RAMA UNIVERSITY

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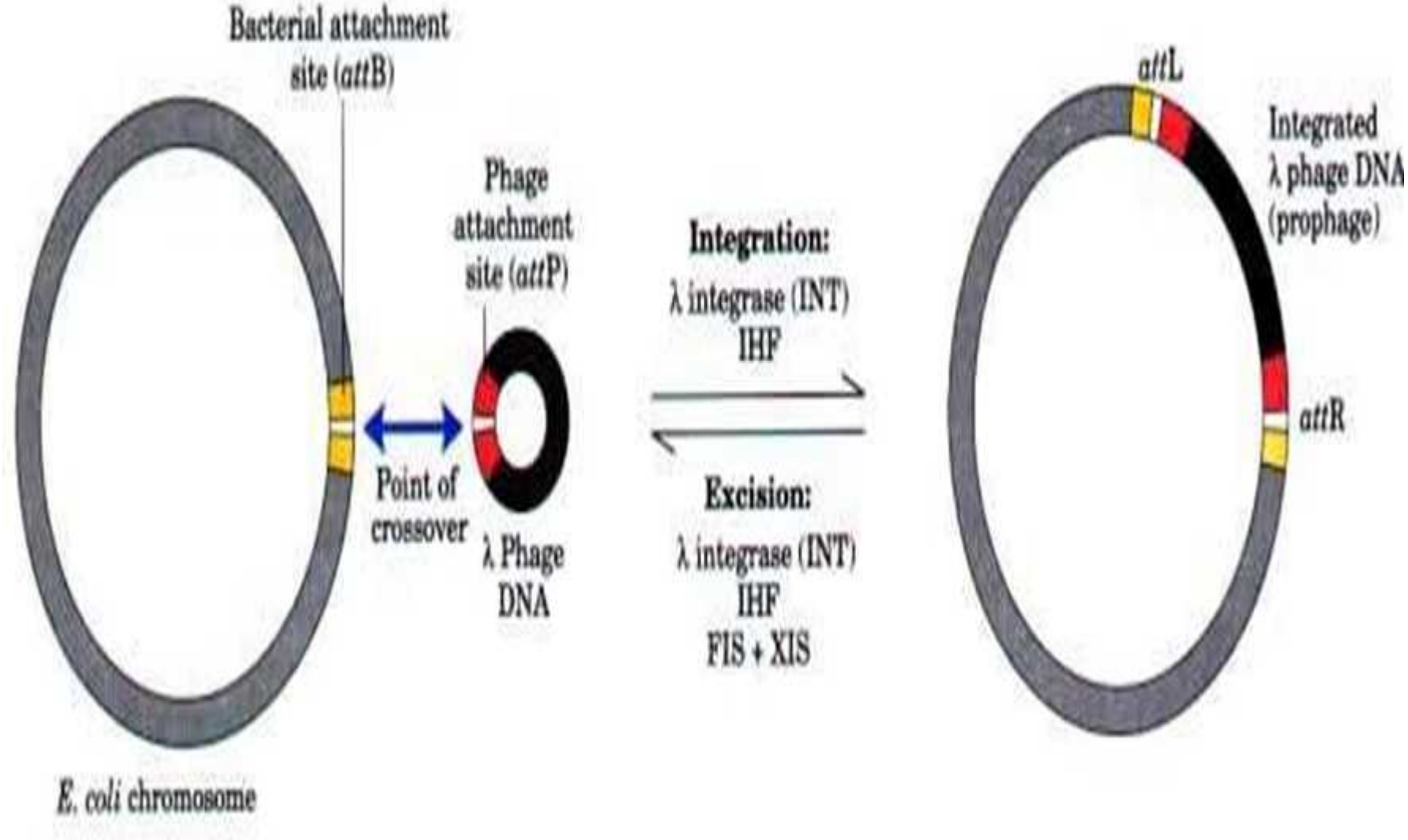
FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

SITE SPECIFIC RECOMBINATION

Conservative 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.
- Bacteriophage genome into bacterial chromosome
- Site-specific recombinase (SSRs) perform rearrangements of DNA segments by recognizing and binding to short DNA sequences (sites), at which they cleave the DNA backbone, exchange the two DNA helices involved and rejoin the DNA strands.

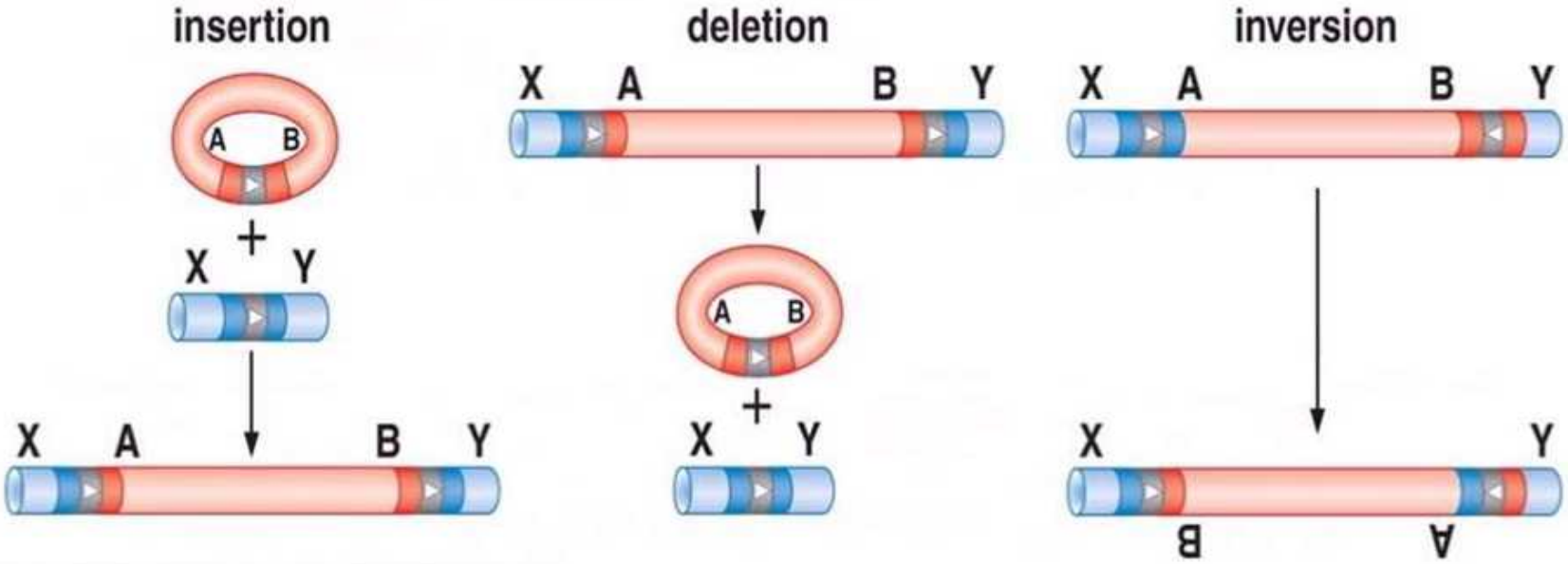

Bacterial attachment site (attB)
Phage attachment site (attP)
Point of crossover
λ Phage DNA
E. coli chromosome
Integration:
λ integrase (INT)
IHF
Excision:
λ integrase (INT)
IHF
FIS + XIS
attL
Integrated λ phage DNA (prophage)
attR

❖They are employed in a variety of cellular processes, including bacterial genome replication, differentiation and pathogenesis, and movement of mobile genetic elements.

❖Recombination sites are typically between 30 and 200 nucleotides in length and consist of two motifs with a partial inverted-repeat symmetry, to which the recombinase binds, and which flank a central crossover sequence at which the recombination takes place. The pairs of sites between which the recombination occurs are usually identical, but there are exceptions

Conservative site-specific recombination

- Depending on the initial arrangement of the parental recombination sites, site-specific recombination has one of three possible outcomes: integration, excision, or inversion



- Based on amino acid sequence homology and mechanistic relatedness most site-specific recombinase are grouped into one of two families:
    1. tyrosine recombinase family
    2. Serine recombinase family

- members of the serine recombinase family were known as resolvase / DNA invertases
- member of the tyrosine recombinases, lambda- integrase, using attP/B recognition sites

1. tyrosine recombinase family

- During strand exchange, the DNA cut at fixed points within the crossover region of the site releases a deoxyribose hydroxyl group
- recombinase protein forms a transient covalent bond to a DNA backbone phosphate.
- This phosphodiester bond between the hydroxyl group of the nucleophilie serine or tyrosine residue conserves the energy that was expended in cleaving the DNA.
- Energy stored in this bond is subsequently used for the rejoining of the DNA to the corresponding deoxyribose hydroxyl group on the other site.

Serine recombinase family

- classical members are gamma-delta and Tn3 resolvase, but also new additions like φC31- Bxb1-, and R4 integrases, cut all four DNA strands simultaneously at points that are staggered by 2bp .
- During cleavage, a protein-DNA bond is formed via a transesterification reaction in which
- a phosphodiester bond is replaced by a
- phosphoserine bond between a 5’ phosphate at the cleavage site and the hydroxyl group of the conserved serine residue