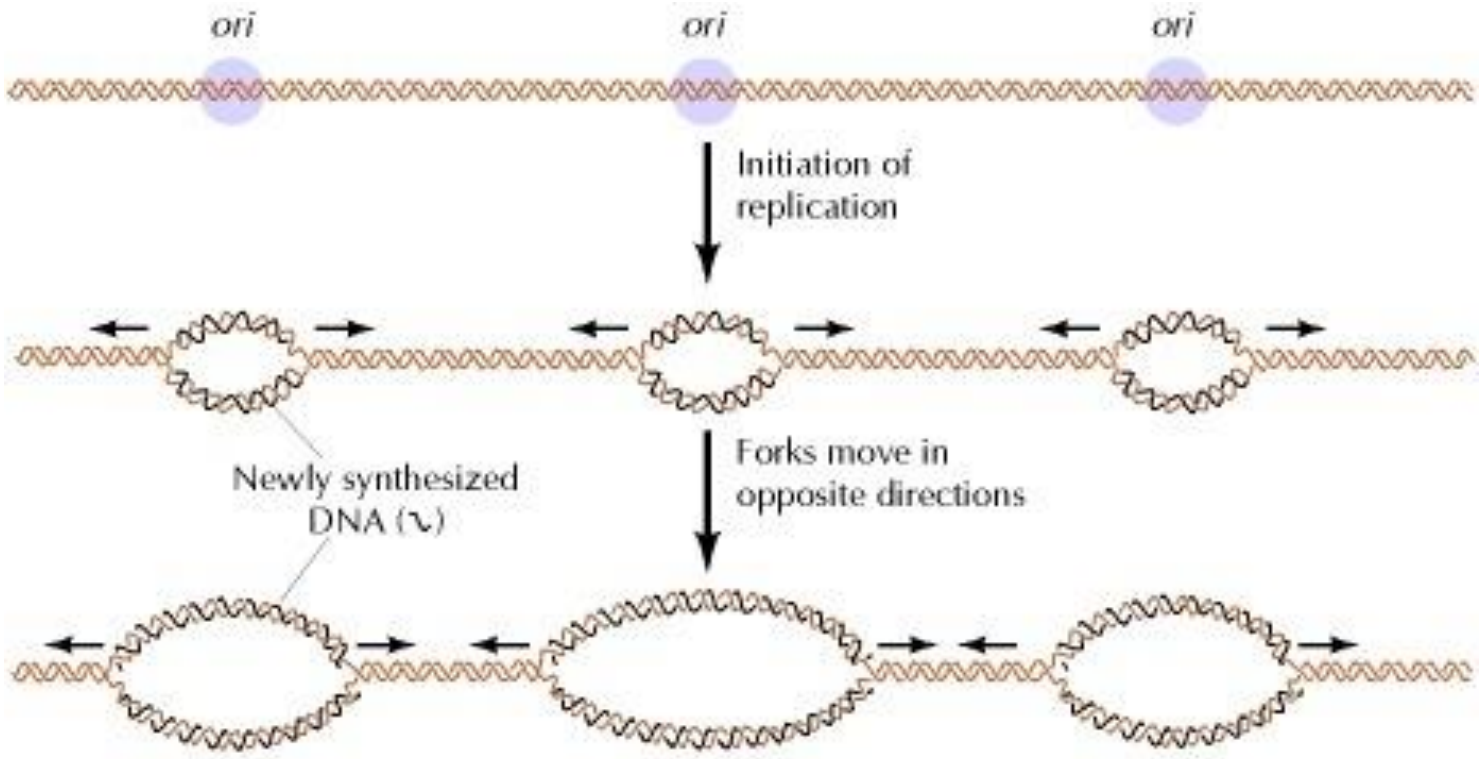
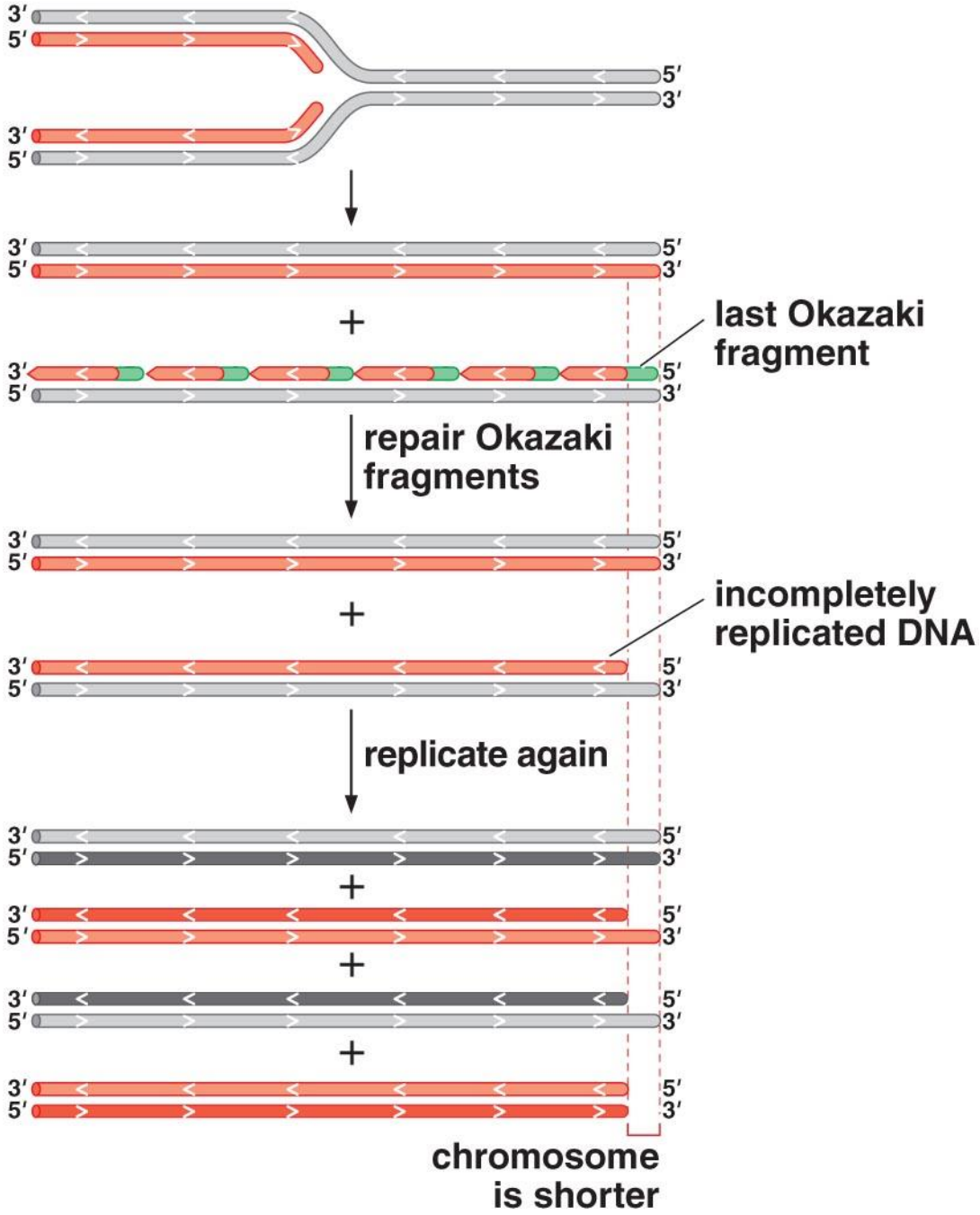
Lecture 19



https://www.ncbi.nlm.nih.gov/books/NBK9940/

End replication problem

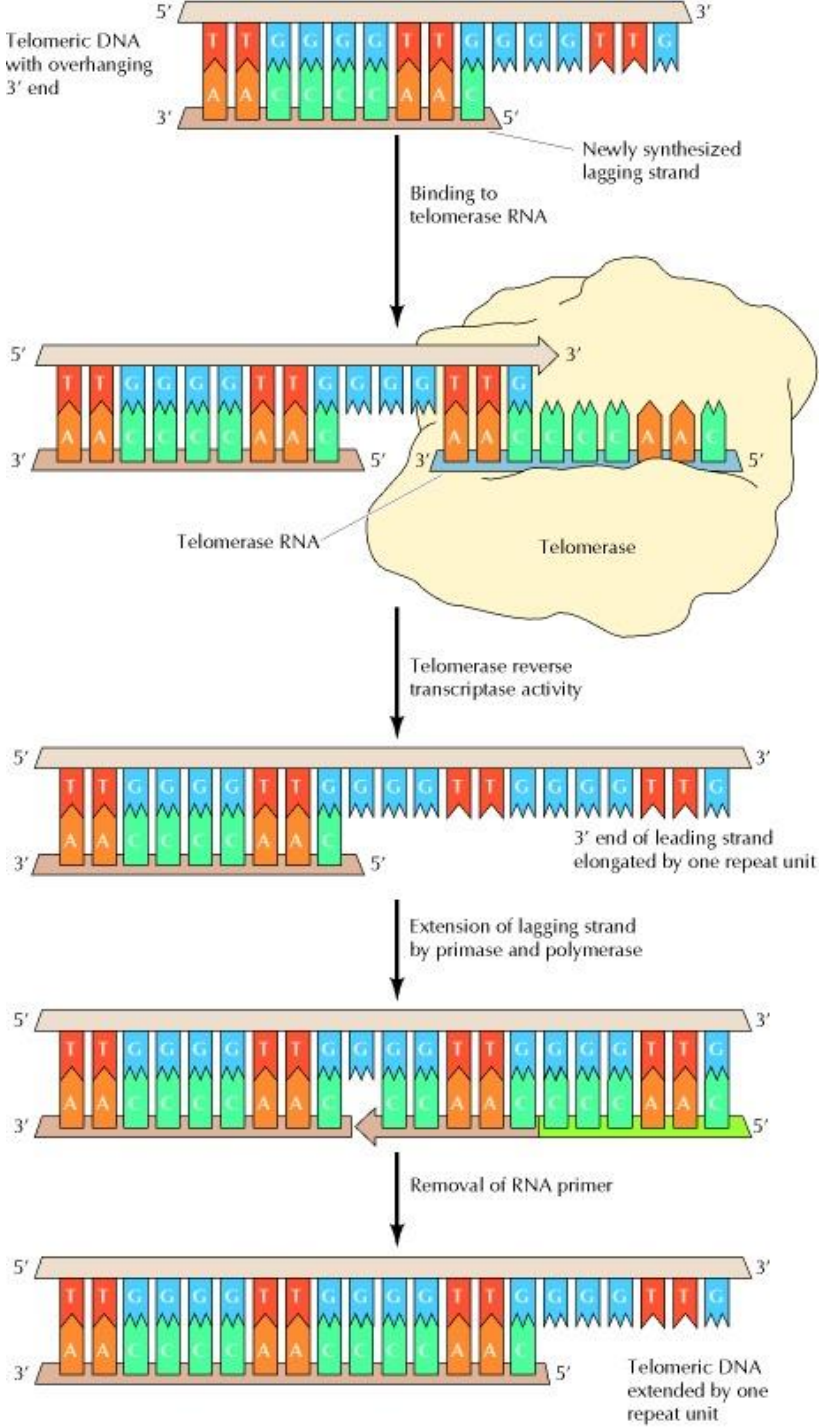


Because DNA polymerases extend primers only in the 5′ to 3′ direction, they are unable to copy the extreme 5′ ends of linear DNA molecules.

Consequently, special mechanisms are required to replicate the terminal sequences of the linear chromosomes of eukaryotic cells.

These sequences (telomeres) consist of tandem repeats of simple-sequence DNA.

They are replicated by the action of a unique enzyme called **telomerase,** which is able to maintain telomeres by catalyzing their synthesis in the absence of a DNA template.



Many organisms have an enzyme called telomerase, which carries out the task of adding repetitive nucleotide sequences to the ends of the DNA.

Telomerase "replenishes" the telomere "cap."

In most multicellular eukaryotic organisms, telomerase is active only in germ cells, some types of stem cells such as embryonic stem cells.

DNA Recombination

The rearrangement of genetic information within and among DNA molecules encompasses a variety of processes, collectively placed under the heading of genetic recombination

Genetic recombination events fall into at least three general classes

Homologous genetic recombination (also called general recombination)
Involves genetic exchanges between any two DNA molecules (or segments of the same molecule) that share an extended region of nearly identical sequence.

The actual sequence of bases is irrelevant, as long as it is similar in the two DNAs.

Site-specific recombination the exchanges occur only at a *particular* DNA sequence.

DNA transposition is distinct from both other classes in that it usually involves a short segment of DNA with the remarkable capacity to move from one location in a chromosome to another.

Why genetic recombination

The functions of genetic recombination systems are as varied as their mechanisms.

They include roles in specialized DNA repair systems, specialized activities in DNA replication, regulation of expression of certain genes, facilitation of proper chromosome segregation during eukaryotic cell division, maintenance of genetic diversity, and implementation of programmed genetic rearrangements during embryonic development.

Homologous Genetic Recombination

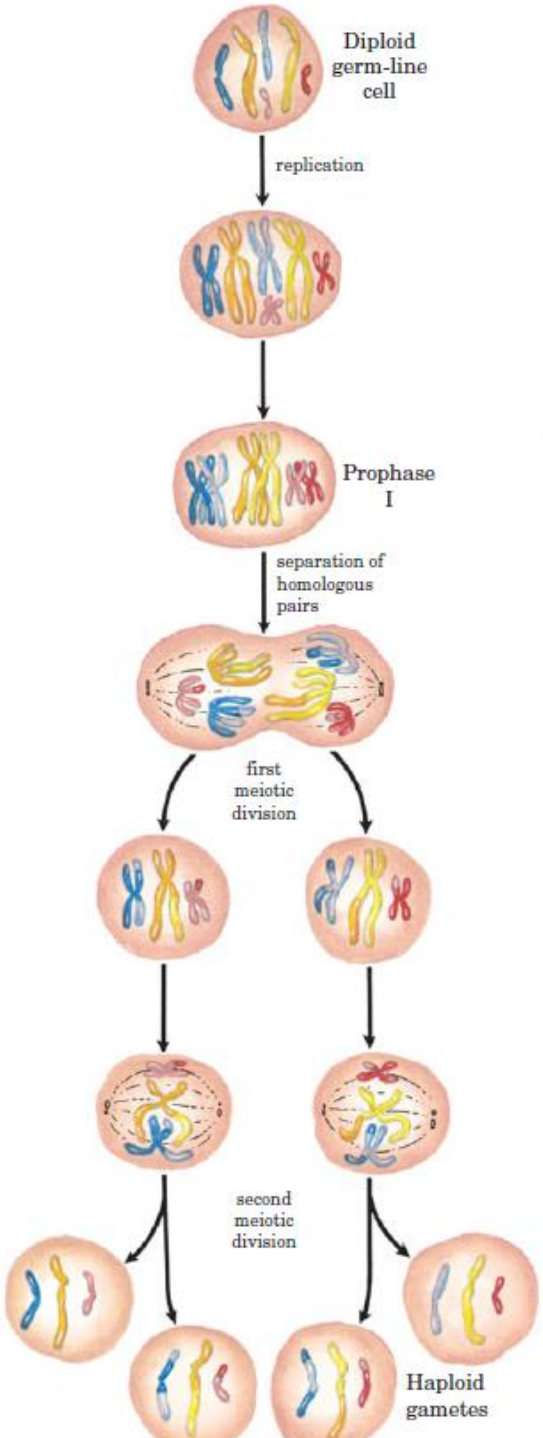
In bacteria, homologous genetic recombination is primarily a DNA repair process and is referred to as **recombinational DNA repair**.

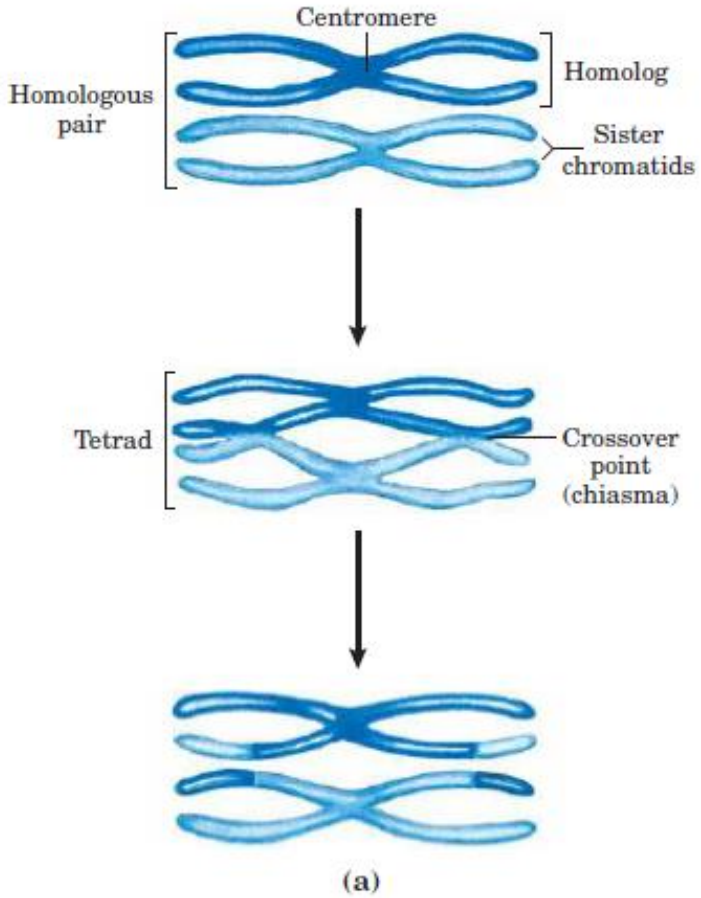
It is usually directed at the reconstruction of replication forks stalled at the site of DNA damage.

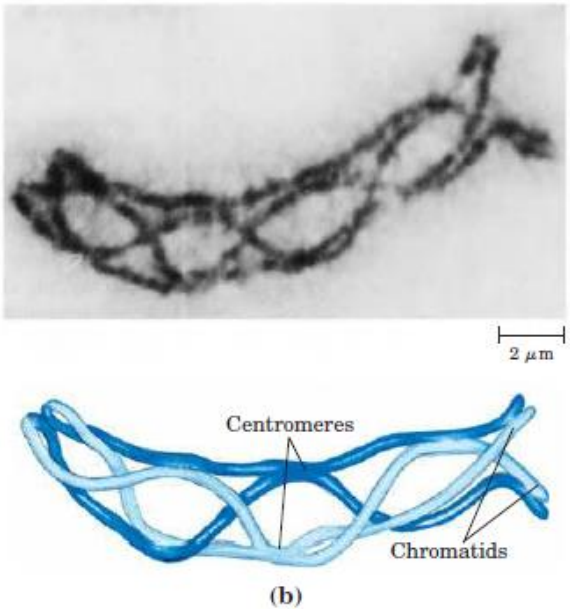
Homologous genetic recombination can also occur during conjugation (mating), when chromosomal DNA is transferred from one bacterial cell (donor) to another (recipient).

In eukaryotes, homologous genetic recombination can have several roles in replication and cell division, including the repair of stalled replication forks.

Recombination occurs with the highest frequency during **meiosis**, the process by which diploid germ-line cells with two sets of chromosomes divide to produce haploid gametes (sperm cells or ova) in animals (haploid spores in plants)—each gamete having only one member of each chromosome pair


Diploid germ-line cell
replication
Prophase I
separation of homologous pairs
first meiotic division
second meiotic division
Haploid gametes


Centromere
Homologous pair
Homolog
Sister chromatids
Tetrad
Crossover point (chiasma)
(a)


2 μm
Centromeres
Chromatids
(b)

After the DNA is replicated during prophase of the first meiotic division, the resulting sister chromatids remain associated at their centromeres.

At this stage, each set of four homologous chromosomes (tetrad) exists as two pairs of chromatids.

Genetic information is now exchanged between the closely associated homologous chromatids by homologous genetic recombination, a process involving the breakage and rejoining of DNA

Crossing over links the two pairs of sister chromatids together at points called **chiasmata** (singular, chiasma).

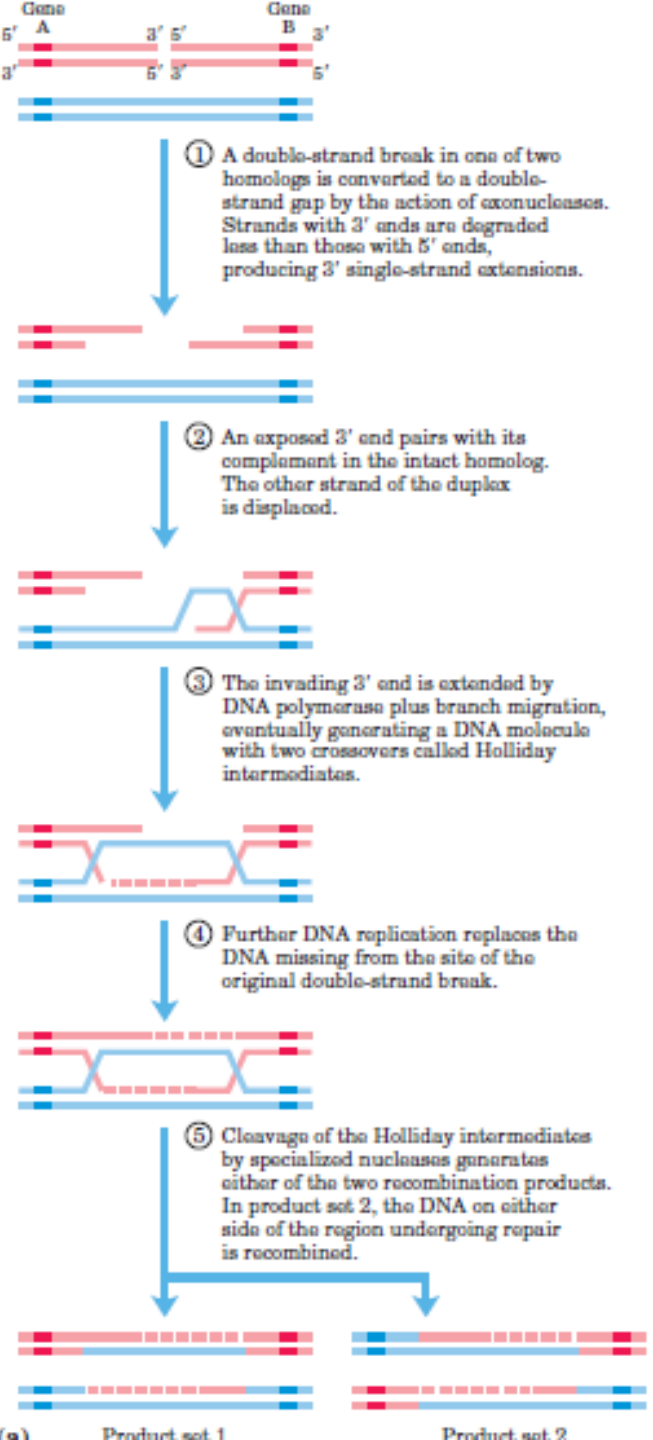
Four key features

First, homologous chromosomes are aligned.

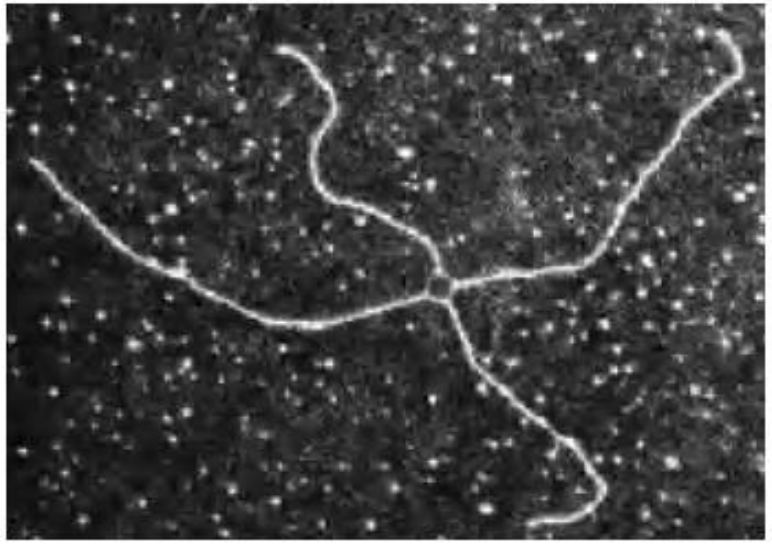
Second, a double-strand break in a DNA molecule is enlarged by an exonuclease, leaving a singlestrand extension with a free 3-hydroxyl group at the broken end.

Third, the exposed 3’ ends invade the intact duplex DNA of the homolog, and this is followed by **branch migration** and/or by replication to create a pair of crossover structures, called **Holliday intermediates**

Fourth, cleavage of the two crossovers creates either of two pairs of complete recombinant products



Holliday intermediates are a feature of homologous genetic recombination pathways in all organisms



Recombination Requires a Host of Enzymes and Other Proteins

Enzymes that promote various steps of homologous recombination have been isolated from both bacteria and eukaryotes.

In *E. coli*, the *recB*, *recC*, and *recD* genes encode the heterotrimeric RecBCD enzyme, which has both helicase and nuclease activities.

The RecA protein promotes all the central steps in the homologous recombination process: the pairing of two DNAs, formation of Holliday intermediates, and branch migration

The RuvA and RuvB proteins (*r*epair of *UV* damage) form a complex that binds to Holliday intermediates, displaces RecA protein, and promotes branch migration at higher rates than does RecA.

Nucleases that specifically cleave Holliday intermediates, often called resolvases, have been isolated from bacteria and yeast.

The RuvC protein is one of at least two such nucleases in *E. coli*.

Site-Specific Recombination

Recombination is limited to specific sequences.

Examples include regulation of the expression of certain genes and promotion of programmed DNA rearrangements in embryonic development or in the replication cycles of some viral and plasmid DNAs.

Each site-specific recombination system consists of an enzyme called a **recombinase** and a short (20 to 200 bp), unique DNA sequence where the recombinase acts (**the recombination site**).

One or more auxiliary proteins may regulate the timing or outcome of the reaction.

Enzymes known as **site-specific recombinases (SSRs**) perform rearrangements of DNA segments by recognizing and binding to short, specific DNA sequences (sites), at which they cleave the DNA backbone, exchange the two DNA helices involved, and rejoin the DNA strands.

In some cases the presence of a recombinase enzyme and the recombination sites is sufficient for the reaction to proceed; in other systems a number of **accessory proteins** and/or accessory sites are required.

Transposons

Transposons, also called transposable elements, are **mobile genetic elements** that generally have only modest target site selectivity and can thus insert themselves into many different DNA sites.

In transposition, a specific enzyme, usually encoded by the transposon and called a ***transposase****,* acts on a specific DNA sequence at each end of the transposon—first disconnecting it from the flanking DNA and then inserting it into a new target DNA site.

There is **no requirement for homology** between the ends of the element and the insertion site.

Most transposons move only very rarely (once in 10^5 cell generations for many elements in bacteria), and for this reason it is often difficult to distinguish them from nonmobile parts of the chromosome.

In most cases, it is not known what suddenly triggers their movement called **transposition**

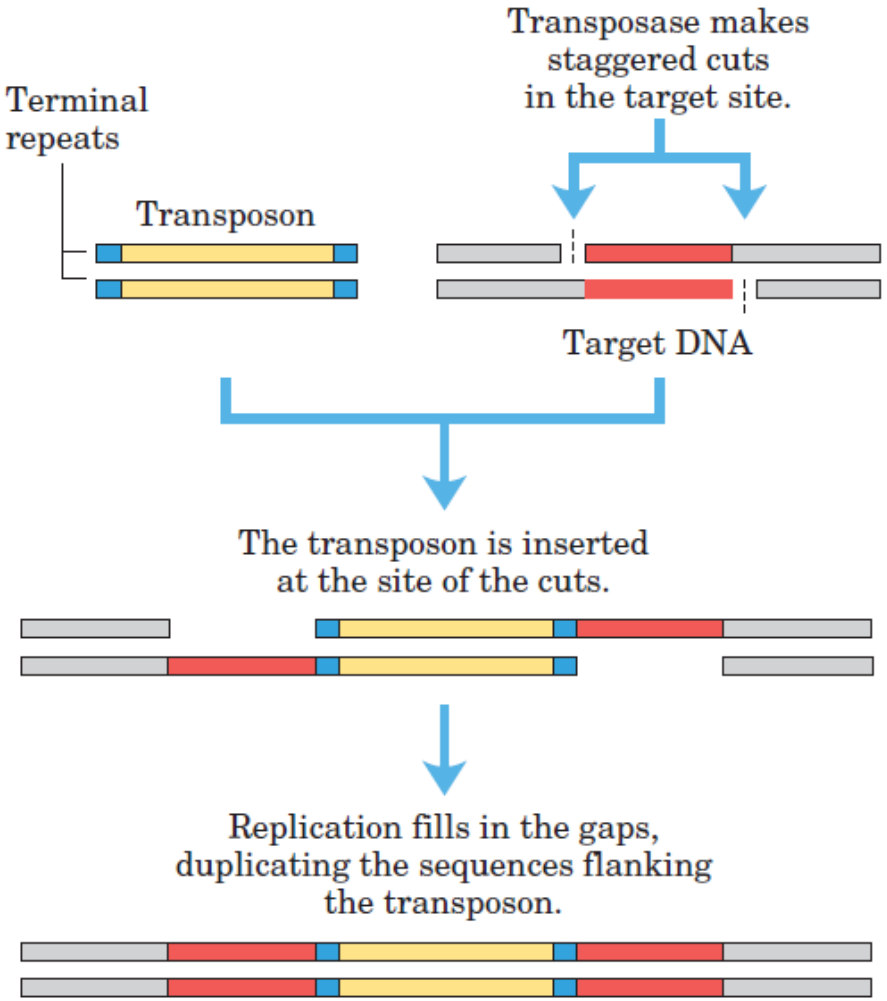
Bacteria have two classes of transposons.

1. Insertion sequences (**simple transposons**) contain only the sequences required for transposition and the genes for the proteins (transposases) that promote the process.

2. Complex transposons contain one or more genes in addition to those needed for transposition.

These extra genes might, for example, confer resistance to antibiotics and thus enhance the survival chances of the host cell.

Short repeat sequences at the end that aid in binding



Excision

Drift

Insertion

There are two general pathways for transposition in bacteria.

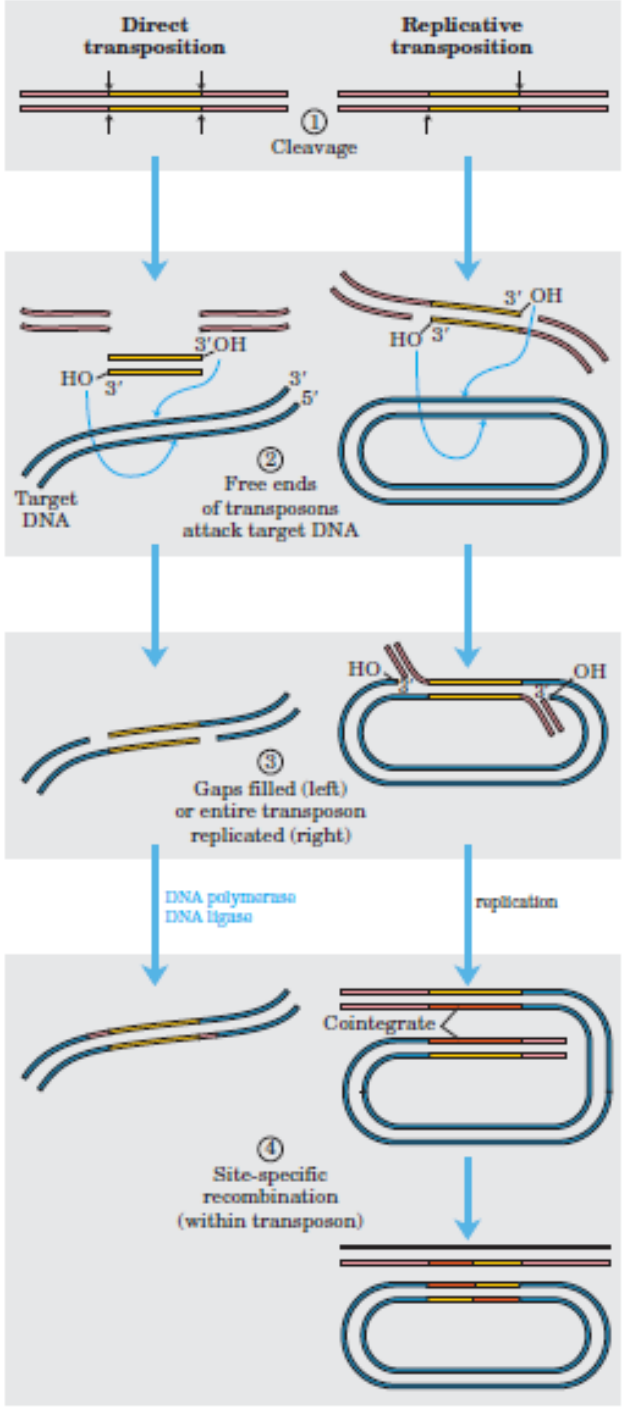
Direct transposition and
Replicative transposition

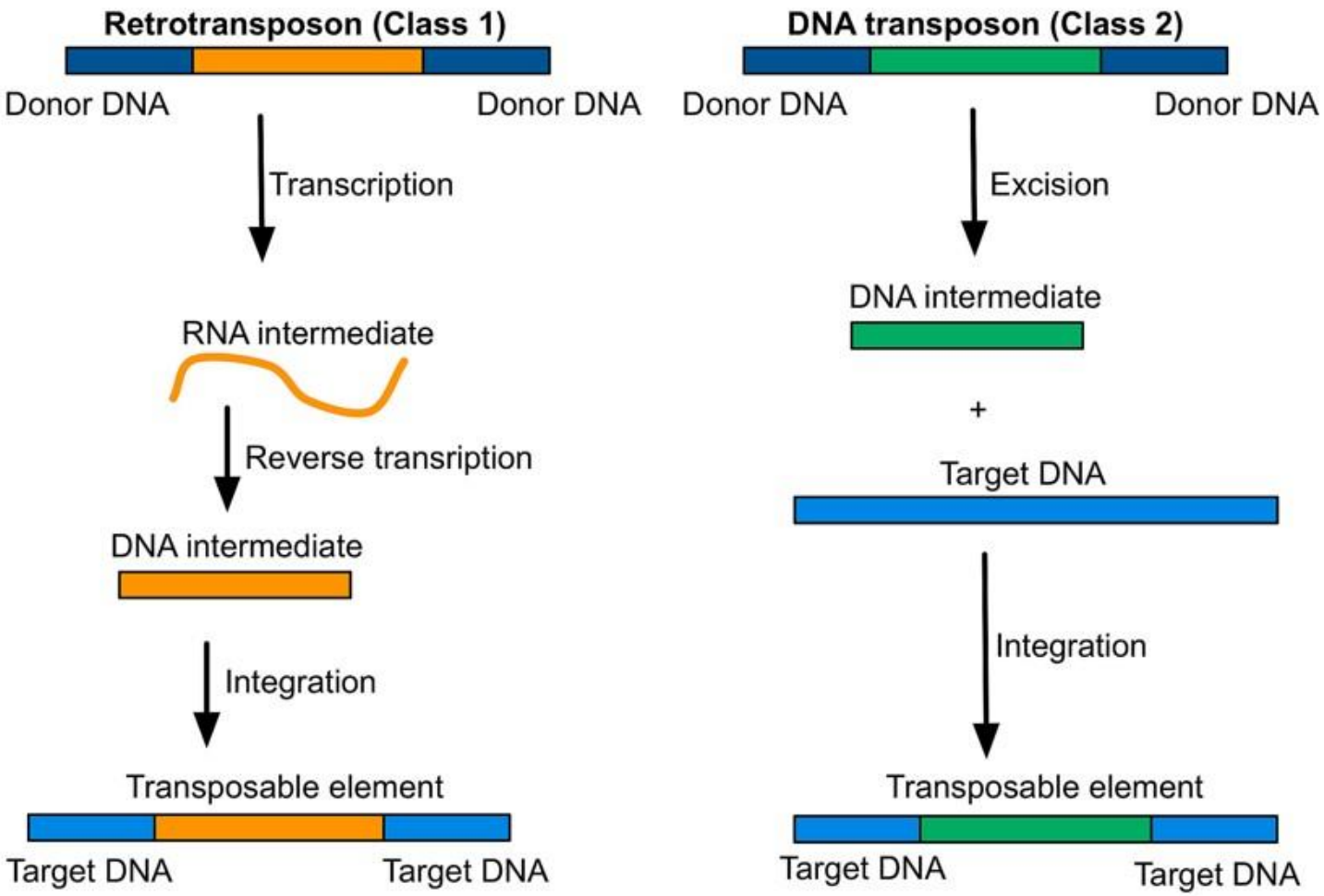
In direct (or simple) transposition, cuts on each side of the transposon excise it, and the transposon moves to a new location.

This leaves a double-strand break in the donor DNA that must be repaired.

At the target site, a staggered cut is made the transposon is inserted into the break, and DNA replication fills in the gaps to duplicate the target site sequence.

In replicative transposition the entire transposon is replicated, leaving a copy behind at the donor location.




Retrotransposon (Class 1)
Donor DNA
Donor DNA
Transcription
RNA intermediate
Reverse transription
DNA intermediate
Integration
Transposable element
Target DNA
Target DNA
DNA transposon (Class 2)
Donor DNA
Donor DNA
Excision
DNA intermediate
+
Target DNA
Integration
Transposable element
Target DNA
Target DNA