

Recombinación sitio-específica

Recombinación en secuencias específicas

Requiere de maquinaria propia

Ejemplos: Recombinasa Cre -sitios *loxP* (fago P1),
Integrasa Int del fago lambda – sitios *att* (*attP* y *attB*)



En la **recombinación homóloga (recíproca o general)** los reordenamientos del DNA tienen lugar entre segmentos de DNA muy similares en secuencias. **Se intercambian los alelos pero el orden de los genes permanece inalterado.** Las enzimas que la catalizan no son específicas para cada secuencia.

Características de la **recombinación sitio-específica**

Consiste en el movimiento de secuencias llamadas **elementos móviles** entre distintos sitios de un mismo cromosoma o diferentes cromosomas

Está mediada por enzimas que reconocen secuencias específicas y cortas de nucleótidos presentes en las moléculas entre las que se produce la recombinación.

Puede alterar el orden de los genes y también agregar nueva información en un genoma.

CSSR: Conservative Site-Specific Recombination

Ciclo de vida del fago λ

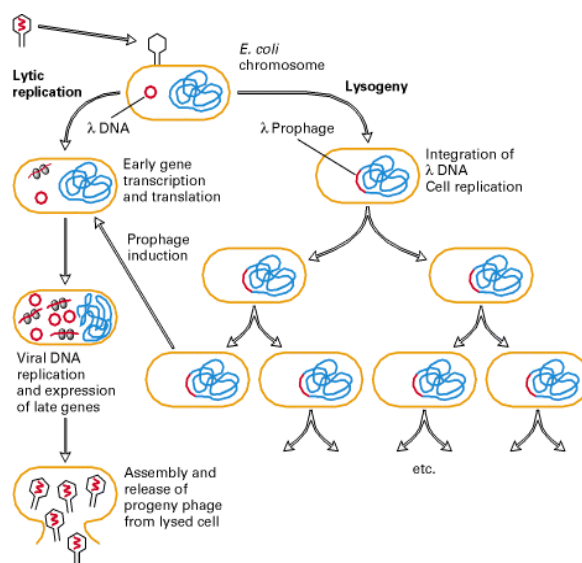
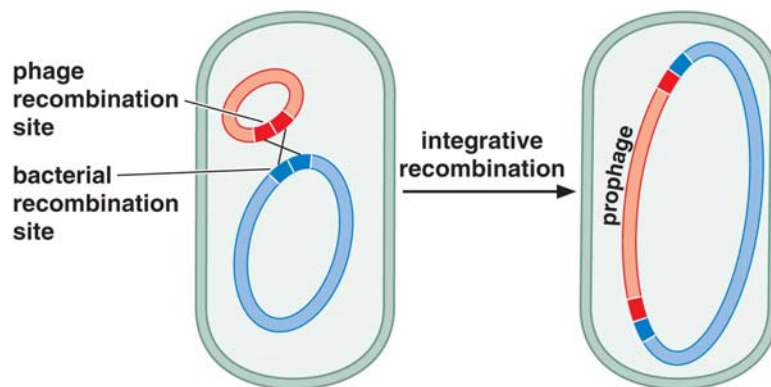


Figure 6-19. A bacteriophage undergoes either lytic replication or lysogeny following infection of *E. coli*. The linear double-stranded DNA is converted to a circular form immediately after infection. (Left) If the nutritional state of the host cell is favorable, most infected cells undergo lytic replication, similar to lytic replication of cells by bacteriophage T4 (see Figure 6-11). (Right) If the nutritional state of the host cell cannot support production of large numbers of progeny phages, lysogeny is established. In this case, viral genes required for the lytic cycle are repressed, and host-cell enzymes synthesize viral proteins that integrate the viral DNA into a specific sequence in the host-cell chromosome where no host-cell genes are disrupted. The prophage DNA then is replicated along with the host-cell chromosome as the lysogenized cell (called a *lysogen*) grows and divides. Repression of the viral genes required for lytic replication is maintained in progeny cells. At infrequent intervals, the prophage in a lysogen is induced, or activated, leading to expression of viral proteins that precisely remove the prophage DNA from the host-cell chromosome and to derepression of the genes required for the lytic cycle. As a result, a normal cycle of lytic replication ensues

CSSR: integración del **genoma del fago λ** en un sitio específico del **DNA de *Escherichia coli***



Recombinación sitio específica en fago λ

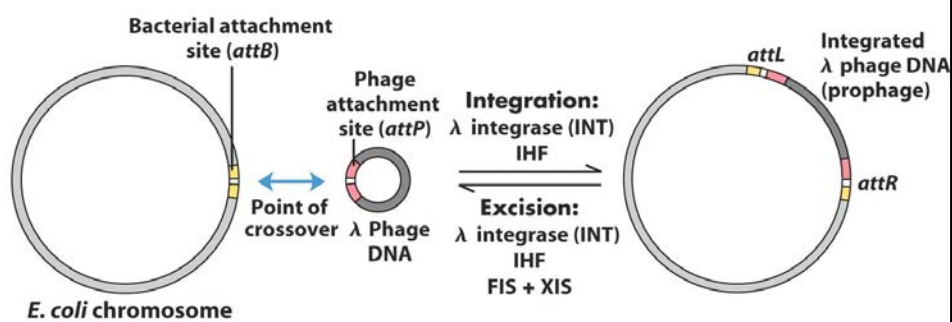
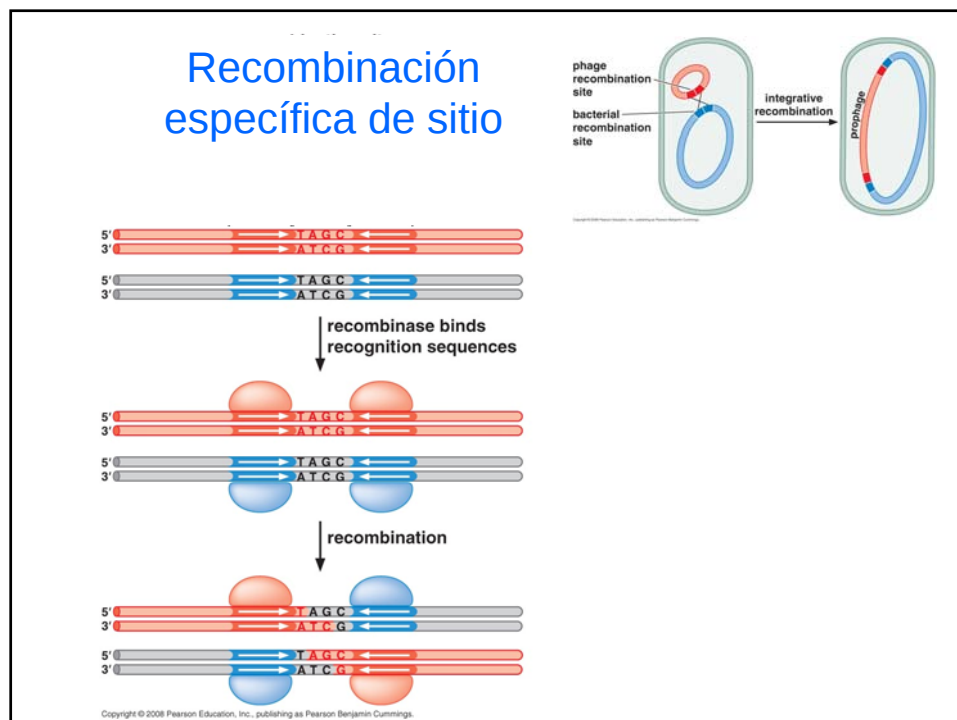
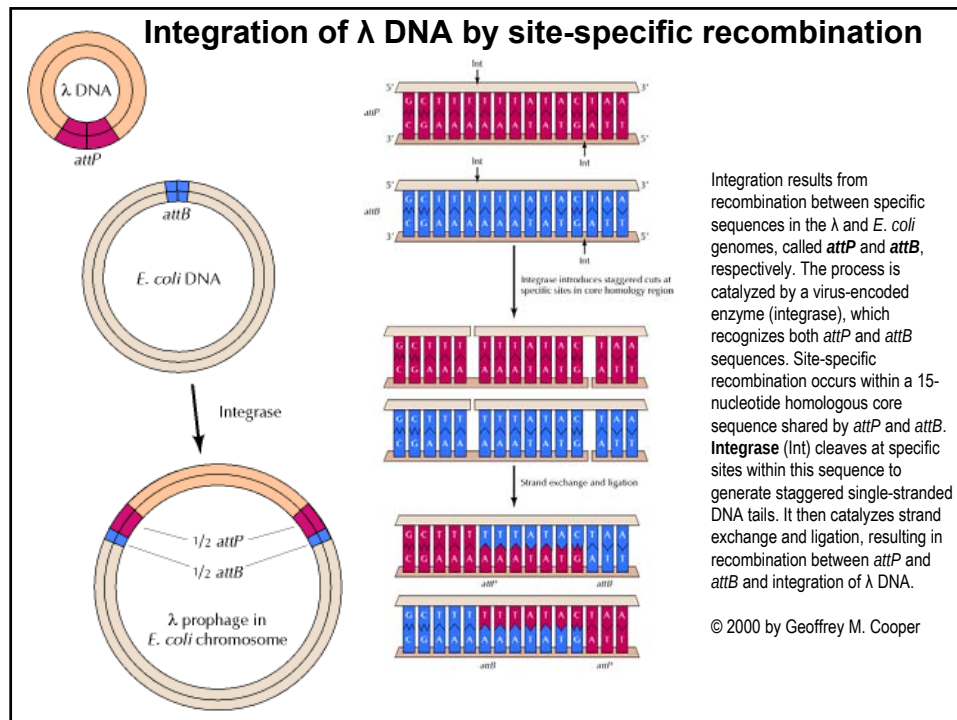
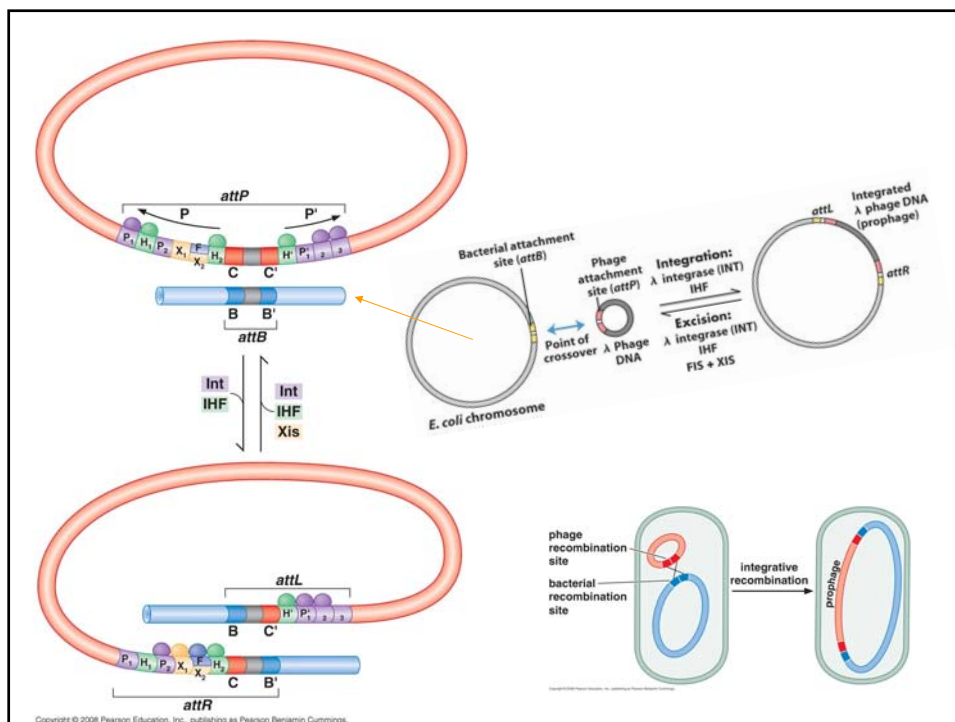
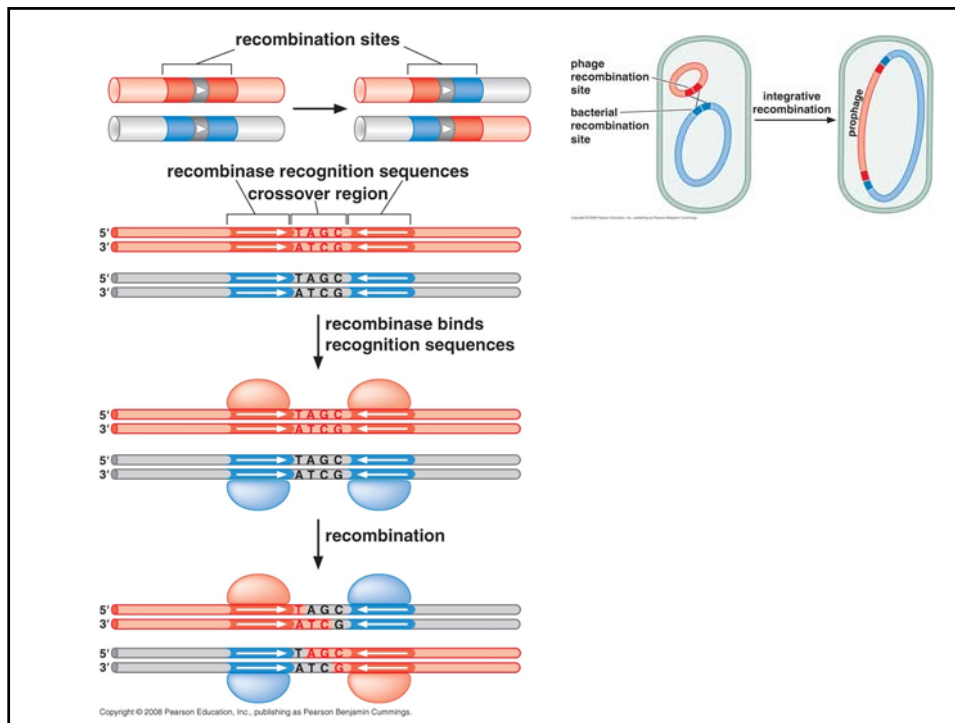


FIGURE 25-40 Integration and excision of bacteriophage λ DNA at the chromosomal target site. The attachment site on the λ phage DNA (*attP*) shares only 15 bp of complete homology with the bacterial site (*attB*) in the region of the crossover. The reaction generates two new attachment sites (*attR*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

The recombinase is the λ 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.





Mecanismo general de acción de la integrasa

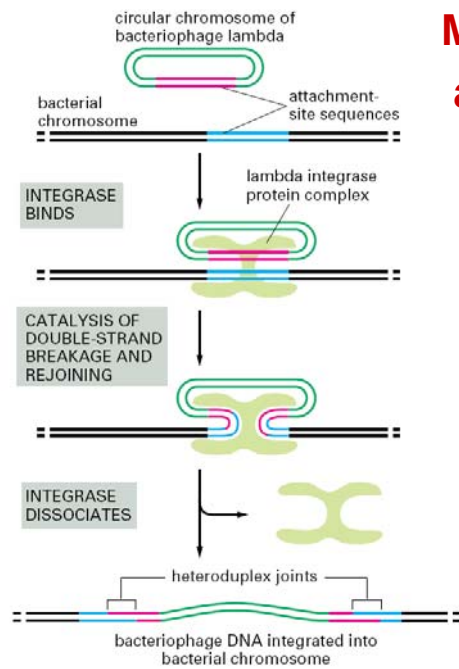
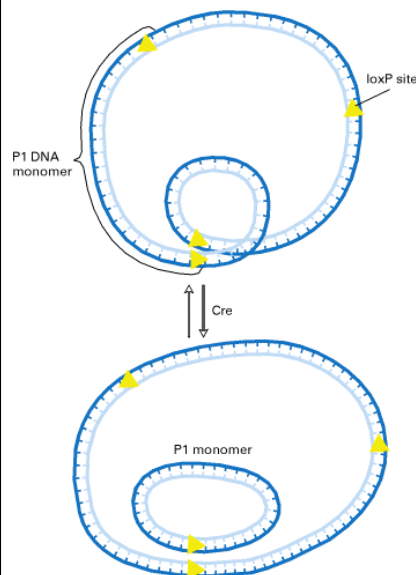


Figure 5-80. The insertion of a circular bacteriophage lambda DNA chromosome into the bacterial chromosome. In this example of site-specific recombination, the lambda integrase enzyme binds to a specific "attachment site" DNA sequence on each chromosome, where it makes cuts that bracket a short homologous DNA sequence. The integrase then switches the partner strands and reseals them to form a heteroduplex joint that is seven nucleotide pairs long. A total of four strand-breaking and strand-joining reactions is required; for each of them, the energy of the cleaved phosphodiester bond is stored in a transient covalent linkage between the DNA and the enzyme, so that DNA strand resealing occurs without a requirement for ATP or DNA ligase.

La recombinasa Cre del bacteriófago P1



During phage P1 DNA replication, long multimeric DNAs are produced; these are resolved into monomeric P1 DNAs by recombination at loxP sites, which separate the P1 DNA monomers composing a multimeric DNA

Cre protein (*Causes recombination*), encoded by phage P1, catalyzes site-specific recombination between loxP sites on multimeric circles of P1 DNA, generating a circular monomeric DNA

13bp **8bp** **13bp**
LoxP ...ATAACTTCGTATAG**CATACATT**TATACGAAGTTAT...

Tyr Recombinasas

Las recombinasas de los bacteriófagos λ y P1 tienen mecanismos de acción idénticos, ambas son **Tyr recombinasas**

(existen Ser recombinasas con mecanismos levemente diferentes)

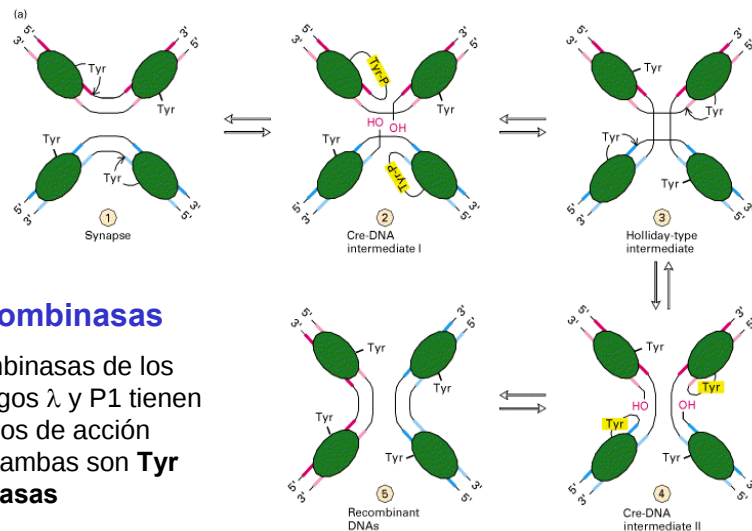
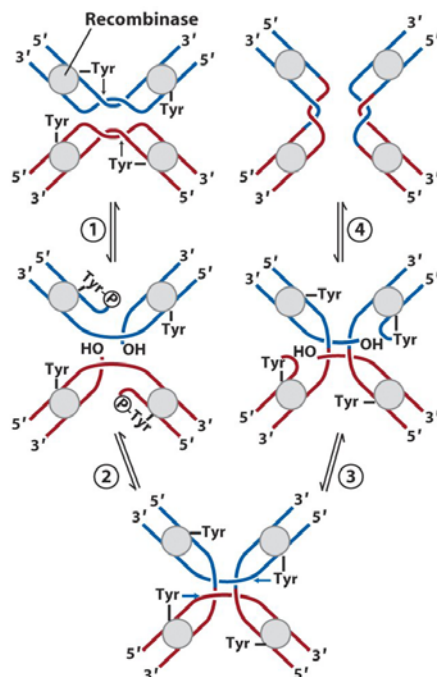
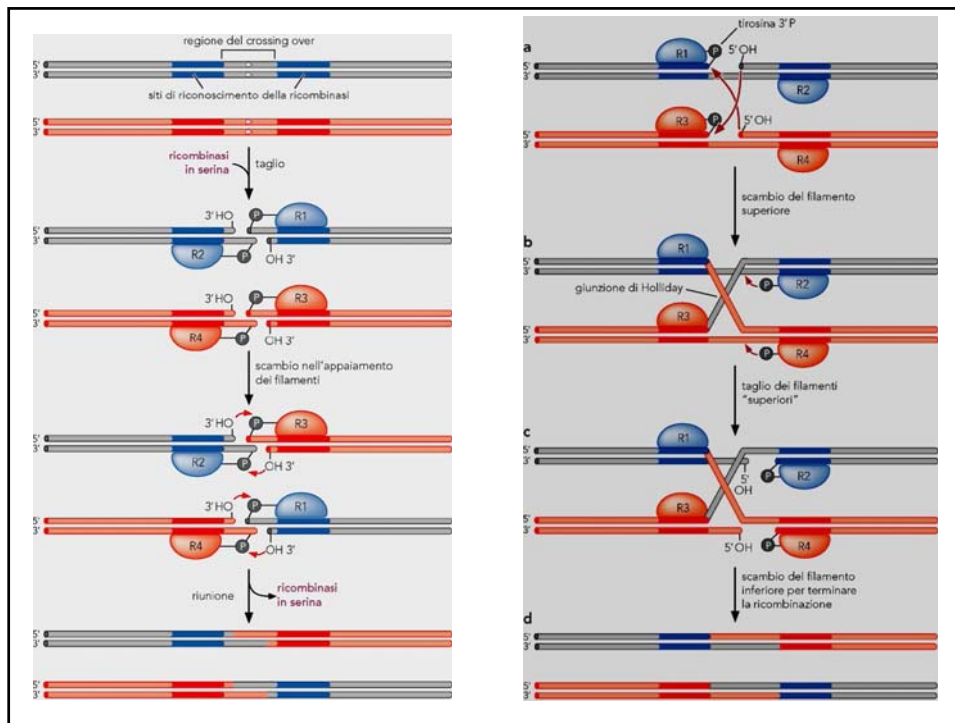


Figure 12-37. Mechanism of Cre-loxP site-specific recombination. Two Cre proteins bind on either side of each of two loxP sites, and then associate together, forming a synapse composed of two DNA molecules and four Cre proteins 1. A tyrosine (Tyr) residue on opposite Cre proteins cleaves the DNA strand to which the proteins are bound, forming two covalent 3-phosphotyrosine bonds and two free 5-hydroxyl ends 2. The free 5-hydroxyl ends in this Cre-DNA intermediate then react with the opposite phosphotyrosine bonds, yielding a Holliday-type intermediate and regenerating native Cre proteins 3. Next, a tyrosine residue on the other two Cre proteins at the synapse cleave the DNA strands to which they are bound, again forming covalent 3-phosphotyrosine bonds and free 5-hydroxyl ends to give a second Cre-DNA intermediate 4. Finally, the free 5-hydroxyl ends of this intermediate attack the opposite phosphotyrosine bonds, yielding the recombinant DNA products still bound to regenerated native Cre proteins 5. [Adapted from F. Guo, D. Gopaul, and G. Van Duyne, 1997, *Nature* 389:40.]





Estruttura del intermediario Cre-DNA

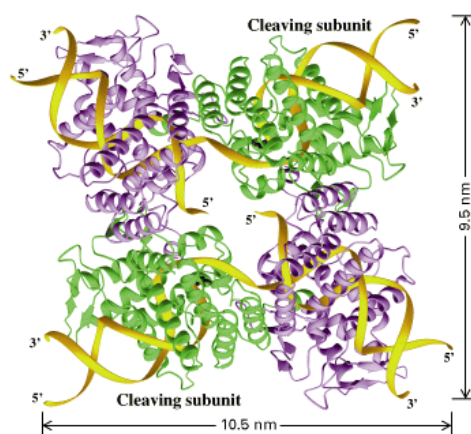
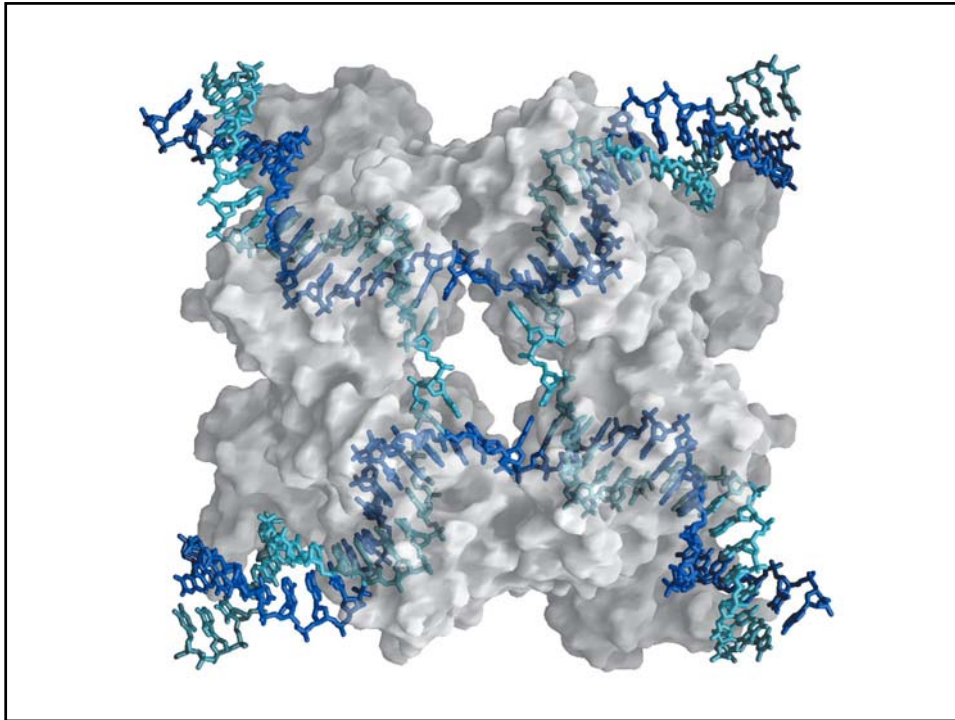
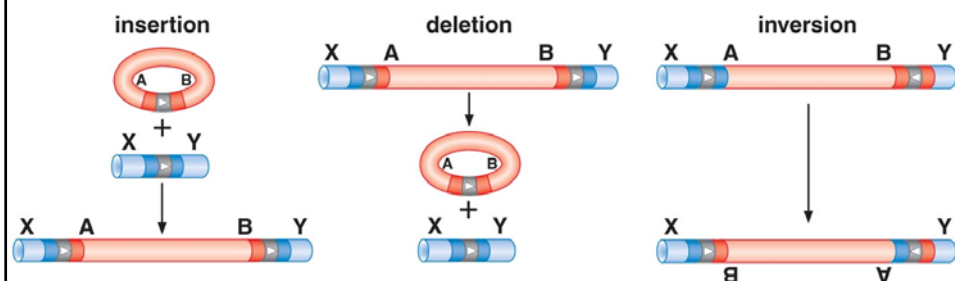


Figure 12-38. Ribbon model based on x-ray crystallography of the Cre-DNA intermediate II in site-specific recombination. Each of the cleaving subunits is covalently linked to a DNA strand by a phosphotyrosine bond. [Courtesy of Dr. G. Van Duyne.]



CSSR: mediante este mecanismo se pueden producir **tres** diferentes tipos de reorganización del DNA



Three types of DNA rearrangements

Distintos tipos de procesos de recombinación (DNA rearrangement)

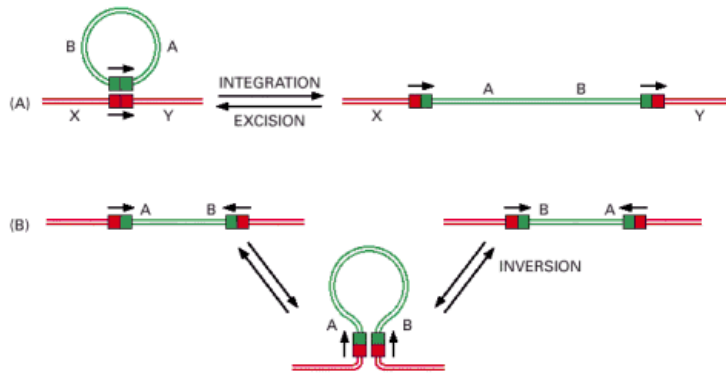


Figure 5-79. Two types of DNA rearrangement produced by conservative site-specific recombination. The only difference between the reactions in (A) and (B) is the relative orientation of the two DNA sites (indicated by arrows) at which a site-specific recombination event occurs. (A) Through an integration reaction, a circular DNA molecule can become incorporated into a second DNA molecule; by the reverse reaction (excision), it can exit to reform the original DNA circle. Bacteriophage lambda and other bacterial viruses move in and out of their host chromosomes in precisely this way. (B) Conservative site-specific recombination can also invert a specific segment of DNA in a chromosome. A well-studied example of DNA inversion through site-specific recombination occurs in the bacterium *Salmonella typhimurium*, an organism that is a major cause of food poisoning in humans; the inversion of a DNA segment changes the type of flagellum that is produced by the bacterium (see [figure 7-64](#)).

Inversión, delección, inserción

(este modelo es aplicable a recombinación homóloga intramolecular cuando las secuencias indicadas en rosado y verde son suficientemente largas).

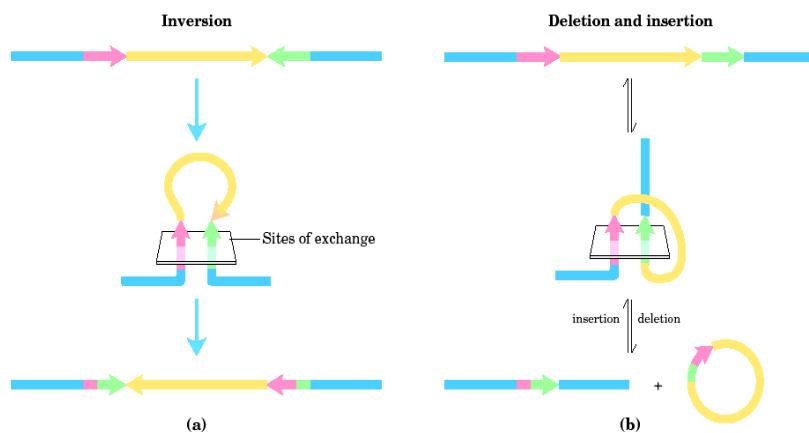
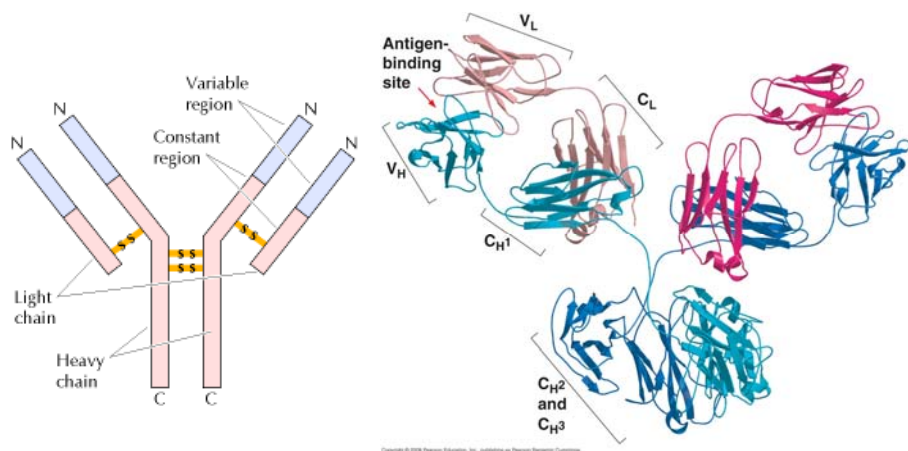
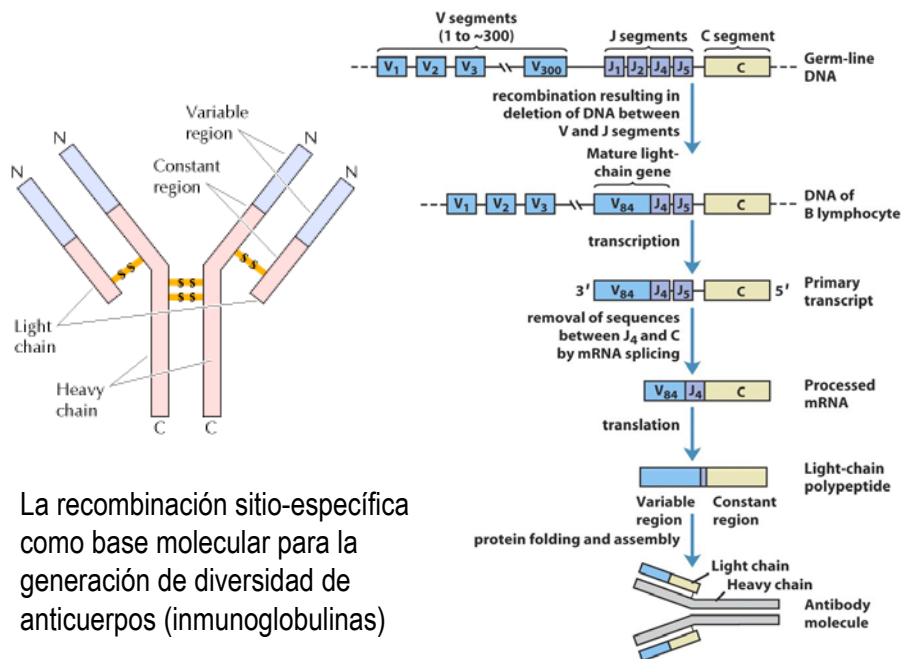


FIGURE 25-39 Effects of site-specific recombination. The outcome of site-specific recombination depends on the location and orientation of the recombination sites (red and green) in a double-stranded DNA molecule. Orientation here (shown by arrowheads) refers to the order of nucleotides in the recombination site, not the 5'→3' direction.

(a) Recombination sites with opposite orientation in the same DNA molecule. The result is an inversion. (b) Recombination sites with the same orientation, either on one DNA molecule, producing a deletion, or on two DNA molecules, producing an insertion.



La recombinación sitio-específica
como base molecular para la
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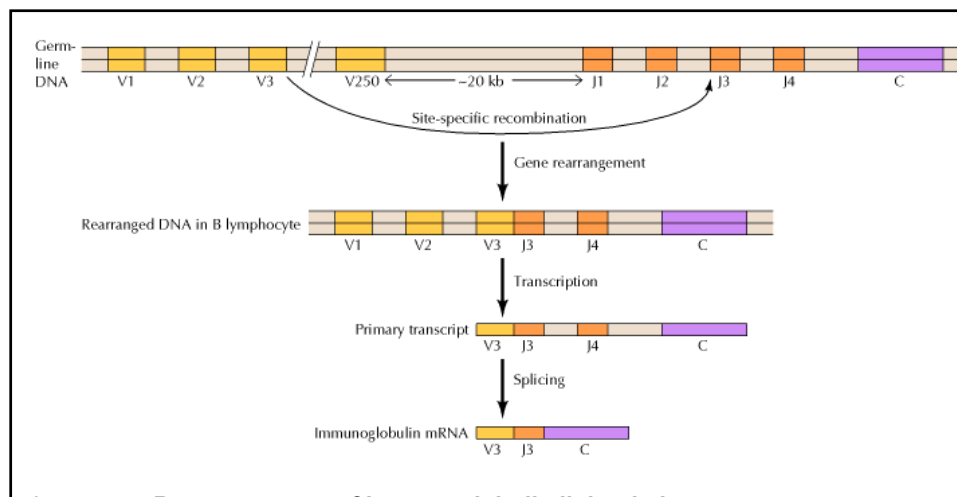
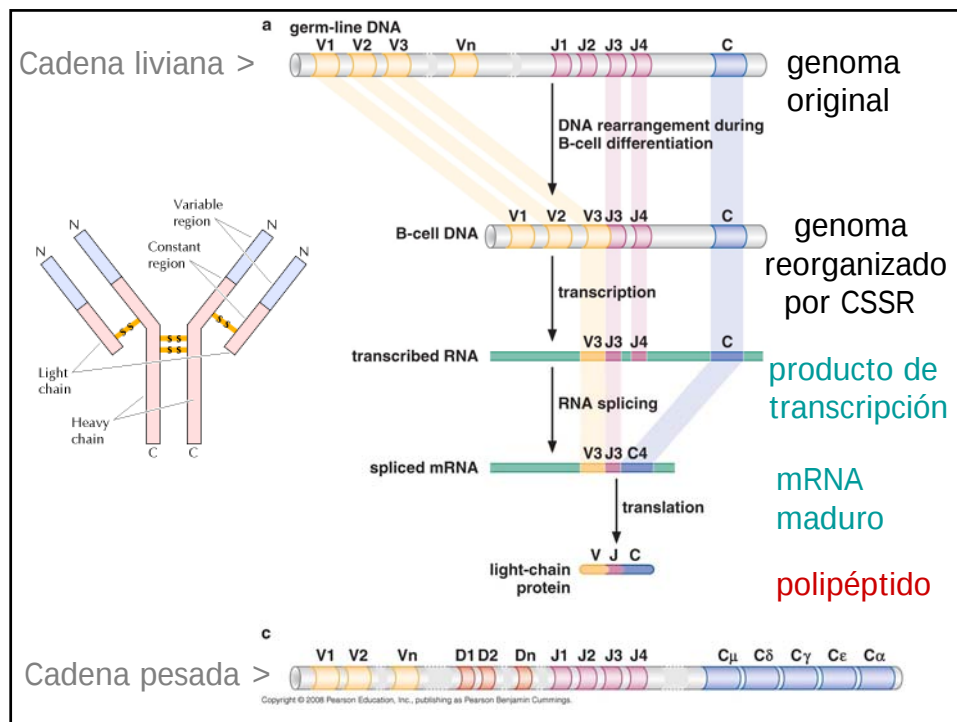


Figure 5.42. Rearrangement of immunoglobulin light-chain genes

Each light-chain gene (mouse κ light chains are illustrated) consists of a constant region (C), a joining region (J), and a variable region (V). There are approximately 250 different V regions, which are separated from J and C by about 20 kb in germ-line DNA. During the development of B lymphocytes, site-specific recombination joins one of the V regions to one of the four J regions. This rearrangement activates transcription, resulting in the formation of a primary transcript containing the rearranged VJ region together with the remaining J regions and C. The remaining unused J regions and the introns between J and C are then removed by splicing, yielding a functional mRNA.

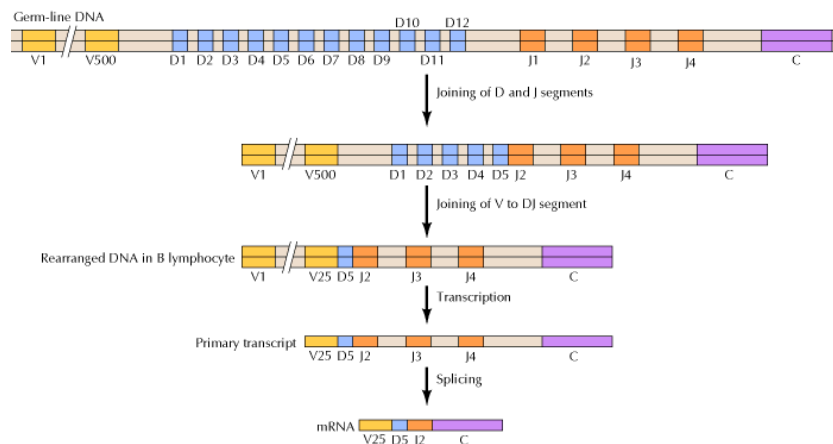


Figure 5.43. **Rearrangement of immunoglobulin heavy-chain genes**

The heavy-chain genes contain D regions in addition to V, J, and C regions. First the D and J segments join. Then a V segment is joined to the rearranged DJ region. The introns between J and C are removed by splicing to yield heavy-chain mRNA.

