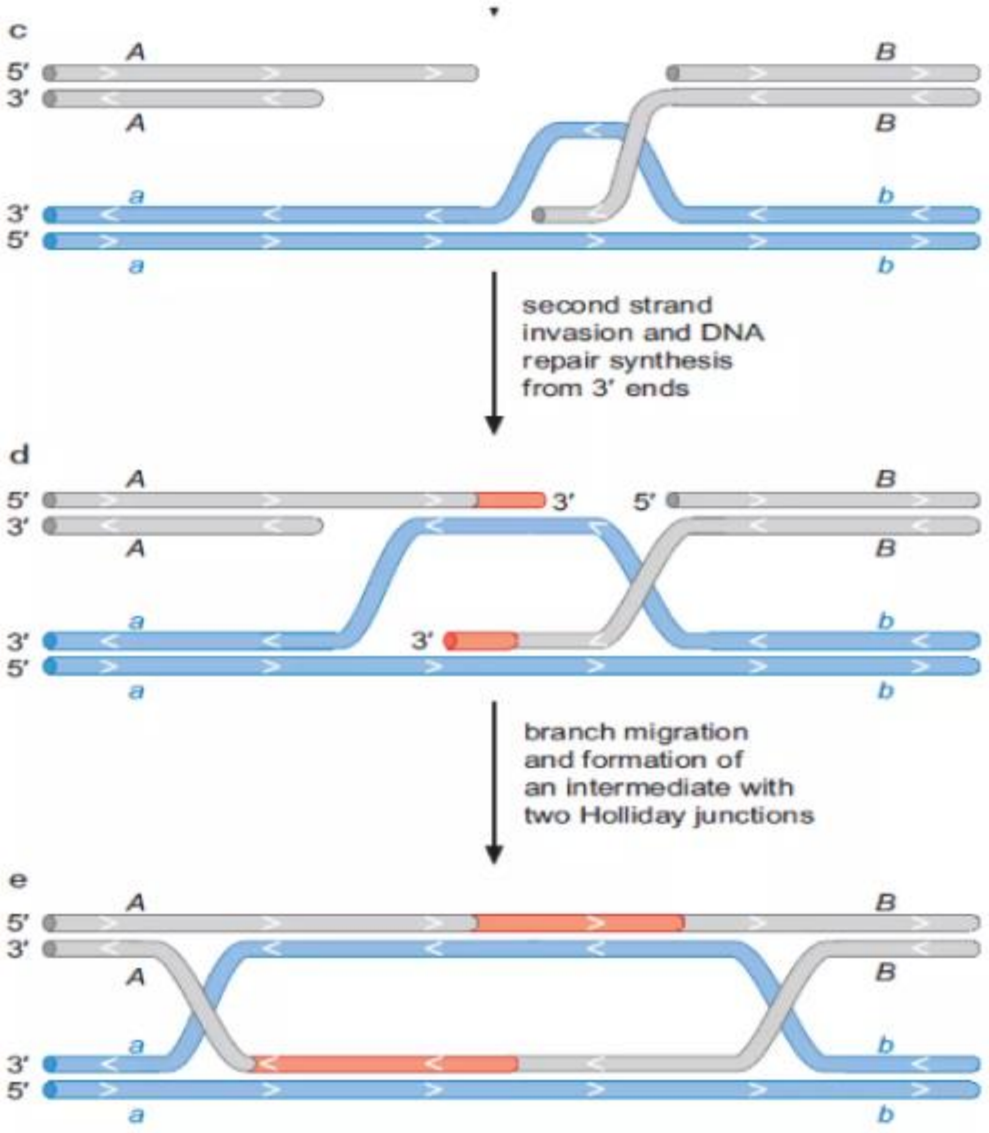
THE DOUBLE-STRAND BREAK REPAIR MODEL

- The initiating event is the introduction of a **double strand break (DSB)** in one of the two DNA molecules.
- After introduction of the DSB, a DNA-cleaving enzyme sequentially degrades the broken DNA molecule to generate regions of single-stranded DNA (ssDNA).
- This processing creates single-strand extensions, known as ssDNA tails, on the broken DNA molecules; these ssDNA tails terminate with 30 ends.



Reference: Molecular biology of the gene (seventh addition) 2012

- The ssDNA tails generated by this process then invade the unbroken homologous DNA duplex.
- The invading strand base-pairs with its complementary strand in the other DNA molecule.
- Because the invading strands end with 3′ termini, they can serve as primers for new DNA synthesis. Elongation from these DNA ends—using the complementary strand in the homologous duplex as a template serves to regenerate the regions of DNA that were destroyed during the processing of the strands at the break site.



- If the two original DNA duplexes were not identical in sequence near the site of the break (e.g., having single-base-pair changes as described above), sequence information could be lost during recombination by the DSB-repair pathway.
- The two Holliday junctions found in the recombination intermediates generated by this model move by branch migration and ultimately are resolved to finish recombination

HOMOLOGOUS RECOMBINATION PROTEIN MACHINES

- Organisms from all branches of life encode enzymes that catalyze the biochemical steps of recombination.
- In some cases, members of homologous protein families provide the same function in all organisms. In contrast, other recombination steps are catalyzed by different classes of proteins in different organisms but with the same general outcome

Prokaryotic and eukaryotic factors that catalyze recombination steps :

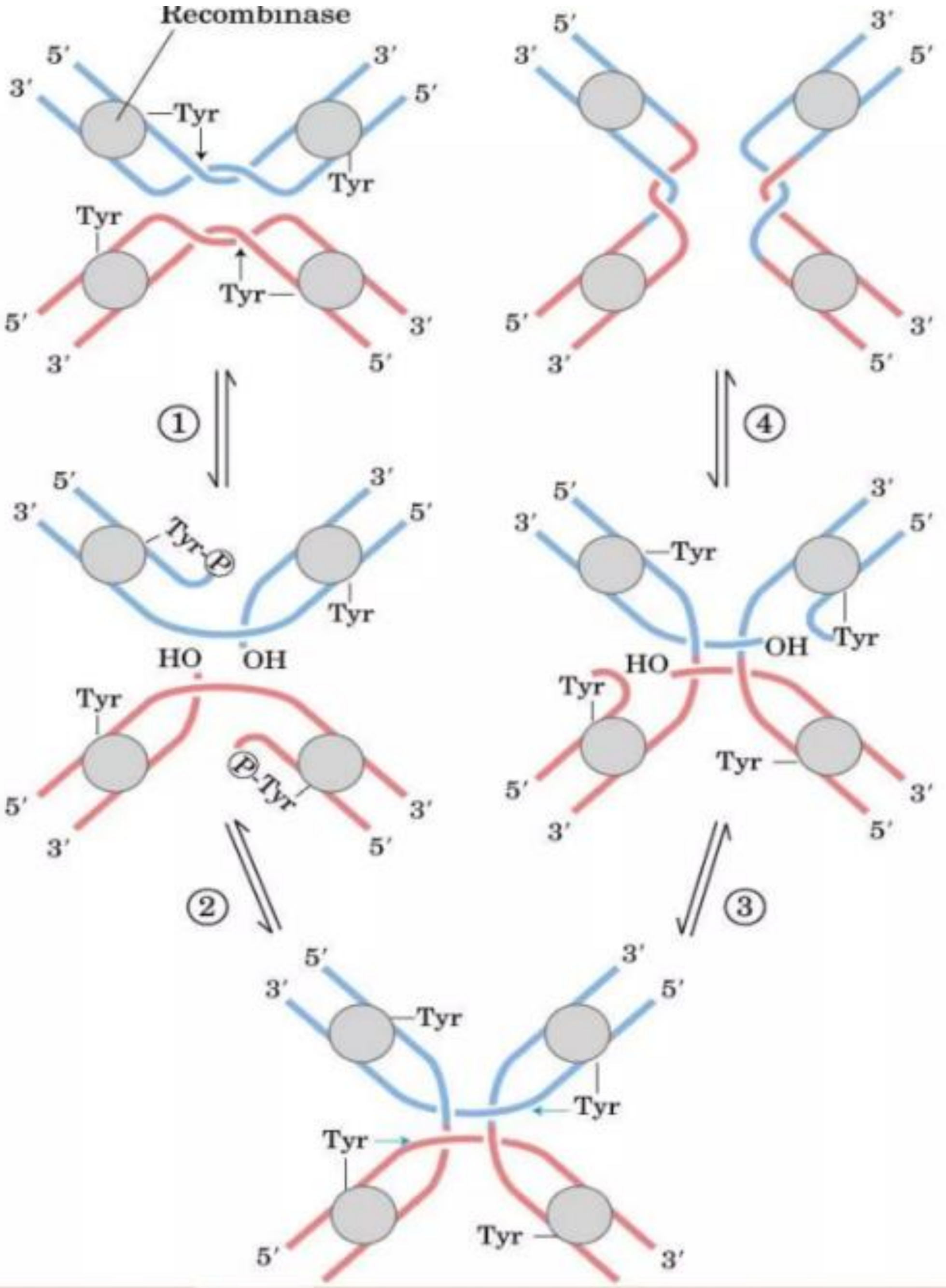
Recobination step	***E.coli*** **protein catalyst**	**Eukaryotic Protein catalyst**
Pairing homologous DNAs and strand invasion	RecA protein	Rad51 Dcm 1 (in meiosis)
Introduction of DSB	None	Spo11 (in meiosis) HO (for mating type switching)
Processing of DNA breaks to generate single strands of invasion	RecBCD Helicase/nuclease	MRX Protein (also called Rad50/58/60 nucleases)
Assembly of strand exchange protein	RecBCD and RecFOR	Rad52 and Rad59
Holiday junction recognition and branch migration	RuvAB complex	unknown
Resolution of holiday junction	Ruvc	Perhaps Mus81 and others

SITE-SPECIFIC RECOMBINATION

- Viruses and transposable elements often integrate their genomes into the host chromosome
- **Site-specific recombination**, is a type of genetic recombination in which DNA strand exchange takes place between segments possessing at least a certain degree of sequence homology
- Site- specific recombination is used by both eukaryotes and prokaryotes to regulate gene expression and to increase the organisms genetic range
- Each site- specific recombination involves:
- Recombinase
- A short (20-200bp) unique DNA sequence where recombinase act

Site specific recombination

FIGURE 25–38 A site-specific recombination reaction. (a) The reaction is carried out within a tetramer of identical subunits. Recombinase subunits bind to a specific sequence, often called simply the recombination site. 1 One strand in each DNA is cleaved at particular points within the sequence. The nucleophile is the OH group of an active-site Tyr residue, and the product is a covalent phosphotyrosine link between protein and DNA. 2 The cleaved strands join to new partners, producing a Holliday intermediate. Steps 3 and 4 complete the reaction by a process similar to the first two steps. The original sequence of the recombination site is regenerated after recombining the DNA flanking the site. These steps occur within a complex of multiple recombinase subunits that sometimes includes other proteins.



MECHANSIM

- There are two general classes of site specific recombination which rely on either Tyr or Ser residues in the active site.
- Recombinase is an important enzyme involved in the site specific recombination
- The recombinase recognizes and binds to each of two recombination sites on two different DNA molecules or within the same DNA

MECHANISM(ctd)

- Step 1- One DNA strand in each site is cleaved at a specific point within the site, and the recombinase becomes covalently linked to DNA at the cleavage site through a phosphotyrosine bond
- Step 2- The transient protein-DNA linkage preserves the phosphodiester bond that is lost in cleaving the DNA. The cleaved DNA strands are rejoined to new partners to form a holliday intermediate, with new phosphodiester bond created at the expense of the protein-DNA linkage
- Step -3 The process is repeated at the second point within each of the two recombination sites in order to complete the reaction

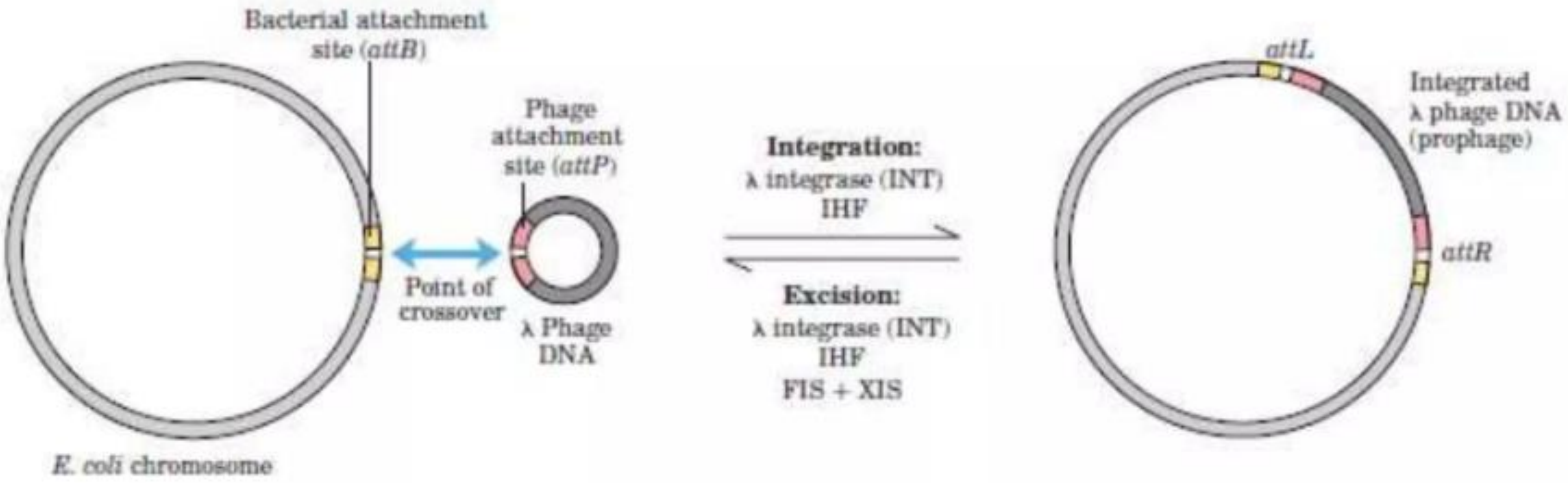


FIGURE 25–40 Integration and excision of bacteriophage *DNA at* the chromosomal target site. The attachment site on the *phage DNA* (attP) shares only 15 bp of complete homology with the bacterial site (attB) in the region of the crossover. The reaction generates two new attachment sites (attR and attL) flanking the integrated phage DNA.

The recombinase is the *integrase (or INT protein). Integration and* excision use different attachment sites and different auxiliary proteins. Excision uses the proteins XIS, encoded by the bacteriophage, and FIS, encoded by the bacterium. Both reactions require the protein IHF (*integration host factor), encoded by the bacterium.*