Recombinación Genética

Recombinación homóloga (general o recíproca): intercambio genético entre dos moléculas de DNA o entre segmentos de la misma molécula de DNA que comparten una **extensa región de secuencia igual o muy similar** (homología). Las secuencias nucleotídicas en sí mismas no son importantes; sólo se requiere que sean iguales o muy similares en ambos segmentos de DNA.

Recombinación Genética

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Recombinación sitio específica: el intercambio genético tiene lugar sólo entre regiones del DNA **relativamente cortas** y con secuencias **particulares y definidas** (específicas).

Transposición: movimiento de segmentos relativamente cortos de DNA desde un lugar del genoma a otro (dejando o no una copia en el lugar original).

Barbara McClintock, 1902–1992

lesiones en el DNA que impiden el avance de la maquinaria de replicación

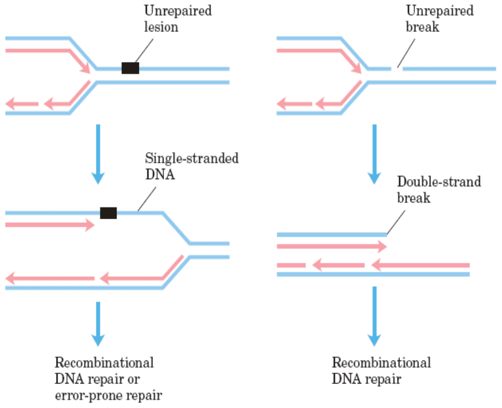

FIGURE 25–28 DNA damage and its effect on DNA replication. If the replication fork encounters an unrepaired lesion or strand break, replication generally halts and the fork may collapse. A lesion is left behind in an unreplicated, single-stranded segment of the DNA; a strand break becomes a double-strand break. In each case, the damage to one strand cannot be repaired by mechanisms described earlier in this chapter, because the complementary strand required to direct accurate repair is damaged or absent. There are two possible avenues for repair: recombinational DNA repair (described in Fig. 25–37) or, when lesions are unusually numerous, error-prone repair. The latter mechanism involves a novel DNA polymerase (DNA polymerase V, encoded by the *umuC* and *umuD* genes) that can replicate, albeit inaccurately, over many types of lesions. The repair mechanism is referred to as error-prone because mutations often result.

La necesidad de la
reparación por recombinación

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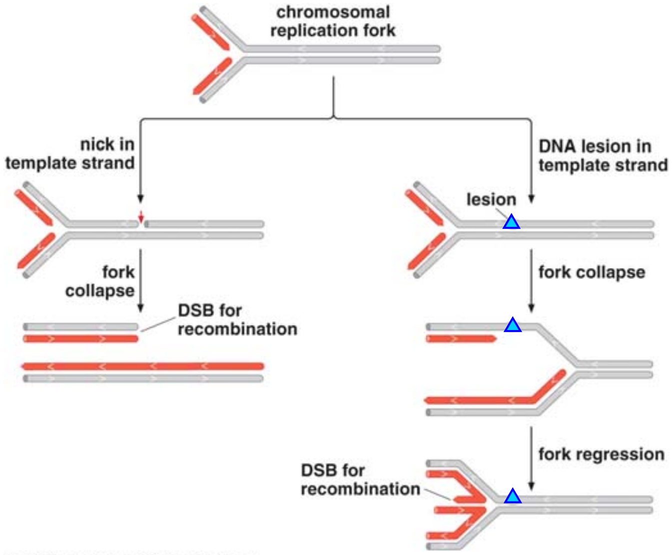
lesiones en el DNA que impiden el avance de la maquinaria de replicación



Recombinación homóloga

(HR = Homologous Recombination)

Mecanismo de **reparación del DNA**:
los mecanismos estudiados hasta ahora en el curso se caracterizan por:

extraer el DNA de la cadena dañada y
usar la **cadena complementaria** como **molde**
para resintetizar el tramo eliminado.

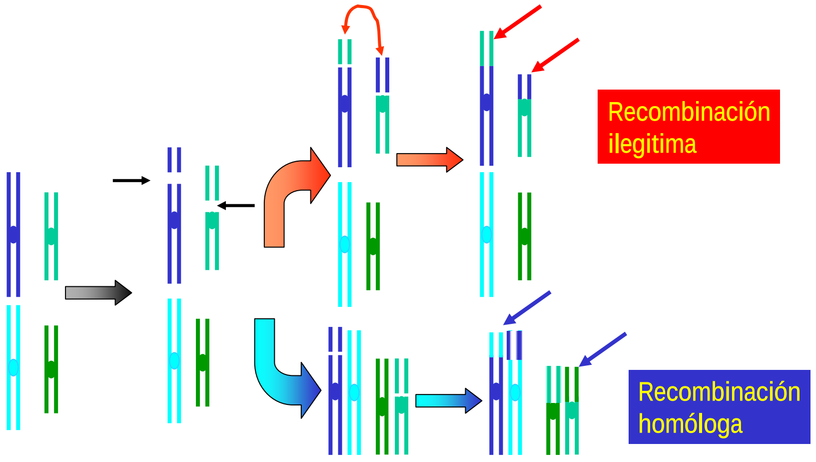
Cuando **ambas cadenas** están dañadas *(double strand break = DSB)***,** o cuando se mantiene una **lesión de cadena simple** porque no funcionaron otros mecanismos, la horquilla de replicación se **atasca**.

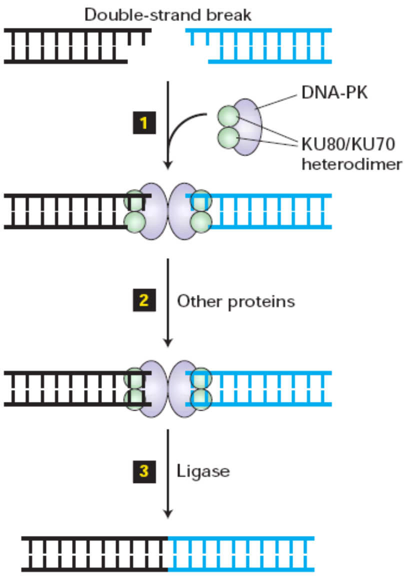
Si el DSB no se repara se pueden producir reordenamientos y aberraciones cromosómicas que llevan a la muerte celular.

El DSB es generalmente causado por **especies reactivas de oxígeno** generadas por el metabolismo celular o por radiaciones ionizantes.

El DSB se repara por **HR** o por la unión no-homóloga de los extremos, *non-homologous end joining* ***(NHEJ).*** Los DSB son recombinogénicos.

Recombinación homóloga y no homóloga





NHEJ

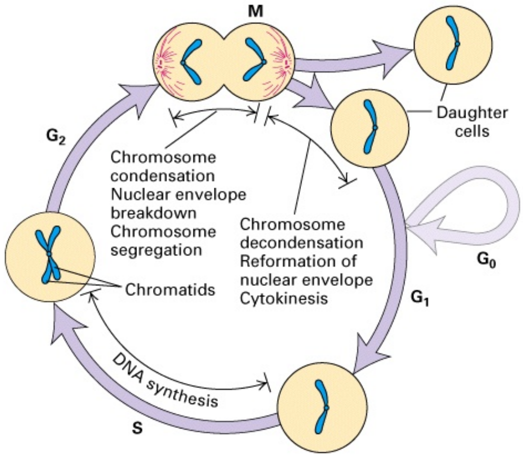
NHEJ es un mecanismo de reparación imperfecto, ya que se pierden algunas secuencias.

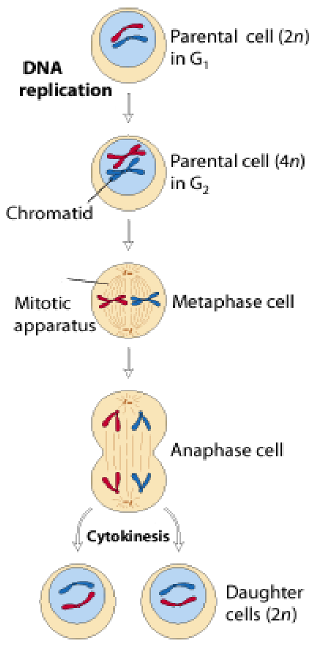
Esto es menos dañino que dejar los extremos libres para que inicien reordenamientos cromosomales.

Esta situación es análoga al resultado del mecanismo SOS (replicación de DNA con muy escasa fidelidad cuado el molde se encuentra dañado)

▲ **FIGURE 23-32 Repair of double-strand breaks by end-joining.** In general, nucleotide sequences are butted together that were not apposed in the unbroken DNA. These DNA ends are usually from the same chromosome locus, and when linked together, several base pairs are lost. Occasionally, ends from different chromosomes are accidentally joined together. A complex of two proteins, Ku and DNA-dependent protein kinase, binds to the ends of a double-strand break (1). After formation of a synapse, the ends are further processed by nucleases, resulting in removal of a few bases (2), and the two double-stranded molecules are ligated together (3). As a result, the double-strand break is repaired, but several base pairs at the site of the break are removed. [Adapted from G. Chu, 1997 *J. Biol. Chem.* **272**:24097; M. Lieber et al., 1997 *Curr. Opin. Genet. Devel.* **7**:99; and D. van Gant et al., 2001, *Nature Rev. Genet.* **2**:196.]

El ciclo celular es una serie ordenada de acontecimientos que conduce a la replicación de las células





◀ **mitosis**

2n

↓ ↓

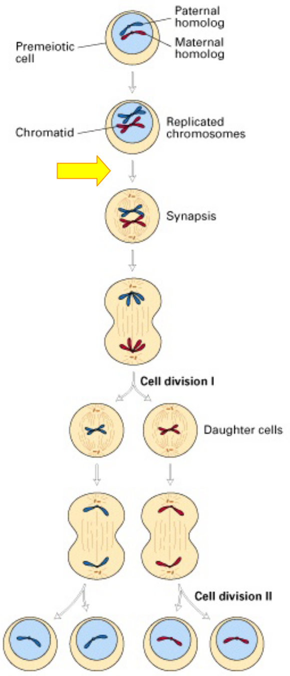
2n 2n

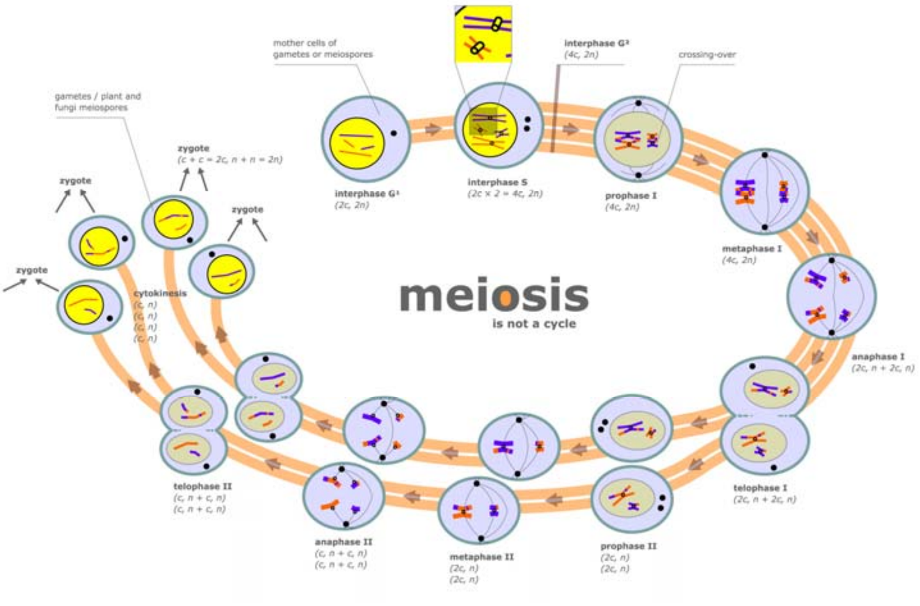
meiosis ▶

2n

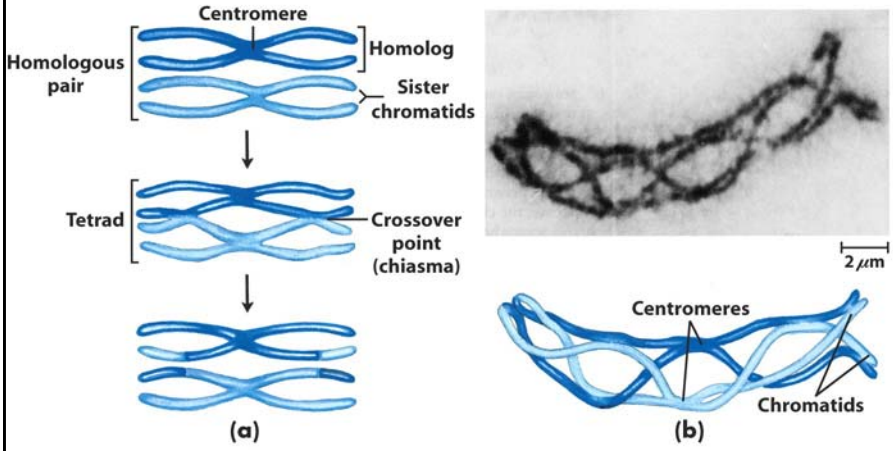
↓ ↓ ↓ ↓

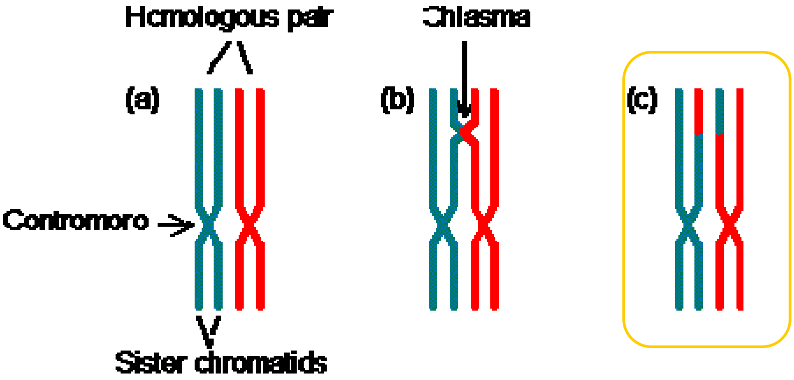
n n n n



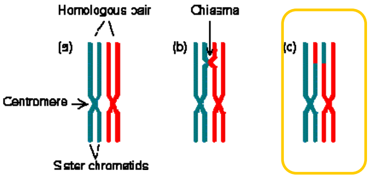


crossing over



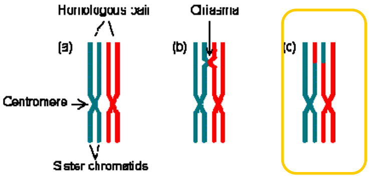


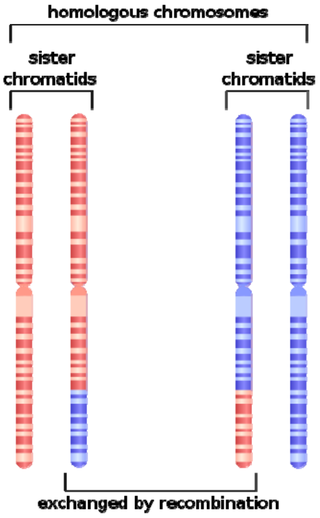
Recombinación homóloga
(durante la meiosis)

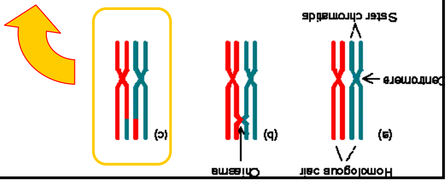


Recombinación homóloga

(durante la meiosis)

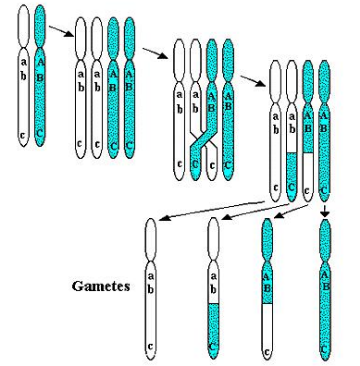




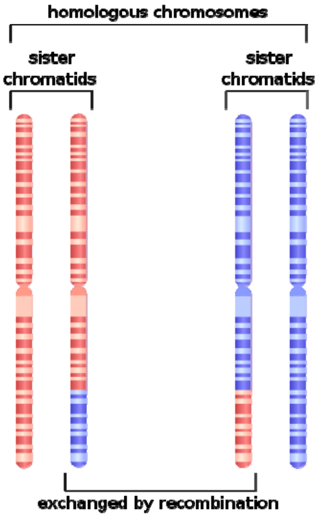


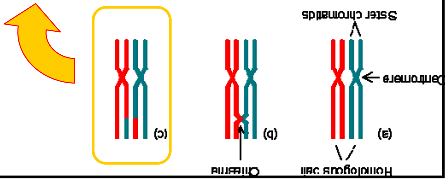
Recombinación homóloga

(durante la meiosis)



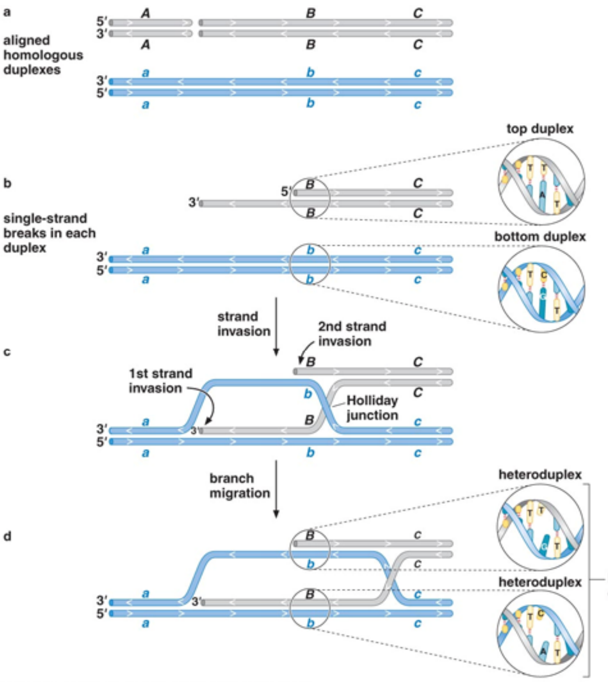
Crossing-over and recombination during meiosis





Recombinación homóloga

(durante la meiosis)

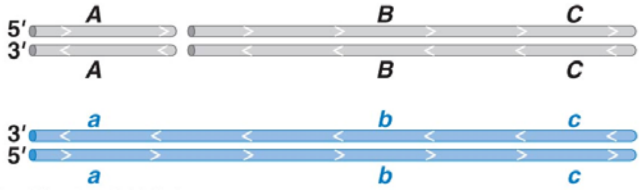


10-1 **Holliday model through the steps of branch migration.** The small arrowheads DNA single strands point in the 5′ to 3′ direction. Note that A and a, B and b, C and c specify differ es, and have slightly different DNA sequences. Therefore, heteroduplex DNA containing these genes in the expanded section in panel d) will have some mismatches.

Modelo de Holliday de recombinación homóloga

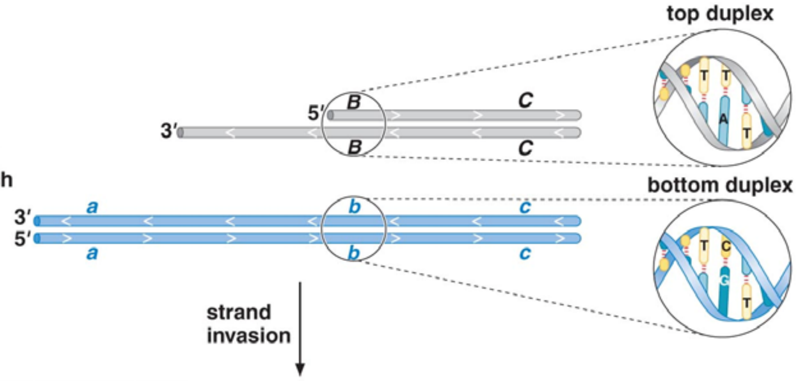
a

aligned homologous duplexes

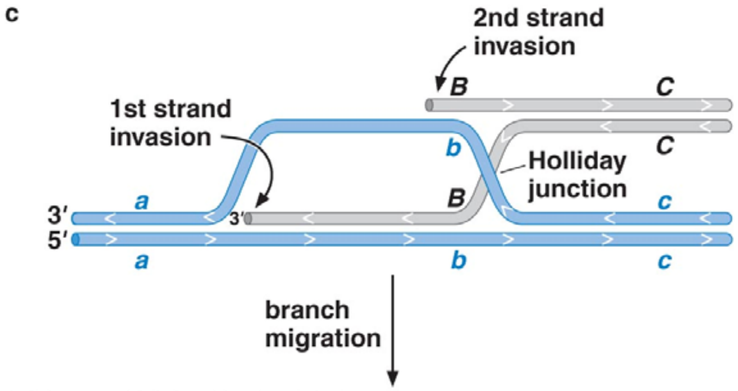


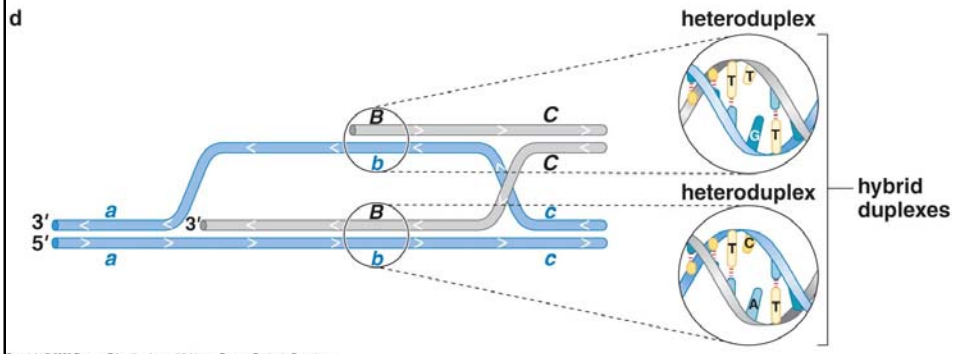
b

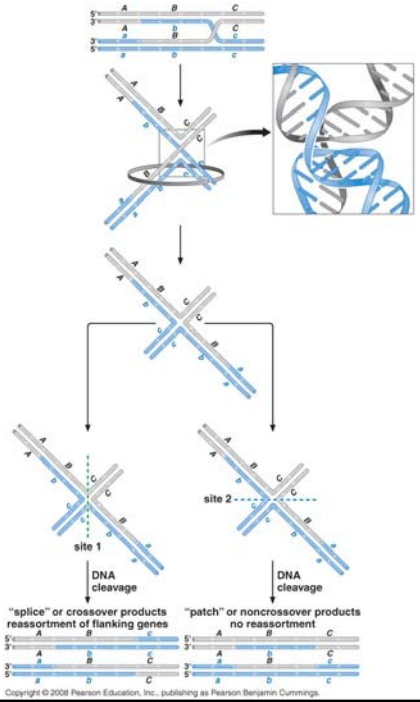
single-strand breaks in each duplex



strand invasion


c
2nd strand
invasion
B
C
1st strand
invasion
b
C
Holliday
junction
3′
a
3′
B
c
5′
a
b
c
branch
migration


d
heteroduplex
B
C
b
C
heteroduplex
hybrid
duplexes
3′
a
3′
B
c
5′
a
b
c
T
T
G
T
T
C
A
T



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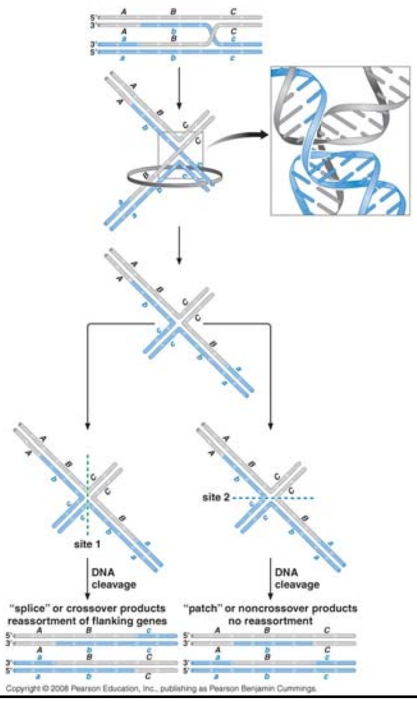
◄ intermediario cruciforme de Holliday

Modelo de Holliday de **recombinación homóloga**



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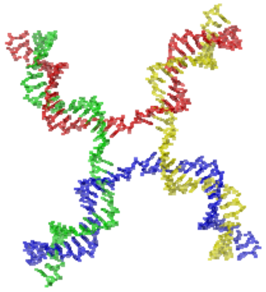
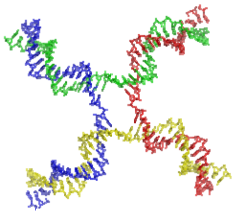
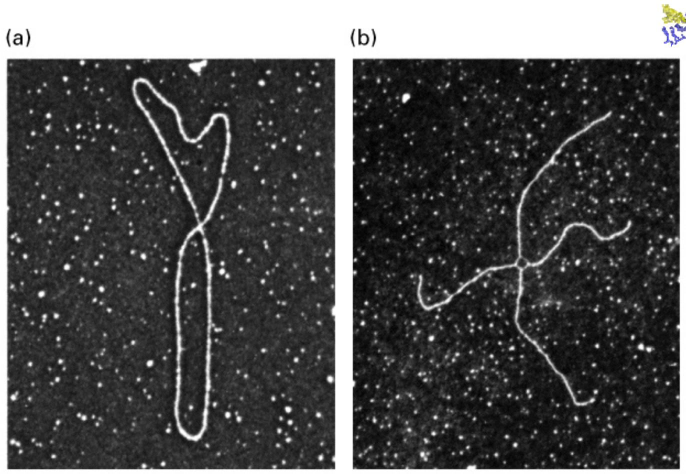


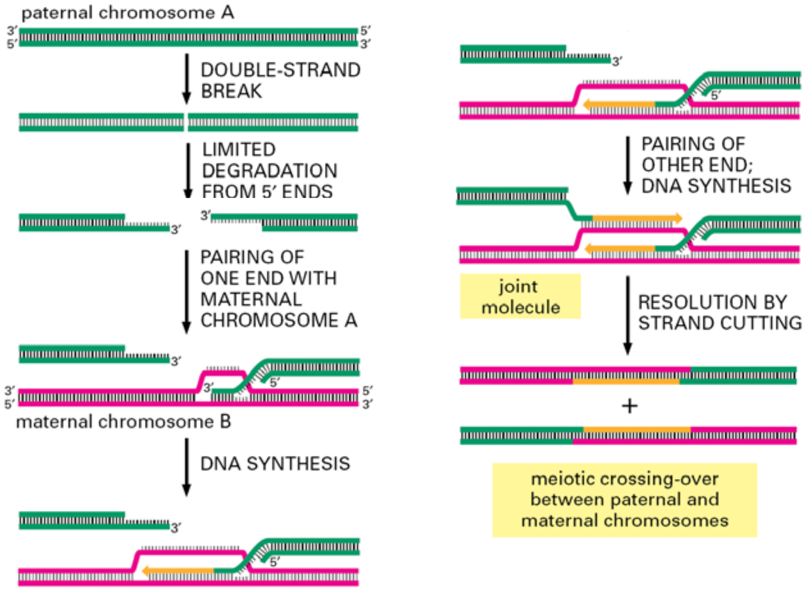
Resolución del intermediario cruciforme de Holliday





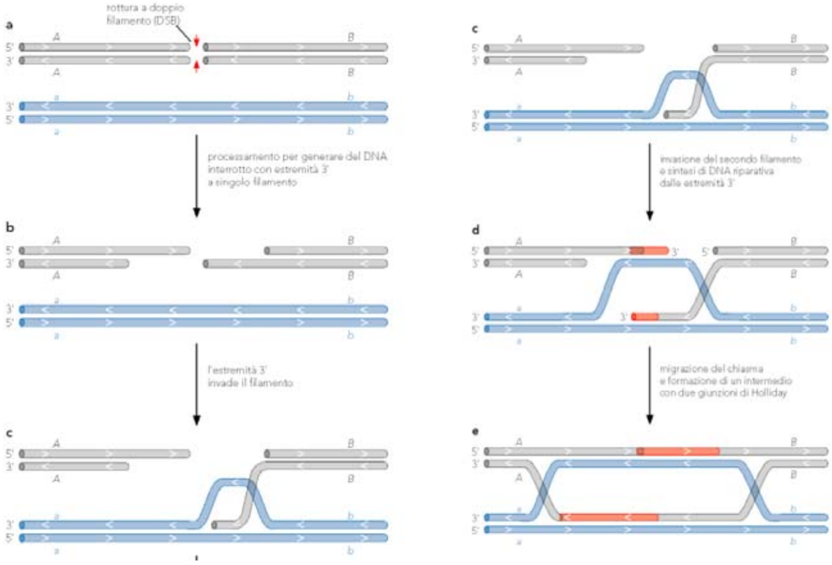
Modelo de recombinación homóloga iniciada por “*double strand break*” (DSB)

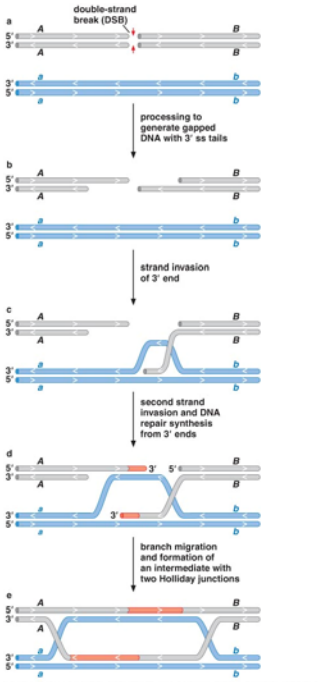
Si para reparar DSB se usa este mecanismo en lugar de NEJ se minimiza la introducción de cambios



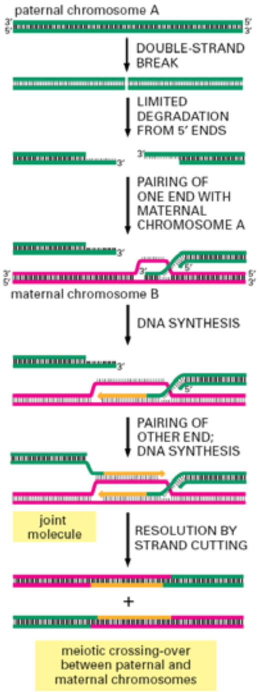
Modelo de recombinación homóloga iniciada por “*double strand break*” (DSB)

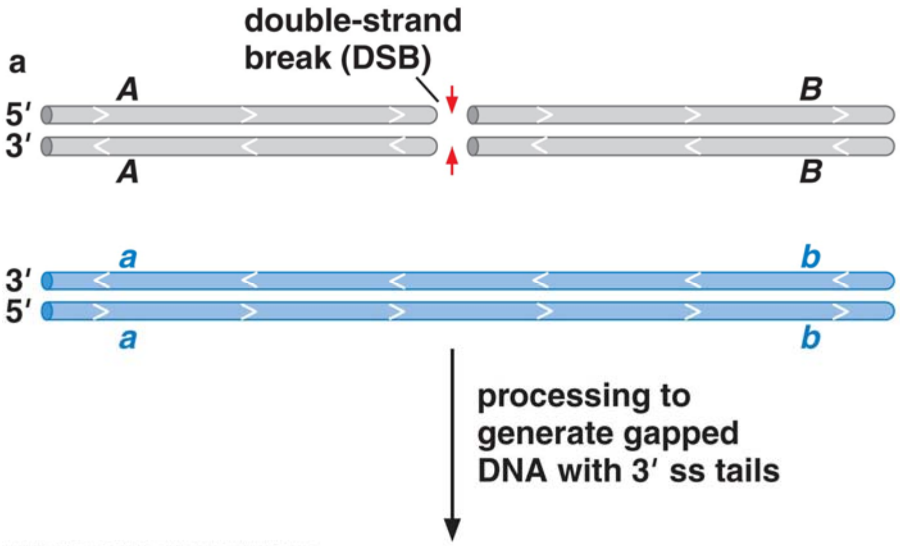
Si para reparar DSB se usa este mecanismo en lugar de NHEJ se minimiza la introducción de cambios





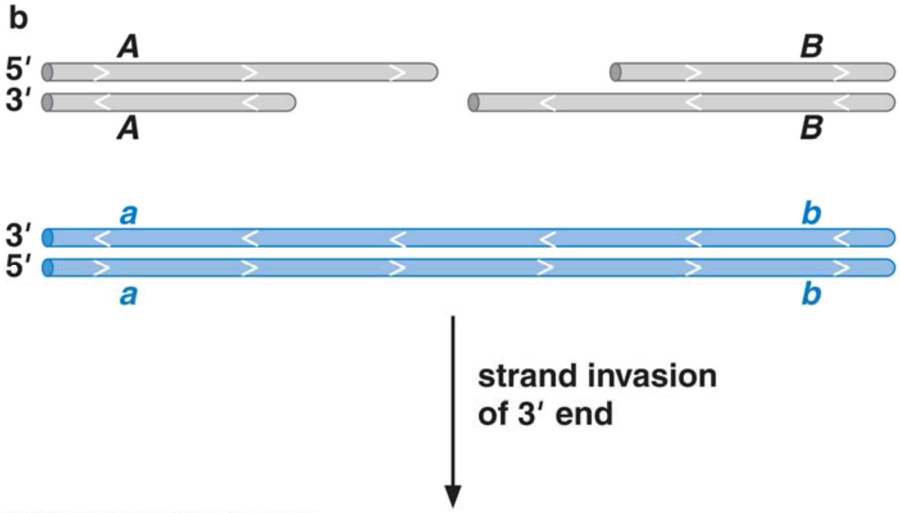
Modelo de recombinación homóloga niciada por “*double strand break*” (DSB)



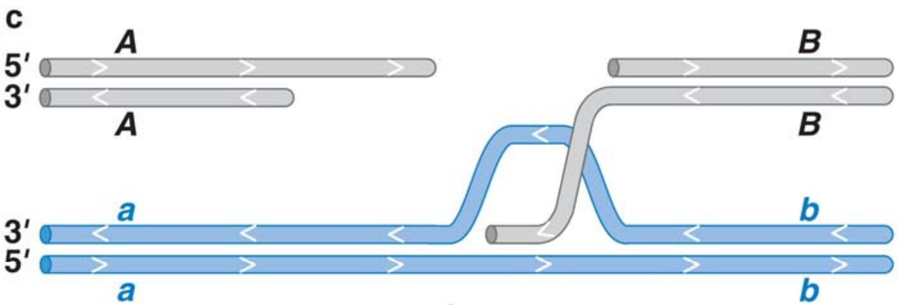


double-strand break (DSB)

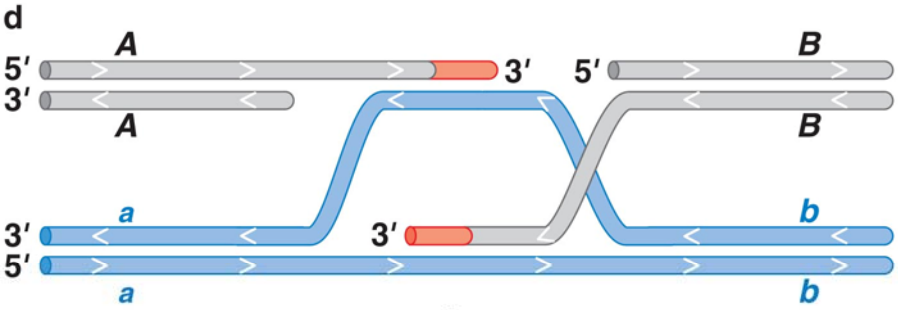
processing to generate gapped DNA with 3′ ss tails



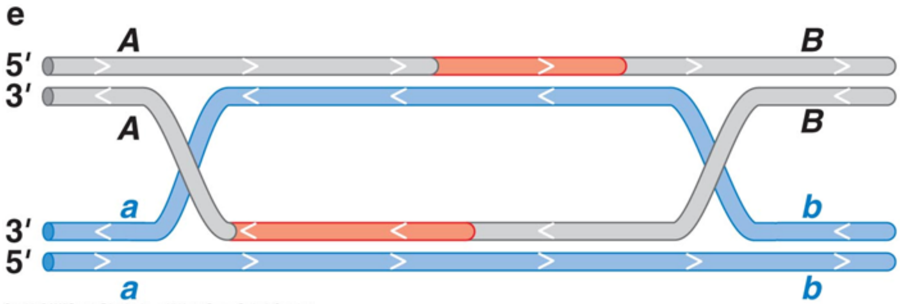
strand invasion of 3′ end



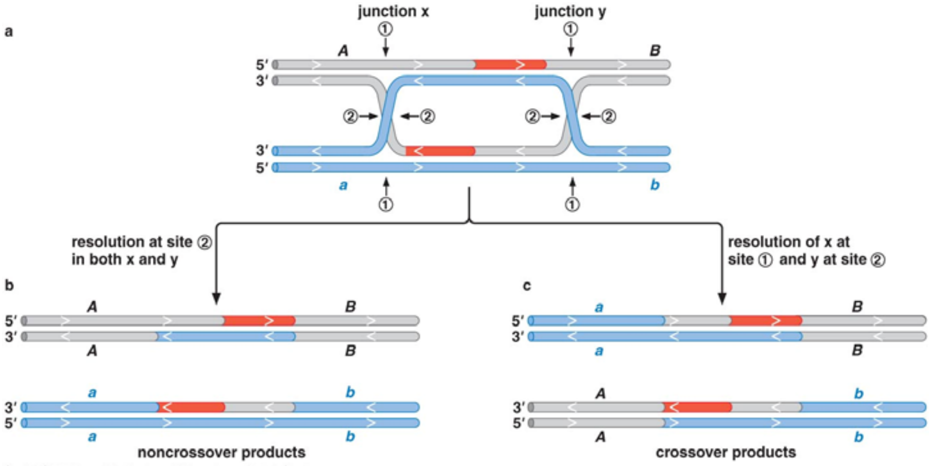
second strand invasion and DNA repair synthesis from 3′ ends



branch migration and formation of an intermediate with two Holliday junctions

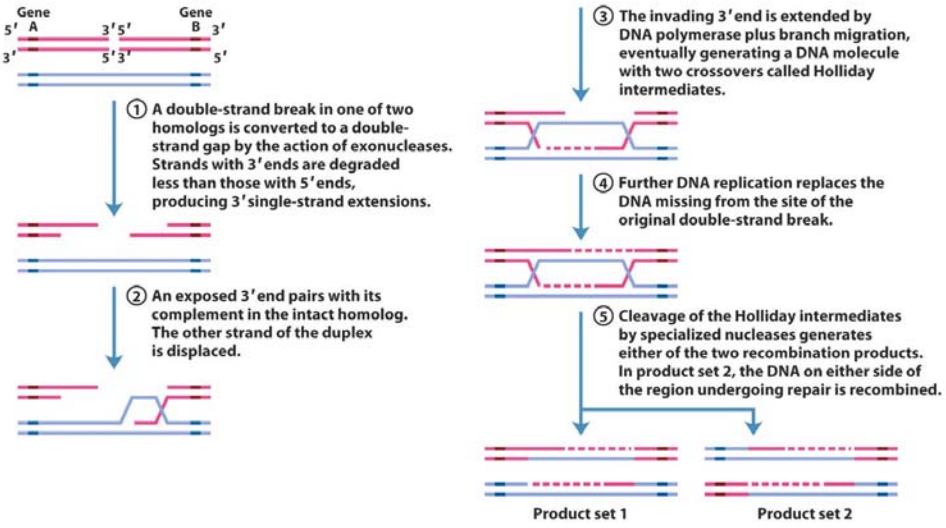


Algunos productos de resolución de los intermediarios del modelo de DSB



BOX 10-1 FIGURE 1 Two possible ways of resolving an intermediate from the DSB-repair pathway. The parental DNA molecules were like those in Figure 10-3. The regions of red DNA are those that were resynthesized during recombination.

Recombinación iniciada por DSB (Fig. Lehninger)



① A double-strand break in one of two homologs is converted to a double-strand gap by the action of exonucleases. Strands with 3′ ends are degraded less than those with 5′ ends, producing 3′ single-strand extensions.

② An exposed 3′ end pairs with its complement in the intact homolog. The other strand of the duplex is displaced.

③ The invading 3′ end is extended by DNA polymerase plus branch migration, eventually generating a DNA molecule with two crossovers called Holliday intermediates.

④ Further DNA replication replaces the DNA missing from the site of the original double-strand break.

⑤ Cleavage of the Holliday intermediates by specialized nucleases generates either of the two recombination products. In product set 2, the DNA on either side of the region undergoing repair is recombined.

Recombinación iniciada por DSB (Fig. Lodish)

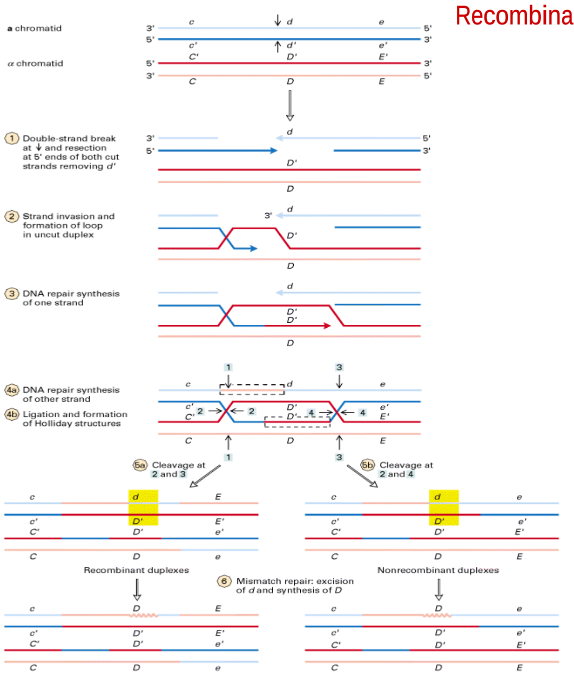


Figure 12-31. Double-strand break model of meiotic recombination developed from studies in the yeasst. A pair of homologous chromatids (double-stranded DNA molecules) are shown, one in blue and the other red. The darker and lighter shades indicate complementary DNA strands. Alleles are indicated by capital and lowercase letters *(D, d).* Complementary DNA strands are also indicated by the presence or absence of prime signs (for instance D and D , and c and c). In this example, the initial double-strand break and resection of 5 ends occurs on the a chromatid, removing the *d* marker 1. This is followed by strand invasion 2 and DNA synthesis with the a chromatid *D* strand as the template 3. Repair synthesis of the other a strand (using its complementary section on the a strand) and ligation result in formation of a Holliday structure with two crossovers 4a and 4b. (Repaired regions are marked by black dashed lines.) Resolution of this crossed-strand intermediate can occur in two ways. Cleavage at sites 2 and 4 (step 5b), or at sites 1 and 3 (not shown here), yields nonrecombinant chromosomes, since all markers surrounding the crossover site (i.e., to the left of *c* and to the right of *E*) are derived from the same initial chromosome. One duplex contains a complementary *D/D* region, but the other contains a heteroduplex mismatched *d/D* region (yellow). In contrast, cleavage (step 5a) at sites 2 and 3, or at sites 1 and 4 (not shown here), yields recombinant double-stranded DNA molecules, since all markers to the left and right of the crossover site have undergone reciprocal recombination. Note that one duplex contains a complementary *D/D* region, but the other contains a heteroduplex, mismatched *d/D* region (yellow). Cells can repair such mismatched heteroduplex regions by excising a single-strand segment containing the mismatch and using the other strand as a template for synthesis of a matching strand 6 (see Figure 12-24). In this example, *d* is removed and *D* is synthesized (jagged red segment), thus "converting" *d* to *D.* The opposite D *d* conversion occurs with equal frequency.

Conversión génica

El fenómeno de DSB se estudió en levaduras porque los cuatro productos meióticos se pueden estudiar en la progenie haploide de esporos.

El paso 6 de la figura anterior muestra que tanto el duplex recombinante como el no recombinante poseen regiones de heteroduplex D-D´.

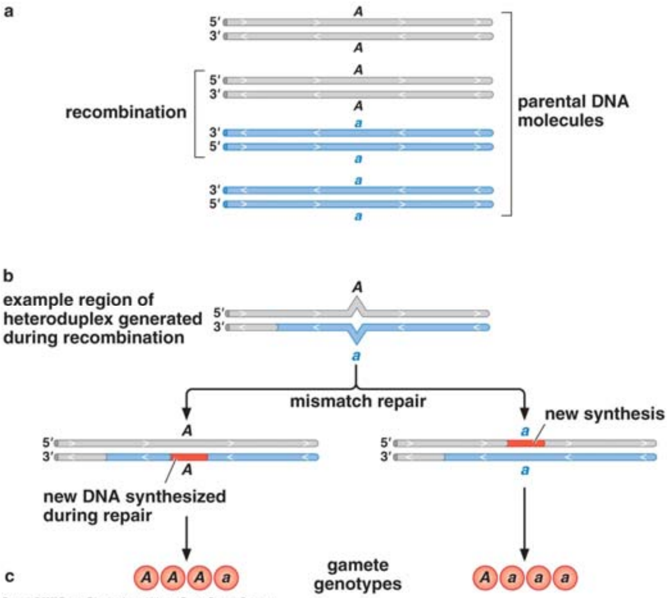
El sistema de reparación *“mismatch”* corregirá D la mitad del tiempo y D´ la otra mitad. De esta forma, tres de los cuatro esporos haploides de las levaduras llevarán el fenotipo D y uno el D´o viceversa.

Los marcadores alélicos alejados del punto de *“cross over”* segregan de acuerdo al cociente 2:2 dado por la segregación independiente de Mendel.

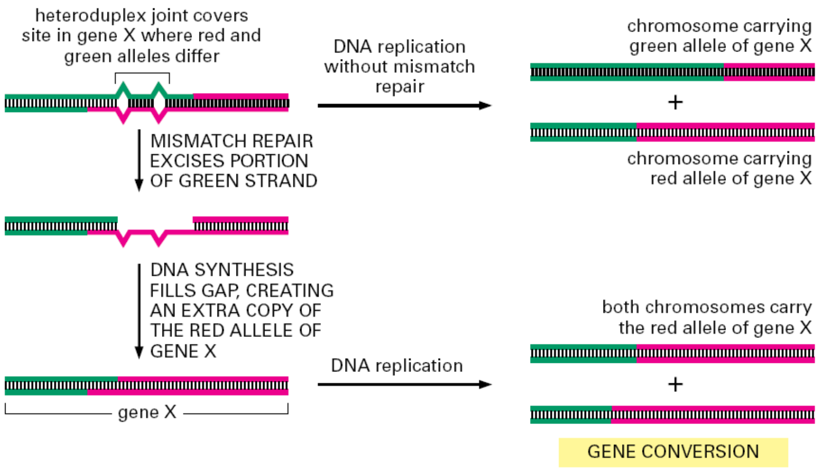
Los marcadores alélicos cerca del punto del “*cross over*” segregan en una relación 3:1 o 1:3.

El fenómeno se denomina conversión génica porque un alelo se “convierte“ en otro.

Conversión génica



Conversión génica



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El fenómeno se denomina **conversión génica** porque un alelo se “convierte“ en otro.

Las proteínas que catalizan la RH

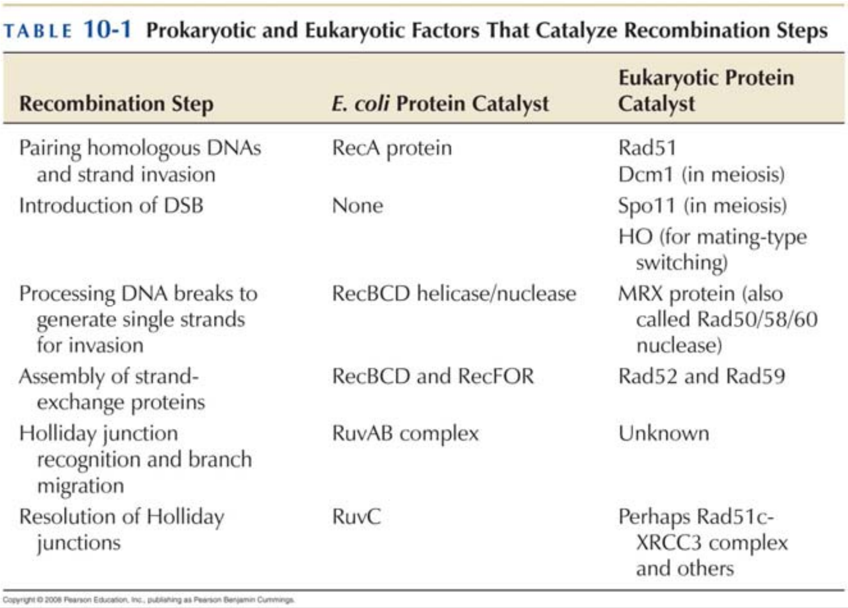
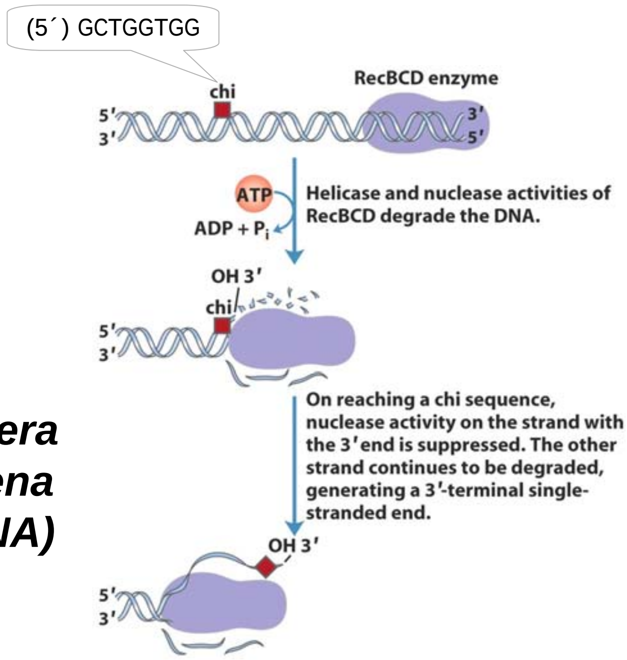


TABLE 10-1 Prokaryotic and Eukaryotic Factors That Catalyze Recombination Steps

Recombination Step	*E. coli* Protein Catalyst	Eukaryotic Protein Catalyst
Pairing homologous DNAs and strand invasion	RecA protein	Rad51 Dcm1 (in meiosis)
Introduction of DSB	None	Spo11 (in meiosis) HO (for mating-type switching)
Processing DNA breaks to generate single strands for invasion	RecBCD helicase/nuclease	MRX protein (also called Rad50/58/60 nuclease)
Assembly of strand-exchange proteins	RecBCD and RecFOR	Rad52 and Rad59
Holliday junction recognition and branch migration	RuvAB complex	Unknown
Resolution of Holliday junctions	RuvC	Perhaps Rad51c-XRCC3 complex and others

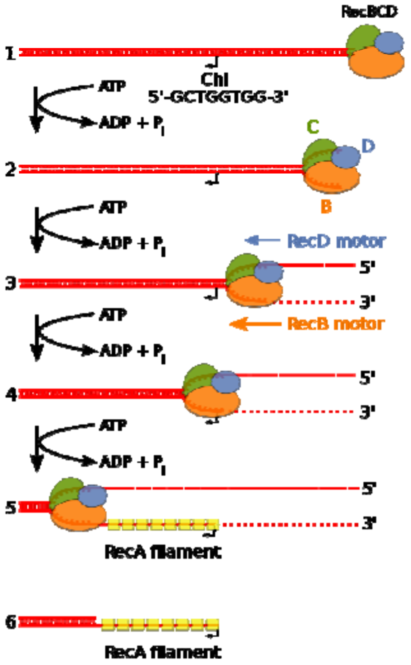


Helicase and nuclease activities of RecBCD degrade the DNA.

On reaching a chi sequence, nuclease activity on the strand with the 3′end is suppressed. The other strand continues to be degraded, generating a 3′-terminal single-stranded end.

Rec*BCD genera DNA de cadena simple (ssDNA)*

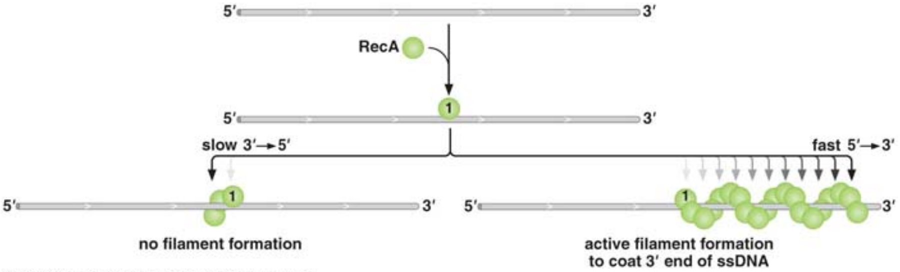
Rec*BCD genera DNA de cadena simple (ssDNA)*

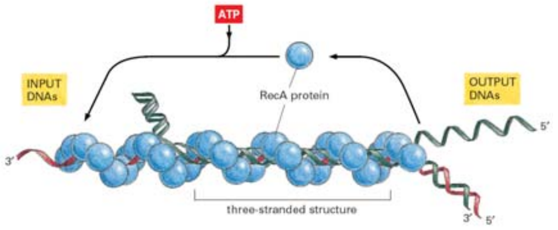


Beginning of the RecBCD pathway.
1: RecBCD binds to a DNA double strand break.
2: RecBCD initiates unwinding of the DNA duplex through ATP-dependent helicase activity.
3: RecBCD continues its unwinding and moves down the DNA duplex, cleaving the 3' strand much more frequently than the 5' strand.
4: RecBCD encounters a Chi sequence and stops digesting the 3' strand; cleavage of 5' is significantly increased.
5: RecBCD loads RecA onto the 3' strand.
6: RecBCD unbinds from the DNA duplex, leaving a RecA nucleoprotein filament on the 3' tail.

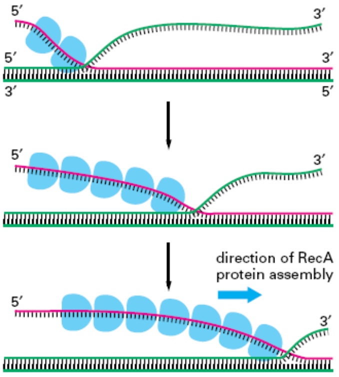
RecA se une al ssDNA

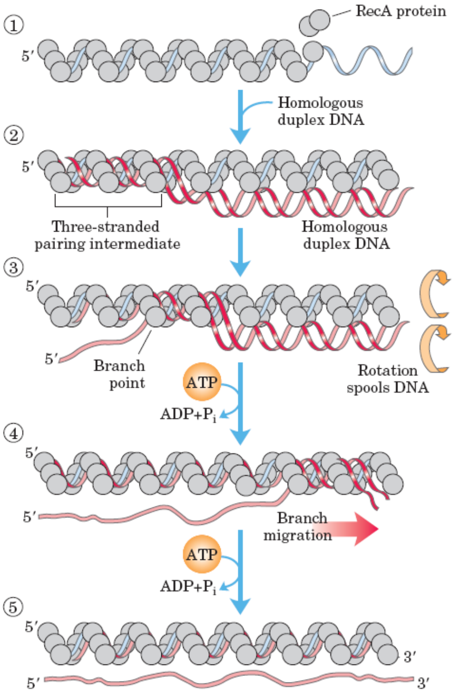
RecA se une al ssDNA





RecA se une al ssDNA y cataliza la invasión de cadenas





intercambio de cadenas catalizado por RecA

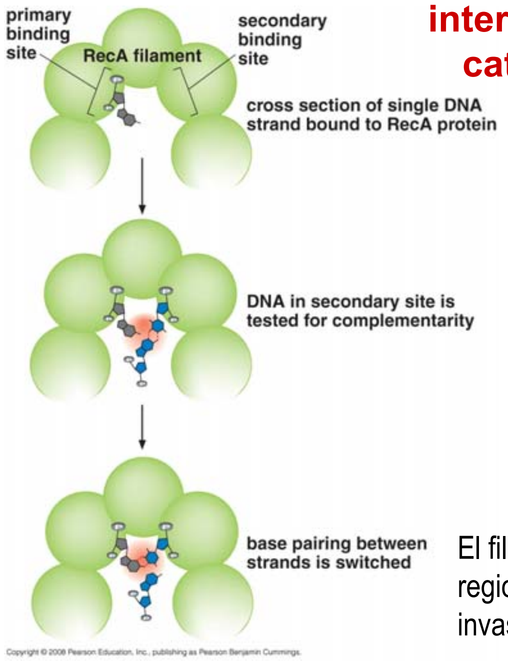


FIGURE 10-10 Model of two steps in the search for homology and DNA strand exchange within the RecA filament. Here the RecA filament is represented from a top down view as in Figure 10-8c. The incoming DNA duplex is shown in blue. (Source: Adapted from Howard-Flanders et al. 1984. *Nature* 309: 215–220. Copyright © 1984 Nature Publishing Group. Used with permission.)

El filamento formado por RecA busca regiones de homología e inicia la invasión e intercambio de cadenas

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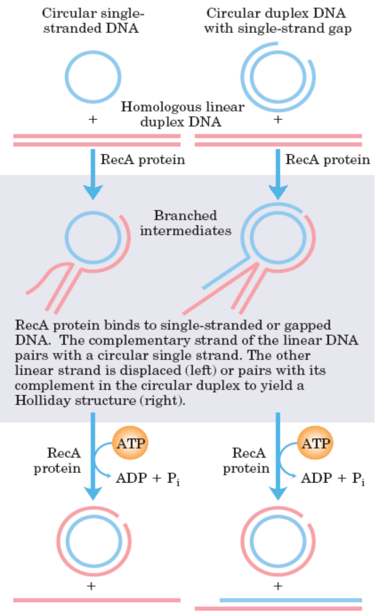
sustratos de RecA

ensayos *in vitro*



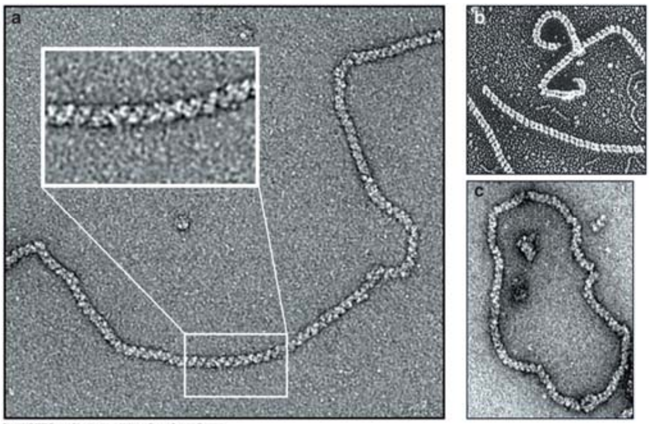
RecA protein binds to single-stranded or gapped DNA. The complementary strand of the linear DNA pairs with a circular single strand. The other linear strand is displaced (left) or pairs with its complement in the circular duplex to yield a Holliday structure (right).

Continued branch migration yields a circular duplex with a nick and a displaced linear strand (left) or a partially single-stranded linear duplex (right).

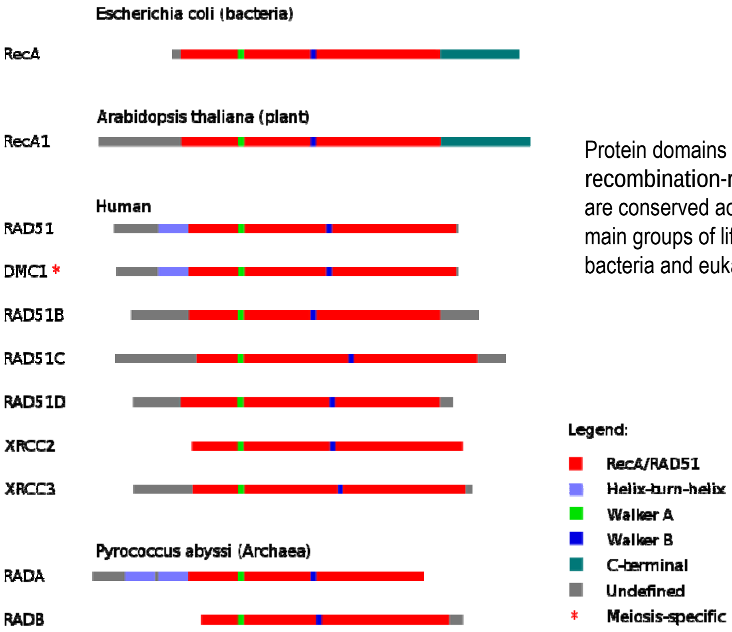
FIGURE 25-35 DNA strand-exchange reactions promoted by RecA protein in vitro. Strand exchange involves the separation of one strand of a duplex DNA from its complement and transfer of the strand to an alternative complementary strand to form a new duplex (heteroduplex) DNA. The transfer forms a branched intermediate. Formation of the final product depends on branch migration, which is facilitated by RecA. The reaction can involve three strands (left) or a reciprocal exchange between two homologous duplexes—four strands in all (right). When four strands are involved, the branched intermediate that results is a Holliday intermediate. RecA protein promotes the branch-migration phases of these reactions, using energy derived from ATP hydrolysis.

análogos de RecA

FIGURE 10-11 RecA-like proteins in three branches of life. Nucleoprotein filaments are shown for (a) human Rad51, (b) *E. coli* RecA, and (c) *A. fulgidus* RadA proteins. The Rad51 and RecA proteins are also shown in Figure 10-8. Notice the similar helical structure of the filaments revealed by the stripes in these EM images. (Source: West S.C. et al. *Nature Reviews in Molecular and Cell Biology* 4: 1–12. Images provided by A. Stasiak, University of Lausanne, Switzerland.)



RecA



Protein domains in homologous recombination-related proteins are conserved across the three main groups of life: archaea, bacteria and eukaryotes

El complejo RuvAB

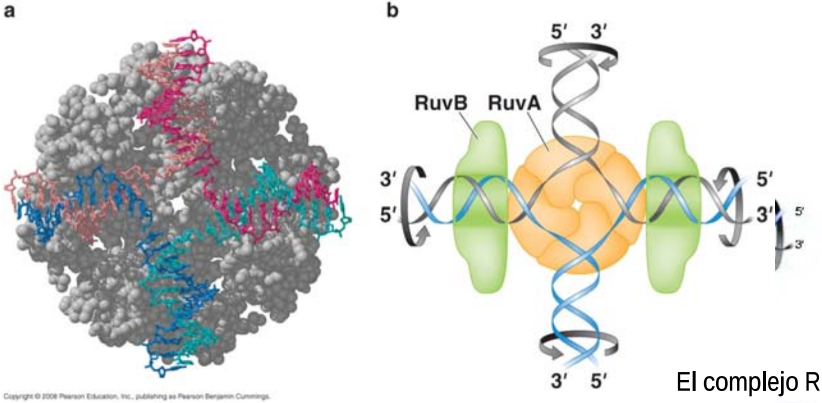


FIGURE 10-12 High resolution structure of RuvA and schematic model of the RuvAB complex bound to Holliday junction DNA. (a) The crystal structure of the RuvA tetramer shows the fourfold symmetry of the protein. (Ariyoshi M., Nishino T., Iwasaki H., Shinagawa H., and Morikawa K. 2000. *Proc. Natl. Acad. Sci. U.S.A.* 97: 8257–8262.) Image prepared with BobScript, MolScript, and Raster 3D. (b) A schematic model of the crystal structure is shown with two RuvB hexamers. Notice how a tetramer of RuvA binds with fourfold symmetry to the junction. Two hexamers of RuvB bind on opposite sides of RuvA, and function as a motor to pump DNA through the junction. The RuvB hexamers are shown in cross-sections, so that the DNA threading through these complexes can be seen. (Source: From Yamada K. et al. Crystal structure of the RuvA-RuvB complex. *Mol. Cell* 10: 677, fig. 4.)

El complejo RuvAB reconoce el intermediario de Holliday y promueve la migración de la ramificación

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RuvC = resolvasa

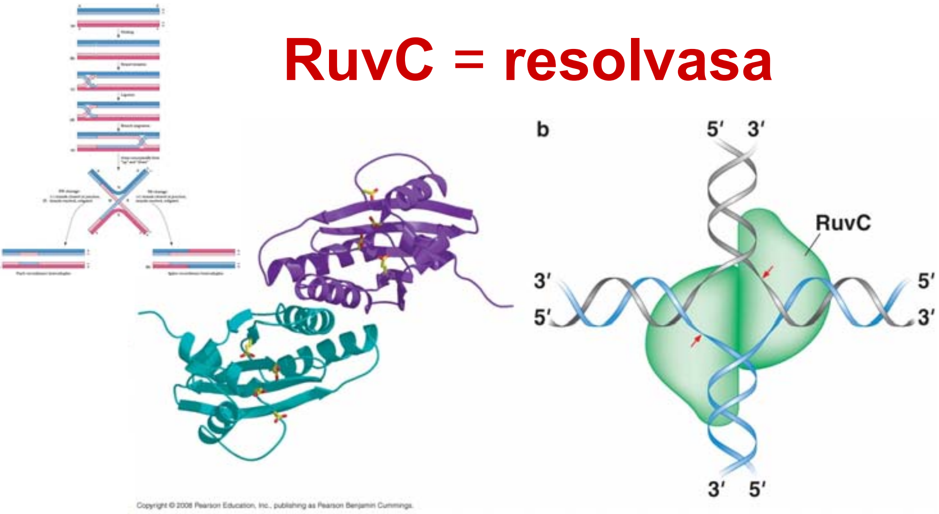
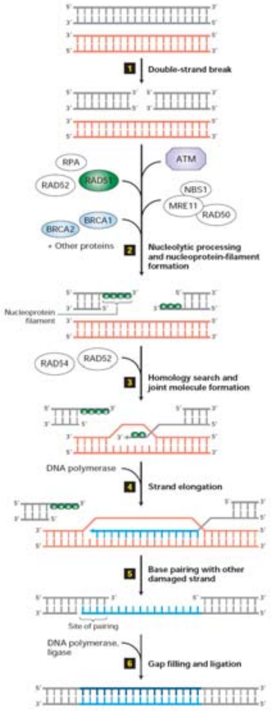


FIGURE 10-13 High resolution structure of the RuvC resolvase and schematic model of the RuvC dimer bound to Holliday junction DNA. (a) The crystal structure of the RuvC protein. (Ariyoshi M., Vassylyev D.G., Iwasaki H., Nakamura H., Shinagawa H., and Morikawa K. 1994. *Cell* 78: 1063–1072.) Image prepared with BobScript, MolScript, and Raster 3D. (b) Model for binding of a RuvC dimer to a Holliday junction. Notice how, in this model, a dimer of RuvC can bind the Holliday junction and introduce symmetrical cleavages into the two identical DNA strands. (Source: Rafferty J.B. et al. 1996. Crystal structure of DNA recombination protein RuvA. *Science* 274: fig. 1b, p. 416, fig. 3e, p. 418. Copyright © 1996 American Association for the Advancement of Science. Reprinted with permission.)

La resolvasa RuvC corta las cadenas de DNA y resuelve el intermediario de Holliday.

La recombinación homóloga es un mecanismo de reparación en procariotas.

En eucariotas también funciona la reparación de DSB.



◀ **FIGURE 23-31 Repair of double-strand breaks by homologous recombination.** During S phase cells copy each chromosome to create two identical sister chromatids that later segregate into daughter cells. The black and red DNAs represent the homologous sequences on these sister chromatids. Step 1: A double-strand DNA break forms in the chromatids. Step 2: The double-strand break activates the ATM kinase (see Figure 23-23); this leads to activation of a set of exonucleases that remove nucleotides at the break first from the 3' and then from the 5' ends of both broken strands, ultimately creating single-stranded 3' ends. In a process that is dependent on the BRCA1 and BRCA2 proteins, as well as others, the Rad51 protein (green ovals) polymerizes on single-stranded DNA with a free 3' end to form a nucleoprotein filament. Step 3: Aided by yet other proteins, one Rad51 nucleoprotein filament searches for the homologous duplex DNA sequence on the sister chromatid, then invades the duplex to form a joint molecule in which the single-stranded 3' end is base-paired to the complementary strand on the homologous DNA strand. Step 4: The replicative DNA polymerases elongate this 3' end of the damaged DNA (green strand), templated by the complementary sequences in the undamaged homologous DNA segment. Step 5: Next this repaired 3' end of the damaged DNA pairs with the single-stranded 3' end of the other damaged strand. Step 6: Any remaining gaps are filled in by DNA polymerase and ligase (light green), regenerating a wild-type double helix in which an entire segment (dark and light green) has been regenerated from the homologous segment of the sister chromatid. [Adapted from D. van Gent et al., 2001, *Nature Rev. Genet.* **2**:196.]