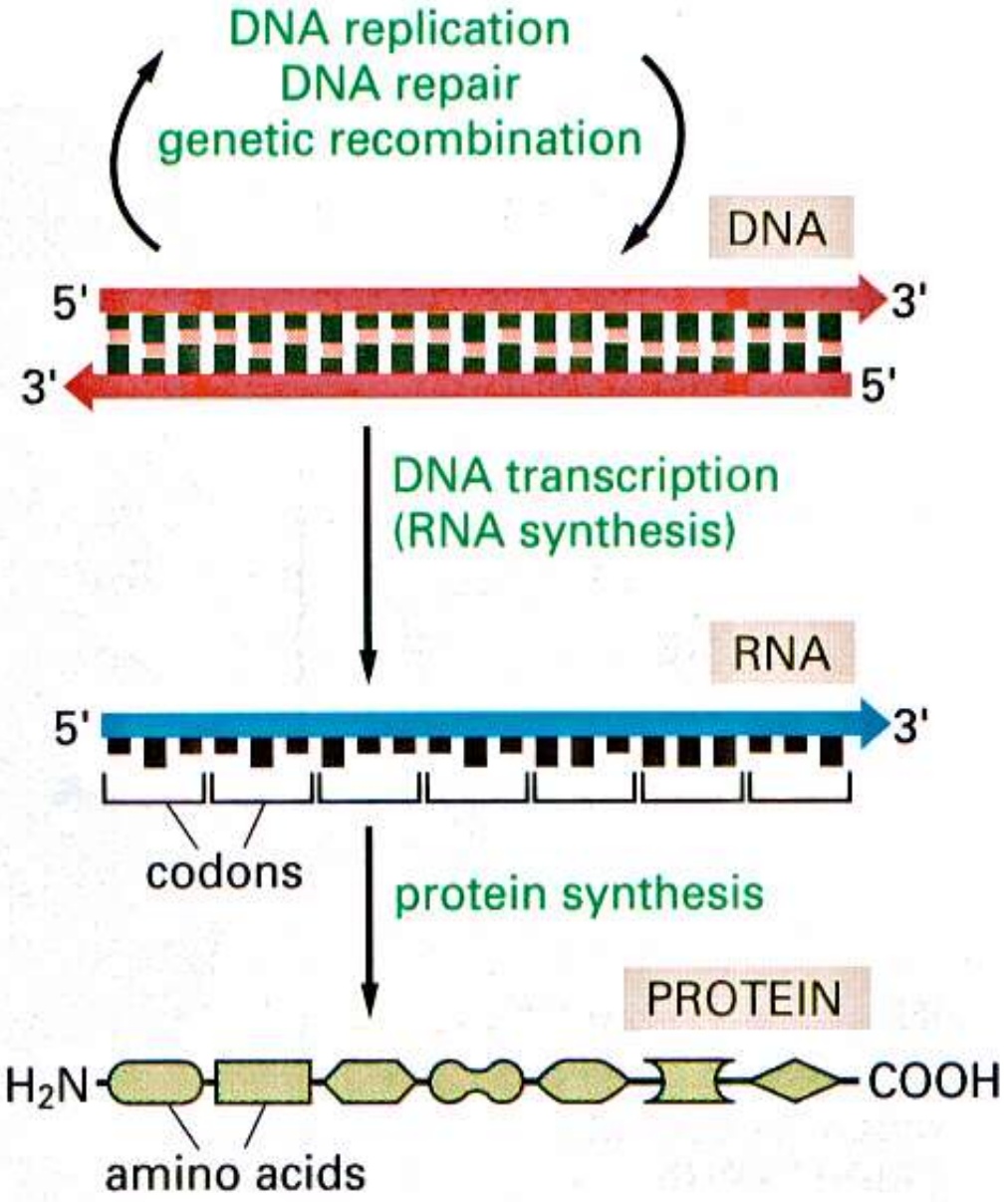
Peter Pristas

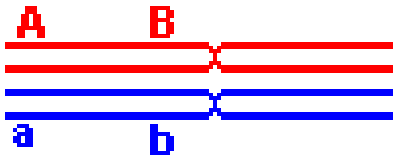
Molecular biology

DNA recombination



The Central Dogma of Molecular Biology

Homologous (or general) rec he heart of



Pur

DN

Genetic diversity

Maintaining proper chromosomal alignment during meiosis

Recombination

Generalized recombination occurs in all genetic exchange processes which generate relatively long pieces of homologous DNA examples include:

- **Chromosomal crossing over**
- **Plasmid-mediated conjugation,**
- **Transduction**
- **DNA transformation**

Three kinds:

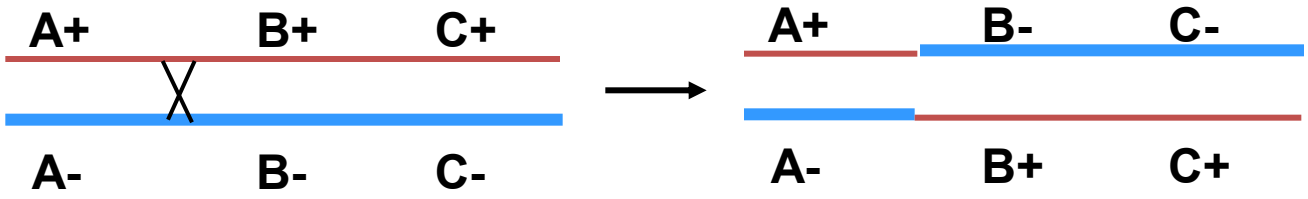
- **Generalized or Homologous recombination**
  Between homologous sequences
- **Site-specific or Specialized recombination**
  between specific pair of sequences
  Lambda integration at the *att* site
- **Illegitimate recombination**
  insertion of DNA without homology - Transpositions

Three models:

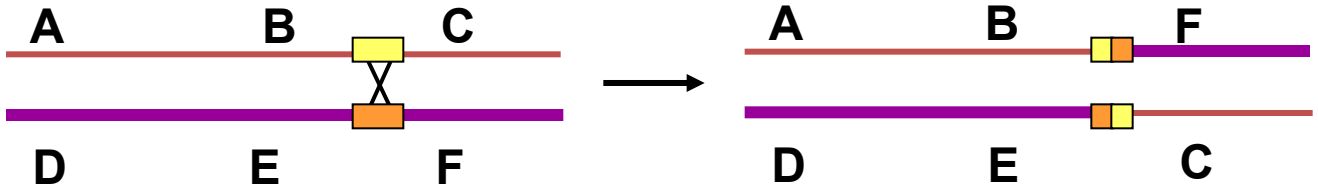
- **Holliday model (Robin Holliday, 1964)**
- **Single-strand invasion model**
- **Double-strand break repair model**

Types of recombination

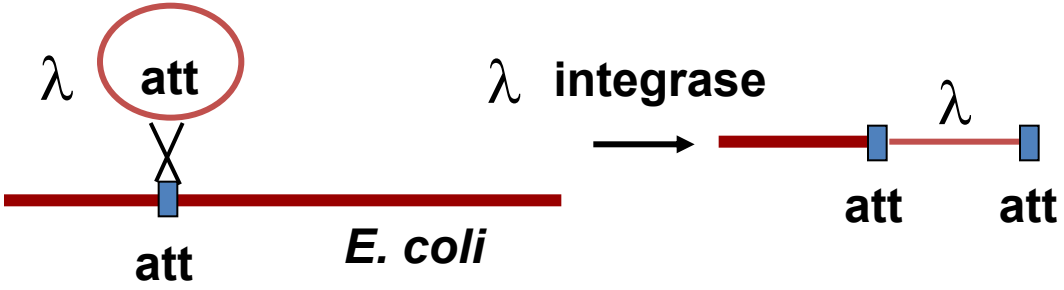
Homologous or general



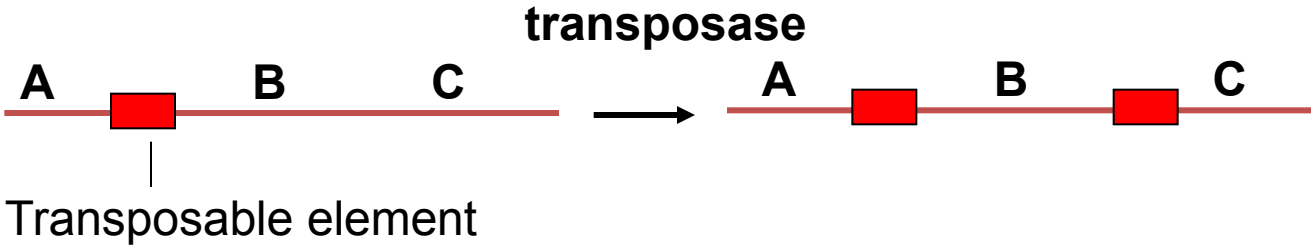
Nonhomologous or illigitimate



Site-specific



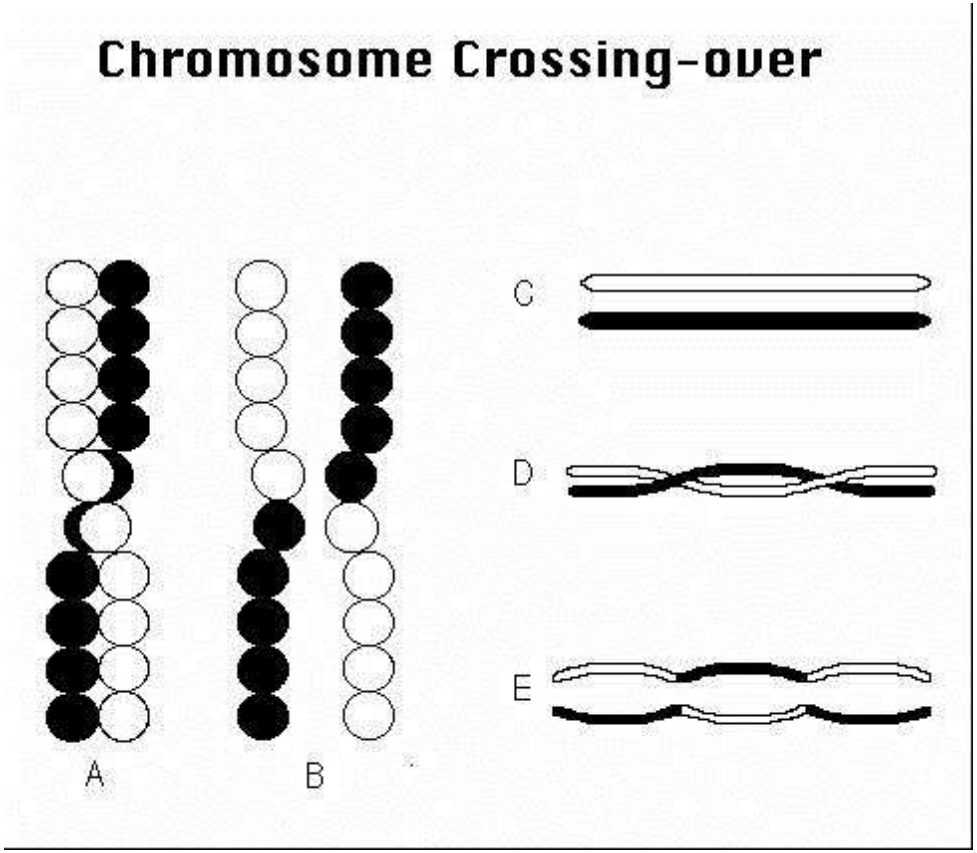
Replicative recombination, transposition



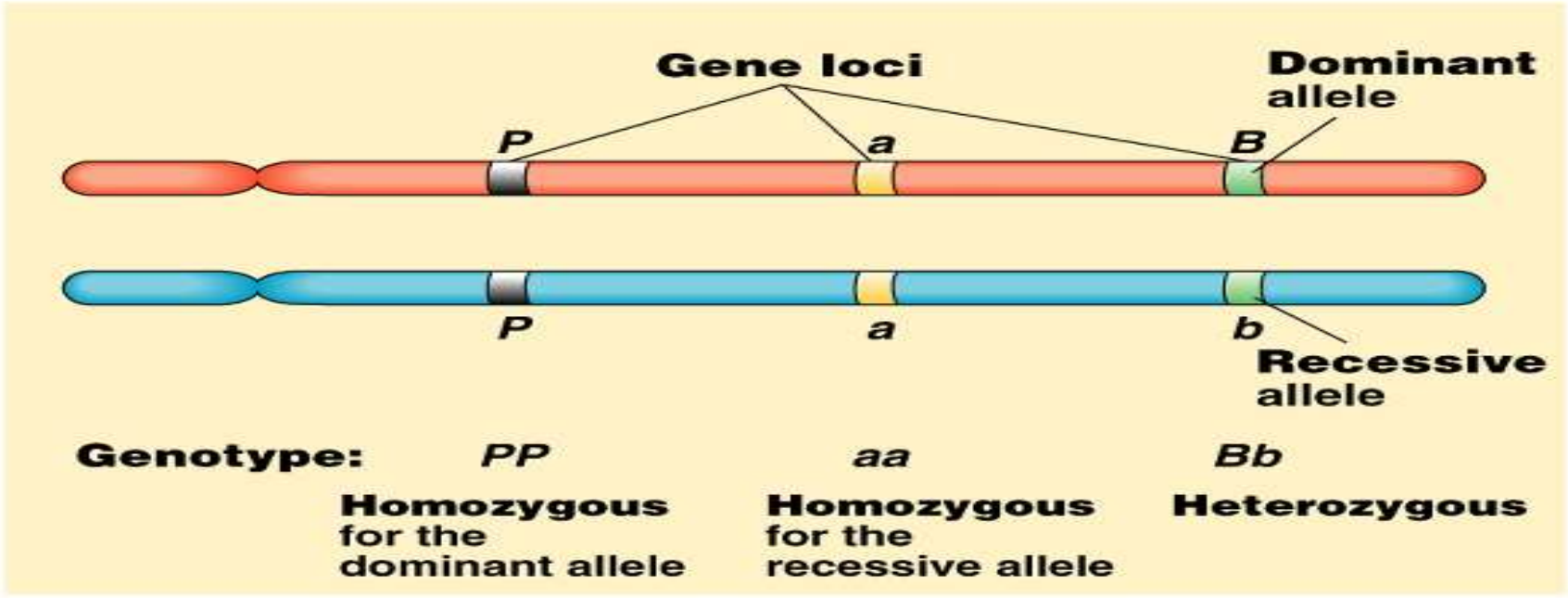
Generalized recombination

Chromosomal Loci Can Switch Position During or After DNA Replication

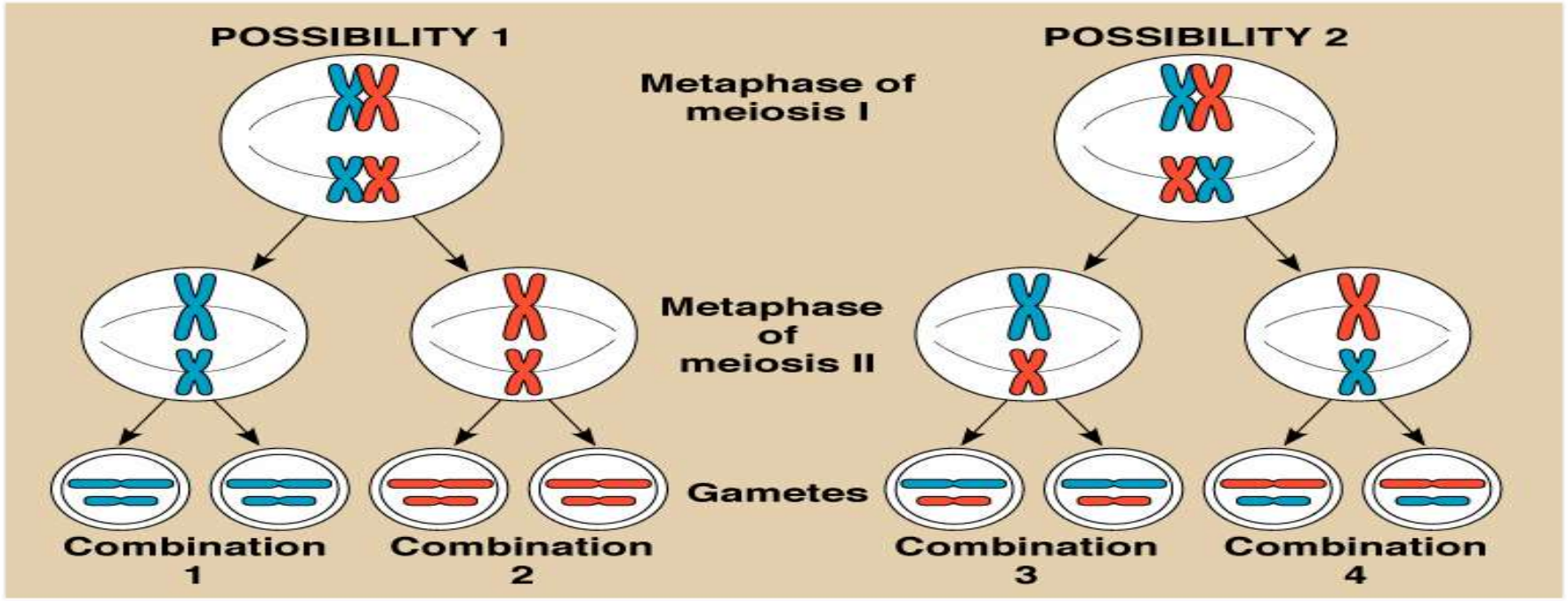
- particularly during meiosis (meiotic recombination = crossing over)



Homologous chromosomes in a diploid nucleus



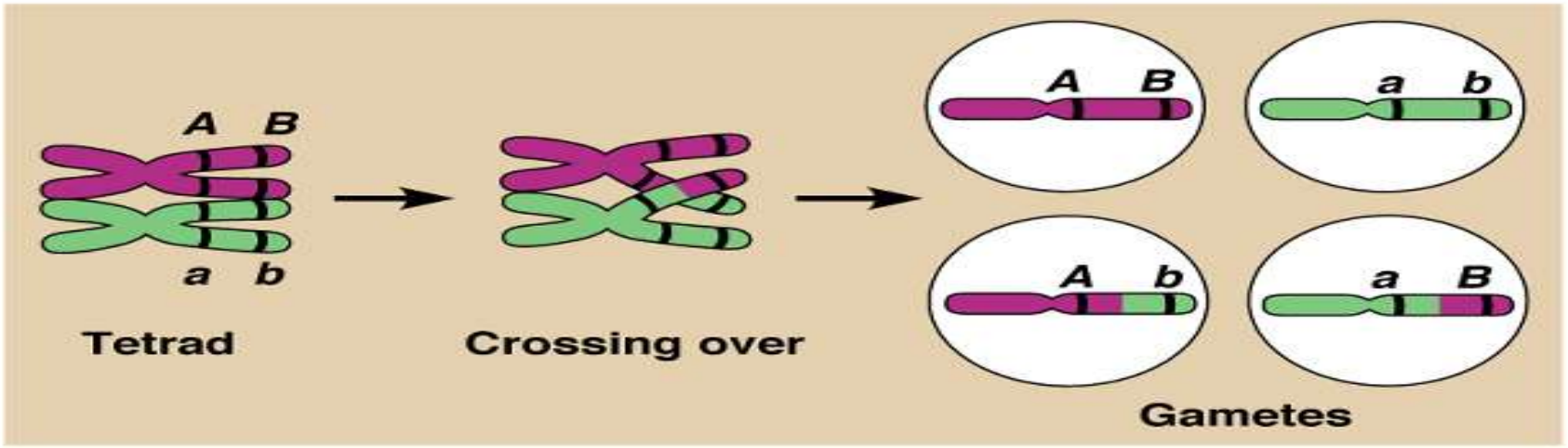
Independent assortment of loci on different chromosomes



Minimum number of gamete types = 2^n In humans, n = 23
Gamete types from mother: 8,388,608
Gamete types from father: 8,388,608

Maximum chance that two children from the same parents will be identical: 1/70 trillion

Recombination and Crossing over

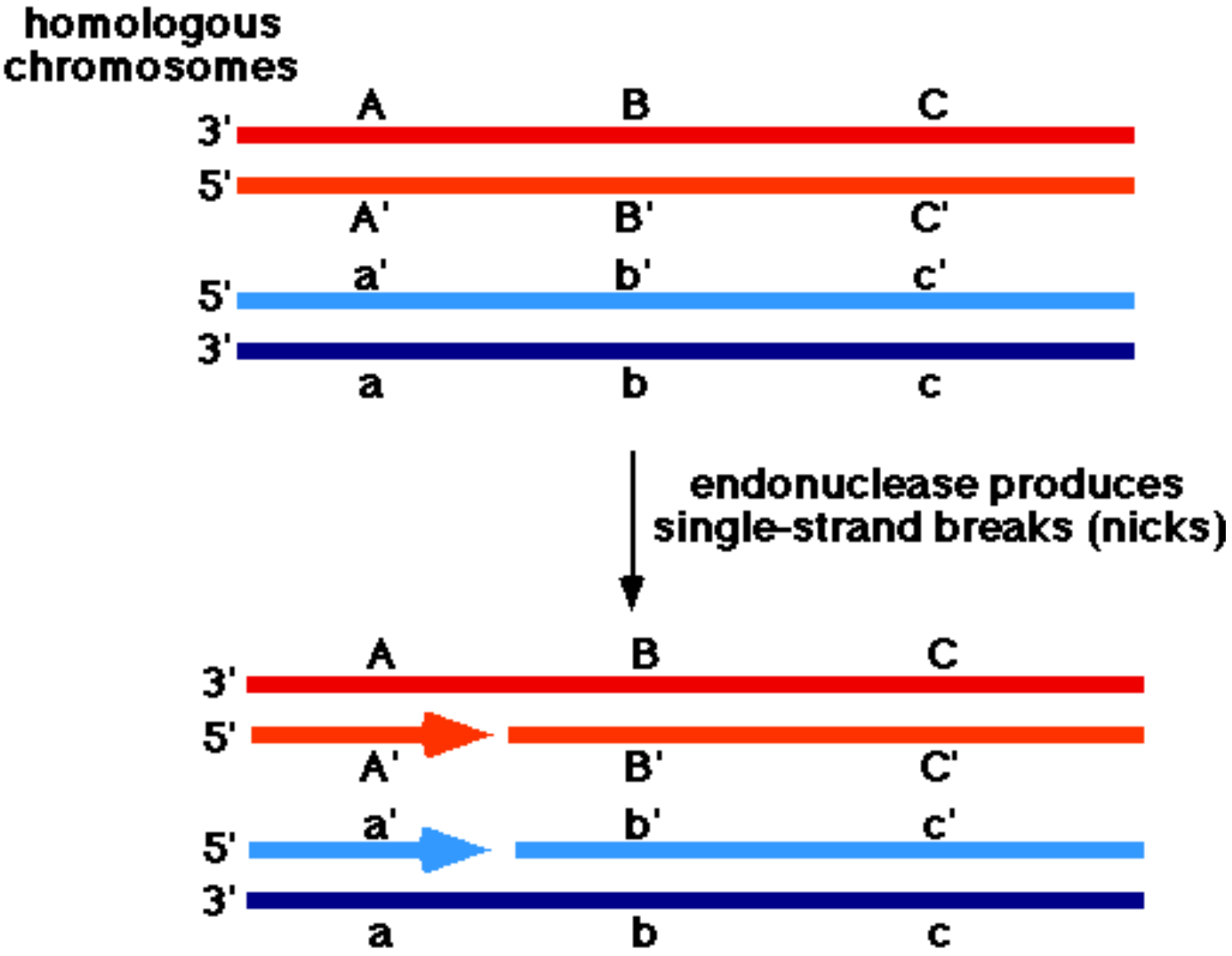


Re-shuffles linked alleles

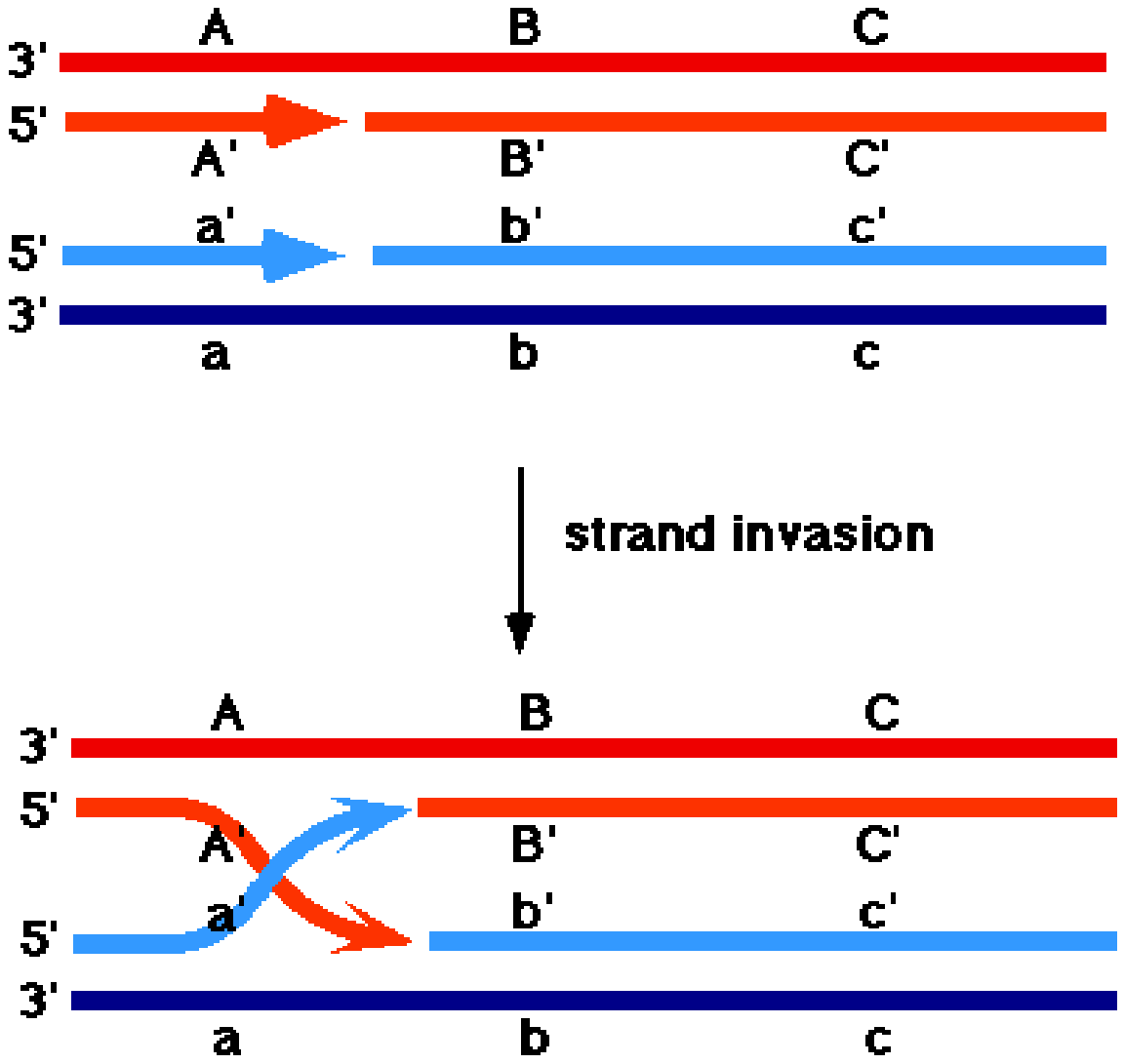
Recombination - HOLLIDAY model

- Pairing: align homologous duplexes
- Single strand invasion:
  - Endonuclease nicks at corresponding regions of the same strands of homologous chromosomes
  - Ends generated by the nicks invade the other, homologous duplex
  - Ligase seals nicks to form a **joint molecule.**
  - **("Holliday intermediate" or "Chi structure")**
- Branch migration expands heteroduplex region.

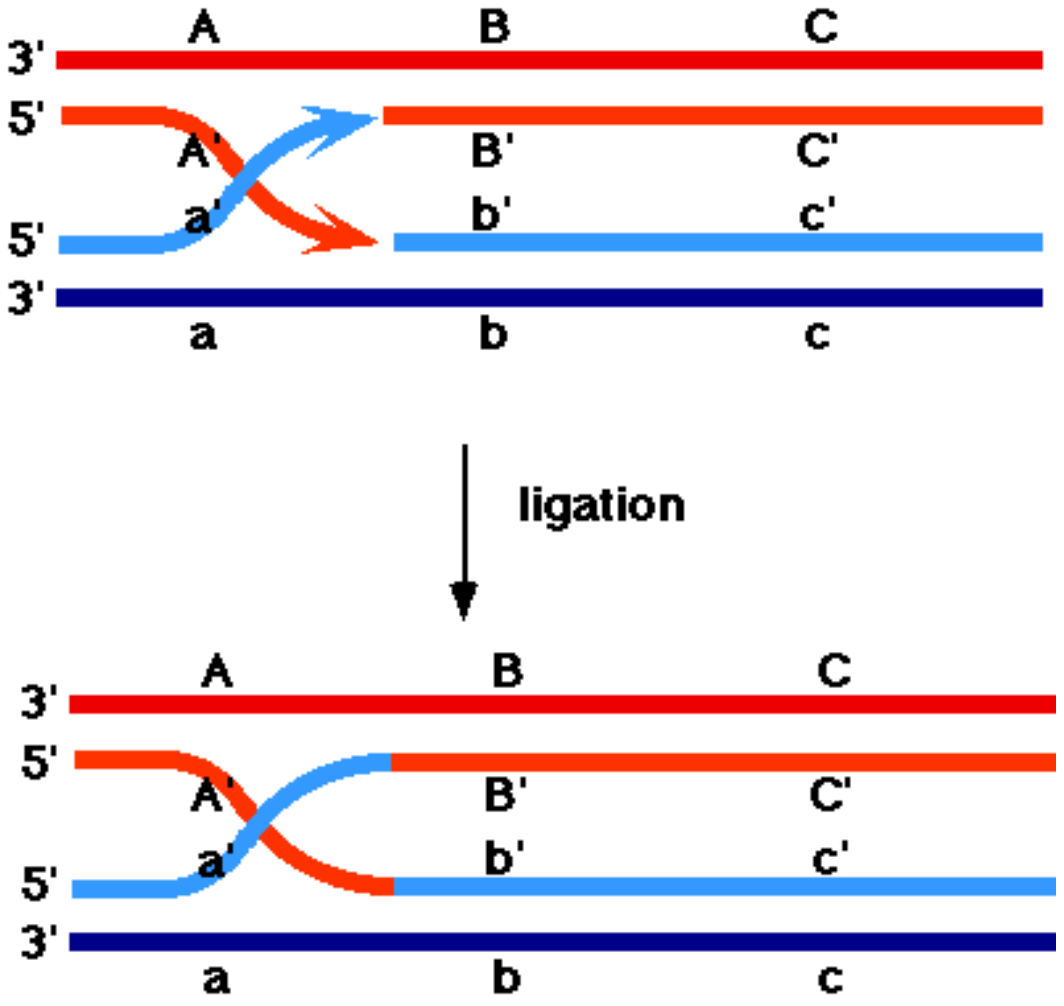
Recombination - HOLLIDAY model



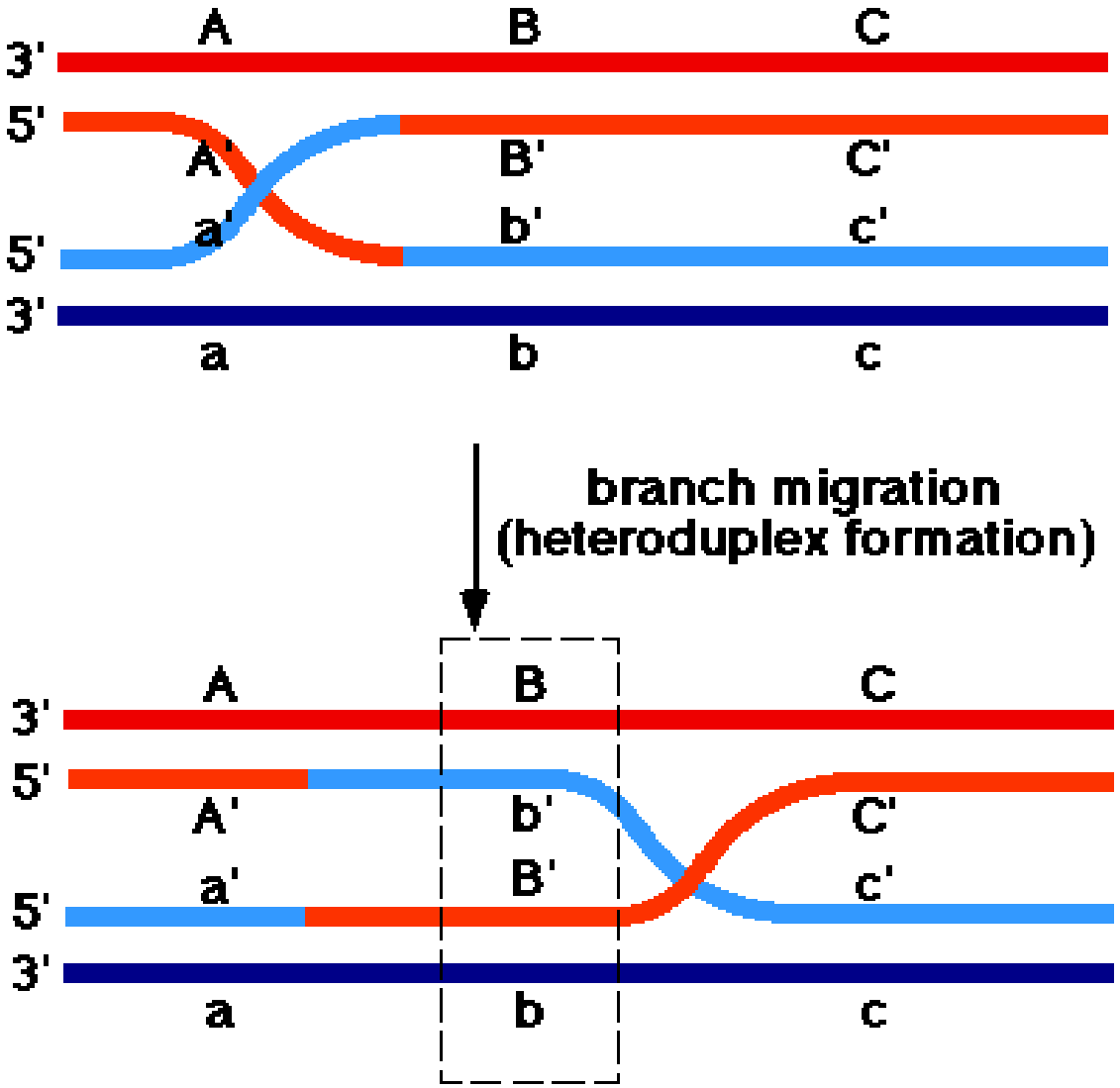
Recombination - HOLLIDAY model



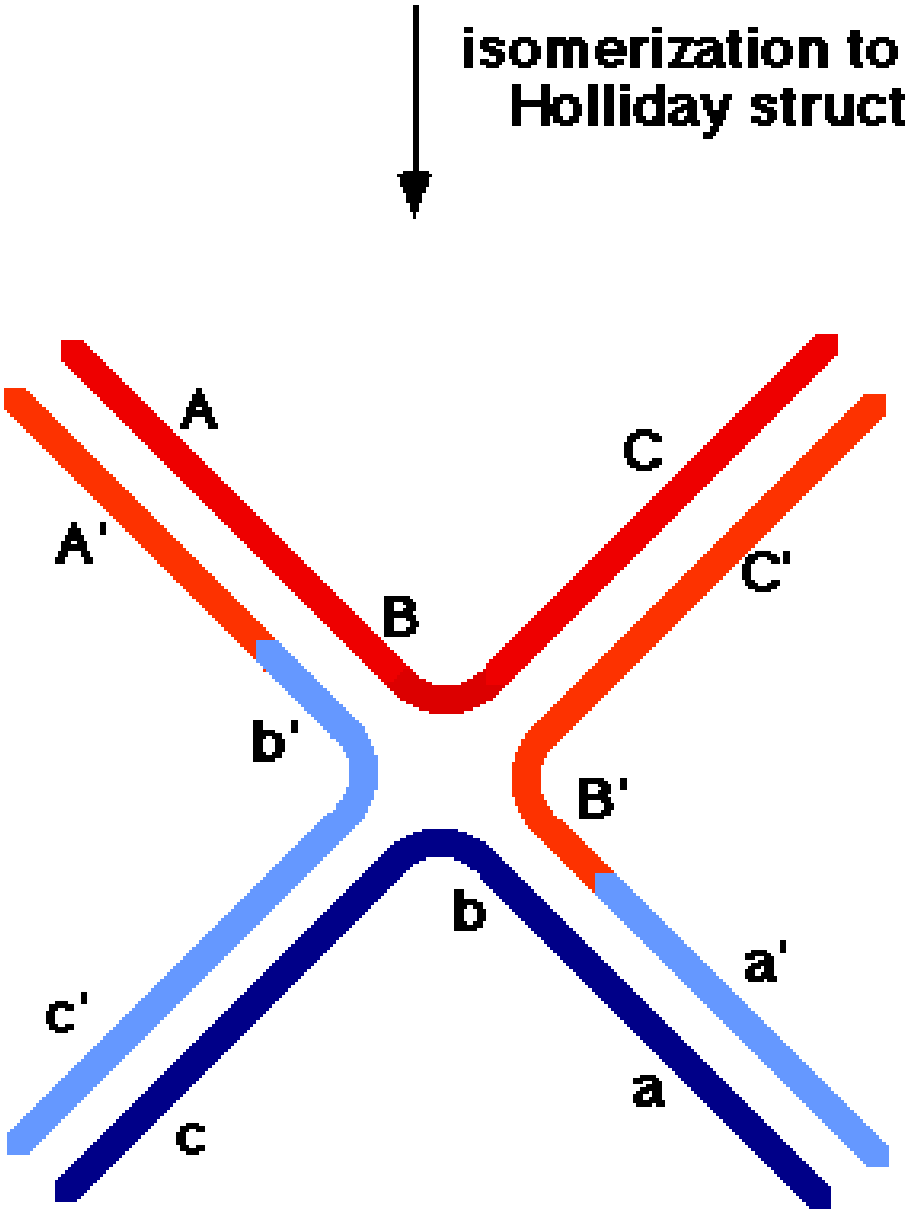
Recombination - HOLLIDAY model



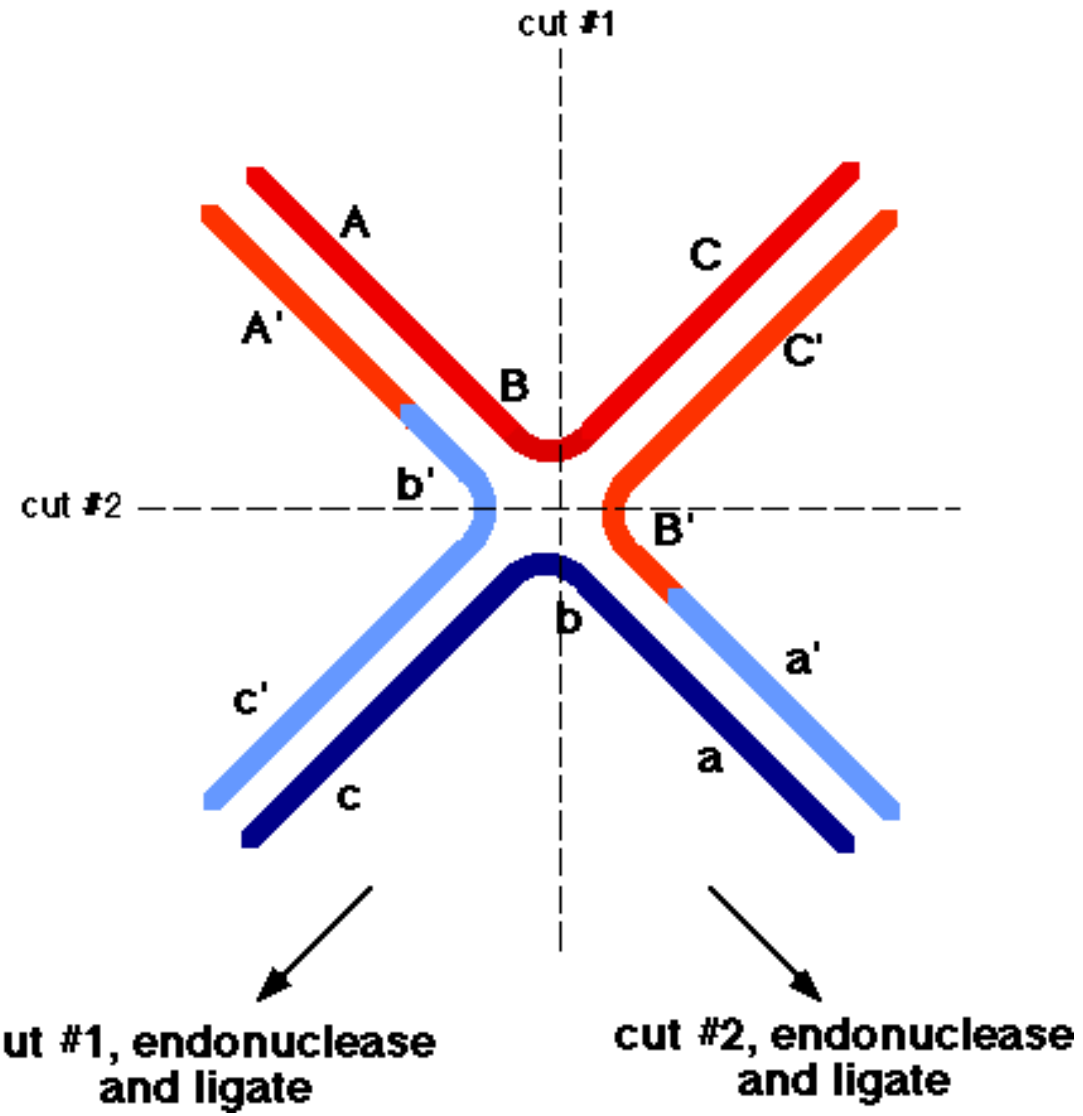
Recombination - HOLLIDAY model



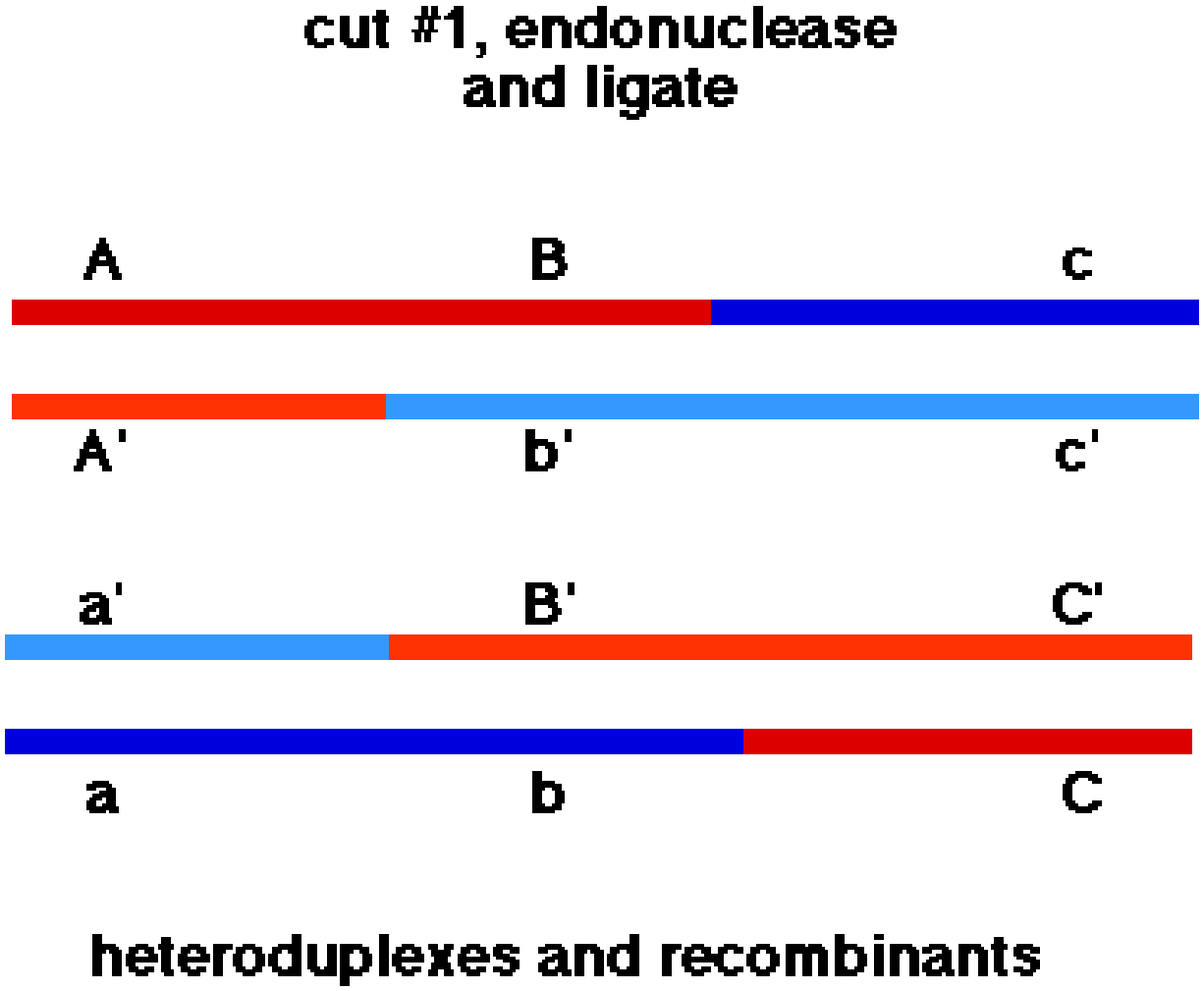
Recombination - HOLLIDAY model



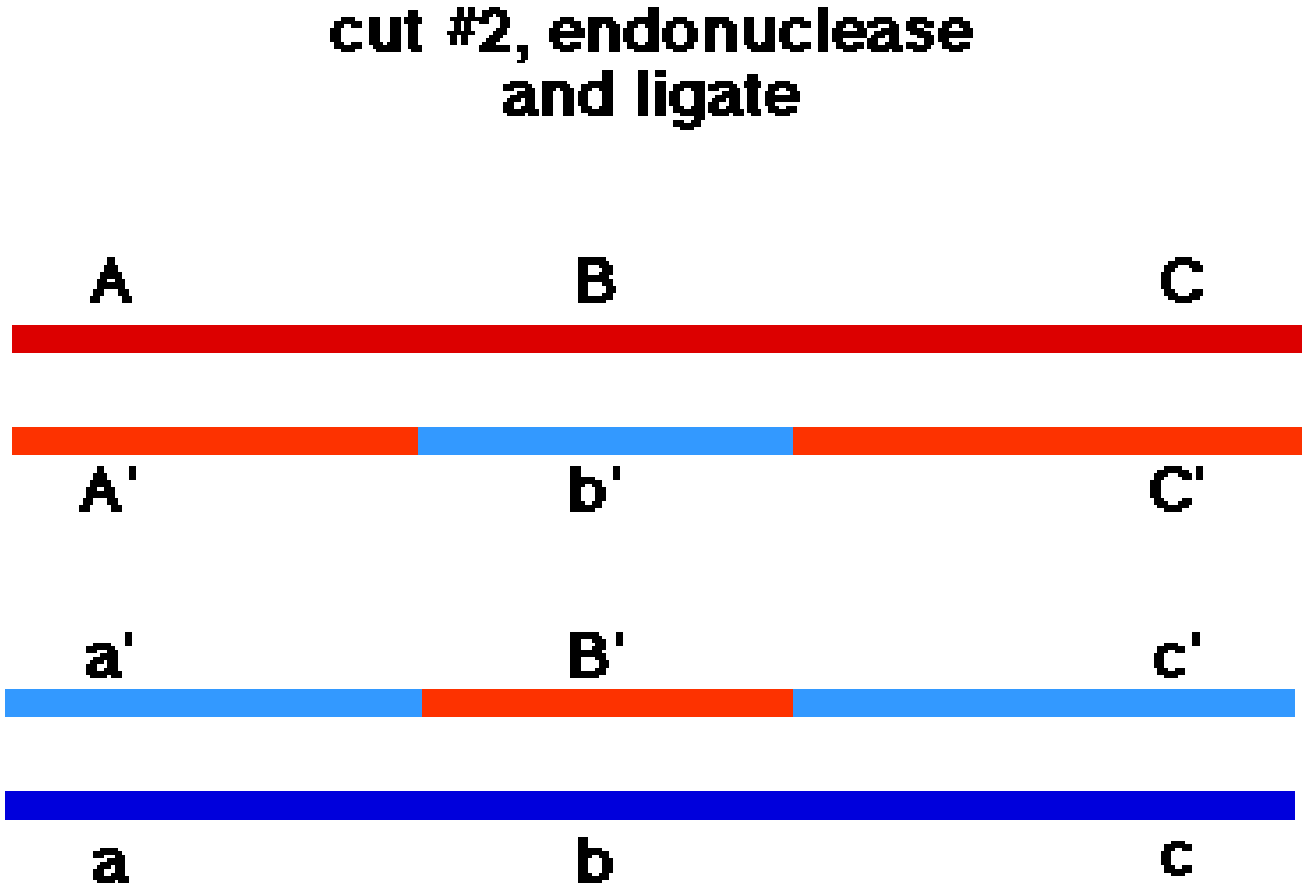
Recombination - HOLLIDAY model



Recombination - HOLLIDAY model



Recombination - HOLLIDAY model

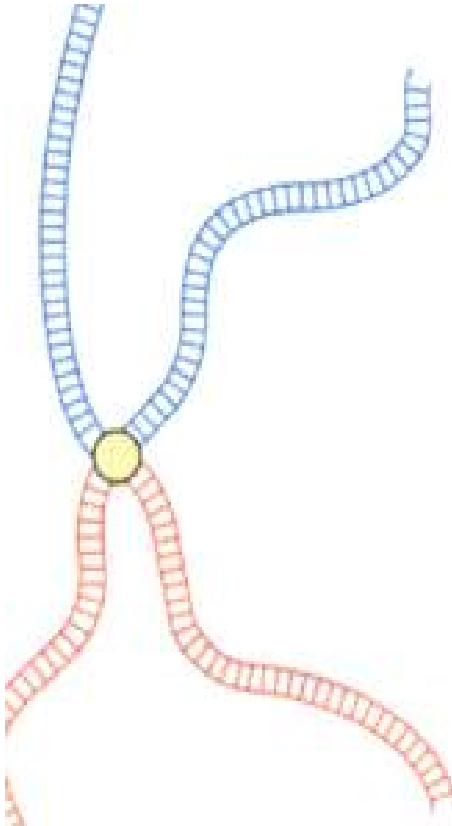


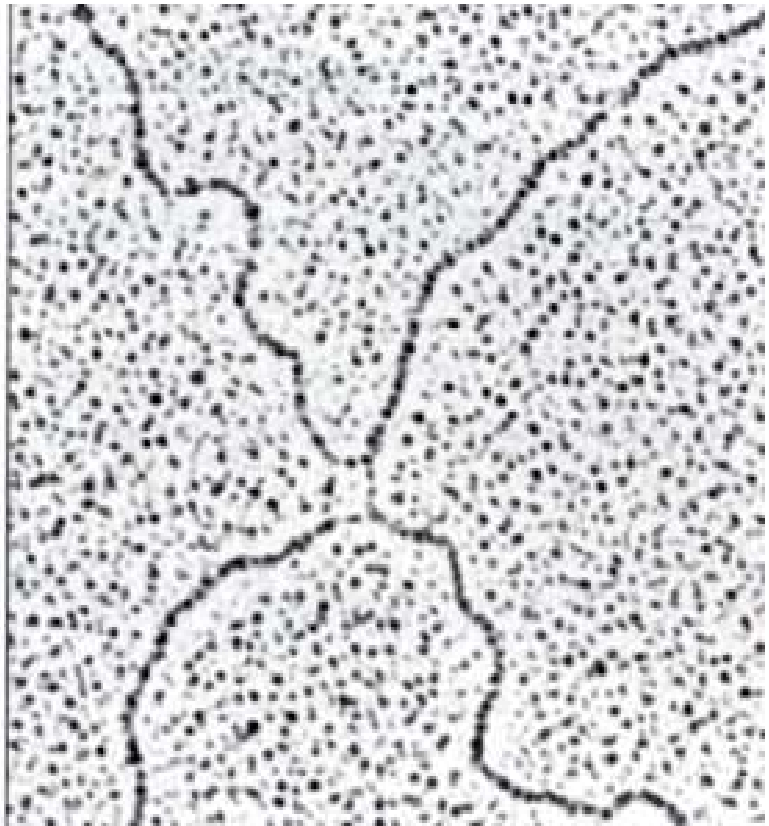
heteroduplexes but no recombinants

DNA Recombination : other models

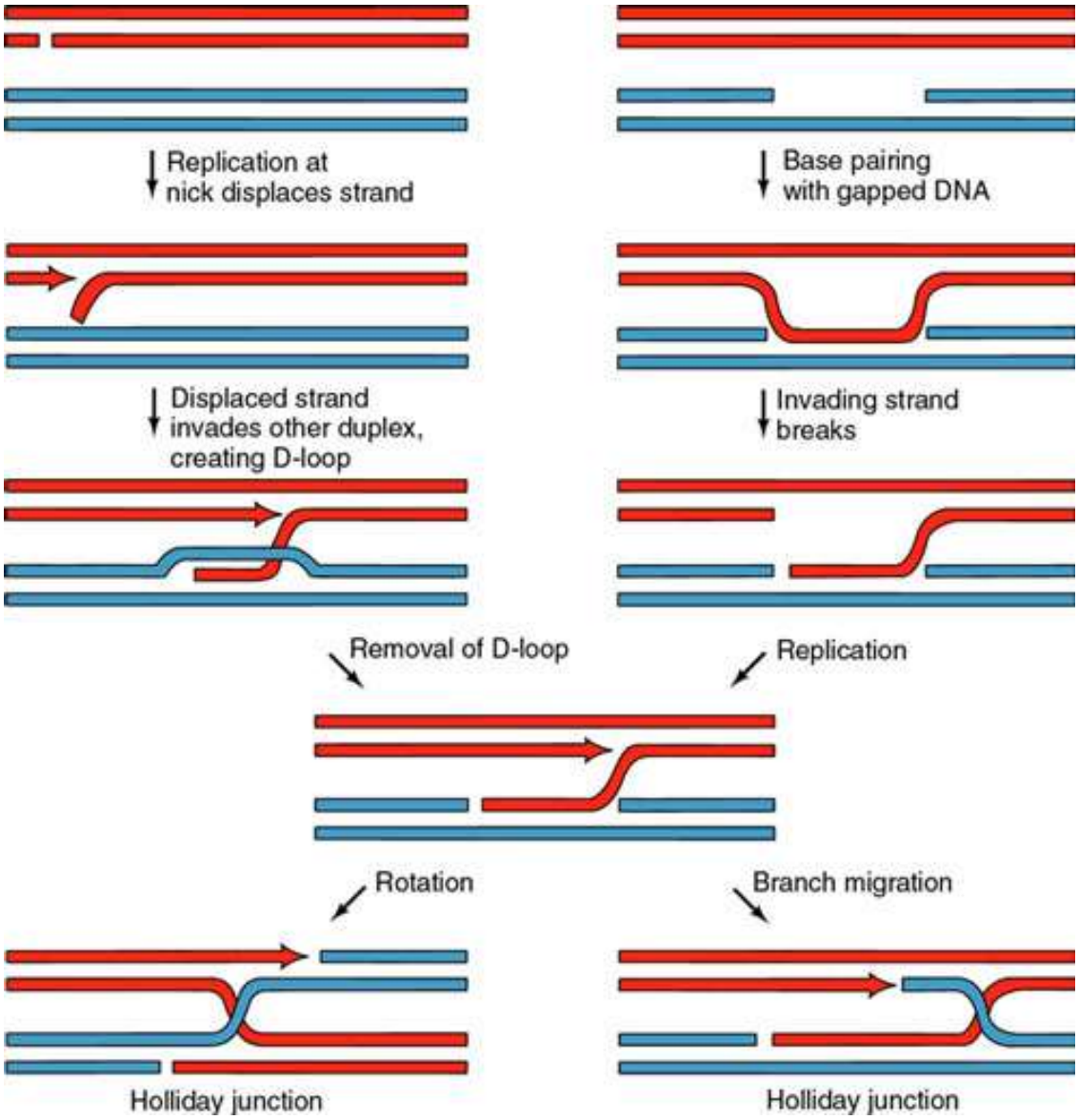
Three models:

Holliday model (Robin Holliday, 1964)
Single-strand invasion model (Meselson Radding)
Double-strand break repair model





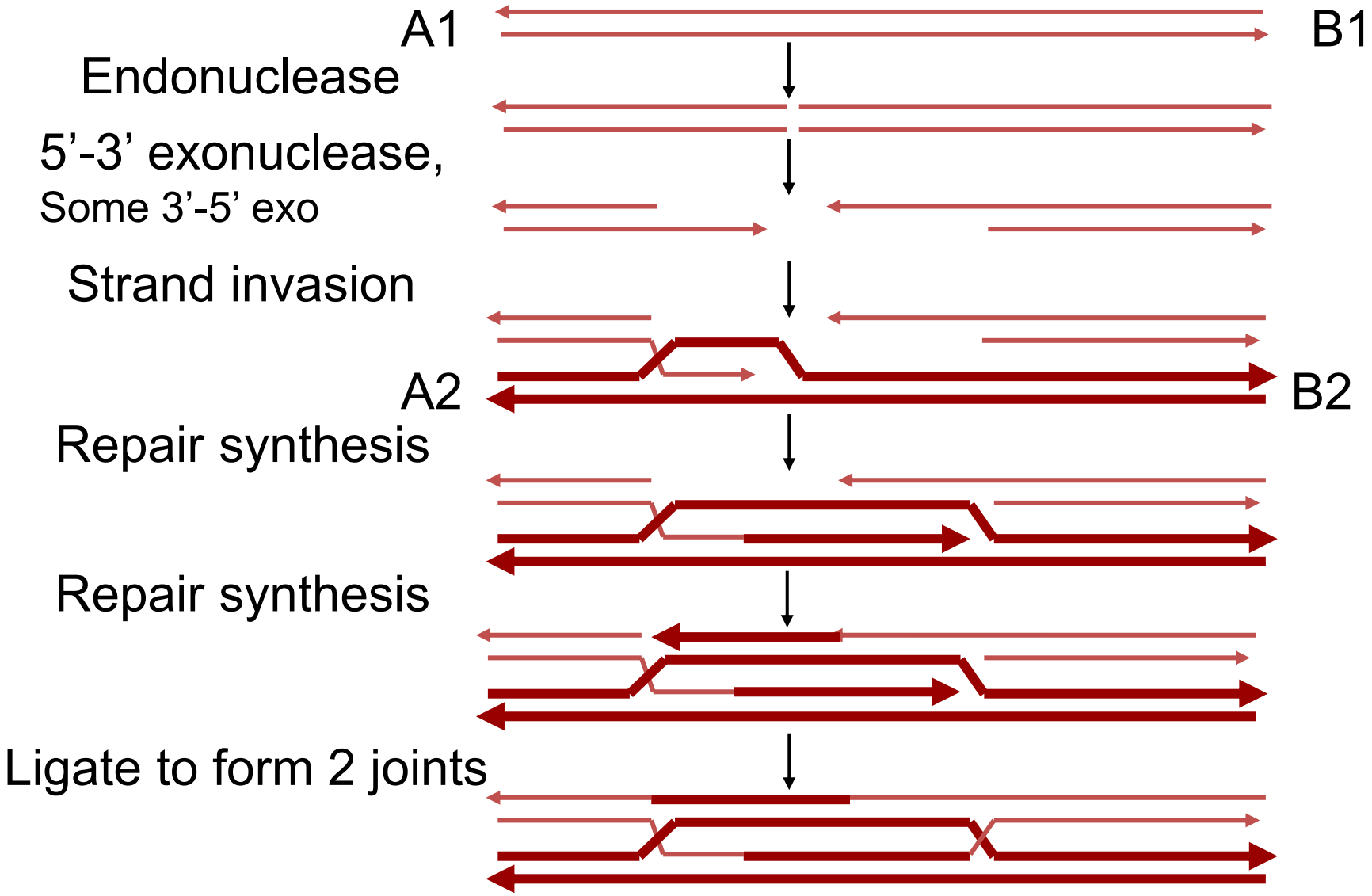
The Meselson-Radding Model



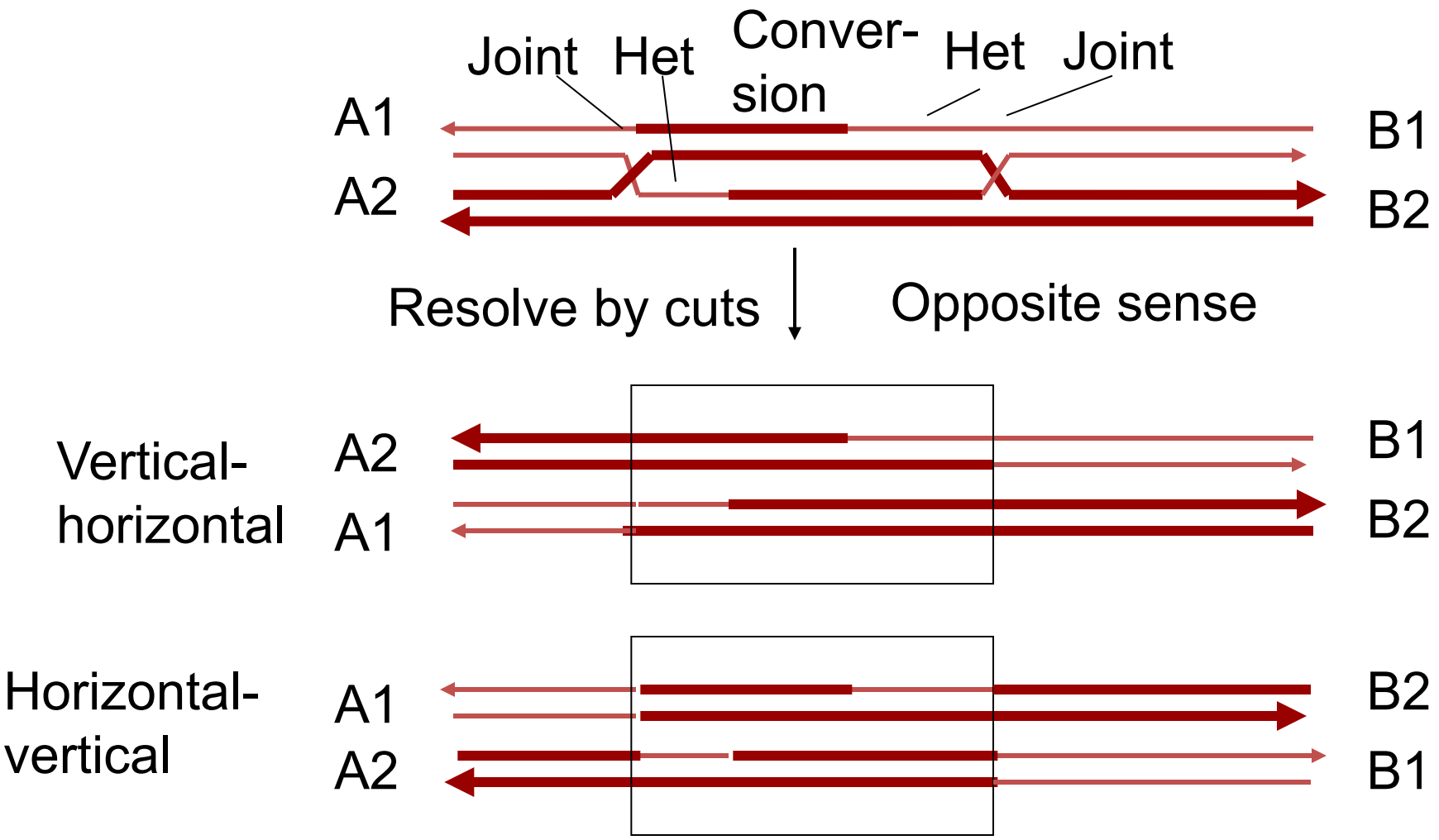
Double strand break model: Evidence

- This model provides a better explanation for recombination events in yeast:
- A double strand break precedes recombination.
- One DNA molecule is used preferentially as the donor of genetic information.
- Gapped substrates can initiate recombination and in the process be repaired

Steps in the double strand break model



Double strand break model: Resolution



See recombination of flanking markers.

Distinguishing features of the models

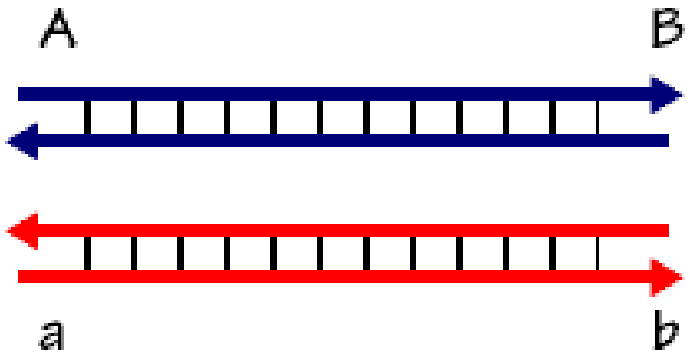
- Double strand break
  - The original gap in the aggressor (recipient) duplex now has the sequence of the donor duplex = conversion
  - Conversion region is flanked by heteroduplex asymmetrically (on “right” on one chromosome, “left” on other)
- Single strand invasion
  - Each chromosome has heteroduplex covering the region of the initial site of exchange to the migrating branch: heteroduplexes are in the same place on each chromosome

Common steps in models

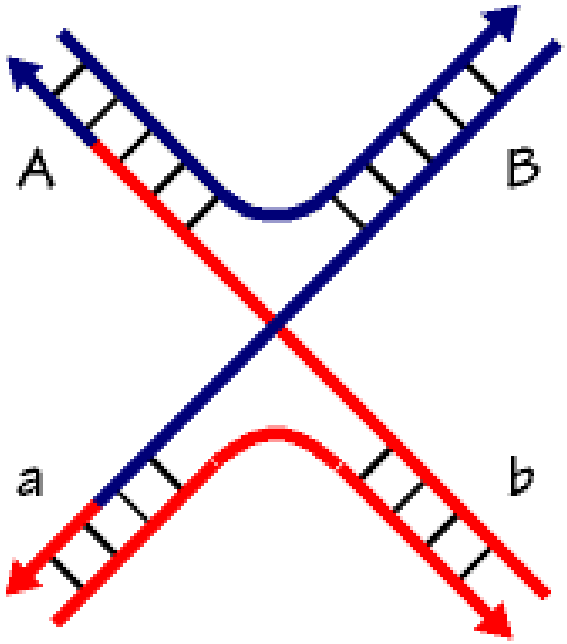
- Generate a single-stranded end
- Search for homology
- Strand invasion to form a joint molecule
- Branch migration
- Resolution

- Enzymes catalyzing each step have been isolated.

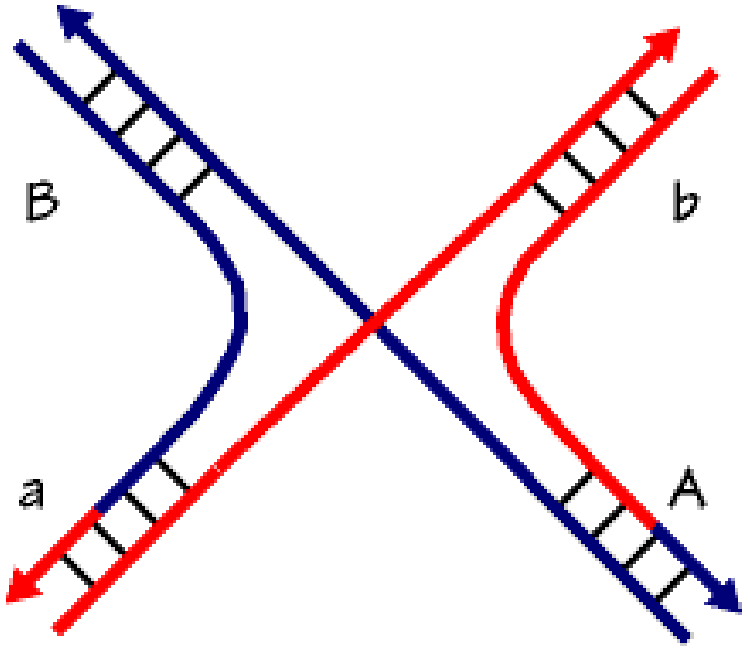
Recombination - HOLLIDAY model



Recombination - HOLLIDAY model



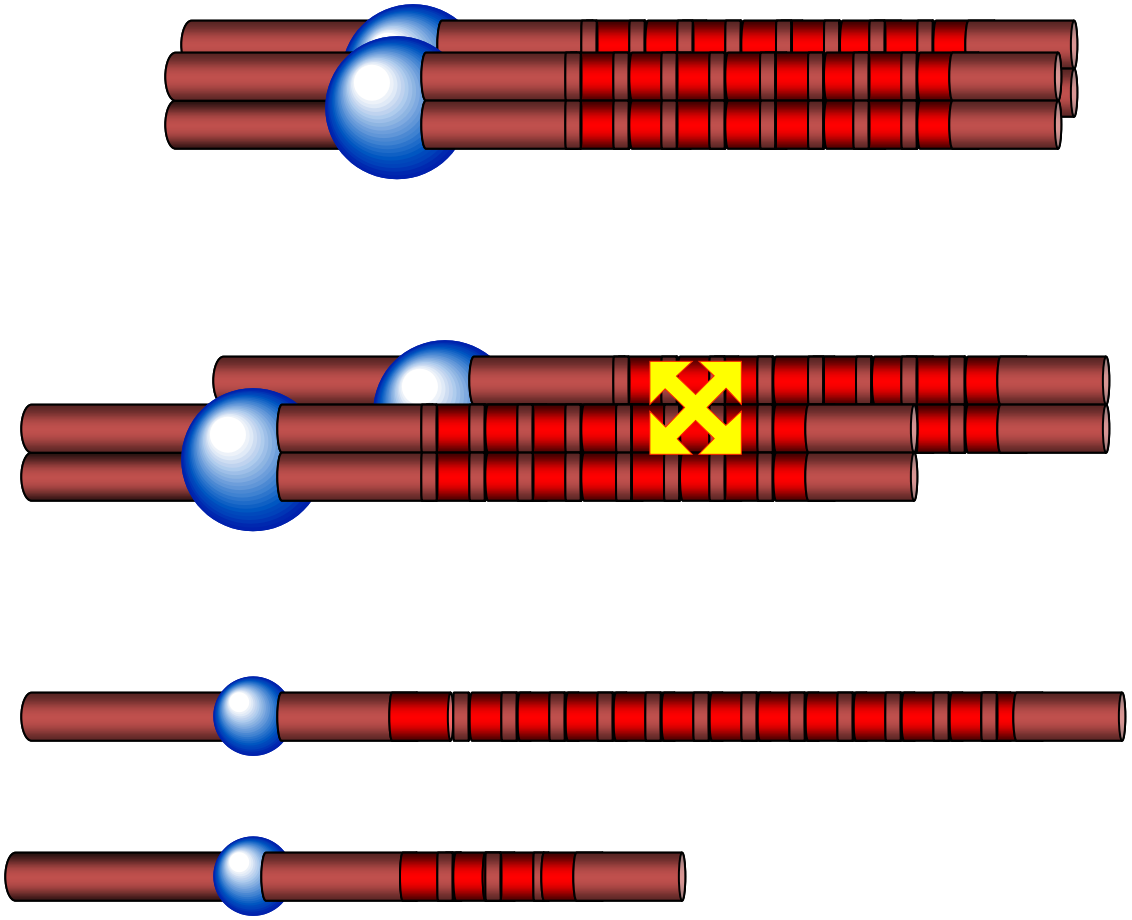
Recombination - HOLLIDAY model



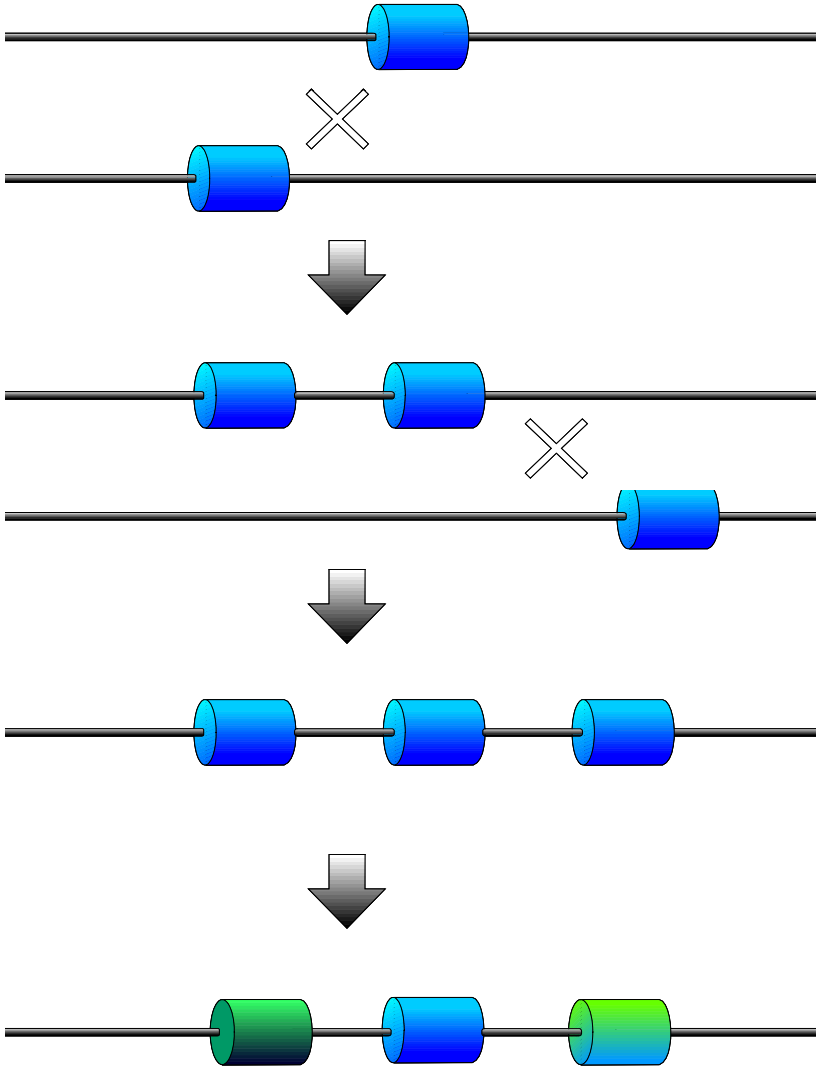
Unequal Crossing Over

- unequal crossing over is frequent in areas of direct repetitive sequences

- Unequal crossing over leads to an expansion/contraction of copy number of repeats



Genome Evolution and the Expansion of Gene Copy Numbers



1. Unequal crossing over between identical genes leads to gene duplication

2. Expansion of the array by additional unequal crossing over events

3. Genetic drift increases sequence divergence between flanking members of the array

Recombination : template, proteins and sites

- **DNA template**

- **Rec proteins: RecA, RecB, RecC and RecD**

- **Ruv proteins: RuvA, RuvB and RuvC**

- ***chi* sites - a nonsymmetrical sequence of 8 bp: 5'..GCTGGTGG..3'**
  - **-Occur naturally about once every 5-10 kb**
  - **- Stimulates recombination within its vicinity, up to 10 kb from the site**
  - **- Target site for RecBCD**

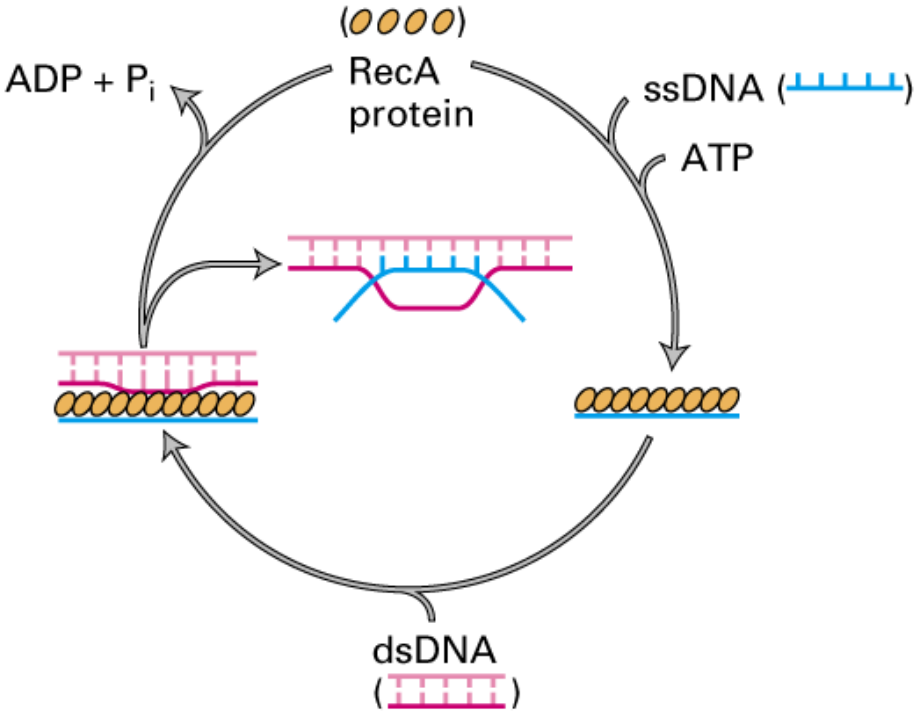
- **Resolution site: Hotspot for Holliday junction resolution, a tetranucleotide: ATTG; target site for RuvC**

Recombination is initiated from gaps, breaks or regions of ss DNA

- agents which promote DNA damage increase the rate of recombination
- **recA** protein recognizes ss structures and pairs with DNA duplex molecule
- **rec B,C** initiate recombination by nicking ds structures at specific **Chi sites**

DNA recombination in bacteria - RecA

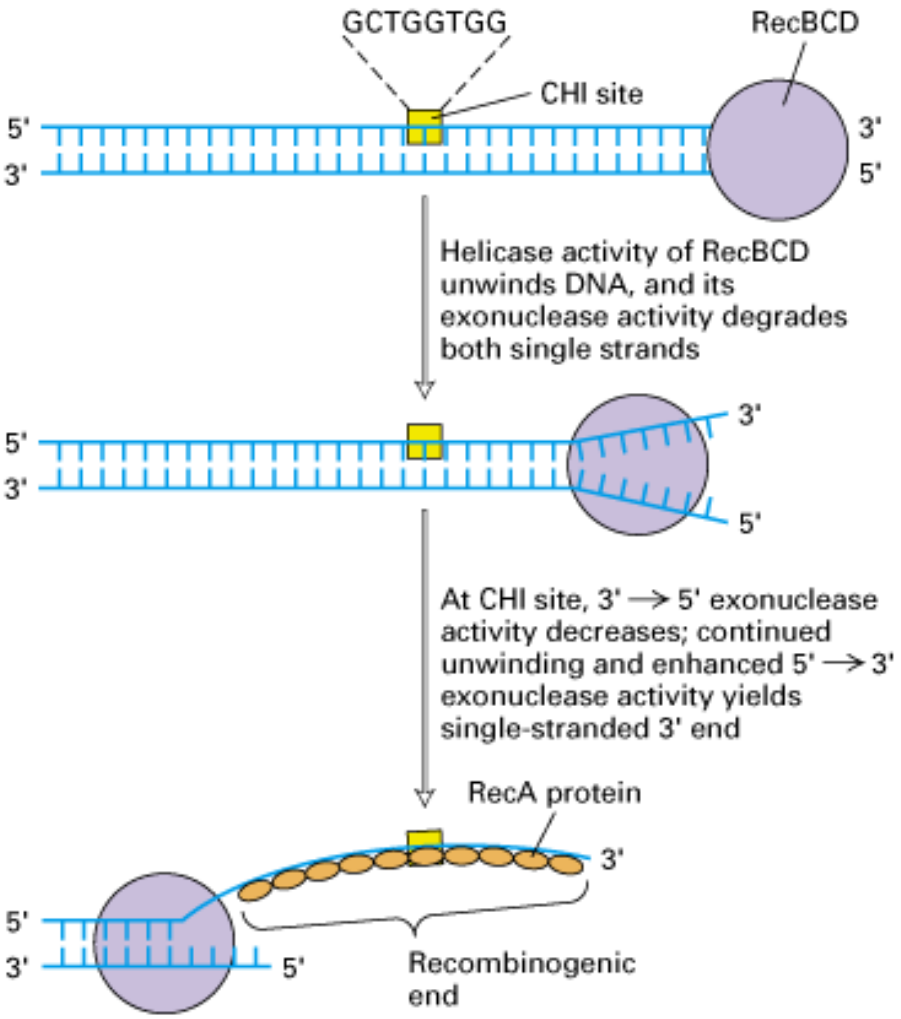
- **A DNA binding protein**
- **ATPase activity, polymerizes and forms filament along DNA**
- **Catalyzes strand exchange**
- **Promotes homologous pairing of the free 3' end released by RecBCD cuts at *chi***



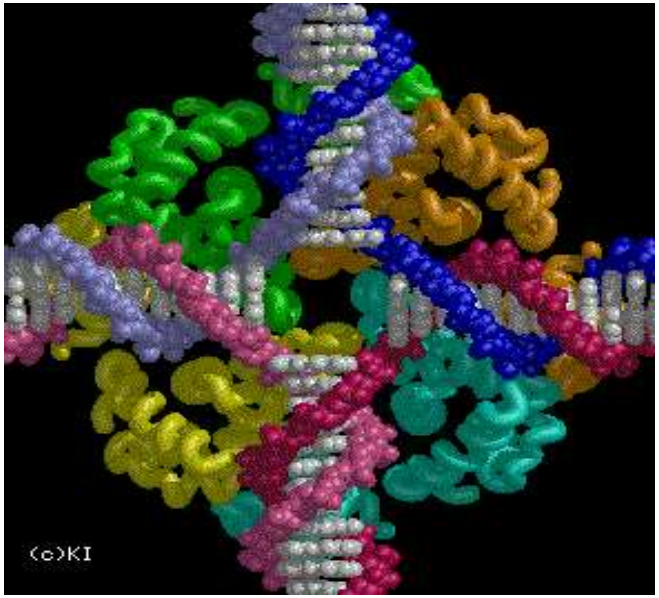
Rec Proteins: RecA, B, C, D

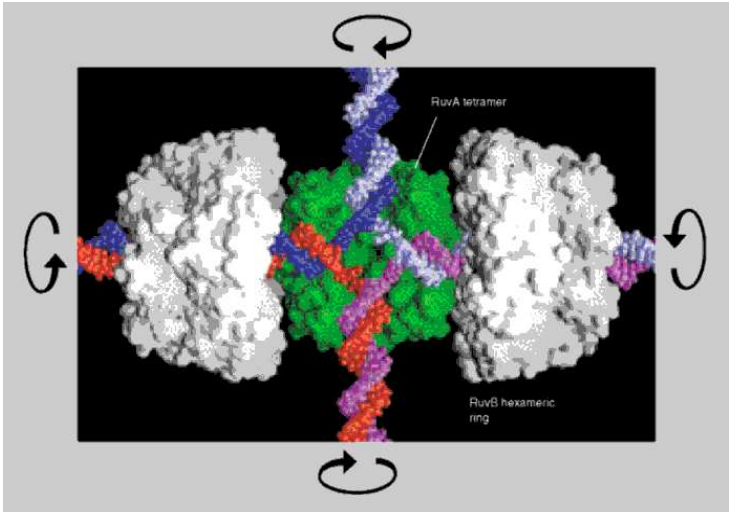
RecB, C, D

- Three proteins 130, 120 and 60 kD; multi-activity complex
- Recognizes CHI sites and binds to the right of this site
- Generates single strand nicks, unwinds the DNA
- Degrades the released single strand
- Upon reaching the *chi* site, RecD falls off thus loosing the nuclease activity
- RecBC continues as helicase



Ruv Proteins





- RuvAB: binds to Holliday structure

RuvA, recognizes the junction, tetramer that contacts all four strands

RuvB, hexamer that promotes branch migration through its helicase activity (motor), ATPase activity (energy)

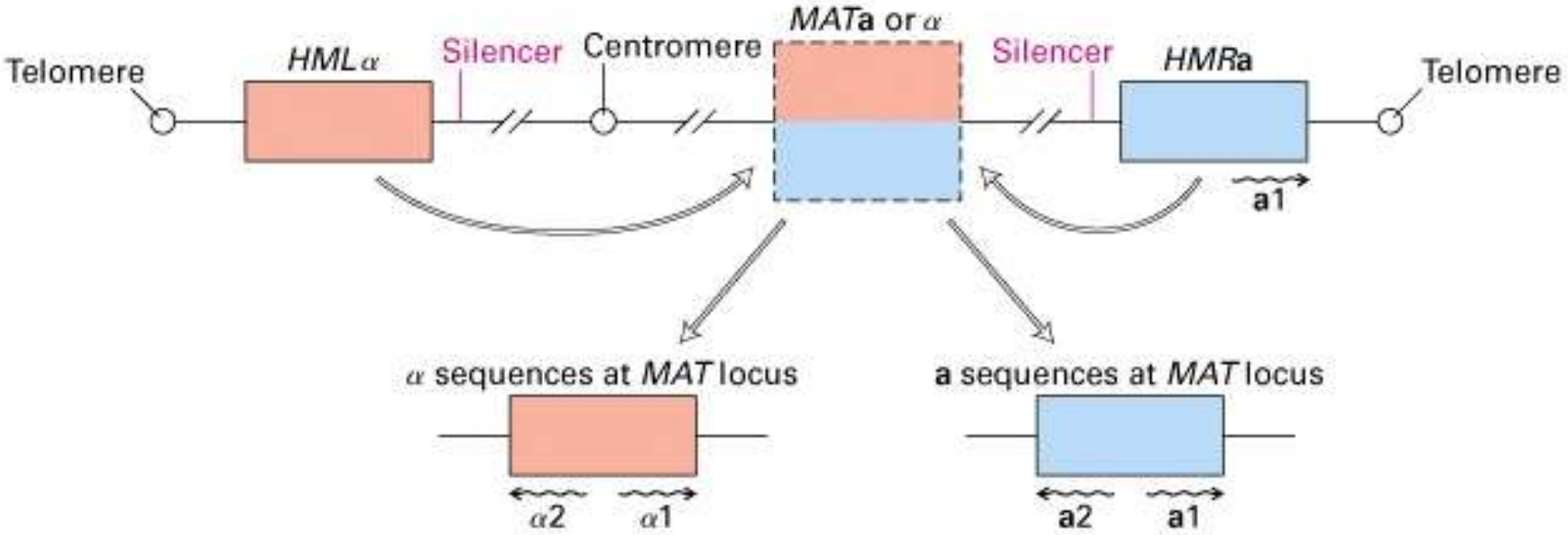
RuvAB cause the branch to migrate at 10-20 bp/s

RuvAB displaces RecA from DNA

RuvC, an endonuclease; hot spot for RuvC is a tetranucleotide, ATTG; resolves the Holliday junction; resolve it in either North-South or West-East pattern

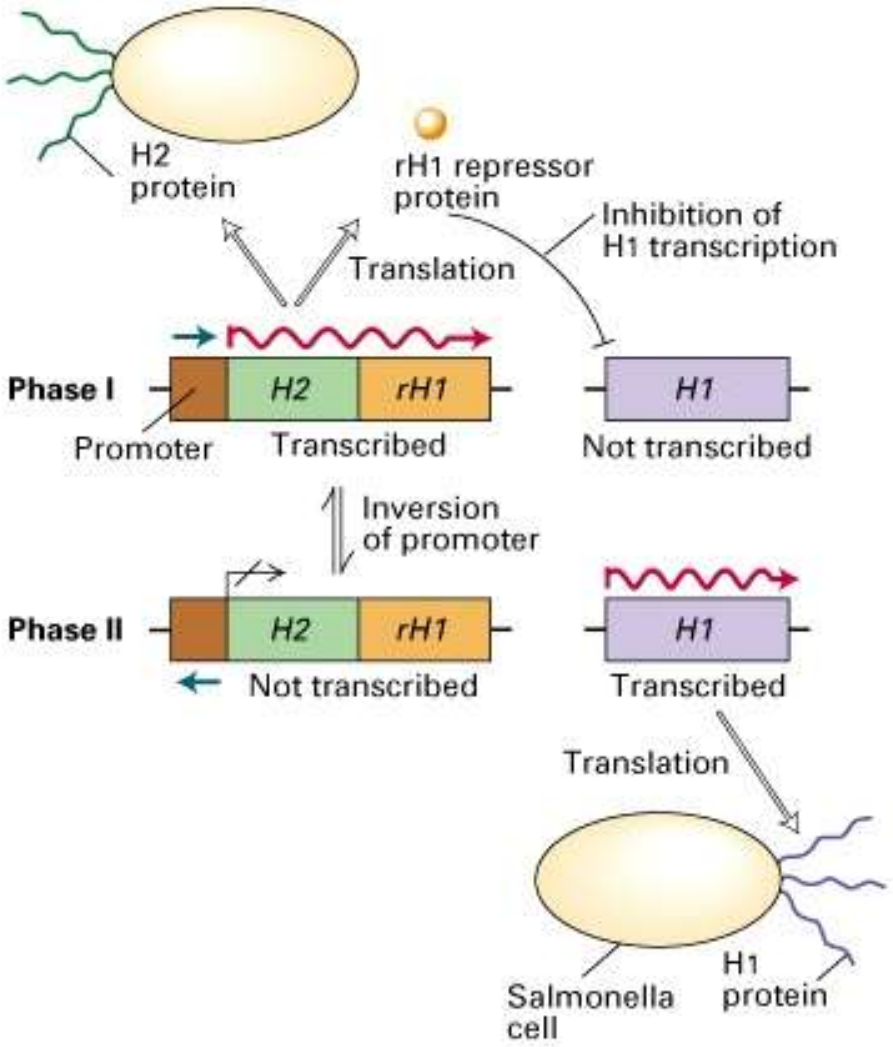
Functional Gene Conversion

- Single-stranded DNA from either HML or HMR invades the MAT locus
- Heteroduplex forms
- Mismatch repair changes allele at MAT



Recombination can control gene expression

- antigen switching in *Salmonella* involves site-specific intragenomic recombination
- somatic cells may undergo substantial genome rearrangements by recombination
  - the generation of antibody specificity



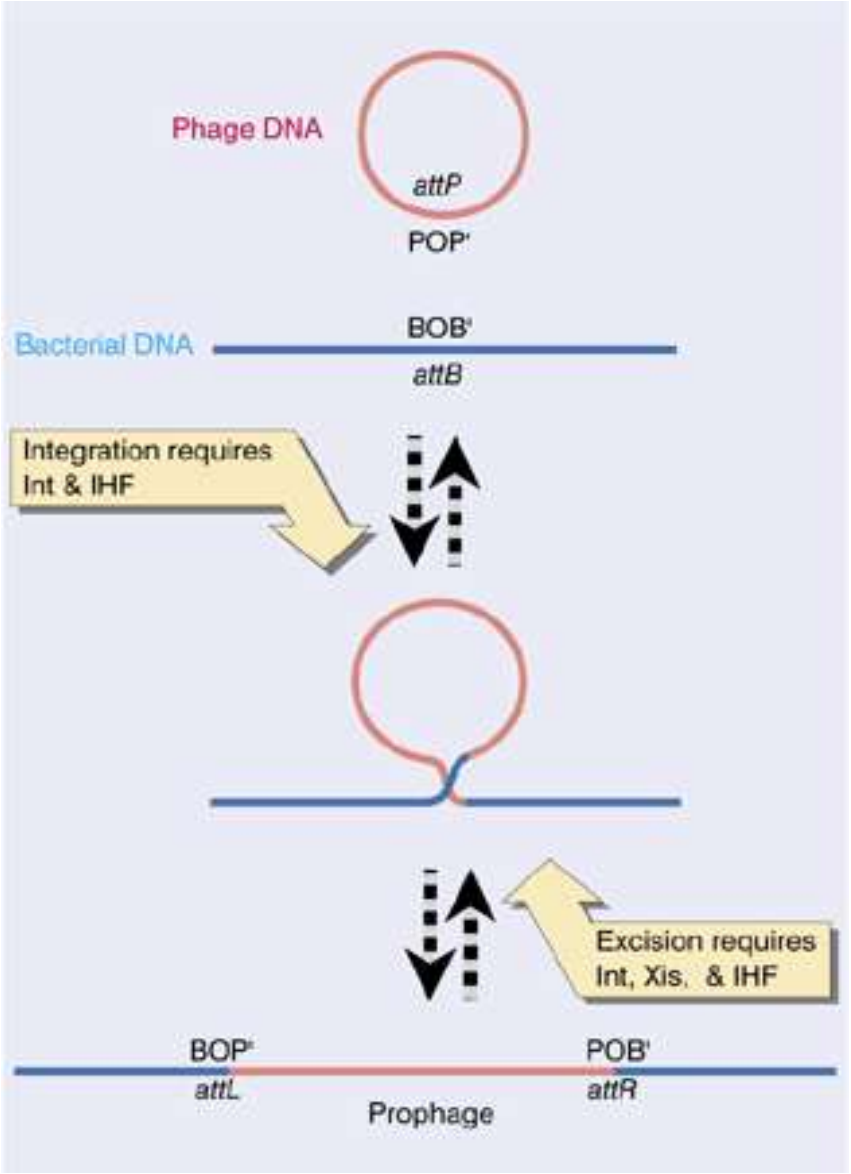
Inverted Repeats Flanking the Promoter Region Lead to Frequent Inversion and Prevent Transcription.

Specialized or site-specific recombination

- DNA sequences are moved by site-specific recombination
- not guided by extensive sequence homology (as in meiotic crossing over) but by short enzyme recognition sequences (recombinases)
- phage *lambda* integration at *att B, P* sites via integrase
- P1 phage recombination via Cre protein at Lox P sites
- RuvA, B, and C proteins in *E. coli*

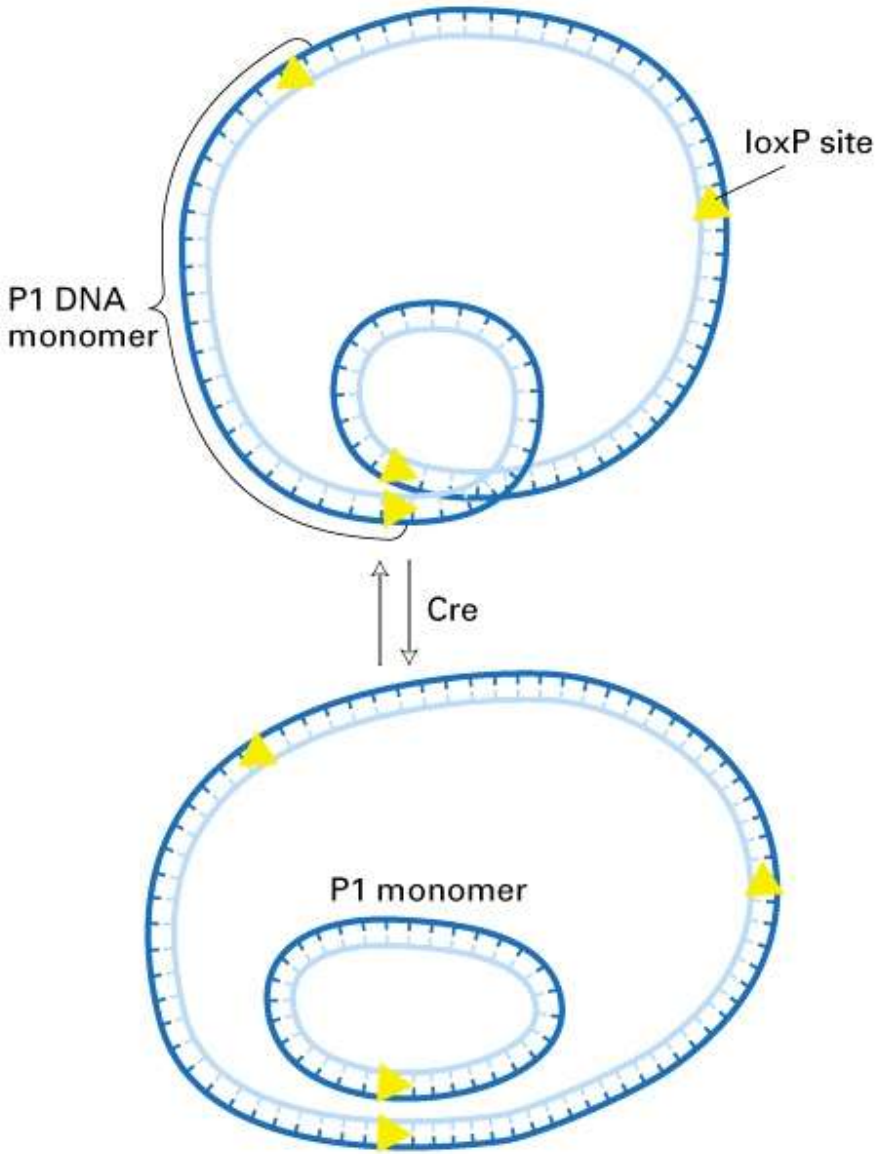
Integration of bacteriophage lambda

Site	Core DNA Sequence
attP	tCAGCTTt tTtatAc tAAGTTGg
attB	cCTGCTTt tTtatAc tAACTTGa
proB	tgcGCTaa tTtatAc gAgGCTac
trpC	gCgtaaTg tTtatAa atgGCgGc
galT	cgcctTTg tTttcAa aAACCTGc
thrA	cggGCTTt tTtctgt gtttCctg
rrnB	ttgGCTat tTtacca cgACTgtc

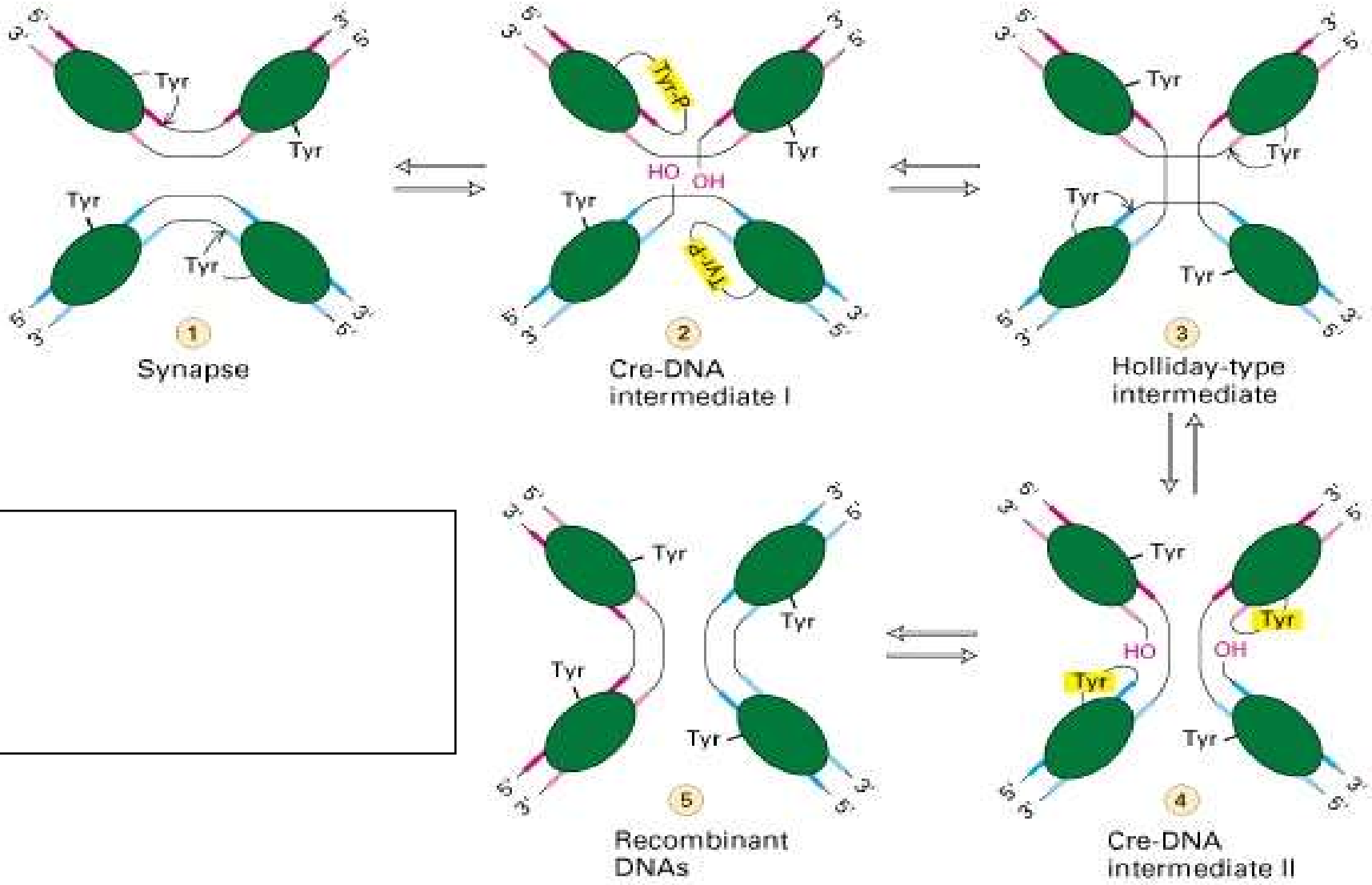


P1 phage recombination

- Cre protein produced by P1 phage
- loxP site recognized by Cre
- Generation of circular monomeric forms from multimers



P1 phage recombination

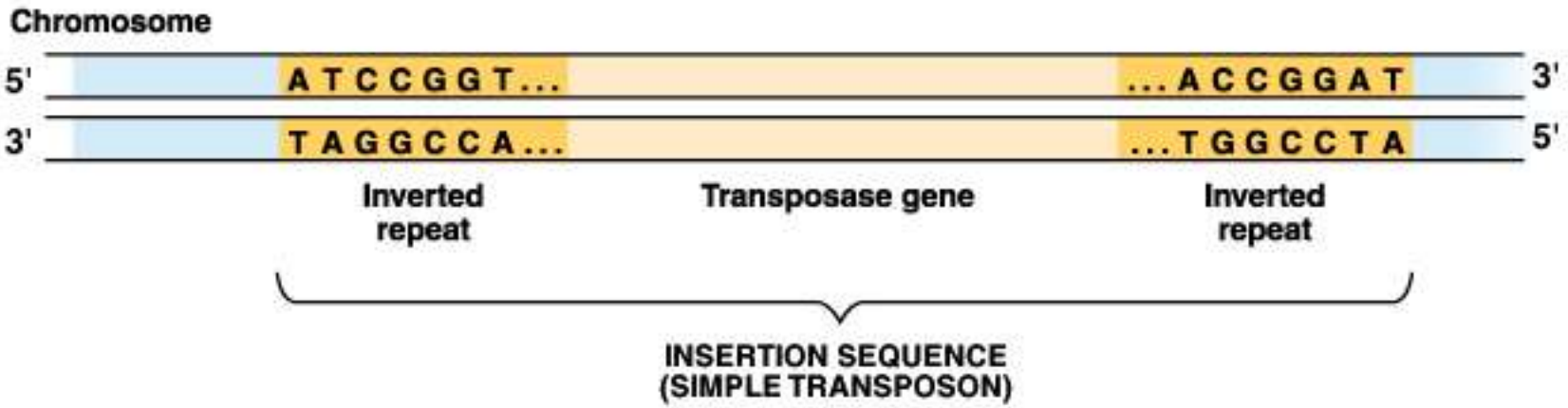


Transposons

Transposons (a transposable genetic element) is a piece of DNA which can move from one location to another in a cell's genome

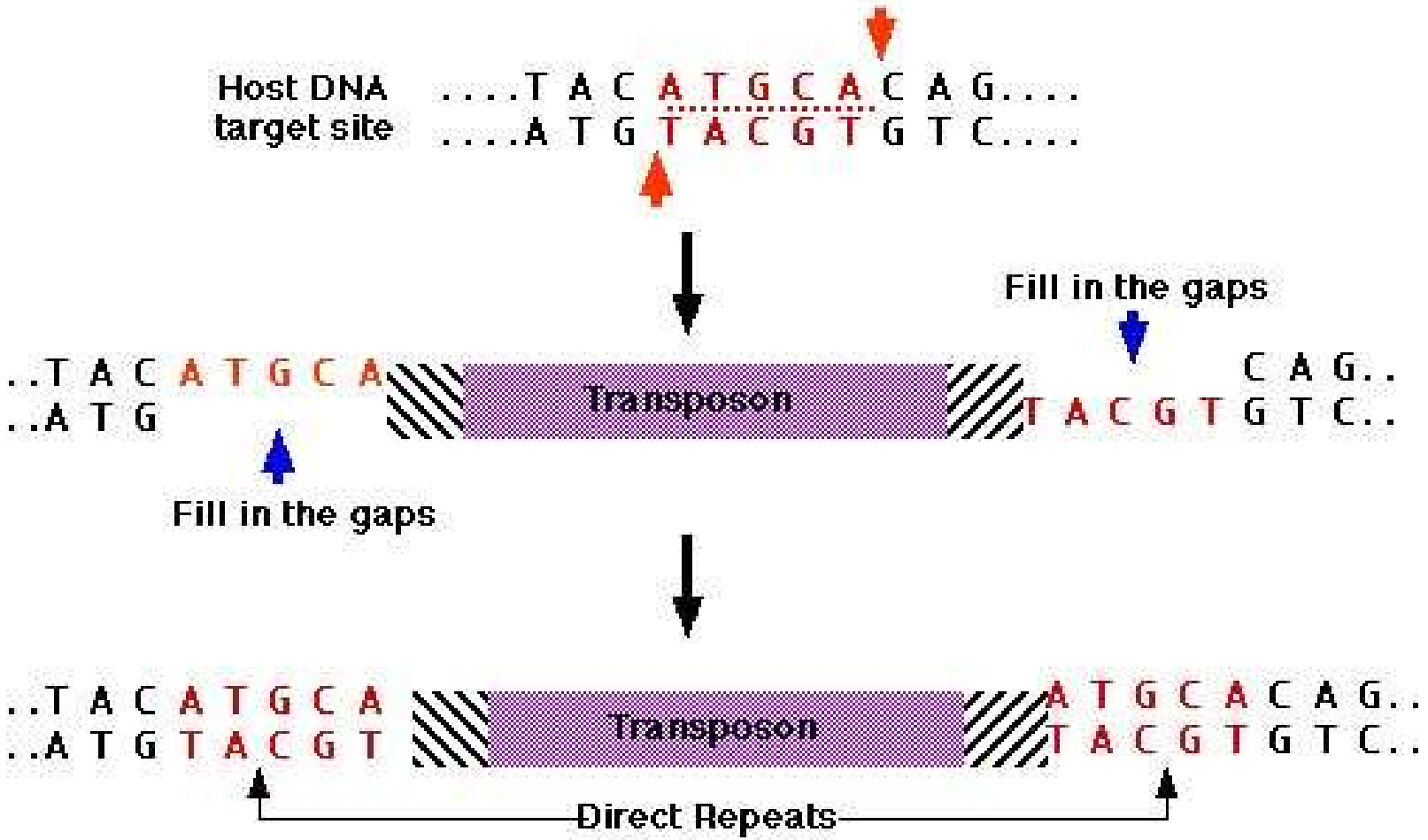
Transposons are characterized by inverted repeats. Many tranposons encode a enzyme called a transposase which is responsible for catalysing DNA cutting and resealing.

Some transposons in bacteria carry antibiotic resistance genes. Multicoloured "Indian" corn is due to transposons.

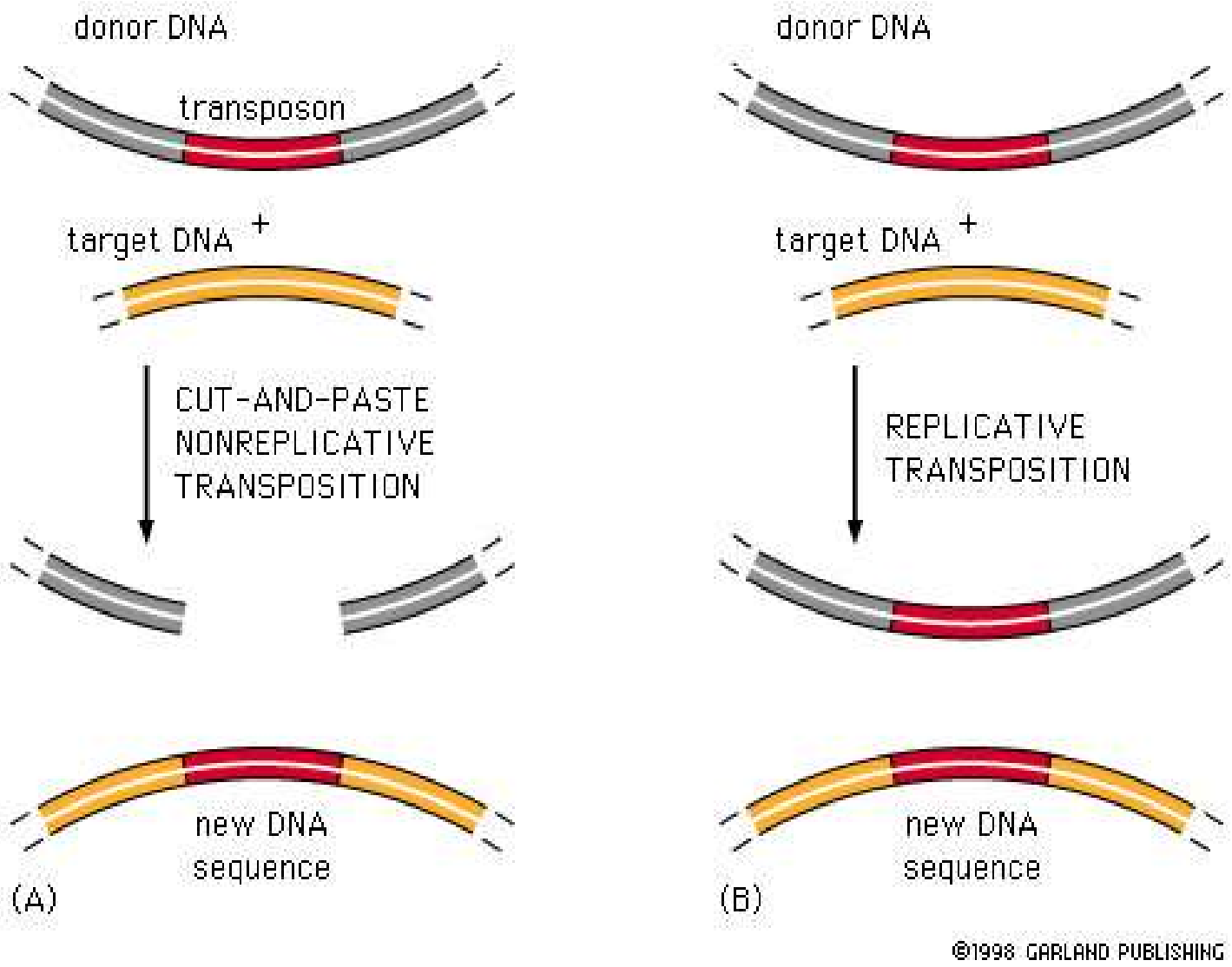


Illegitimate recombination - transposons

duplication of DNA ends



Transposonsition mechanisms



DNA recombination in bacteria

Asexual proces – transfer DNA from one bacterium to another

- **CONJUGATION: DNA is transferred from a "donor" bacteria to a "recipient" cell in a process that requires cell contact; usually plasmid mediated.**
- **TRANSFORMATION: the cell takes up DNA sequences from the environment**
- **TRANSDUCTION: bacterial DNA is carried into the cell by a phage particle.**

Conjugation

- **Transmission of a single strand of DNA between cells in contact with each other (DNAse resistant).**
- **Dependent on systems encoded by "conjugative elements"; frequently transmissible plasmids. All include genes that allow intercellular contacts to be established, and DNA to be transported from one cell to another.**
- **Gm(-) bacteria contain many transmissible (Tra^+) plasmids; extremely common in Enteric (*E. coli, Shigella, Salmonella*, etc.) and *Pseudomonas*. All express conjugative pili (various types) as an aid to establishing cell contact.**
- **Gm(+) systems establish cell contact differently (no pili). Conjugative plasmids have been described for *Enterococcus, Bacillus, Staphylococcus, Streptococcus*, and others. In some cases (*Streptococcus; Enterococcus; Staphylococcus*), transfer systems encoded by a "conjugative transposon" have also been found.**
- **Bacteria can transfer DNA by conjugation to many types of cells, including bacteria in different genera. F plasmid mediated conjugative transfer of DNA from *E. coli* to yeast has been demonstrated. Conjugation is used by *Agrobacterium* to transfer tumor-inducing genes to plants.**

Conjugation - the F factor (F plasmid)

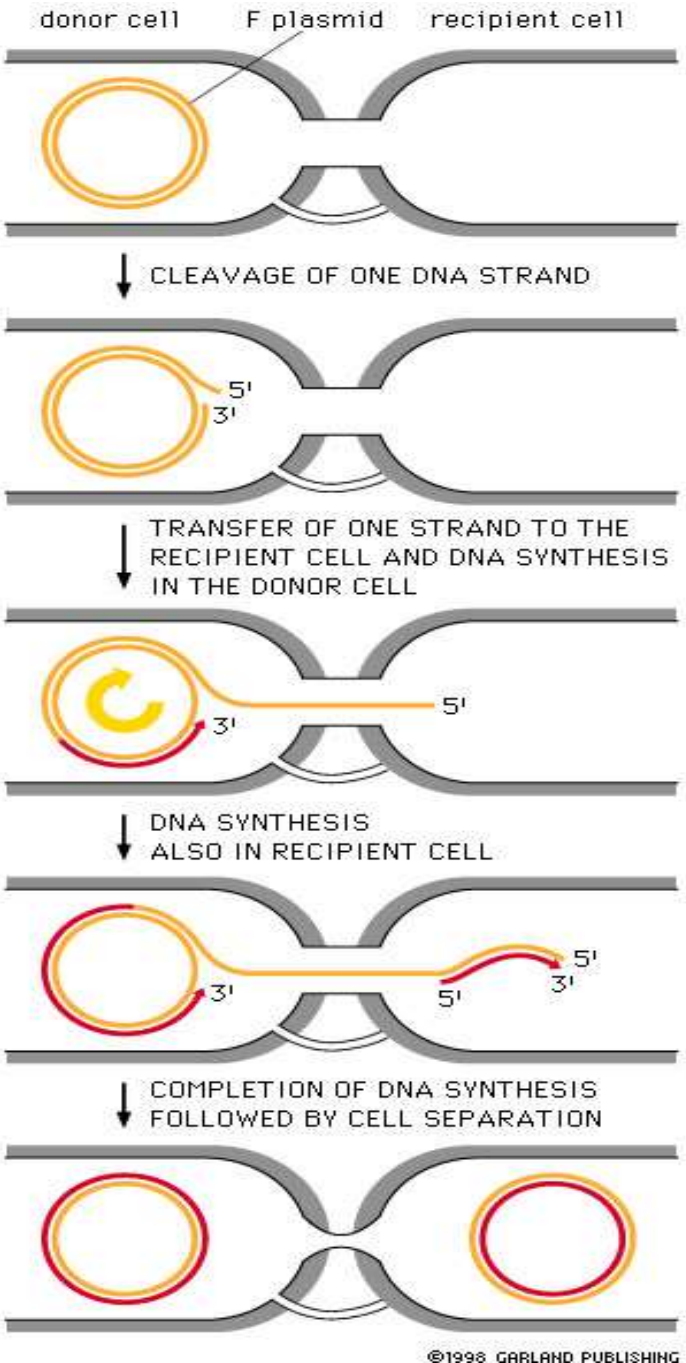
- **First conjugative plasmid identified (found in *E. coli* K12; many F-like plasmids found since).**
- **F is a 100 kb circular dsDNA, low copy number (1-2/cell).**
- **The F transfer system is encoded by a 34 kb region; expression is "derepressed" due to spontaneous mutation.**

Conjugation mechanism (F^+)

- **Contact stage:**
  - **1. F-pilus synthesized by donor (F^+).**
  - **2. The tip of the F-pilus contacts the F^- cell surface.**
  - **3. The cells come into surface contact and a mating channel is formed through pilus retraction.**
- **DNA transfer stage:**
  - **1. A nick is introduced in one strand of F DNA at a specific site: the F origin of transfer (*oriT*).**
  - **2. Starting at the 5' end, the nicked single strand is displaced, transported to the recipient and recircularized.**
  - **3. DNA replication in the donor replaces exported strand; in the recipient the complementary strand is made. The recipients become F^+ "transconjugants".**
- **Note: In a mixed population of F+ and F- cells, "epidemic spread" will soon convert all cells to F+ donors.**

Conjugation

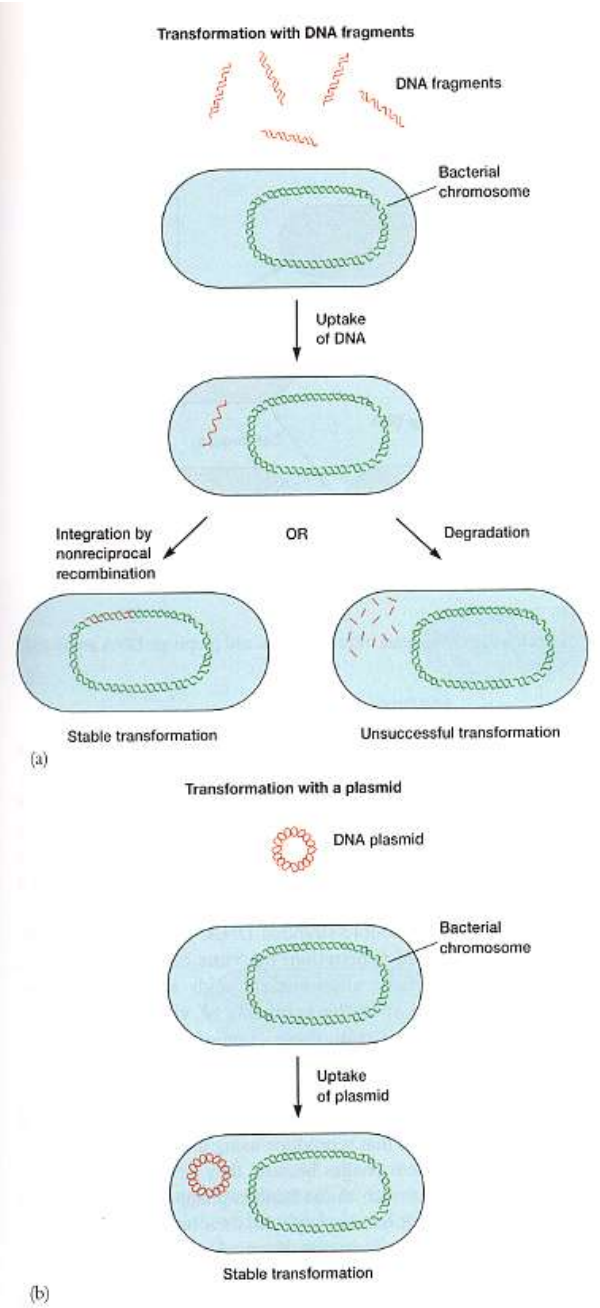
Synthesis and transfer of the F plasmid is accomplished by the rolling circle mechanism of DNA replication.



Transformation

1. Uptake of linear, naked DNA
- **-some bacteria are naturally competent and will take up DNA**
- **-sometimes the DNA recombines into host**
  - **Example: *Neisseria pil* genes**
- **-sometimes DNA gets degraded.**

2. Uptake of circular, naked DNA
- **-usually more efficiently maintained particularly in *E. coli***
- **-not degraded as readily**
- **-can replicate autonomously**

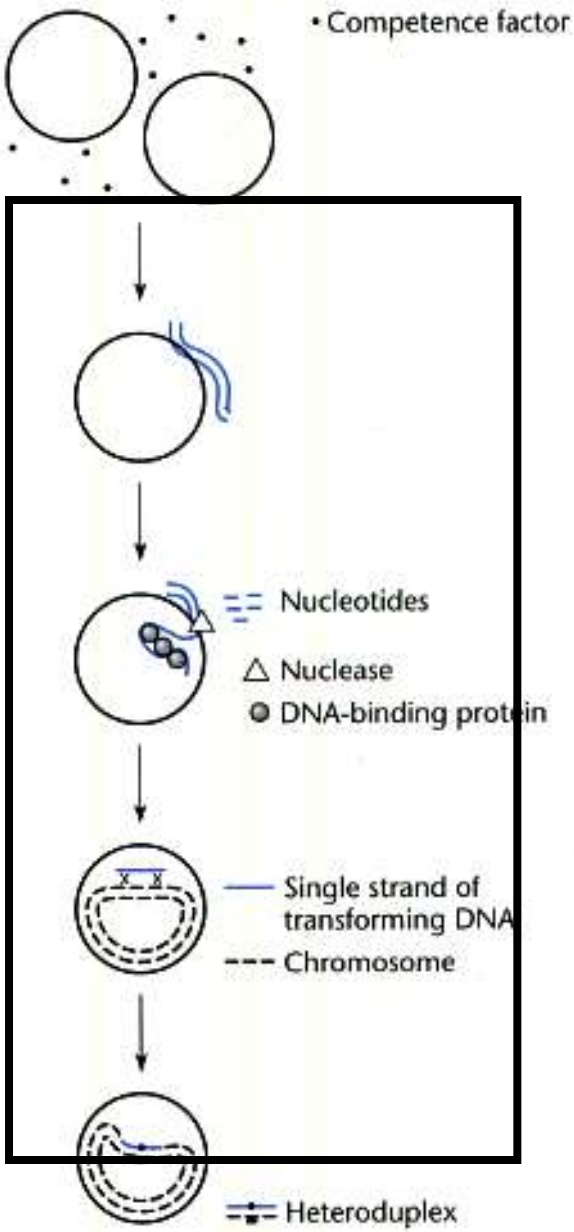


Transformation

- **Transformation systems differ among bacterial genera.**
  - **Competence: Ability to bind DNA and take it up.**
    - ***Streptococcus pneumoniae, Bacillus subtilis, Haemophilus influenzae, Neisseria gonorrhoeae* are naturally able to take up DNA under particular culture conditions.**
  - **Artificial competence: High [Ca^{2+}] treatment renders *E. coli* transformable and allows efficient uptake of double-stranded, closed circular DNA.**
  - **Electroporation allows DNA to enter many types of cells**
    - **Exposure to electric field forms transient "holes" that DNA can traverse**

Transformation

- **Gm(+) transformation systems (*Streptococcus pneumoniae*)**
  - **Cells reach competence at density 10^7-10^8/ml as competence factor (CF) accumulates, inducing transformation system.**
  - **Any type of double stranded DNA binds reversibly, then irreversibly to the cell surface.**
  - **As DNA is transported into cell, it is cut (in the membrane) into smaller pieces; one strand is denatured, so only single stranded fragments enter.**
  - **These strands can displace a strand of homologous DNA; recombinants (transformants) are formed.**



Transformation

- **Gm(-) transformation systems**
  - **Conditions for competence vary; no extracellular factors.**
  - **The DNA may be required to contain certain sequences (indicative of species relationship). *Haemophilus influenzae* only takes up DNA with sequence AAGTGCGGTCA.**
  - **Linear ds-DNA fragments are transported into the cell; recombination with homologous sequences occurs.**
  - **Antigenic variation of *Neisseria gonorrhoeae* pili can stem from transformation and recombination with *pil* genes from other *N. gonorrhoeae.***

Transduction

- **DNA is transferred from one bacteria to another in a bacteriophage particle.**
- **Two types: generalized transduction & specialized transduction.**
- **These differ in mechanism and outcome.**

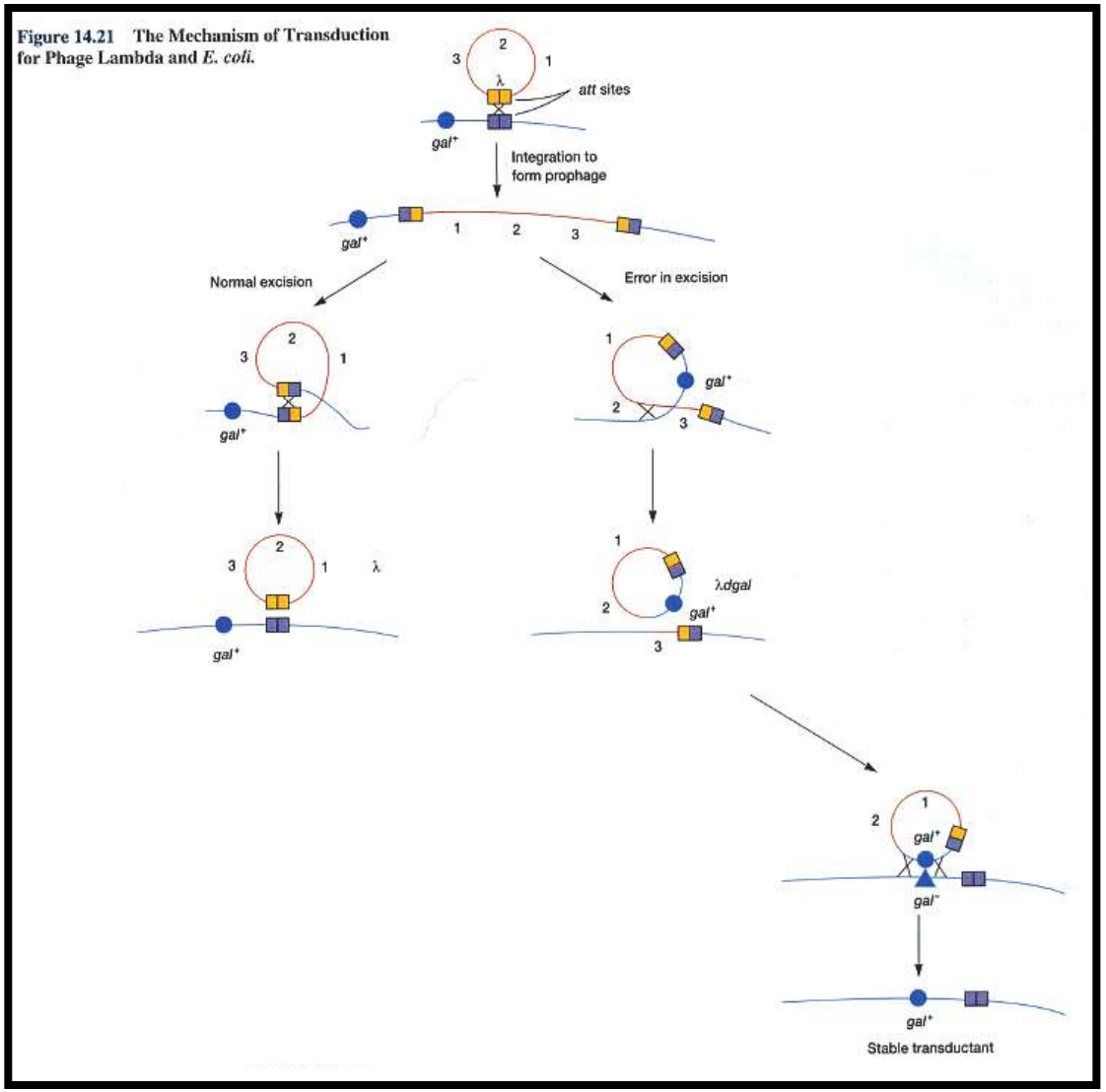
Specialized transduction

- **Genetics**
  - **Only genes near the phage attachment site (*att*) can be transduced.**
  - **The genes carried by the "transducing phage" become integrated with the phage. Thus they are added to, and do not replace chromosomal genes. The transductant may be "merodiploid".**
  - **The transduced genes are now a part of the phage genome. When transductants are induced, correct excisions lead to multipication and production of transducing phages. The lysate will be a "high frequency transducing" (HFT) lysate.**
  - **HFT's are a source of specific pieces of host DNA.**
  - **HFT's can be used in complementation studies.**
  - **The amount of bacterial DNA on a specialized transducing phage is limited by the size of DNA that can be packaged; some bacterial DNA can be added to lambda without loss of essential lambda genes. If more is added, essential lambda genes are lost, but a "helper" phage can provide these functions.**
  - **Genetic engineers use λ cloning vectors; DNA fragments are ligated to the vector, packaged into phage particles *in vitro*, and used to infect *E. coli* cells.**

Specialized Transduction: phage λ

Imprecise excision of lysogenic phage DNA results in host genes being carried by phage l

Carried chromosomal gene can transduce recipient.



Generalized transduction

- **Does not require lysogeny. The phage DNA does not integrate in the chromosome: a phage particle DNA packaging error is involved, and fragments from anywhere on the chromosome can be transduced. Example: phage P1**
- **Mechanism:**
  - **1. The capsid packaging system for some phage DNA ís can permit a phage capsid to occasionally be filled with bacterial DNA instead of phage DNA (about 1 P1 phage particle in 10^3 contains bacterial instead of phage DNA).**
    - **Due partly to the less active nuclease activity of P1**
  - **2. After lysis of the host (the transductional donor) this "transducing particle" adsorbs and releases its DNA into a new host.**
  - **3. The second host (transductional recipient) is "infected" with a piece of bacterial DNA rather than with bacteriophage DNA.**

Generalized transduction

- **Either a plasmid or a chromosomal gene fragment can be transmitted by generalized transduction. A plasmid would replicate in the new host. A chromosomal fragment can recombine with the chromosome of the recipient bacterium. Selection for recipient bacteria that express donor markers allow identification of transductants.**
- **The size of the DNA fragment contained in the transducing particle is limited by the size of the phage capsid. About 2% of the *E. coli* chromosome can be carried by P1[about 1 in 10^5 progeny P1 particles will carry a particular gene].**

Generalized transduction

Phage infects

Degrades host DNA, but not to completion

Phage and chromosomal DNA (of appropriate size) is packaged

Chromosomal DNA is now "infectious"

Can transduce gene(s)