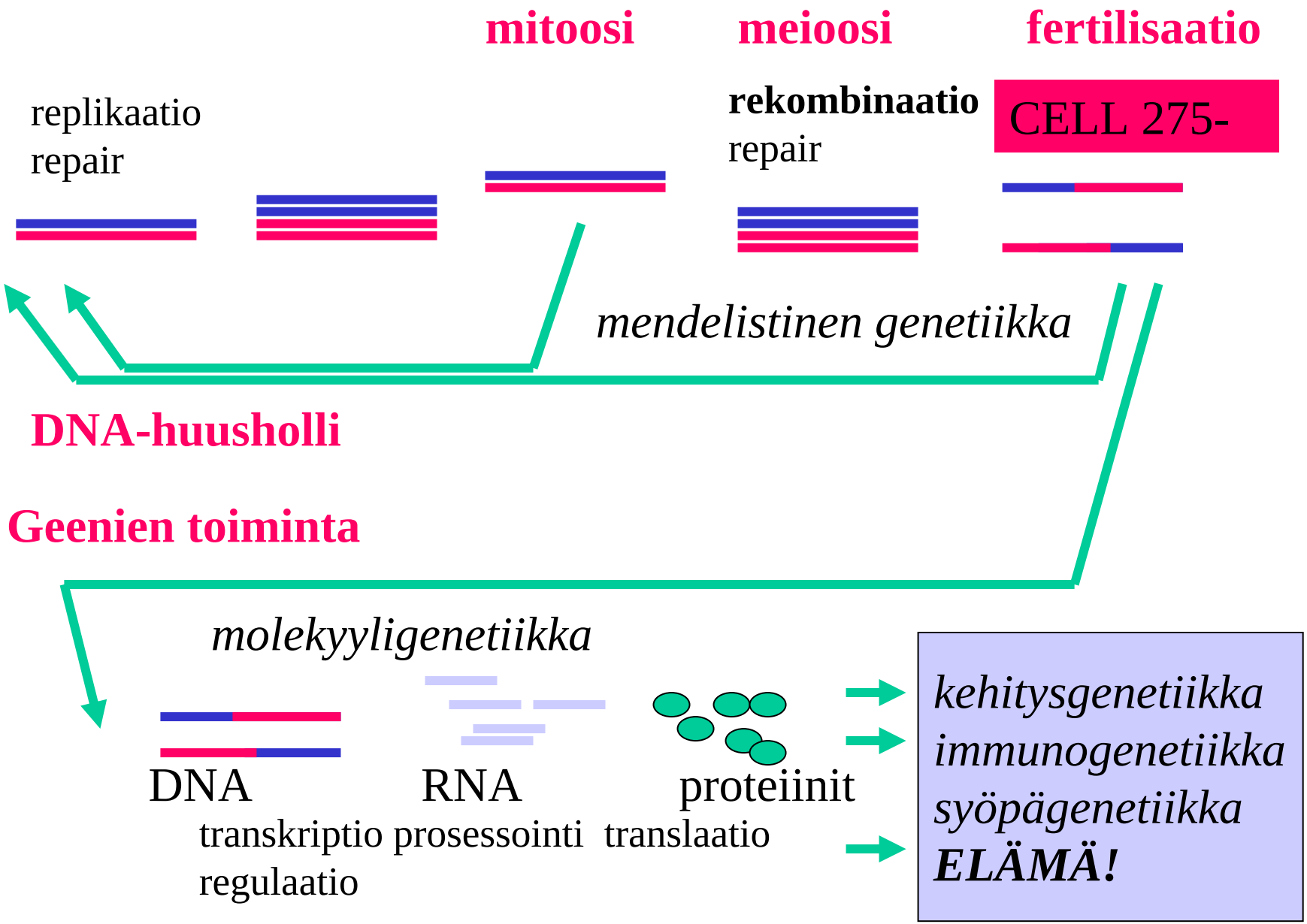
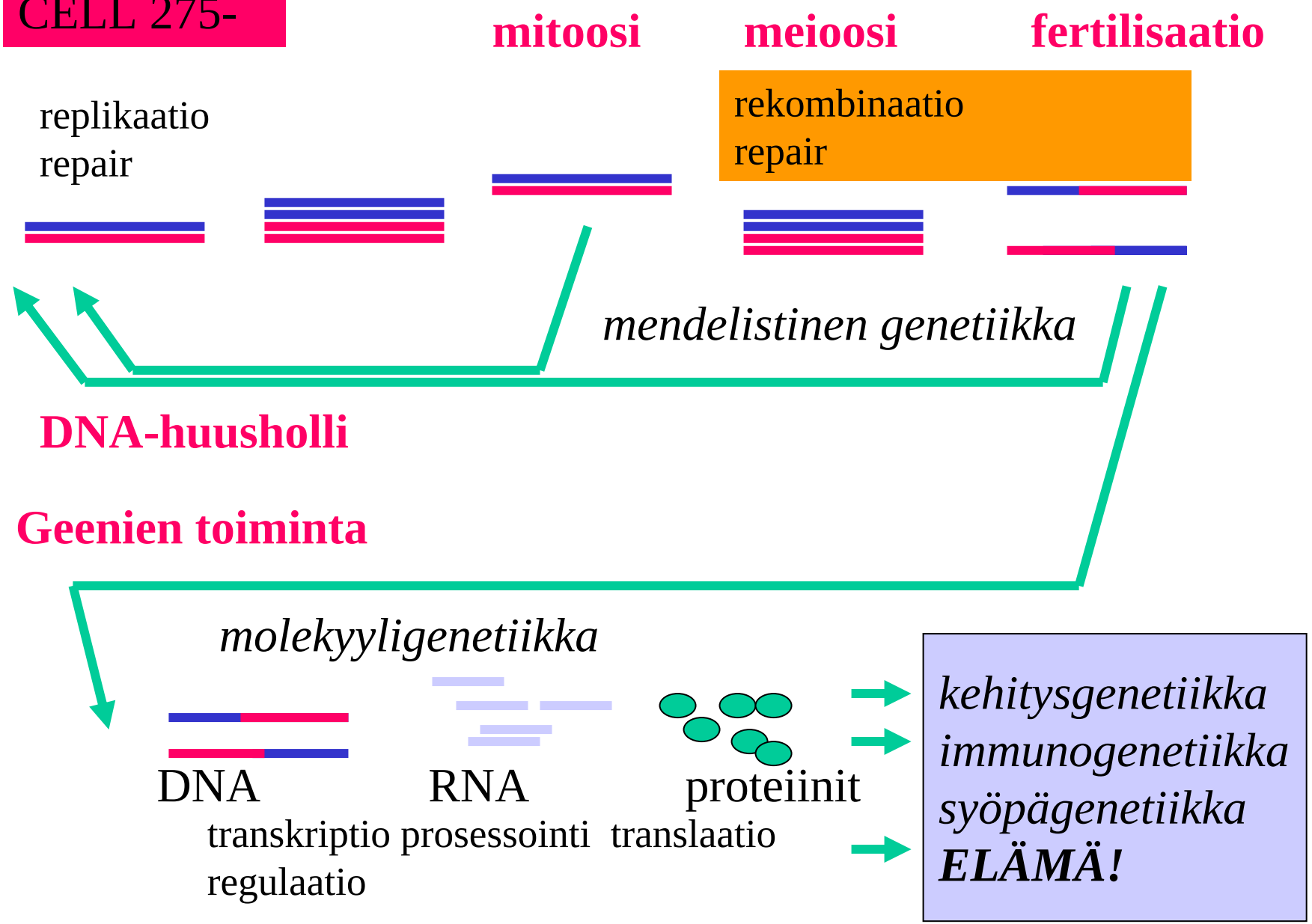



Genetiikan perusteiden miellekartta

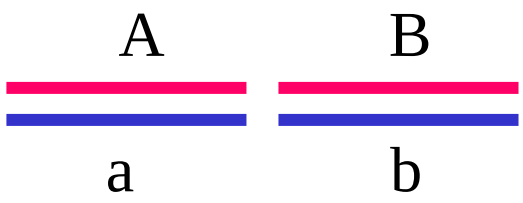
CELL 275-

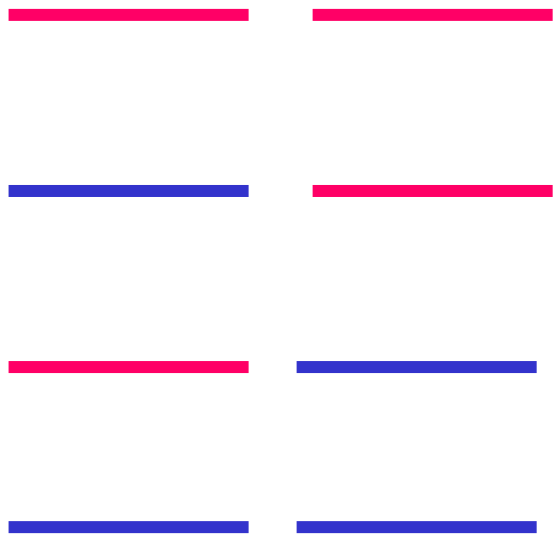



Genetiikan perusteiden miellekartta: tänään rekombinaatio

# Rekombinaatio eli perintöaineksen uudelleenjärjestäytyminen

Kromosomien *välinen* uudelleenjärjestäytyminen ei tietenkään ole mikään pulma (tärkeää kylläkin)



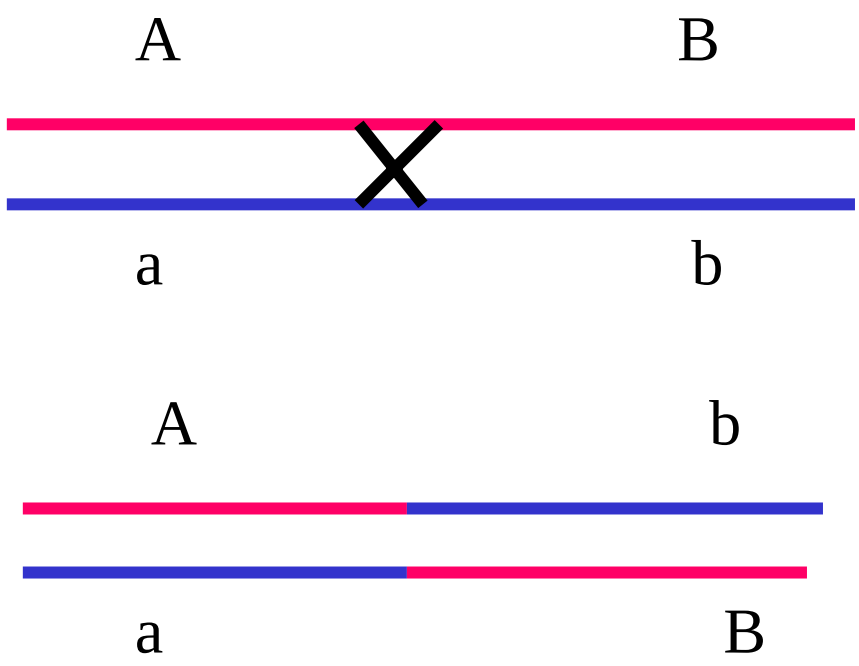



Genotyyppi tuottaa gameetteja, joissa eri kromosomit yhdistyvät vapaasti.

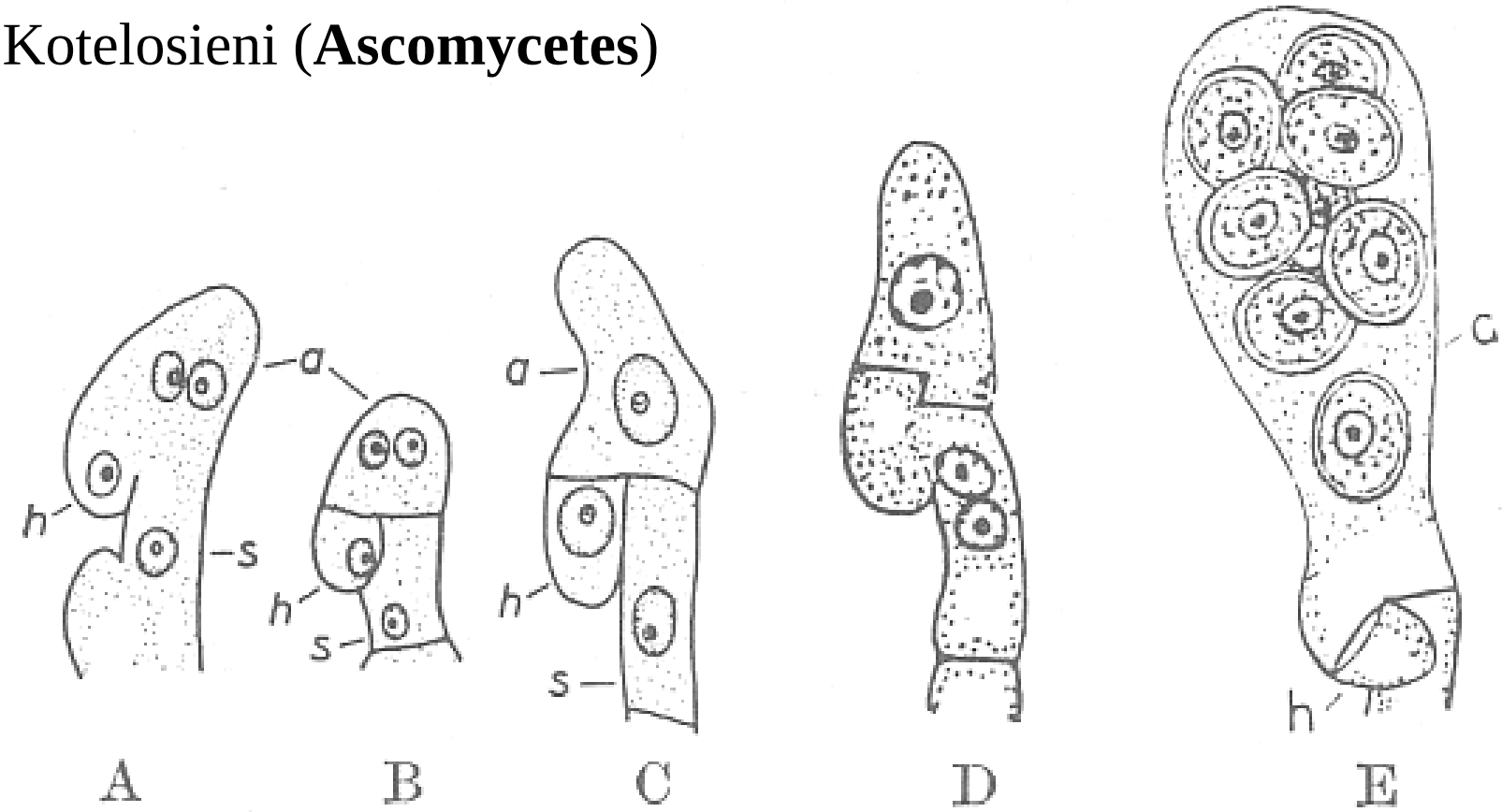
Yhdistelmävaihtoehtoja on $2^n$ (n = kromosomien haploidi lkm). Ihmisellä on 23 kromosomiparia, $2^{23}$ = 8 388 608

# Kromosomin *sisäinen* rekombinaatio eli crossing over

**Mendelistinen malli: punainen viiva ja sininen viiva katkaistaan ja liitetään yhteen**
**Tämä *köyhä* malli riittää selittämään 99% risteytyskokeissa ja perhekaavioissa havaittavista asioista**



Kotelosieni (**Ascomycetes**)




Hyyfit ovat kohdanneet ja kasvavat kaksitumaisina.
Ulkoinen merkki johtaa tumien yhtymiseen (D), jota sitten heti seuraa meioosi-mitoosi, tuottaen 8 itiötä

*Neurospora crassa* on yksi kuuluisa kotelosieni, ja korvasieni toinen.

# Tetradianalyysi

*Neurospora* elelee haploidina ja suorittaa joskus rihmastojen fuusion, joka tuottaa hetkellisesti diploideja soluja

*Neurospora*-sienellä kotelopullo on niin ahdas, että meioosin ensimmäisen jakautumisen tuotteet eivät pääse enää toistensa ohi, joten sentromeerin asema jää yhdeksi markkeriksi (4+4)

Tässä koejärjestelyssä saatiin selville, että crossing over tapahtuu replikaation jälkeen eli neljän juosteen kesken (seuraava kuva)

*pro* on merkkialleeli (proliiniauksotrofi)

Lajilla tapahtuu yksi tavallinen mitoosi meioosin jakautumisten jälkeen, jolloin saadaan 8 itiötä

Eläimillä kaikki sukusolut menevät vaivatta sekaisin eikä tällaista tetradianalyysia voi tehdä; merkkialleelit jotka toimisivat haploidivaiheessa ovat myös harvinaisia

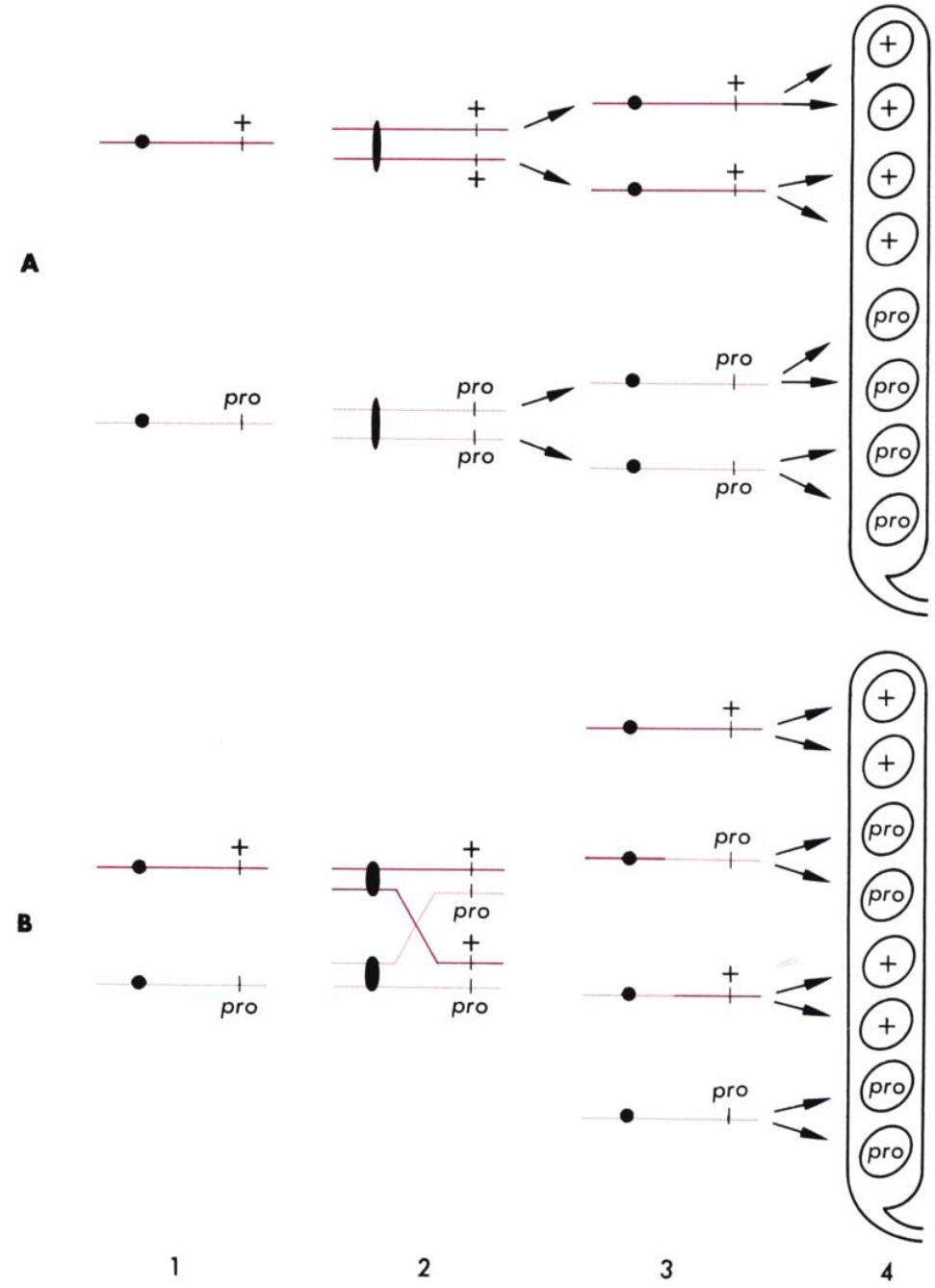



FIGURE 6–8. *Alternative arrangements of ascospores in ascus of* Neurospora. *In (A) crossing-over does not occur or does not involve genes (+) and pro; in (B) crossing-over occurs between the centromere and the (+) pro alleles (B 2). Only if crossing-over occurs in the four-strand stage, involving nonsister chromatids, can the sequence of ascospores shown at (B 4) be attained. In 1 of both (A) and (B), the chromosomes contributing to the zygote by each parental strain are shown; in 2, replication has taken place and it is at this stage that synapsis and crossing-over (if any) occurs; in 3, the chromosomes of each of the meiospores resulting from meiosis are depicted; in 4, a mature ascus and the genotypes of each of its ascospores are shown. Meiosis is taking place between 1 and 3; mitosis occurs between 3 and 4.*

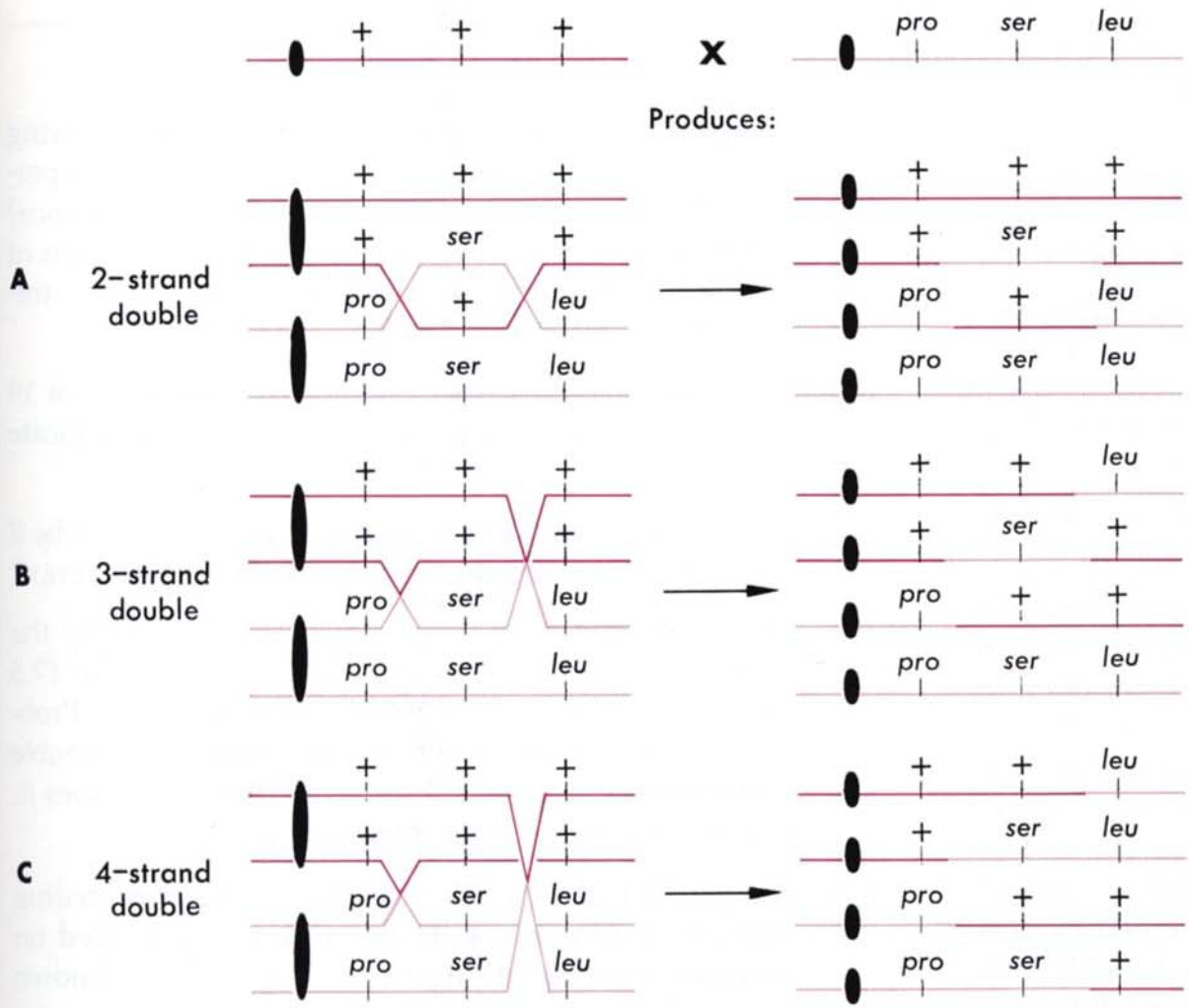


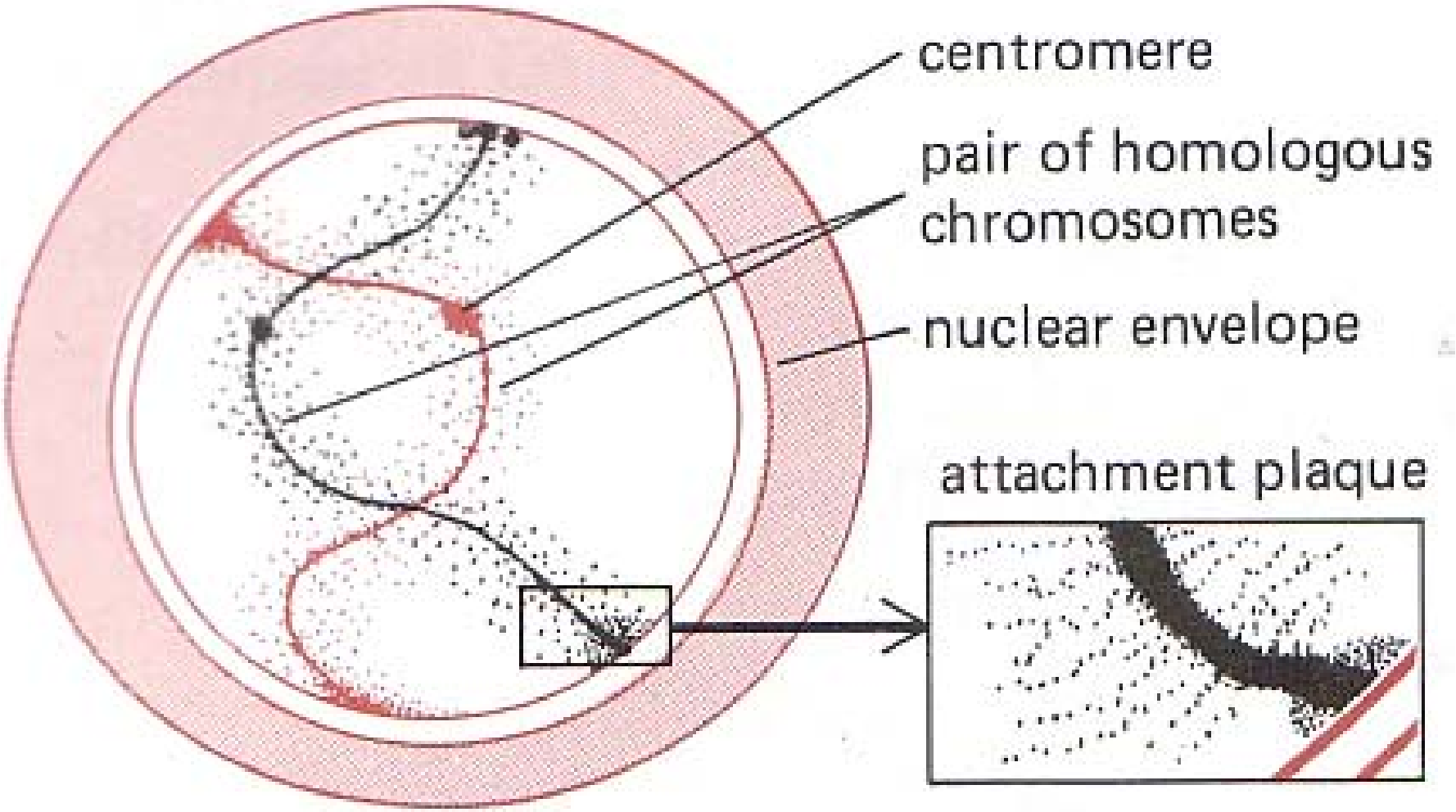

**FIGURE 6–9.** *Diagram showing possible types of double crossing-over involving (A) two chromatids only, (B) three chromatids, (C) all four chromatids, as inferred from tetrad analysis.*

Tällainen koe kolmella merkkialleelilla (proliini-, seriini- ja leusiiniauksotrofit) todisti, että crossing over tapahtuu solun ollessa nelijuosteisessa tilassa (eli replikaatio tapahtuu ennen meioosia).

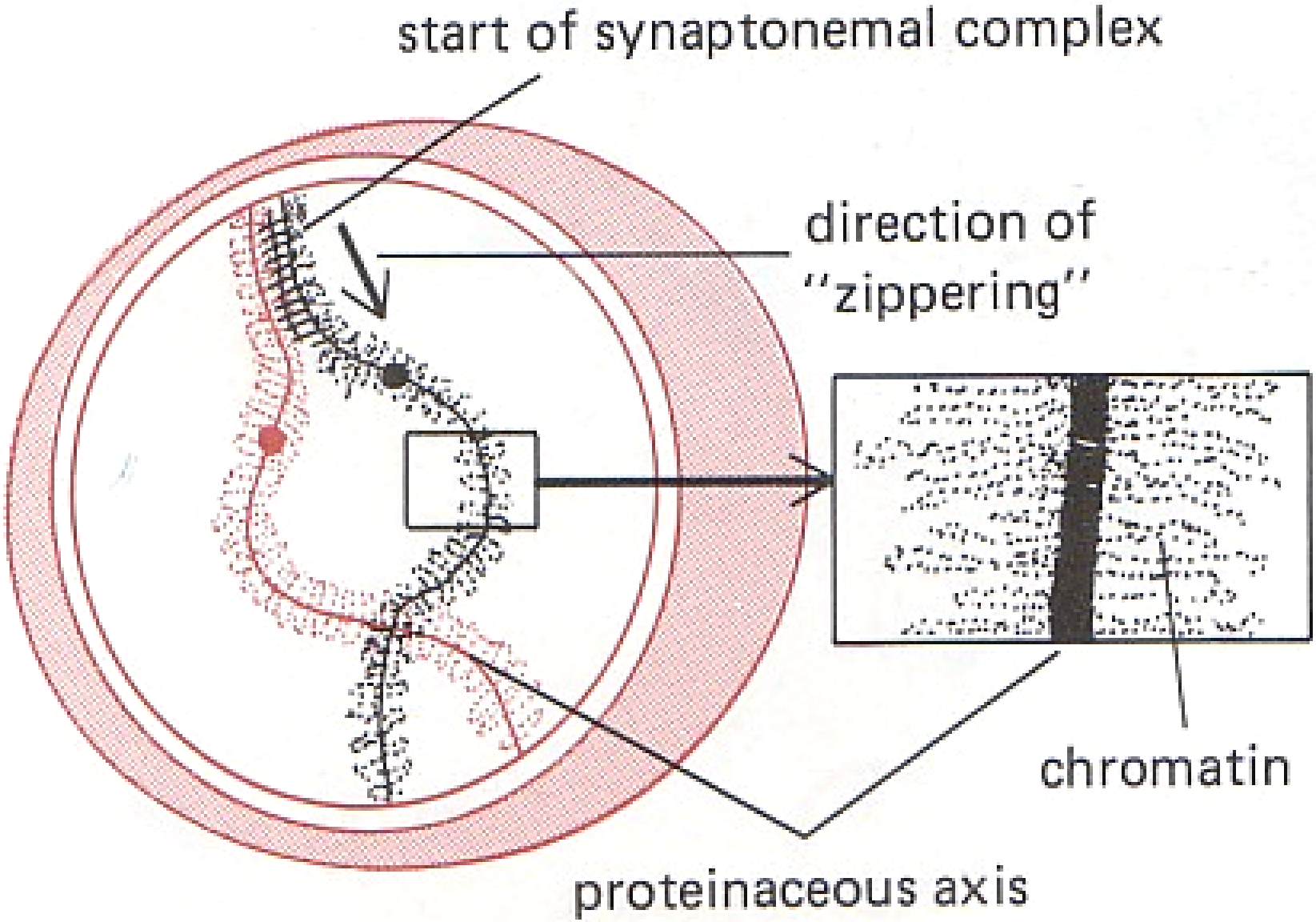
**Kromosomin *sisäinen* rekombinaatio eli crossing over**

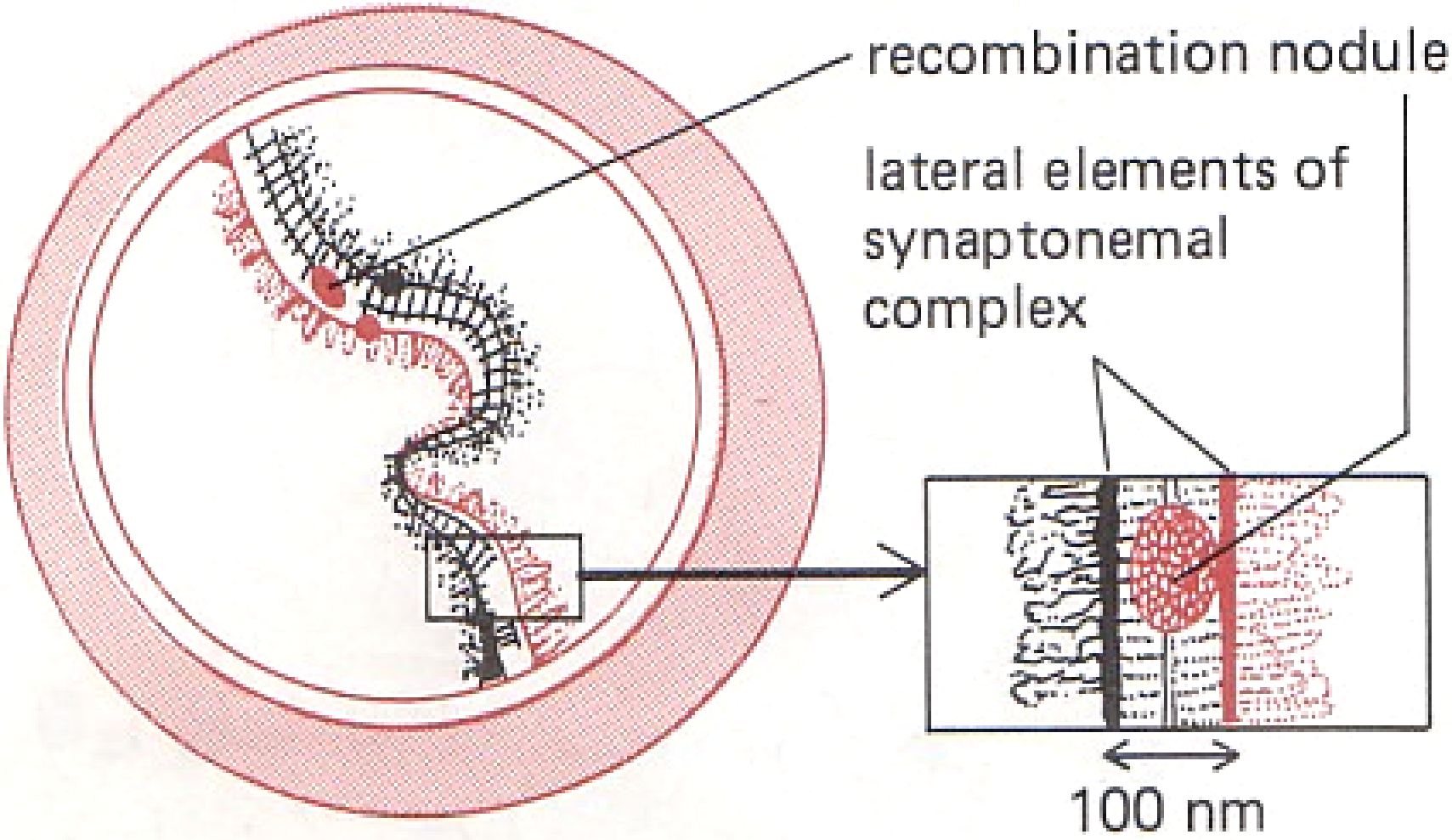
Mendelistinen malli: punainen viiva ja sininen viiva katkaistaan ja liitetään yhteen

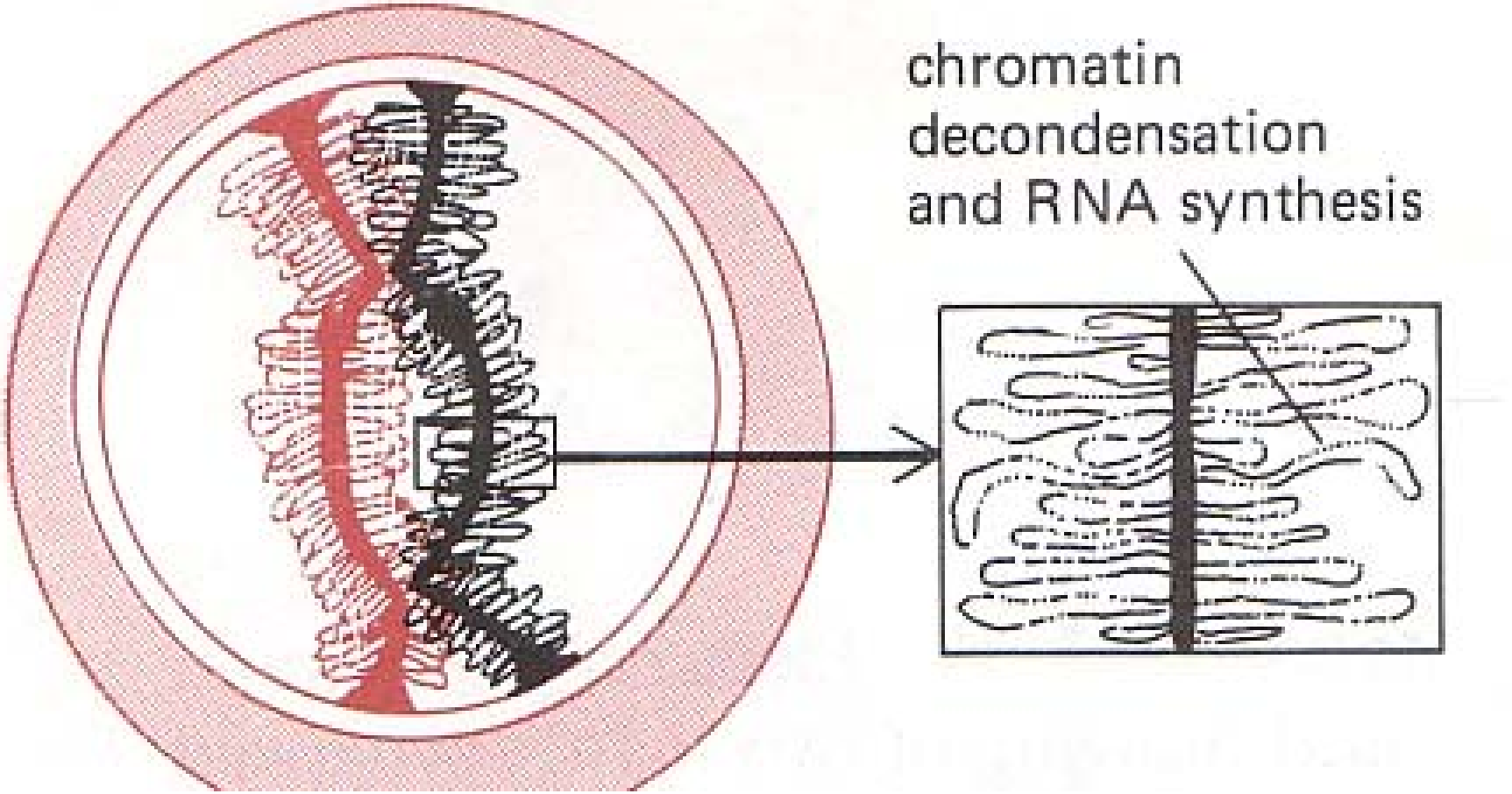
**Askelta hienostuneempi, *sytologinen* malli: muistetaan, että juosteita on neljä ja tapahtuman paikka ja aika *synaptonemaalikompleksi* meioosin I profaasissa**



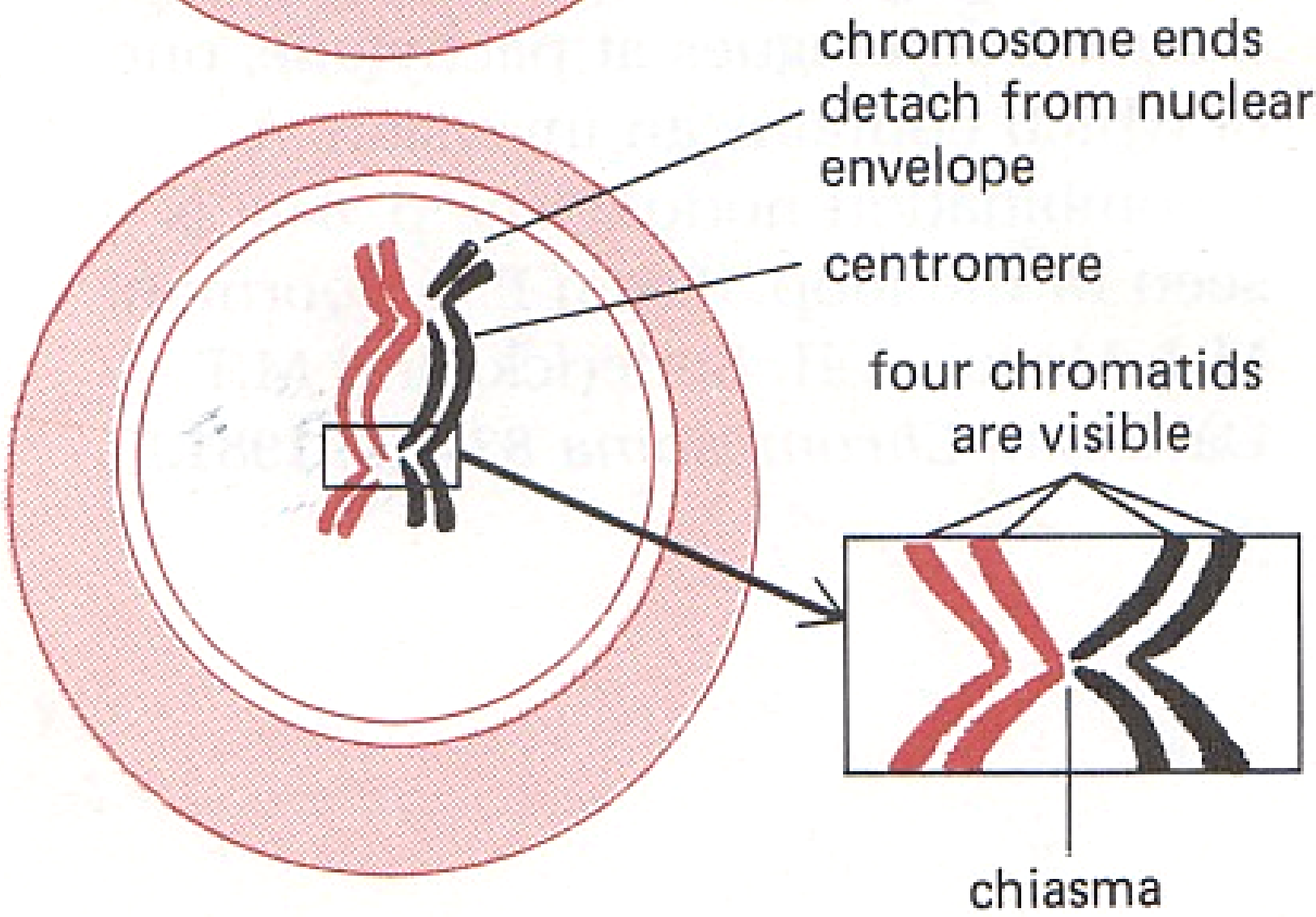

Meioosia varten homologisten kromosomien pitäisi pariutua (*synapsis*). Molemmat ovat replikoituneet, mutta se ei näy: sisarkromatidit ovat tiukasti yhdessä, kenties vielä sekaisinkin (afrikkalaispalmikolla)


start of synaptonemal complex
direction of
"zippering"
chromatin
proteinaceous axis

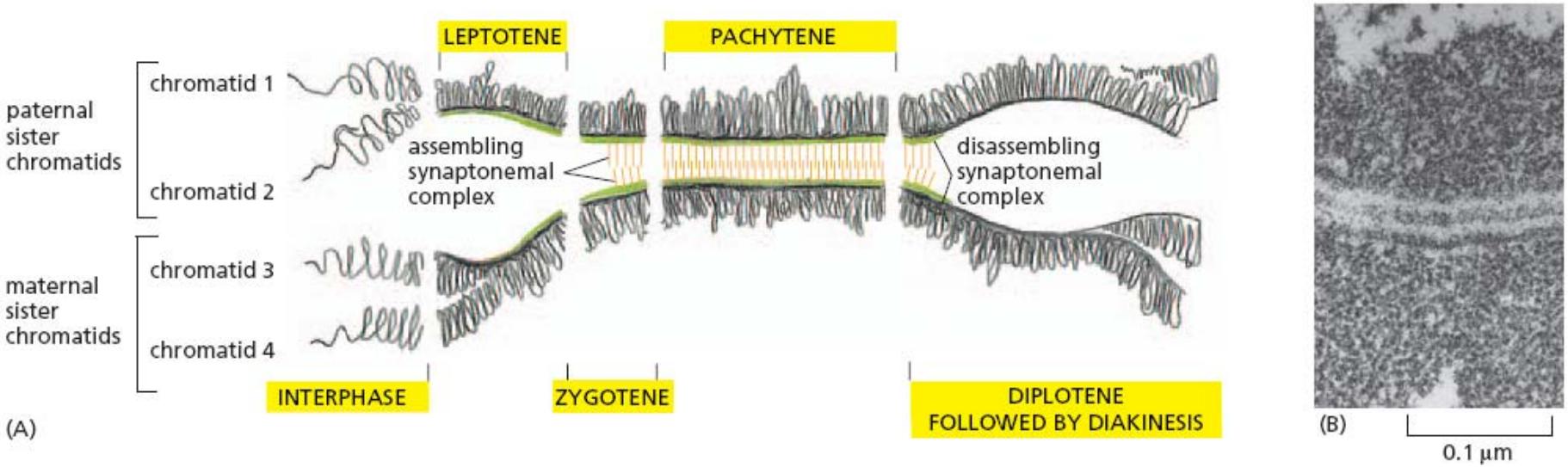

recombination nodule
lateral elements of synaptonemal complex
100 nm




Kiasmat, joissa DNA-juosteet (tai ainakin kromosomit) menevät ristiin, ovat merkkinä siitä että crossing over on tapahtunut. Kiasma voi siirtyä ja jakautumisen alkaessa se siirtyykin. Yleisesti ajatellaan, että kiasmat ovat välttämättömia kromosomien ohjaamiseksi (parin yhdessäpitämiseksi), mutta on tapauksia, joissa meioosi sujuu ilmankin (akiasmaattinen meioosi, esim naarasperhosilla?)




Tästä voidaan jatkaa, **profaasi** on päättynyt. Crossing over **on tapahtunut** ja ristiin menevät juosteet pitävät nelikon yhdessä

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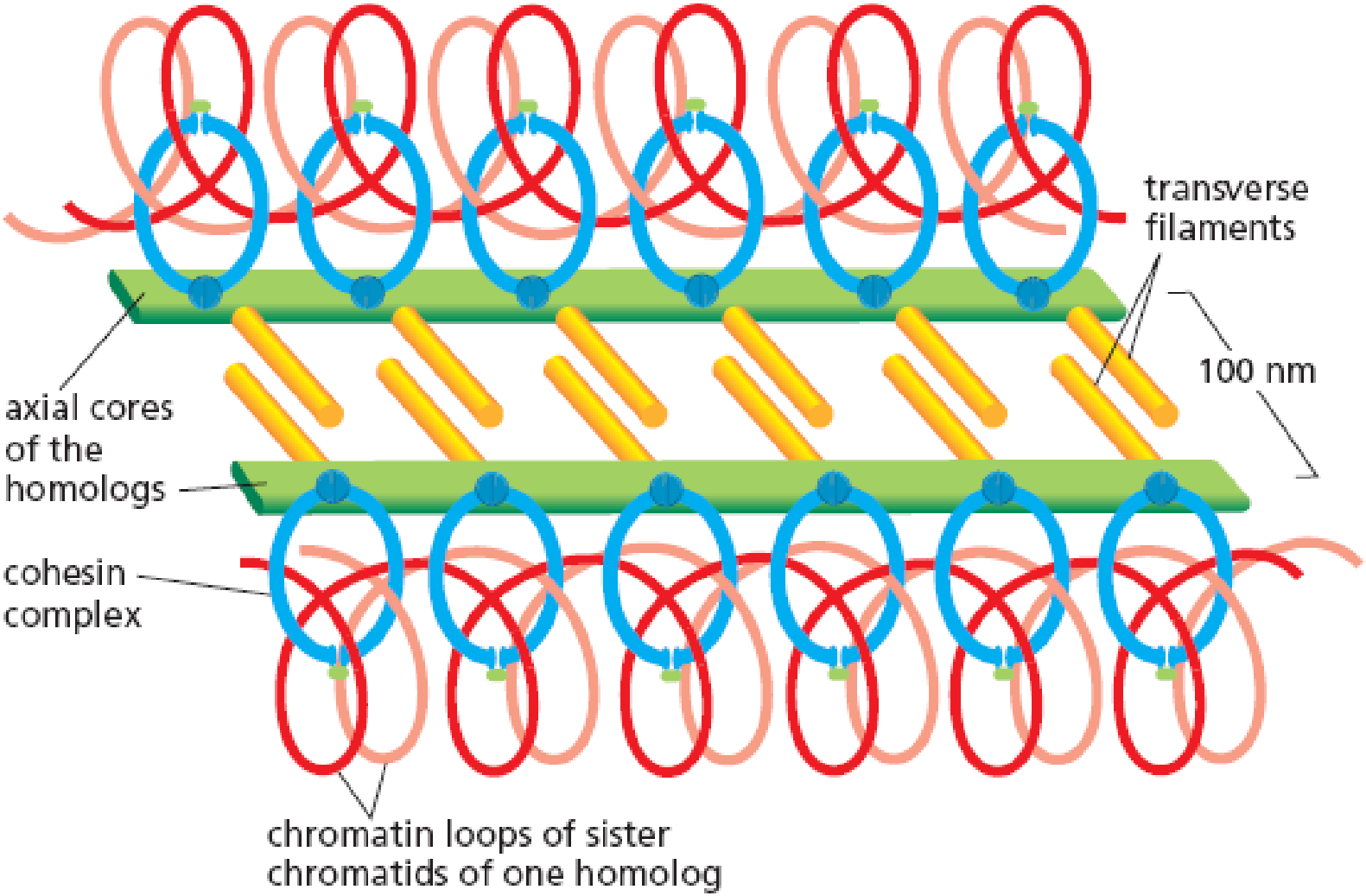


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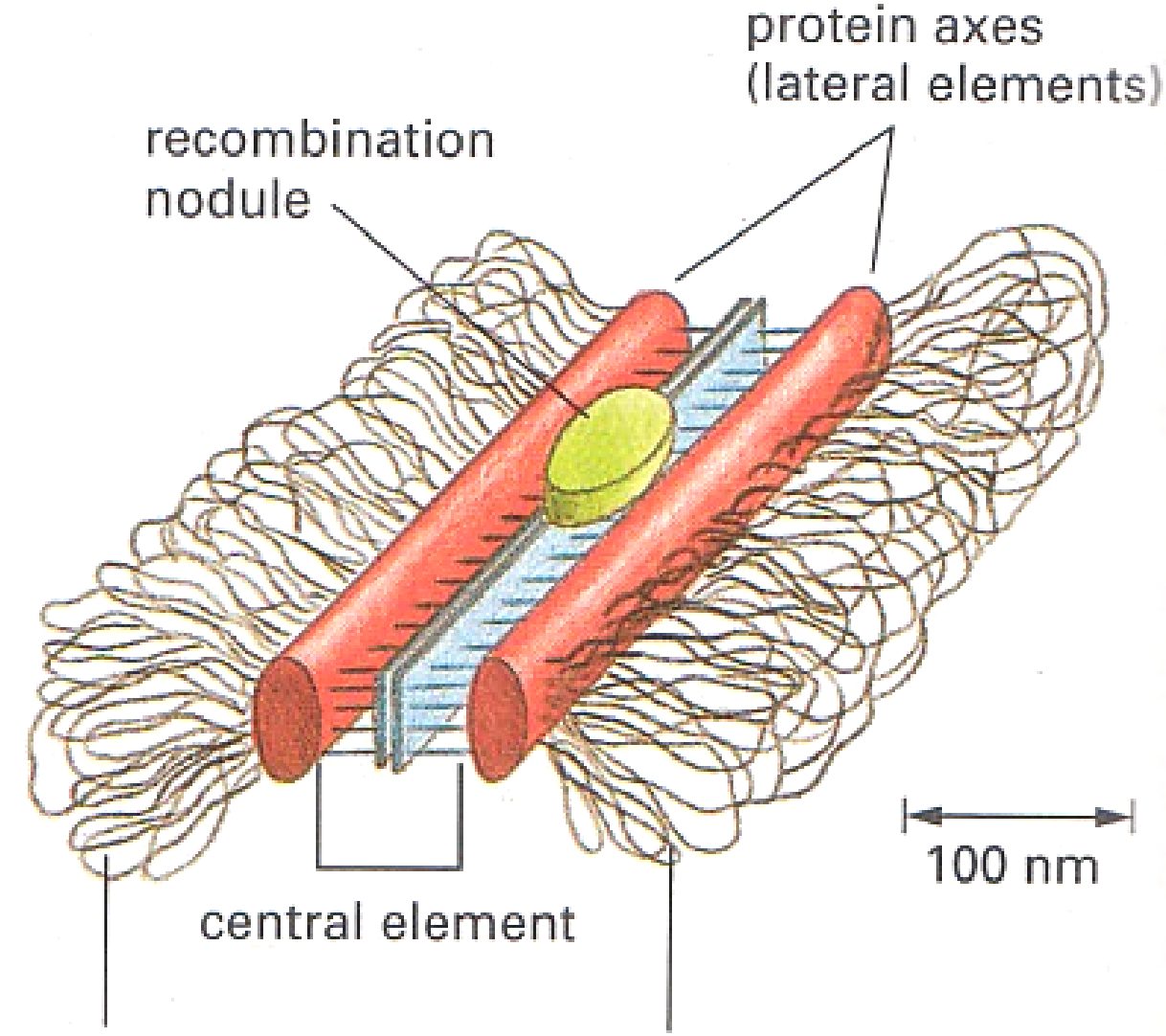


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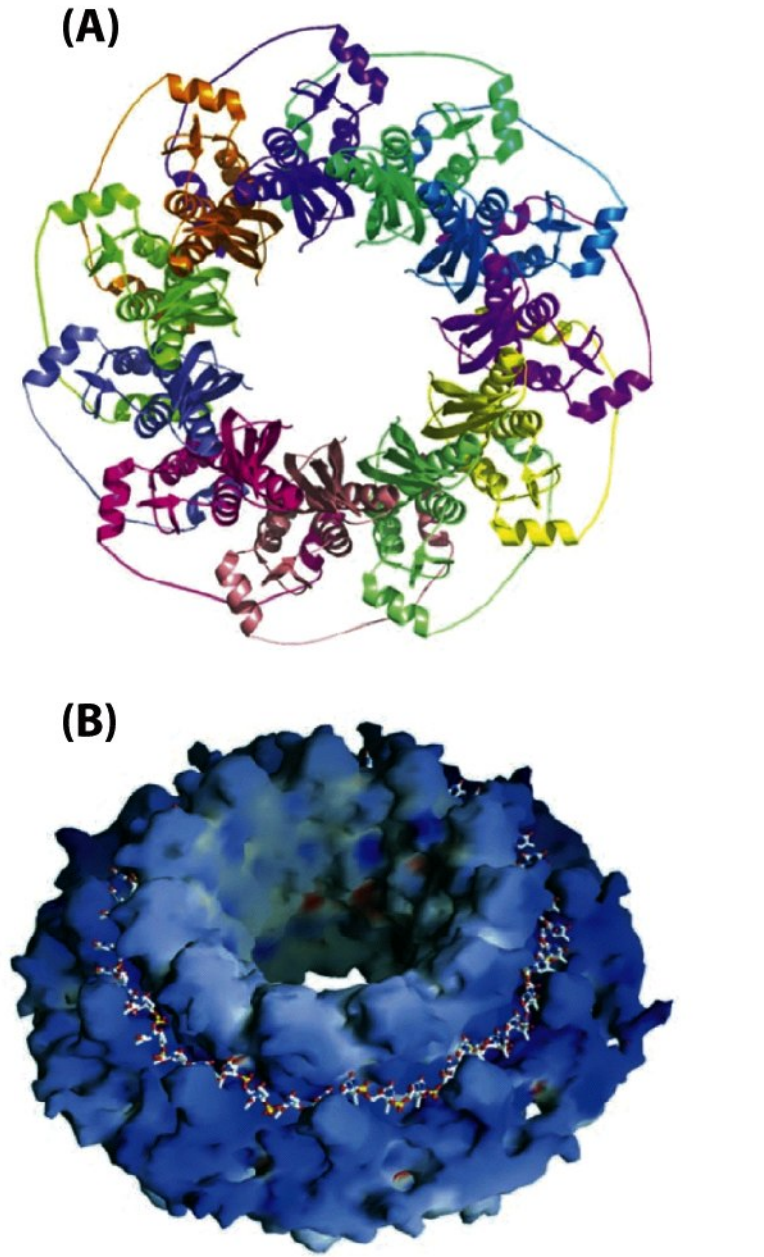



Figure 5-57 Molecular Biology of the Cell 5/e (© Garland Science 2008)

**Rad52** proteiini koostuu 11 osasta

Alemmassa kuvassa pilkistää DNA, ja laitteen on ehdotettu välittävän kahden komplementaarisen single strandin kohtaamista

**CELL 308**

**Kromosomin *sisäinen* rekombinaatio eli crossing over**

Kromosomien *välinen* uudelleenjärjestäytyminen ei tietenkään ole mikään pulma

Mendelistinen malli: punainen viiva ja sininen viiva katkaistaan ja liitetään yhteen: no problems!

Askelta hienostuneempi: muistetaan, että juosteita on neljä ja paikka ***synaptonemaalikompleksi*** meioosin I profaasissa (ei vieläkään muuta kuin lisää termejä)

**Molekyylitasolla rekombinaatio on näennäisesti mutkikkaampi, ja molekyylitasollahan se tietenkin tapahtuu!**

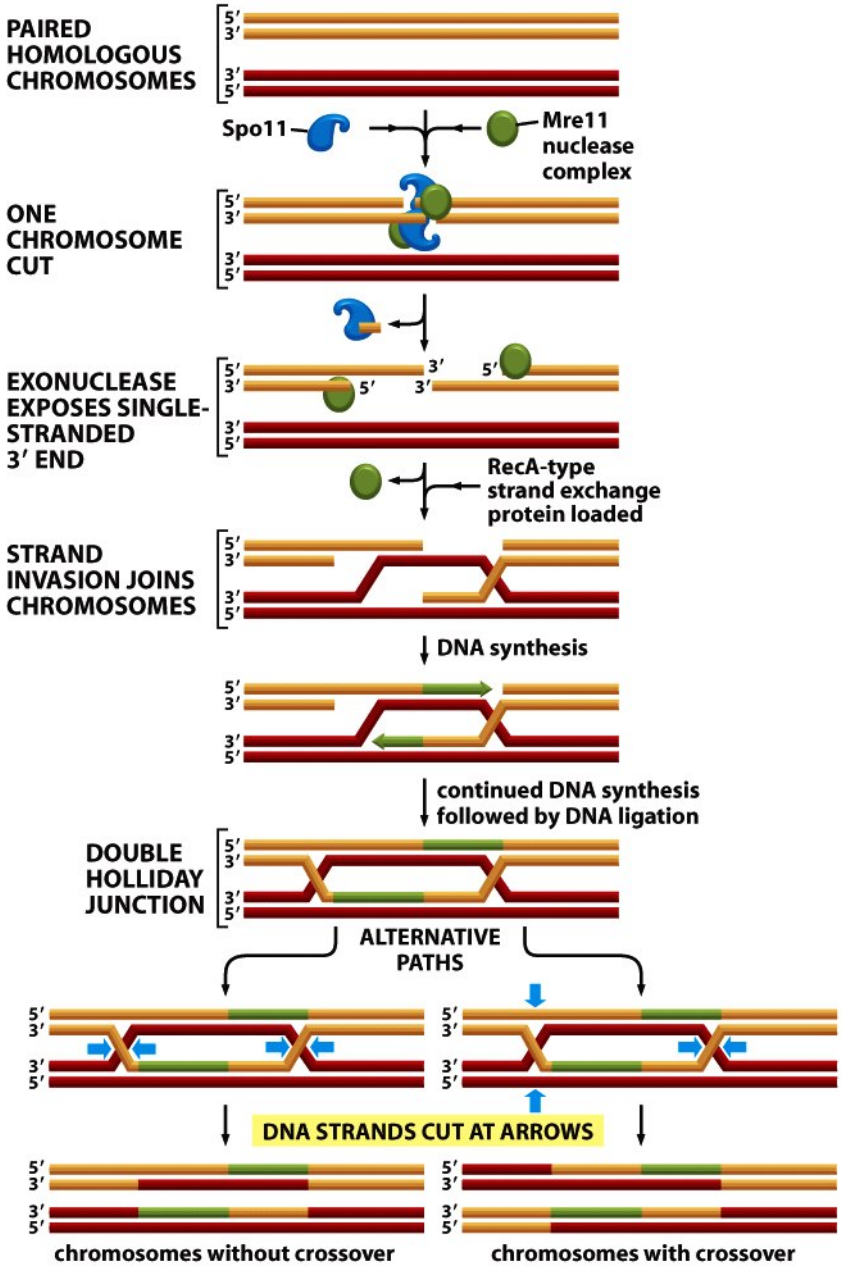



Figure 5-64 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 313

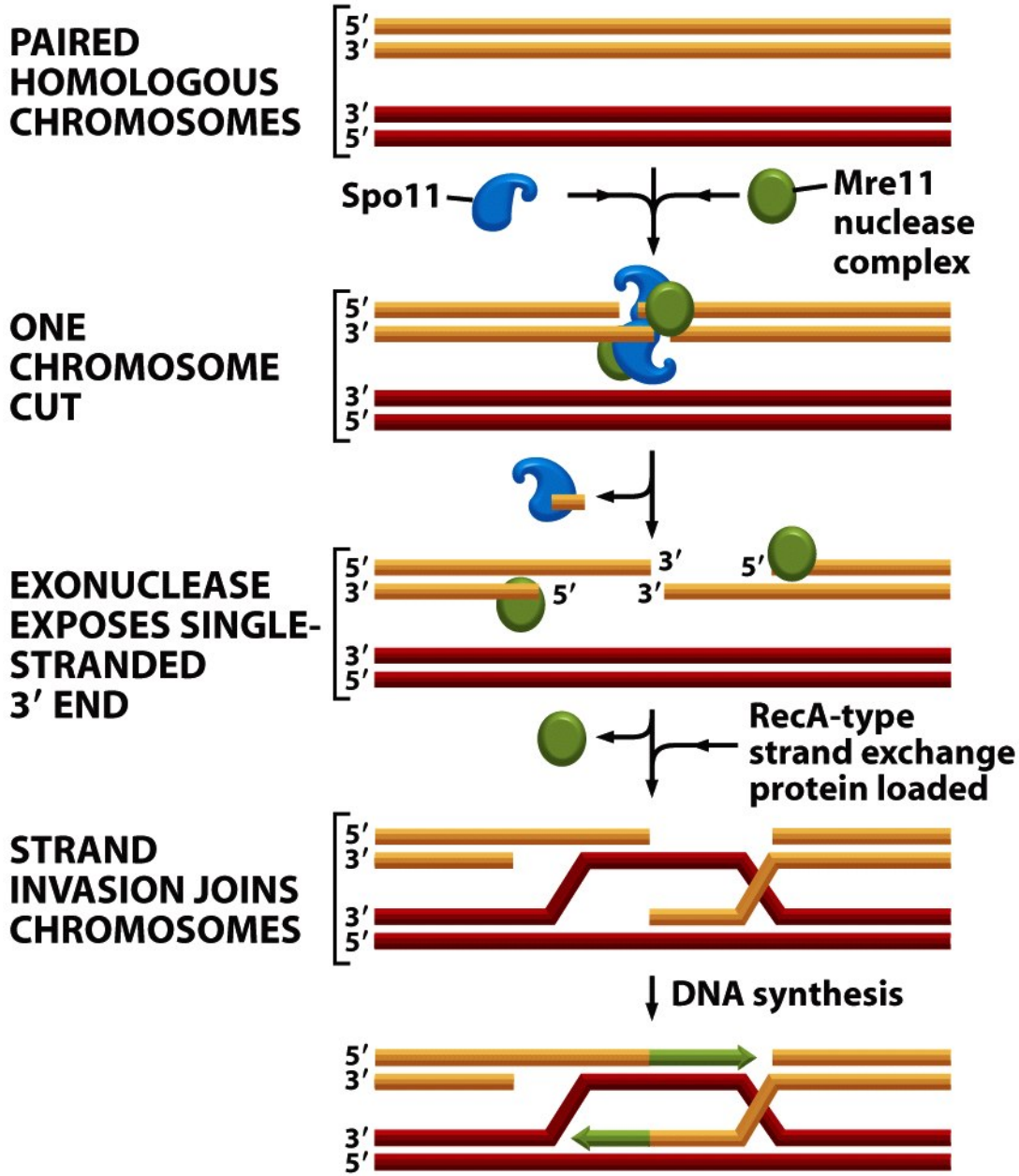



Figure 5-64 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 313

CELL 313

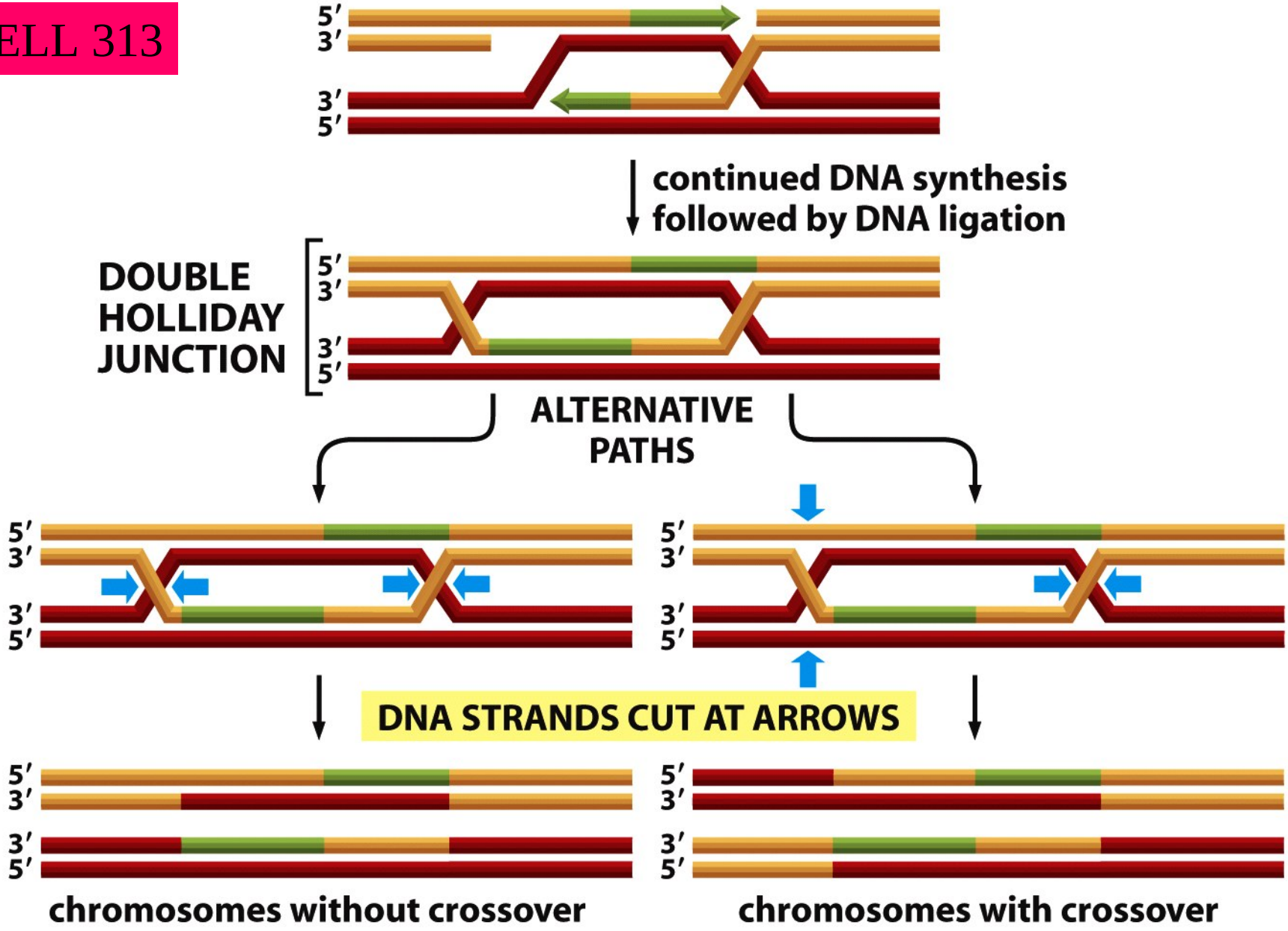



Figure 5-64 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

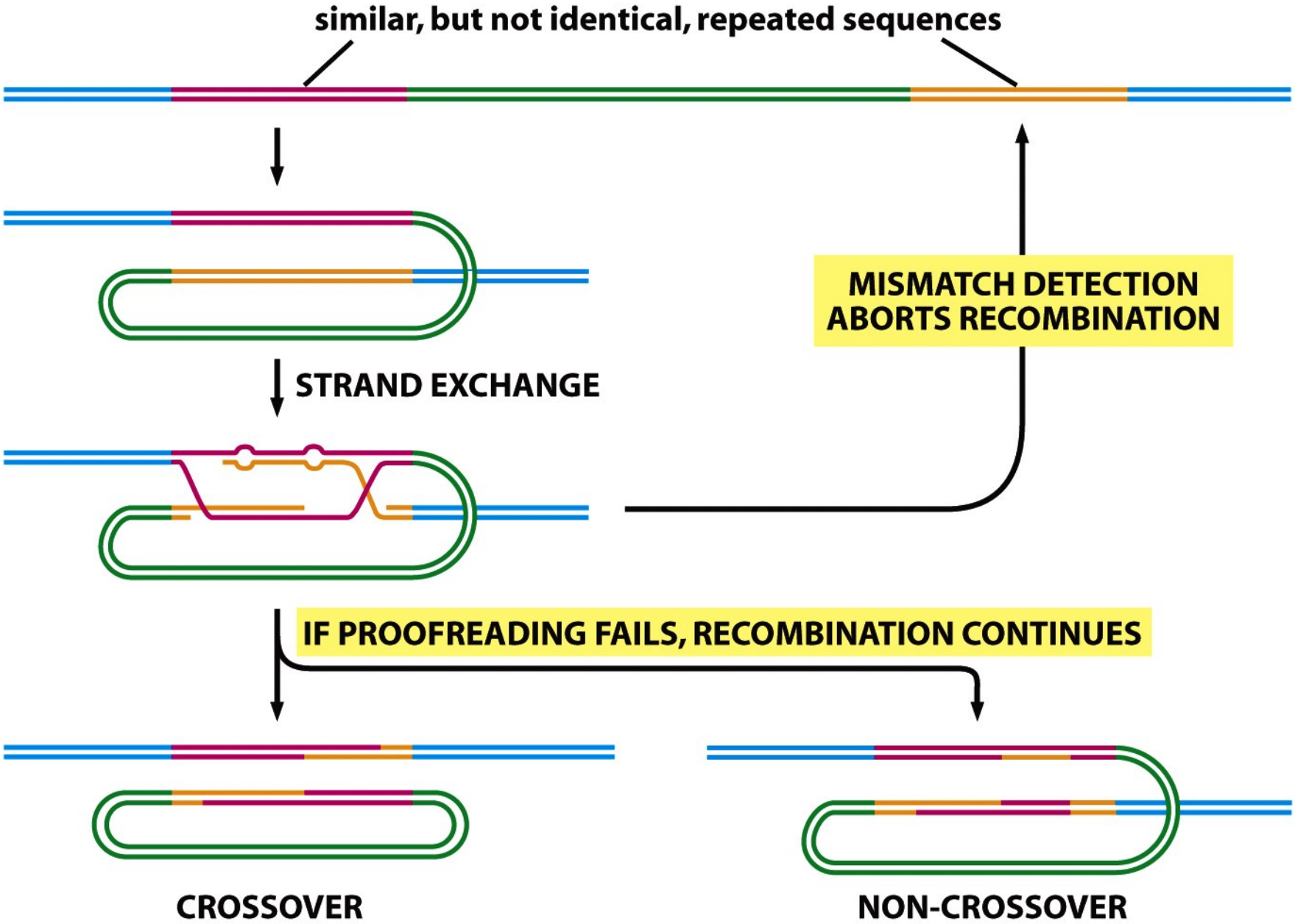



Figure 5-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

## Meiotic Recombination Is Initiated by Double-strand DNA Breaks

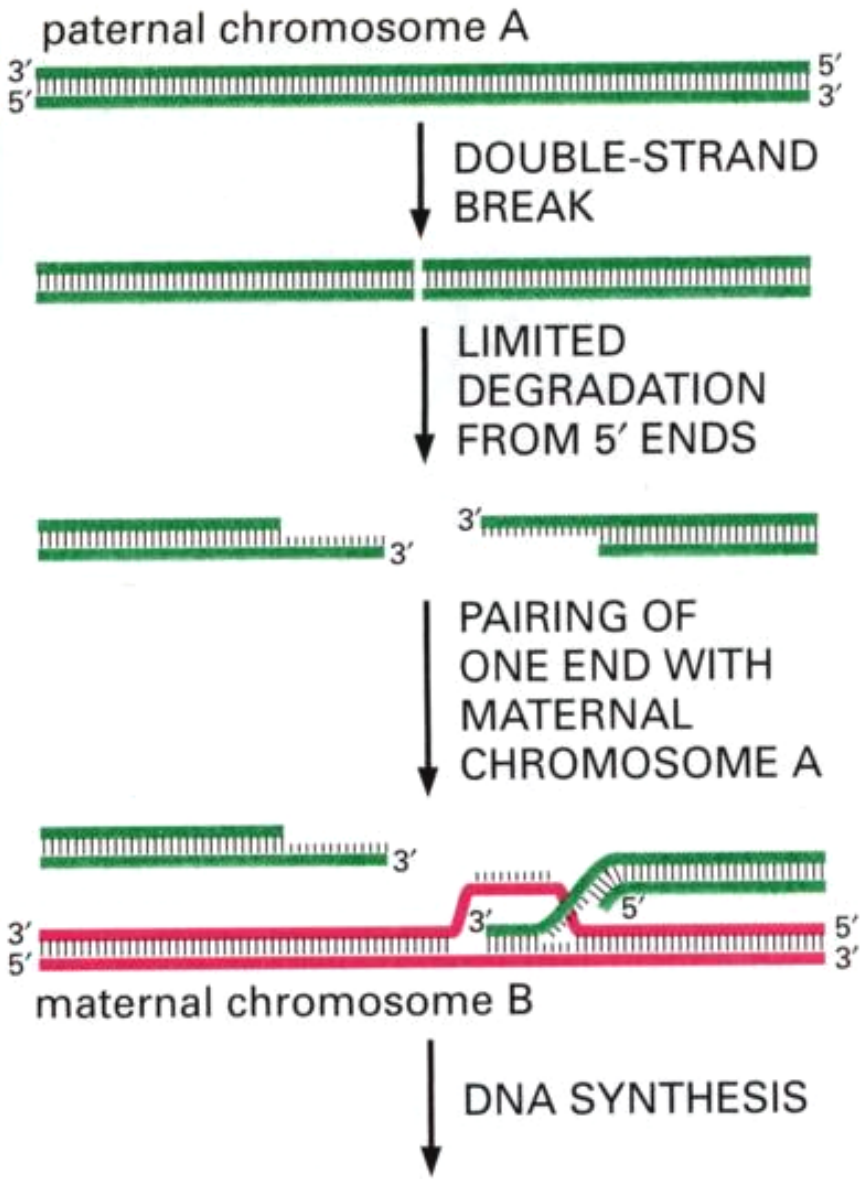
CELL 2002, 277

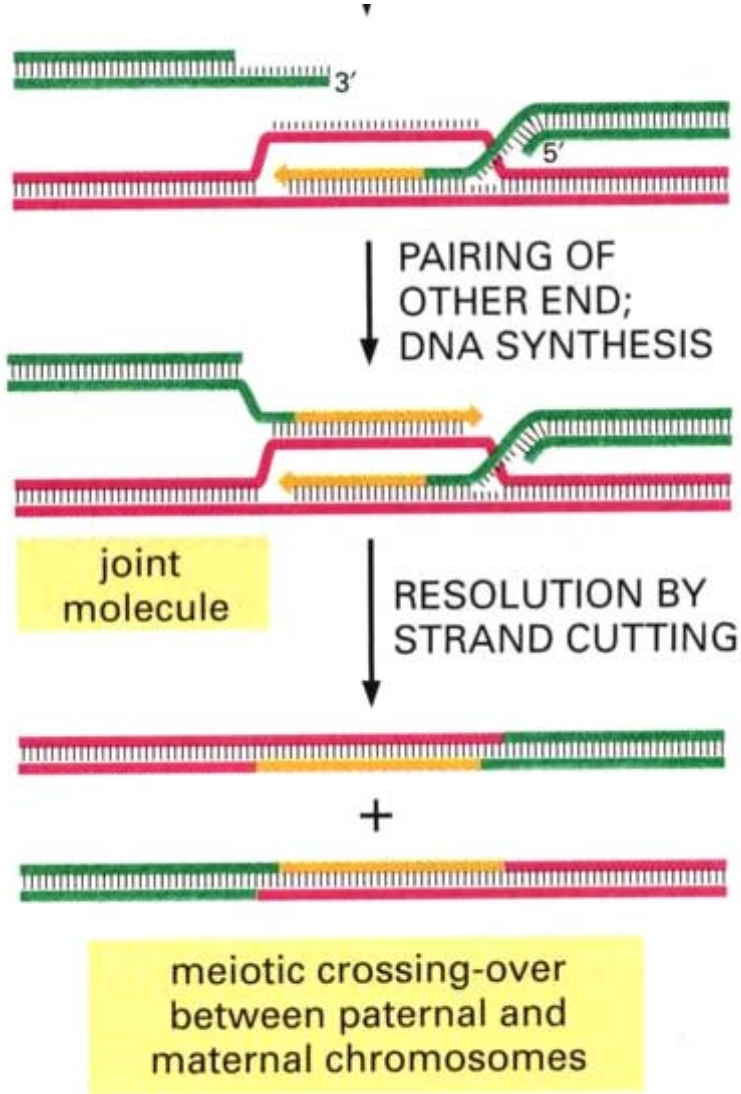
Extensive base-pair interactions cannot occur between two intact DNA double helices. Thus, the DNA synapsis that is critical for general recombination in meiosis can begin only after a DNA strand from one DNA helix has been exposed and its nucleotides have been made available for pairing with another DNA helix. In the absence of direct experimental evidence, theoretical models were proposed based on the idea that a break needed to be made in just one of the two strands of a DNA helix to produce the exposed DNA strand required for DNA synapsis. This break in the phosphodiester backbone was thought to allow one of the nicked strand ends to separate from its base-paired partner strand, freeing it to form a short heteroduplex with a second intact DNA helix—thereby beginning synapsis. Models of this type are reasonable in theory, and they have been described in textbooks for nearly 30 years.

In the early 1990s, sensitive biochemical techniques became available for determining the actual structure of the recombination intermediates that form in yeast chromosomes at various stages of meiosis. These studies revealed that general recombination is initiated by a special endonuclease that simultaneously cuts *both* strands of the double helix, creating a complete break in the DNA molecule. The 5′ ends at the break are then chewed back by an exonuclease, creating protruding single-stranded 3′ ends. It is these single strands that search for a homologous DNA helix with which to pair—leading to the formation of a "joint molecule" between a maternal and a paternal chromosome (Figure 5–56).

In the next section, we begin to explain how a DNA single strand can "find" a homologous double-stranded DNA molecule to begin DNA synapsis.

CELL vuonna 2002 277



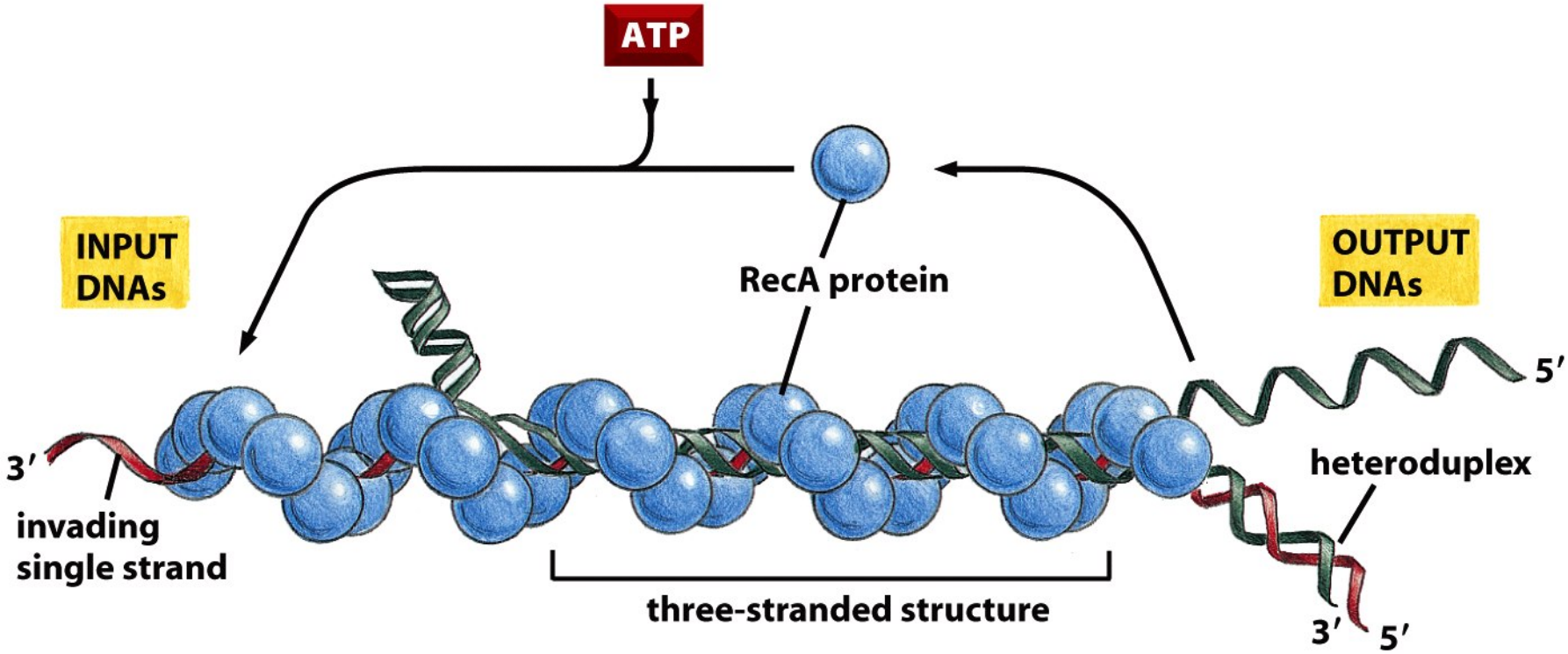



Figure 5-56 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 307

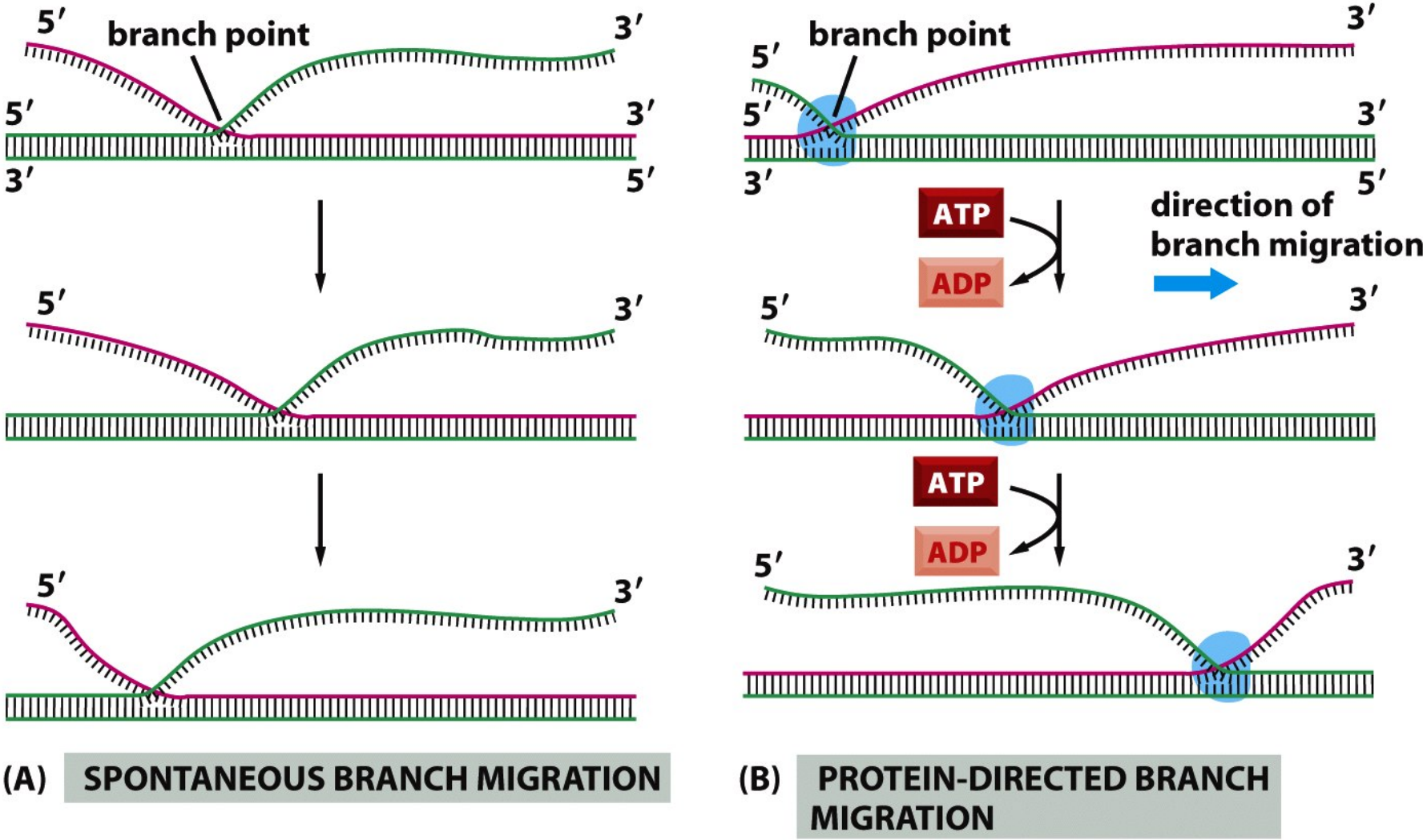



Figure 5-58 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 308

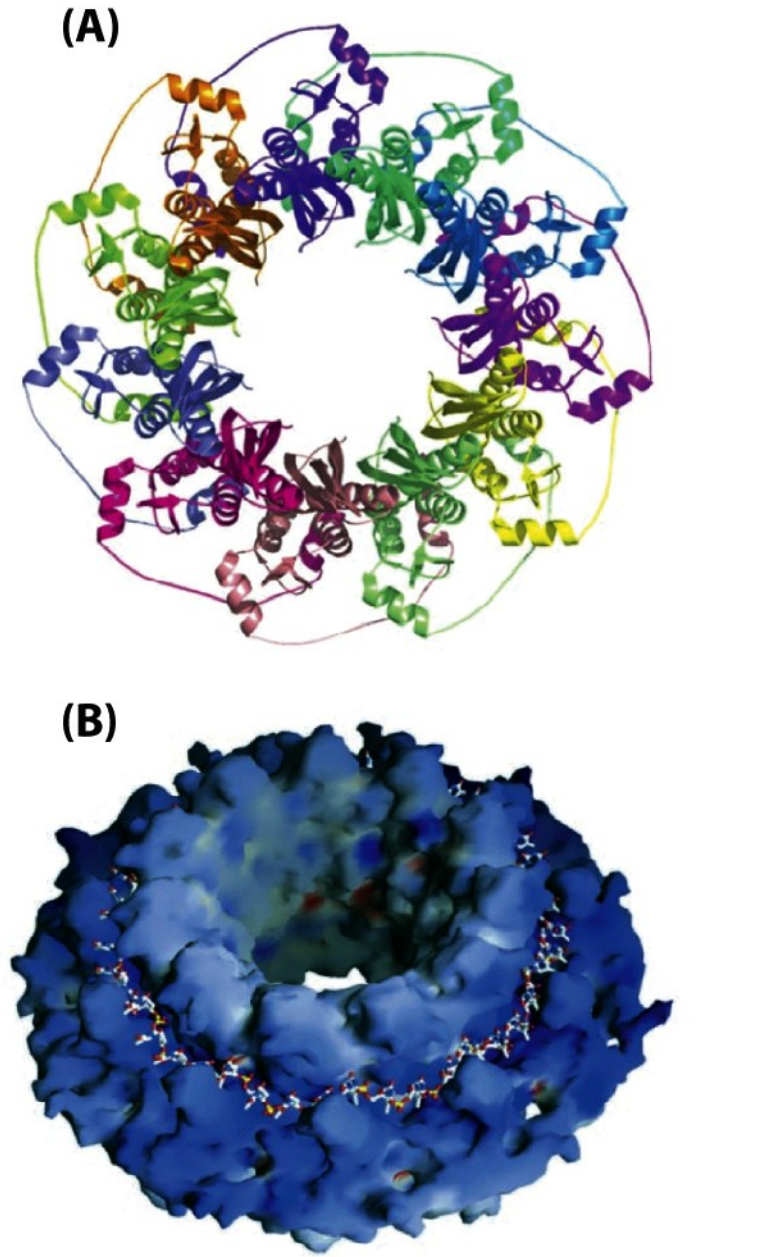



Figure 5-57 Molecular Biology of the Cell 5/e (© Garland Science 2008)

**Rad52** proteiini koostuu 11 osasta

Alemmassa kuvassa pilkistää DNA, ja laitteen on ehdotettu välittävän kahden komplementaarisen single strandin kohtaamista

**CELL 308**

# Bakteerin transformaatio

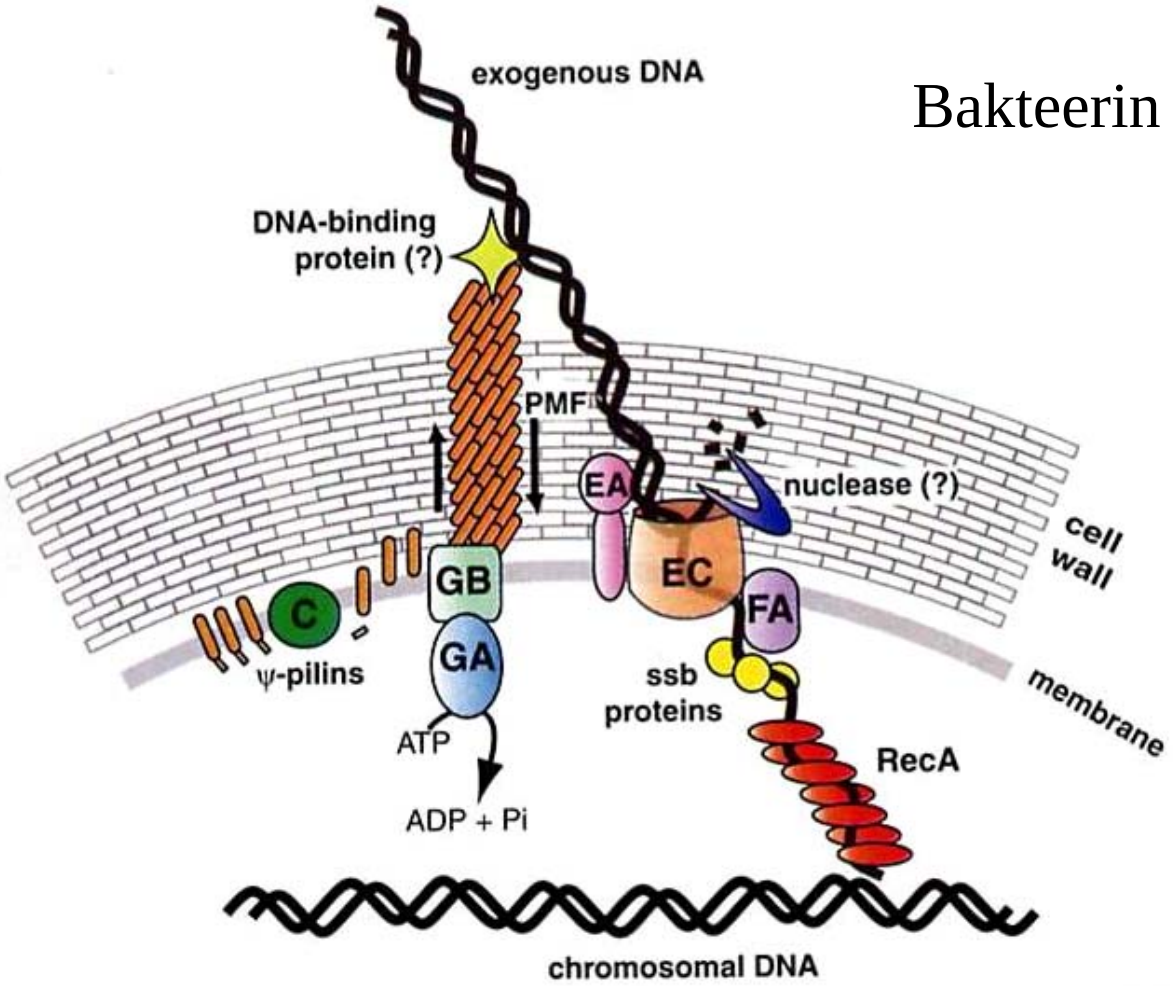
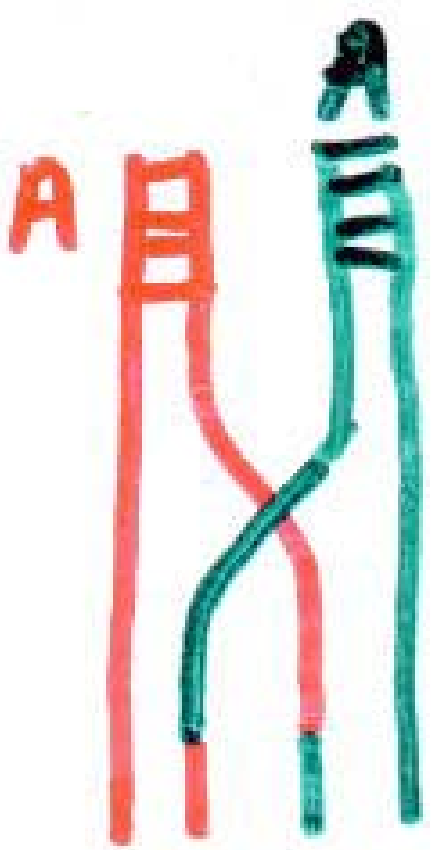



**Fig. 2.** DNA uptake during transformation in *B. subtilis*. The uptake machinery is preferentially located at the cell poles. The Ψ-prepilins are processed by the peptidase and translocate to the outer face of the membrane. With the aid of the other ComG proteins, the major Ψ-pilin ComGC assembles into the Ψ-pilus, which attaches exogenous DNA via a hypothetical DNA binding protein. Retraction of the Ψ-pilus, driven by the proton motive force, and DNA binding to the receptor (ComEA) are required to transport one strand of DNA through the membrane channel (ComEC) while the other is degraded by an unidentified nuclease. The helicase/DNA translocase (ComFA) assists the process, along with ssDNA binding proteins that interact with the incoming DNA. RecA forms a filament around the ssDNA, and mediates a search for homology with chromosomal DNA. ADP, adenosine diphosphate; Pi, inorganic phosphate; PMF, proton motive force; ssb, single-stranded DNA binding protein.

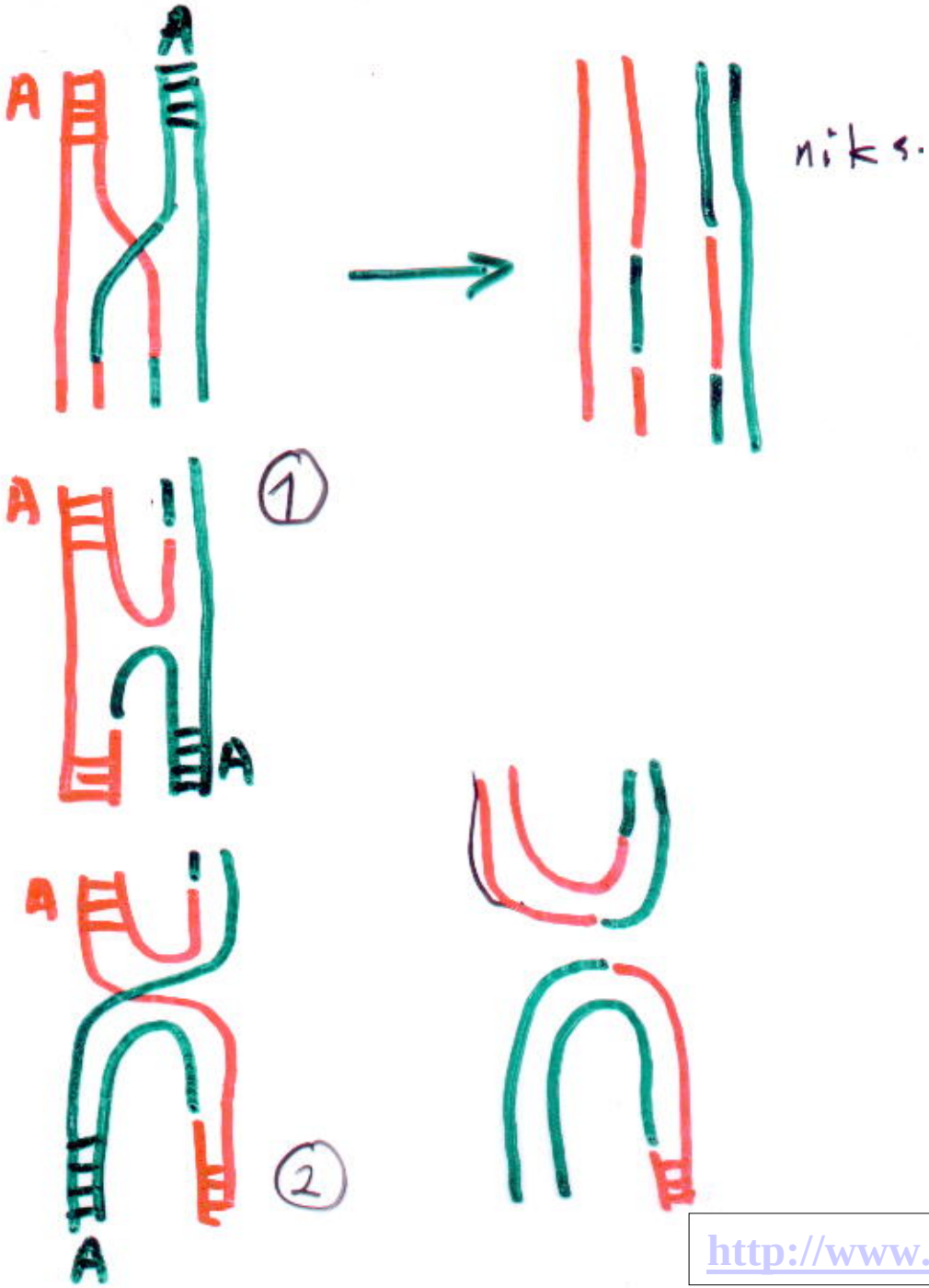
Science December 2 2005




No, juosteet ovat vaihtaneet osia, hallitusti ja tarkasti.

Nyt ne on oikeastaan solmussa, jonka nimi on Hollidayn liitos (junction)

CELL 311




Miten juosteet sitten pääsevät irti toisistaan?

Se ei olekaan aivan helppoa, eikä varmasti taaskaan tapahdu “itsestään”.

Kts **CTAG** CELLIN videoista

http://www.youtube.com/watch?v=gQFKdA3VgEg

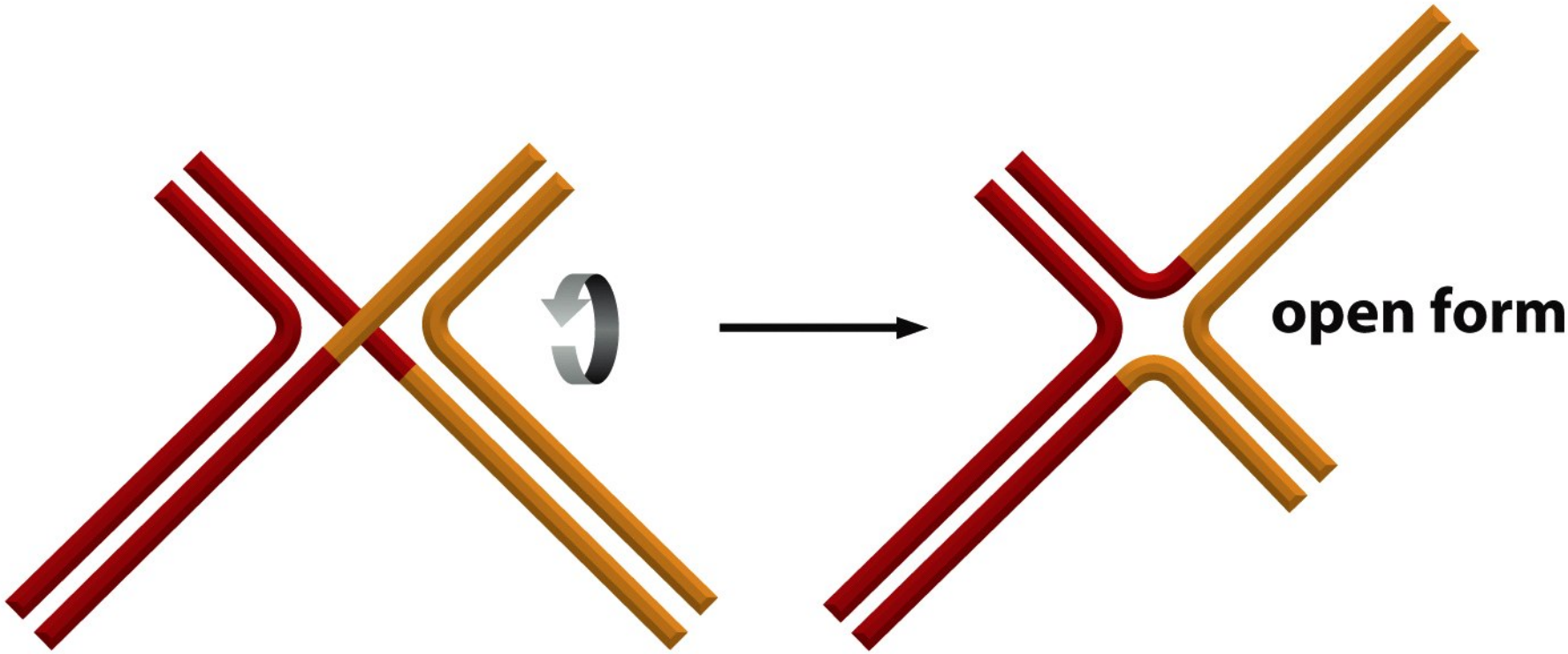
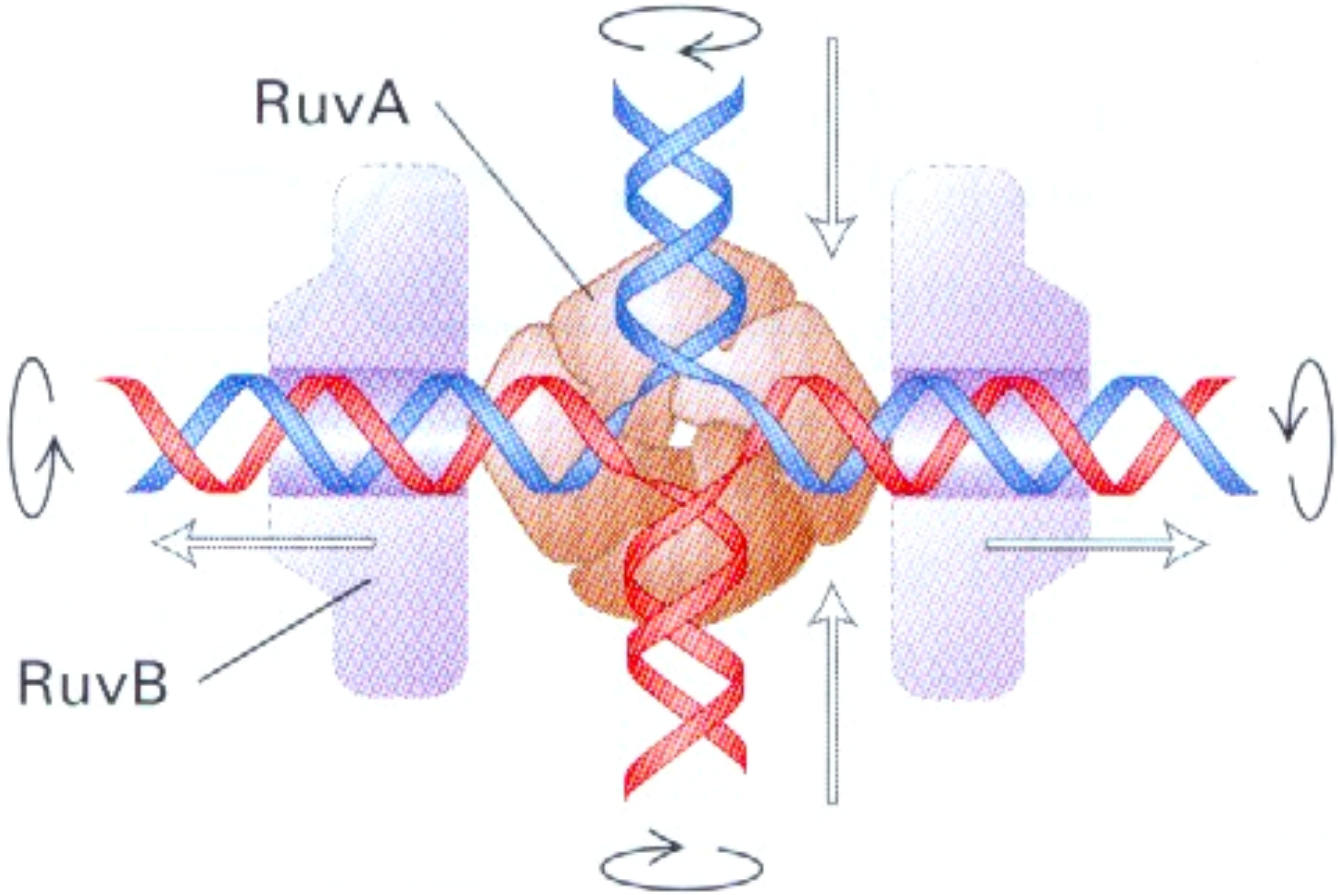



Figure 5-61 Molecular Biology of the Cell 5/e (© Garland Science 2008)




CELL 312

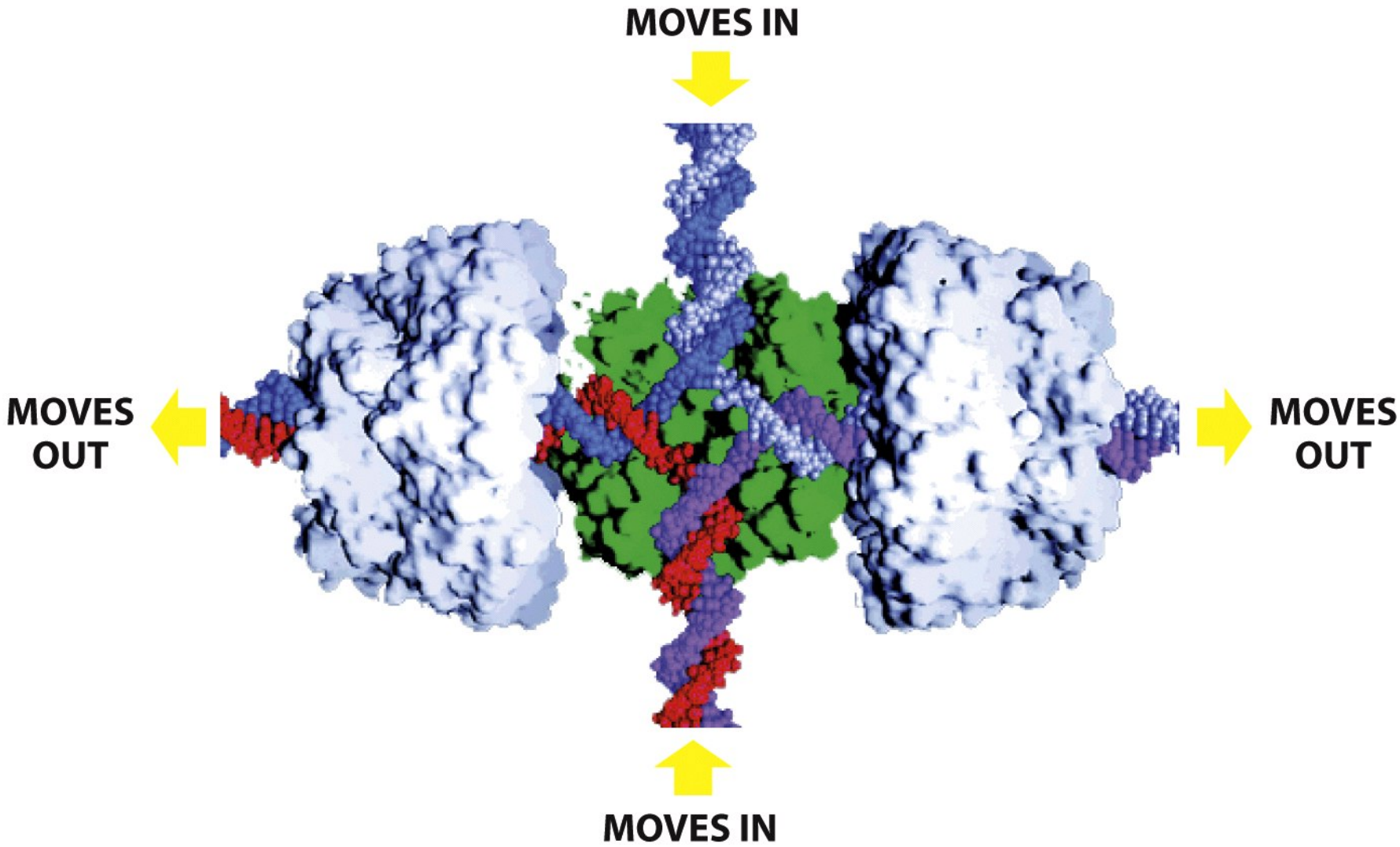
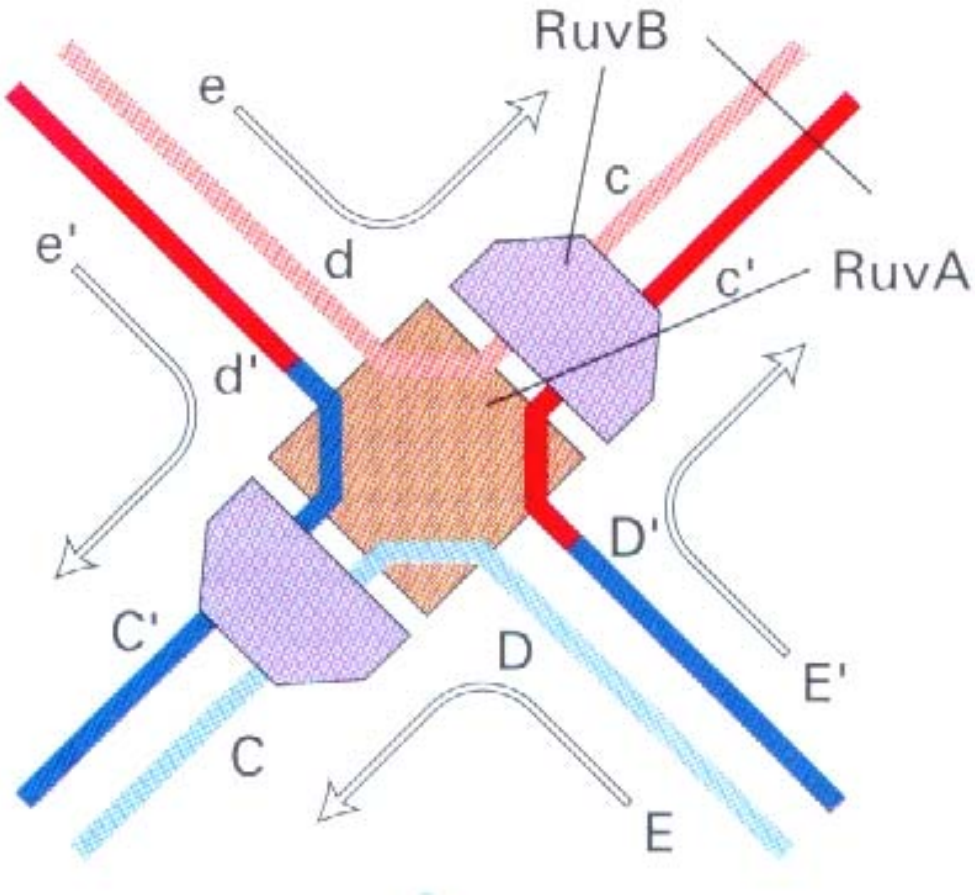
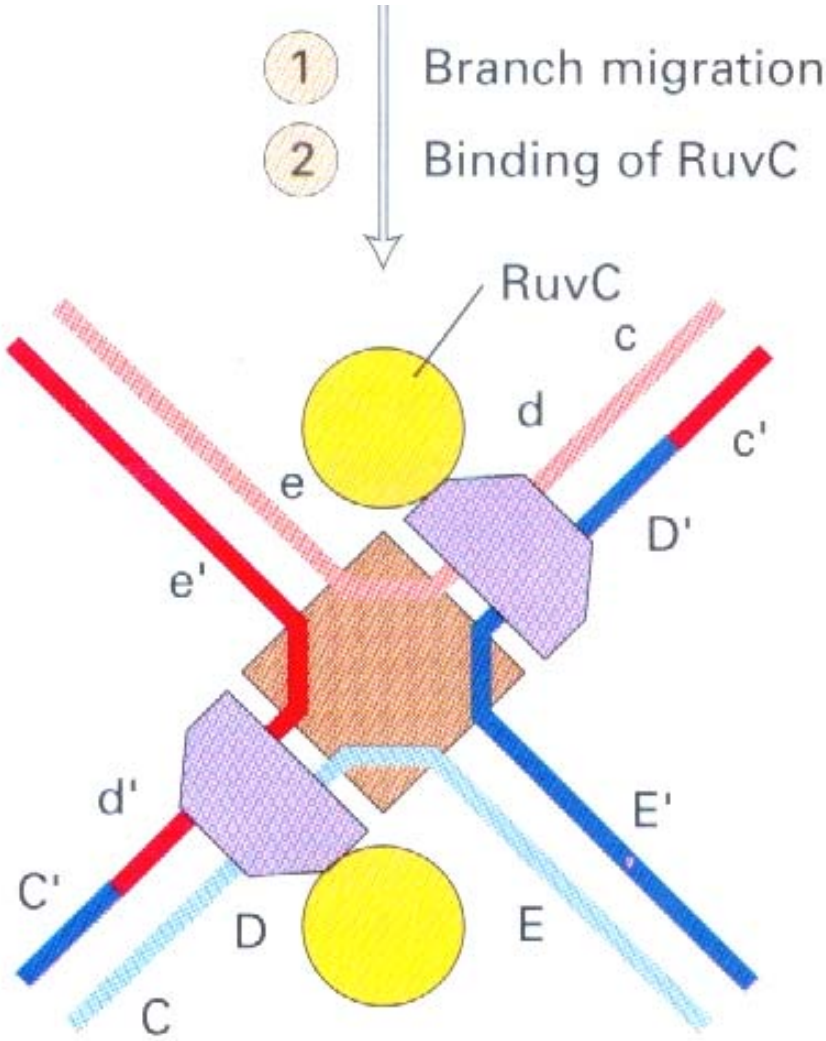


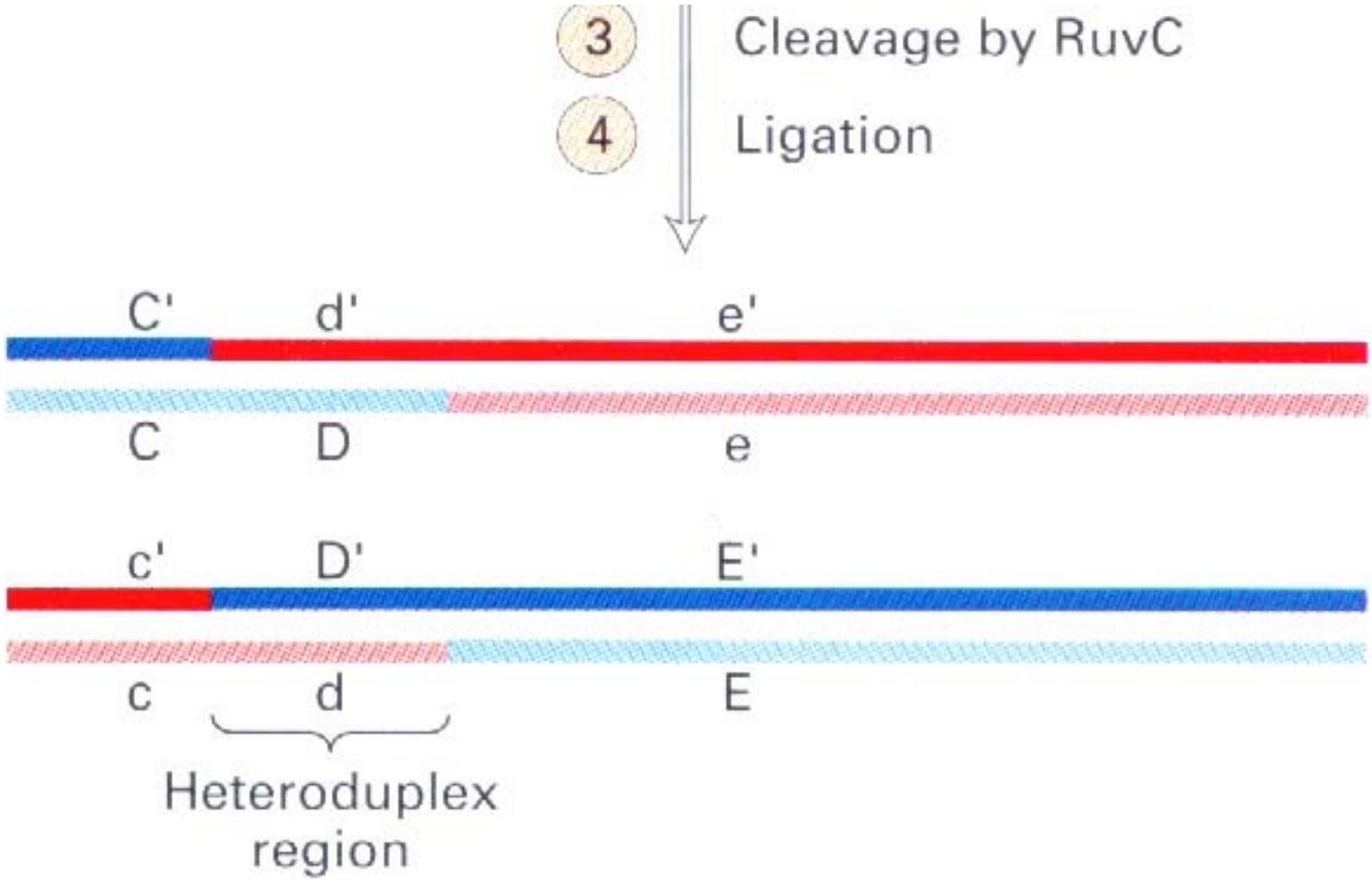

Figure 5-62 Molecular Biology of the Cell 5/e (© Garland Science 2008)

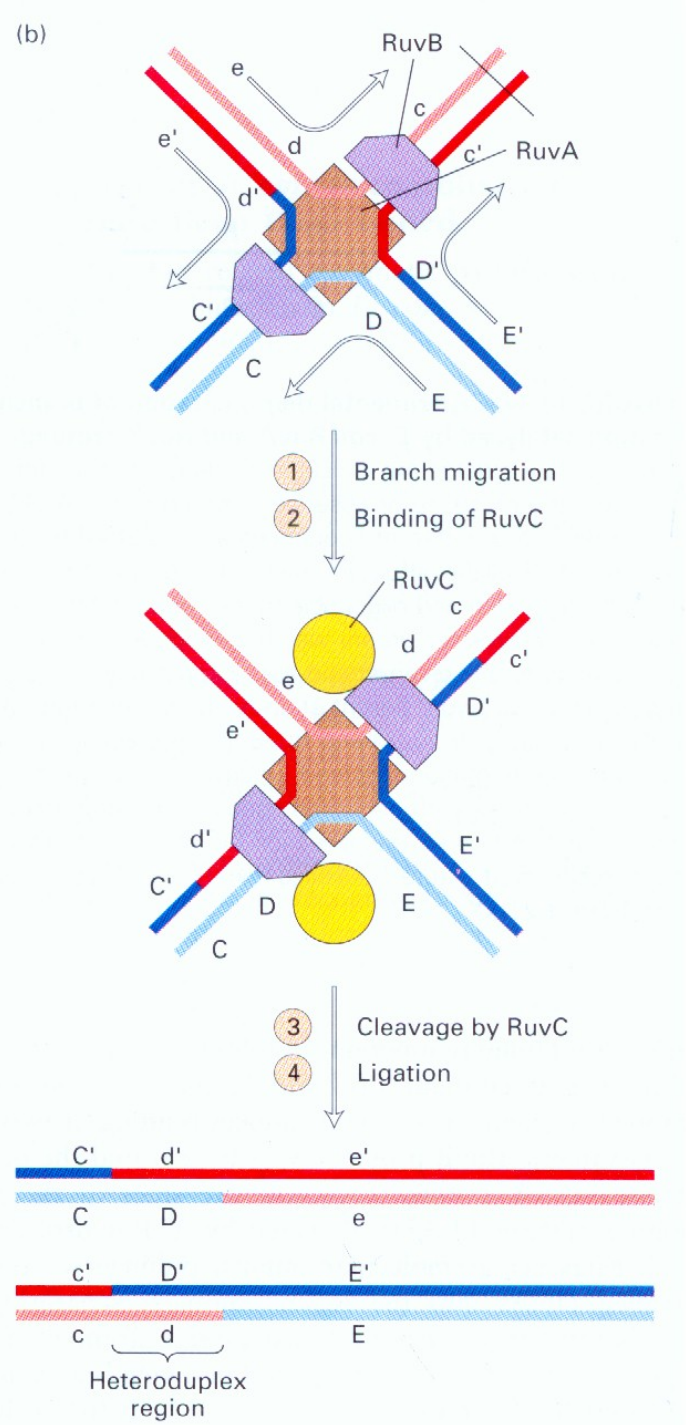
CELL 312

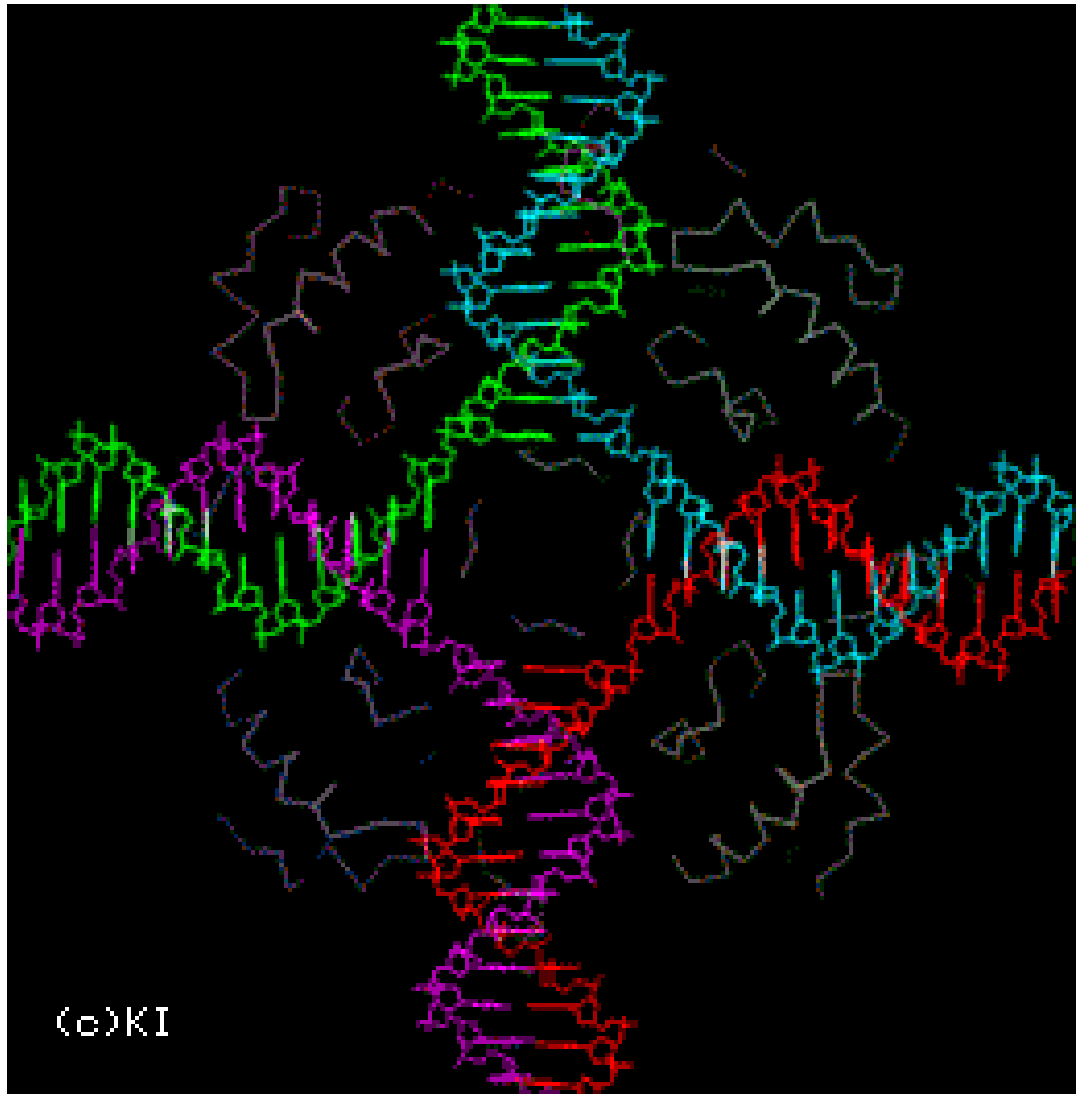
(b)




1
Branch migration
2
Binding of RuvC
RuvC
c
d
c'
e
D'
e'
d'
E'
C'
D
E
C


3
Cleavage by RuvC
4
Ligation
C'
d'
e'
C
D
e
c'
D'
E'
c
d
E
Heteroduplex
region


(b)
RuvB
RuvA
e
e'
d
d'
c
c'
C'
C
D
D'
E'
E
1 Branch migration
2 Binding of RuvC
RuvC
3 Cleavage by RuvC
4 Ligation
C'
d'
e'
C
D
e
c'
D'
E'
c
d
E
Heteroduplex region


(c)KI

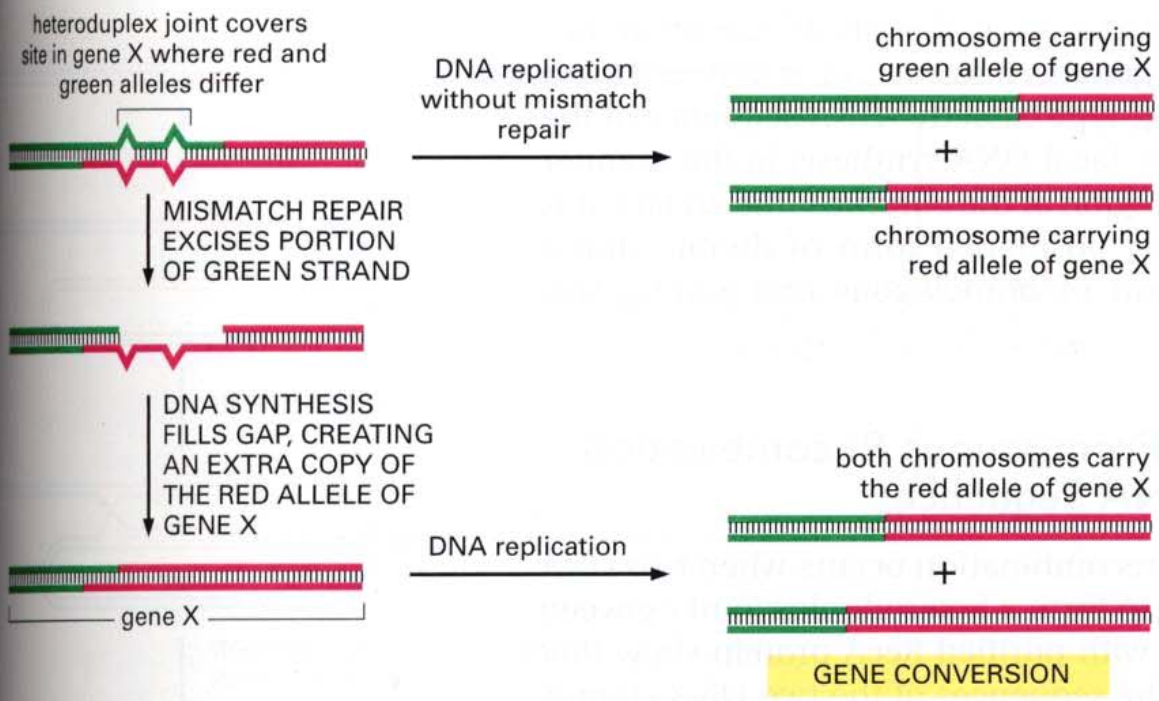



**Figure 5–66 Gene conversion by mismatch correction.** In this process, heteroduplex joints are formed at the sites of the crossing-over between homologous maternal and paternal chromosomes. If the maternal and paternal DNA sequences are slightly different, the heteroduplex joint will include some mismatched base pairs. The resulting mismatch in the double helix may then be corrected by the DNA mismatch repair machinery (see Figure 5–23), which can erase nucleotides on either the paternal or the maternal strand. The consequence of this mismatch repair is gene conversion, dectected as a deviation from the segregation of equal copies of maternal and paternal alleles that normally occurs in meiosis.

**Geenin konversio** on yksi varhain havaituista (harvinaisista) poikkeamista mendelistisistä 1:1 lukusuhteista, joka löydettiin niissä *Neurospora crassan* **tetradianalyyseissa**. Muunlaisissa tilanteissa on hyvin vaikea havaita pieniä poikkemia 1:1 lukusuhteesta. Joskus takaisinmutaation näköiset tapaukset selitetään g:lla.

CELL 315

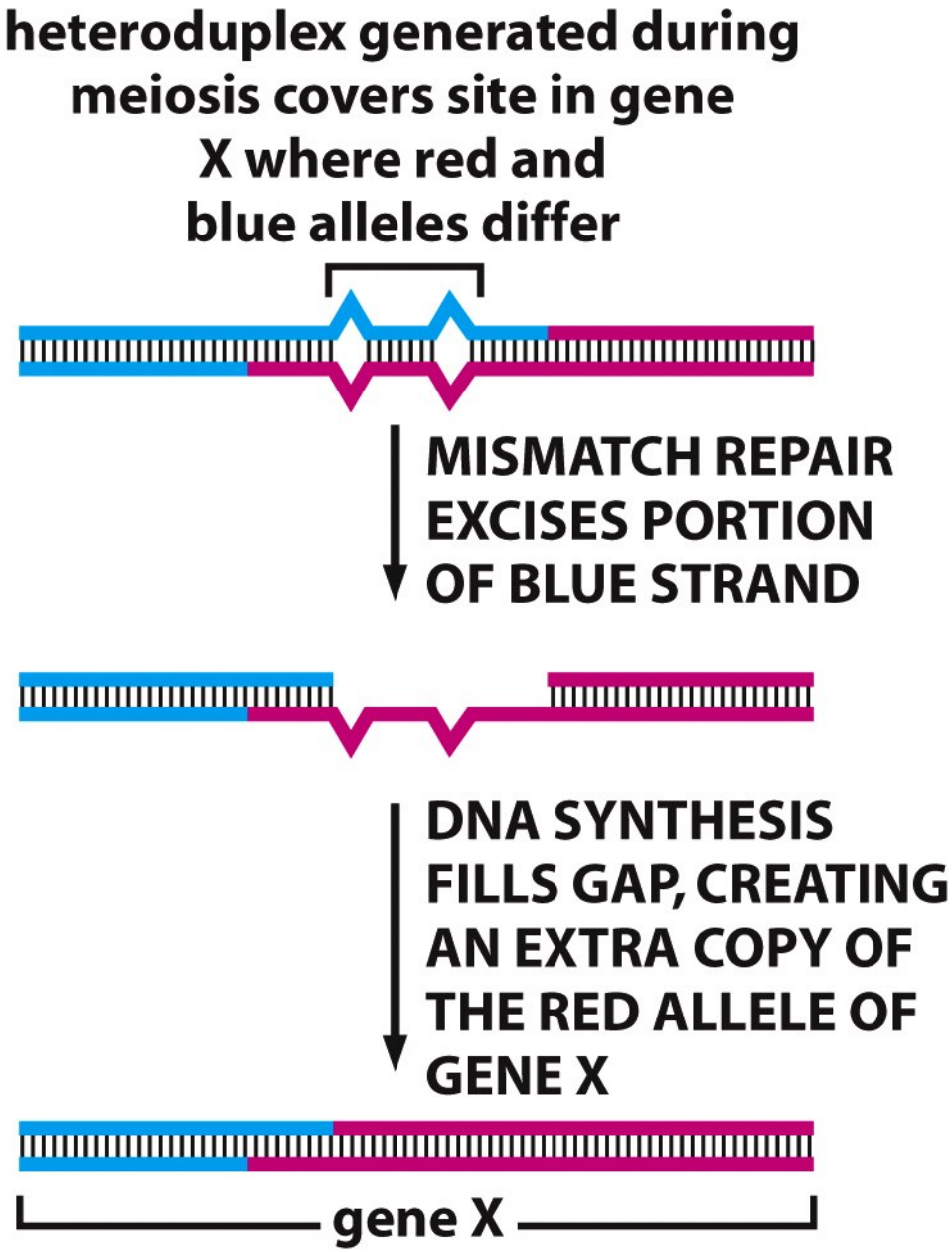



Figure 5-66 Molecular Biology of the Cell 5/e (© Garland Science 2008)

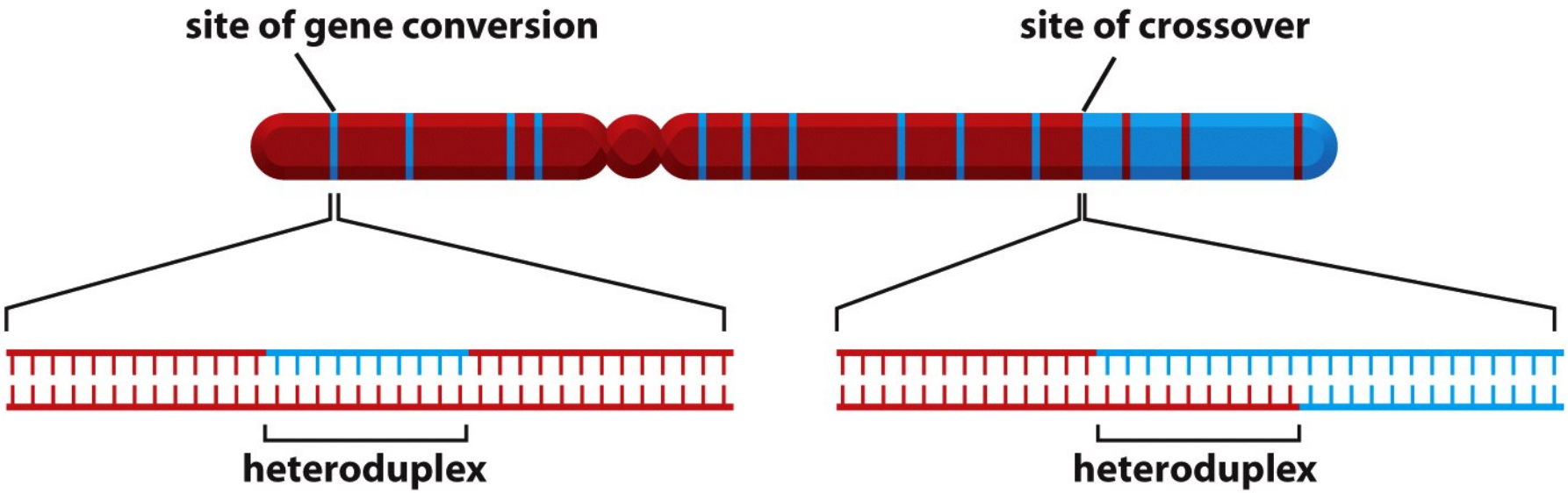



Figure 5-65 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Geenin konversiot ovat pieniä alueita tai yksittäisiä nukleotideja

**DNA –huushollin tapahtumat olivat**

- replikaatio,
- repair,
- rekombinaatio

**Meioosin re-re-re muistisääntö oli**

- replikaatio
- rekombinaatio
- reduktio

Muunlaisia rekombinaatioita kuin meioosin I profaasiin liitettävät

# Transposoni-ilmiöt

## Site-specific Recombination Enzymes Move Special DNA Sequences into and out of Genomes [46]

**Site-specific genetic recombination,** unlike general recombination, is guided by a recombination enzyme that recognizes specific nucleotide sequences present on one or both of the recombining DNA molecules. Base-pairing between the recombining DNA molecules need not be involved, and even when it is, the heteroduplex joint that is formed is only a few base pairs long. By separating and joining double-stranded DNA molecules at specific sites, this type of recombination enables various types of mobile DNA sequences to move about within and between chromosomes.

CELL 316-

**Table 5–3 Three Major Classes of Transposable Elements**

| CLASS DESCRIPTION AND STRUCTURE | SPECIALIZED ENZYMES REQUIRED FOR MOVEMENT | MODE OF MOVEMENT | EXAMPLES |
|---|---|---|---|
| **DNA-only transposons** | | | |
|  short inverted repeats at each end | transposase | moves as DNA, either by cut-and-paste or replicative pathways | P element (*Drosophila*)<br>Ac-Ds (maize)<br>Tn3 and Tn10 (*E. coli*)<br>Tam3 (snapdragon) |
| **Retroviral-like retrotransposons** | | | |
|  directly repeated long terminal repeats (LTRs) at each end | reverse transcriptase and integrase | moves via an RNA intermediate produced by a promoter in the LTR | Copia (*Drosophila*)<br>Ty1 (yeast)<br>THE1 (human)<br>Bs1 (maize) |
| **Nonretroviral retrotransposons** | | | |
|   Poly A at 3′ end of RNA transcript; 5′ end is often truncated | reverse transcriptase and endonuclease | moves via an RNA intermediate that is often produced from a neighboring promoter | F element (*Drosophila*)<br>L1 (human)<br>Cin4 (maize) |

These elements range in length from 1000 to about 12,000 nucleotide pairs. Each family contains many members, only a few of which are listed here. In addition to transposable elements, some viruses can move in and out of host cell chromosomes by transpositional mechanisms. These viruses are related to the first two classes of transposons.

CELL 323

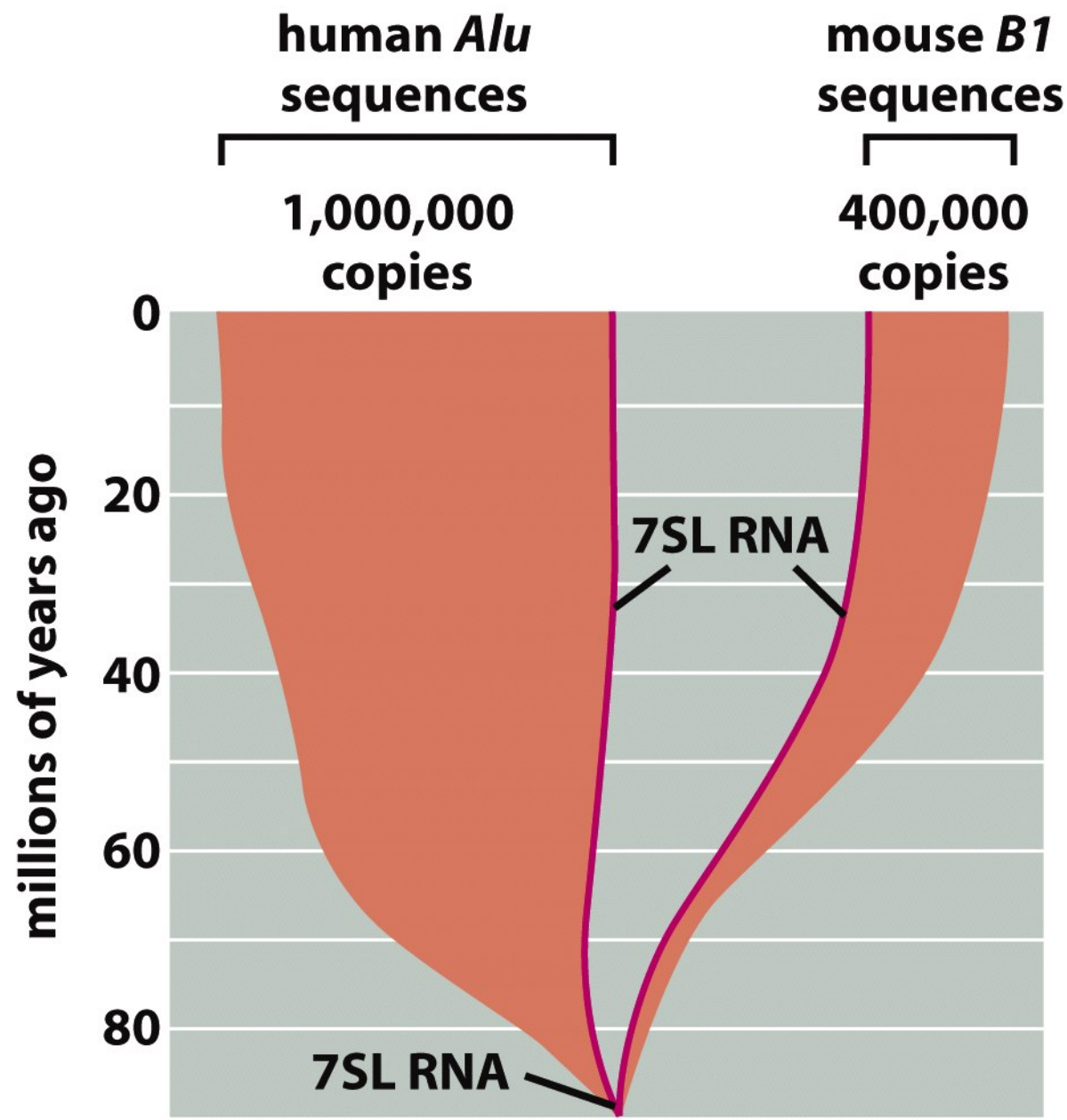



Figure 5-75 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Geeni 7SL RNA:
CELL 728-9
Ohjaa translaatiotuotetta suoraan ER-kalvostoon

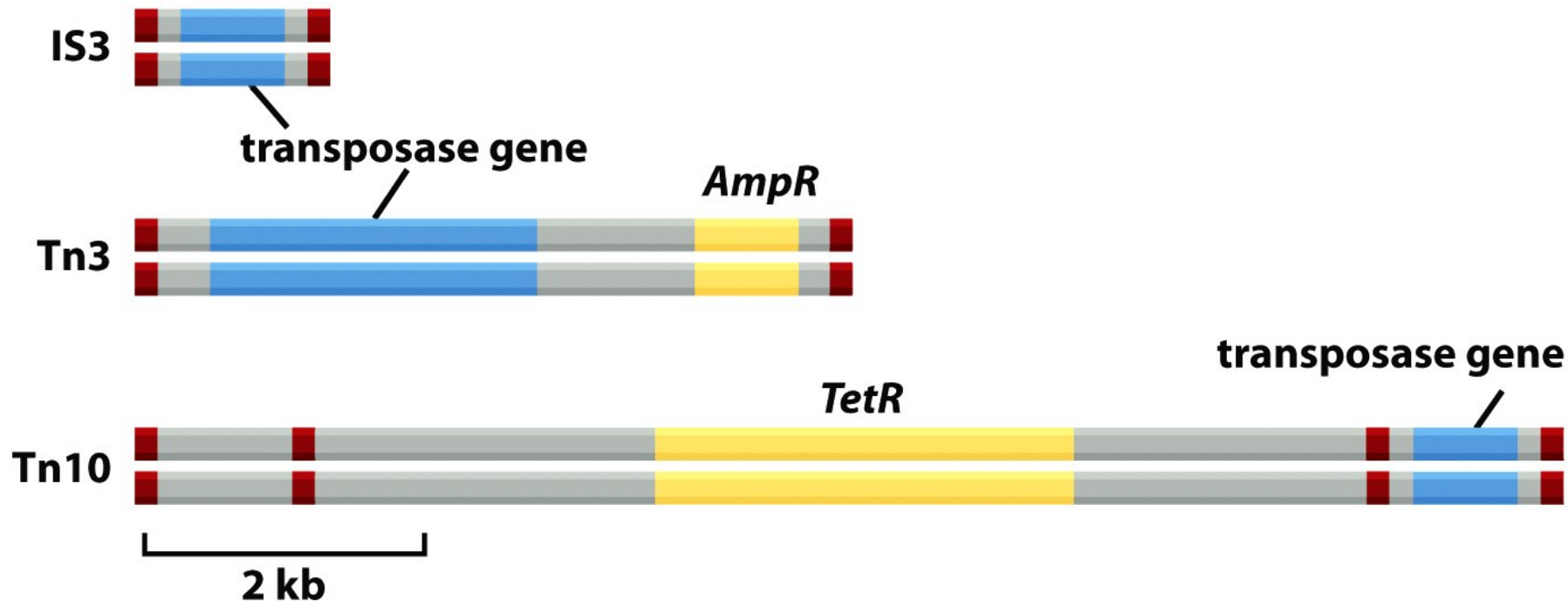



Figure 5-68 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Bakteerien DNA-transposoneja.

Tetrasykliiniresistenssigeeni alimman keskellä on haitallisesti monistunut ja levinnyt kahden naapuritransposonin avulla

CELL 318

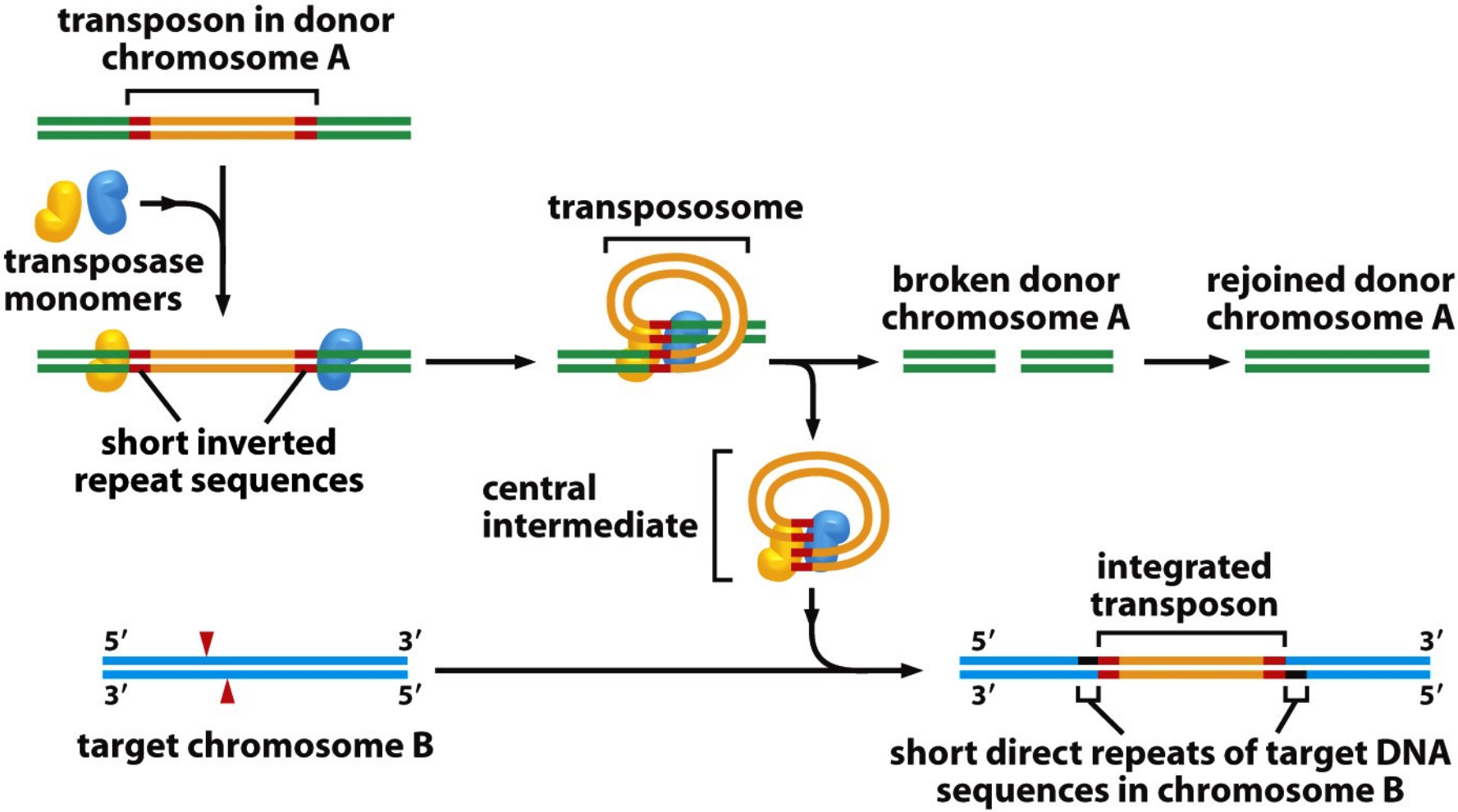



Figure 5-69 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 320

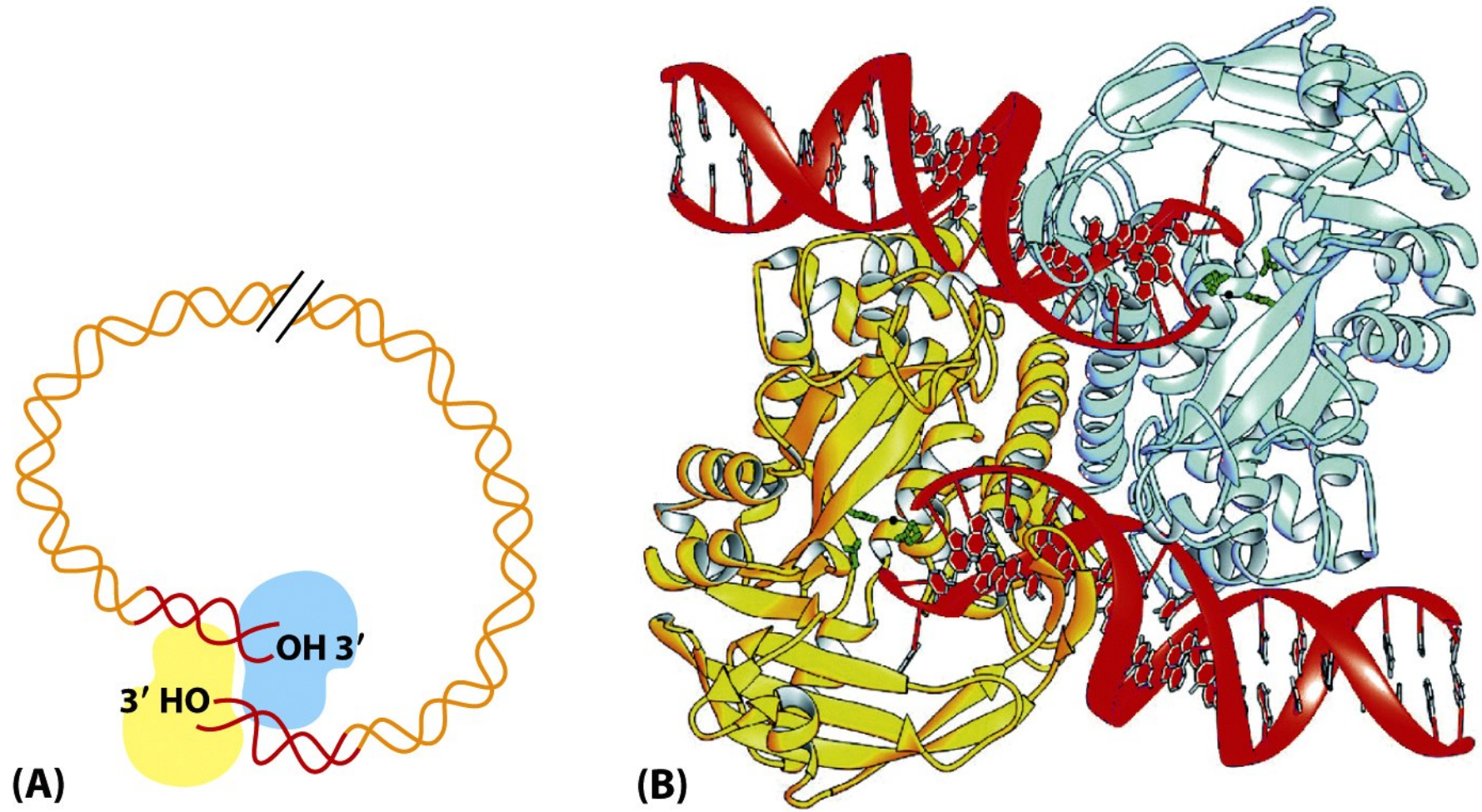



Figure 5-70 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Cut-and-paste transposase -entsyymi

CELL 320

# Retroviruksen integraatio

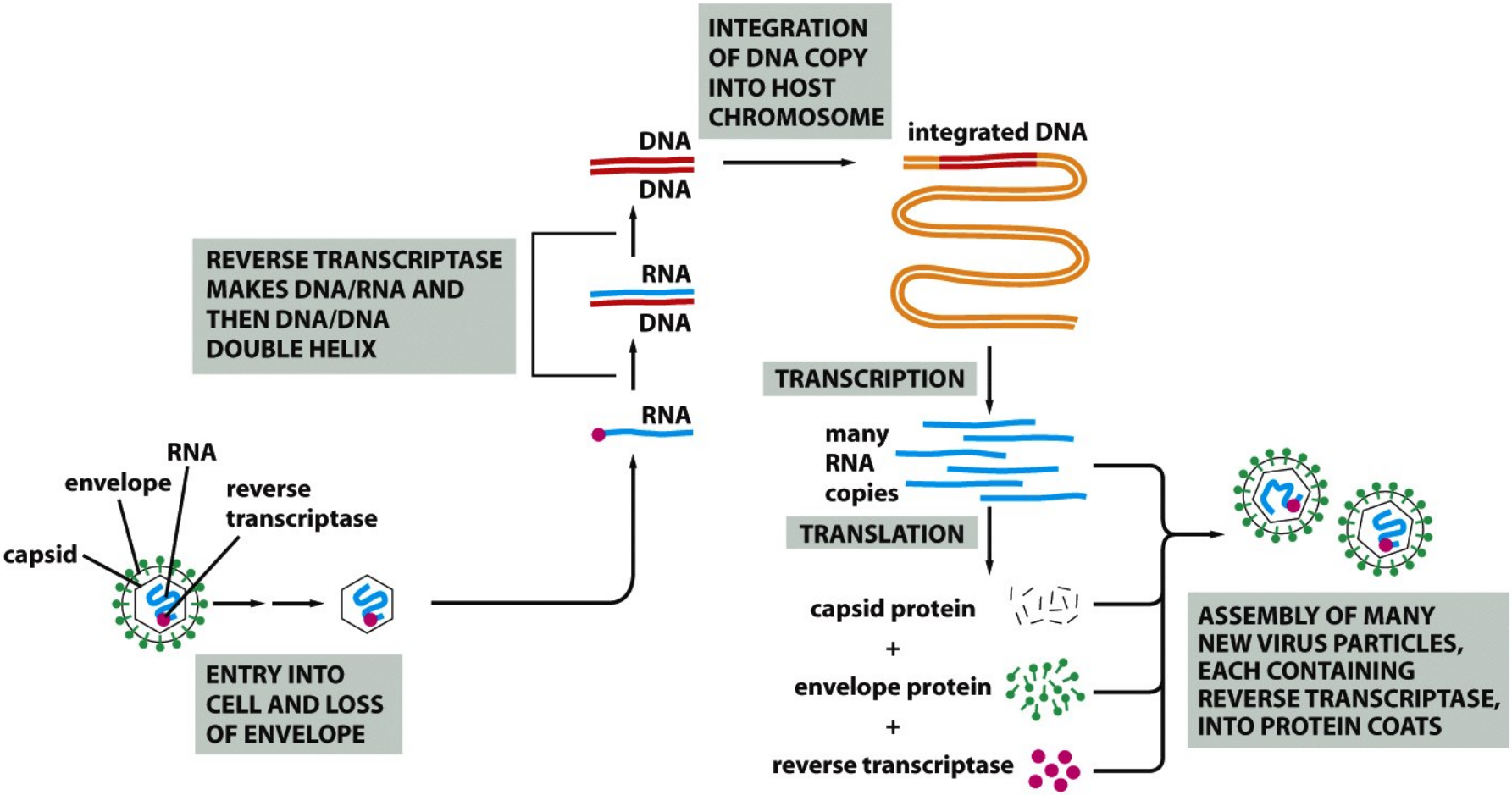
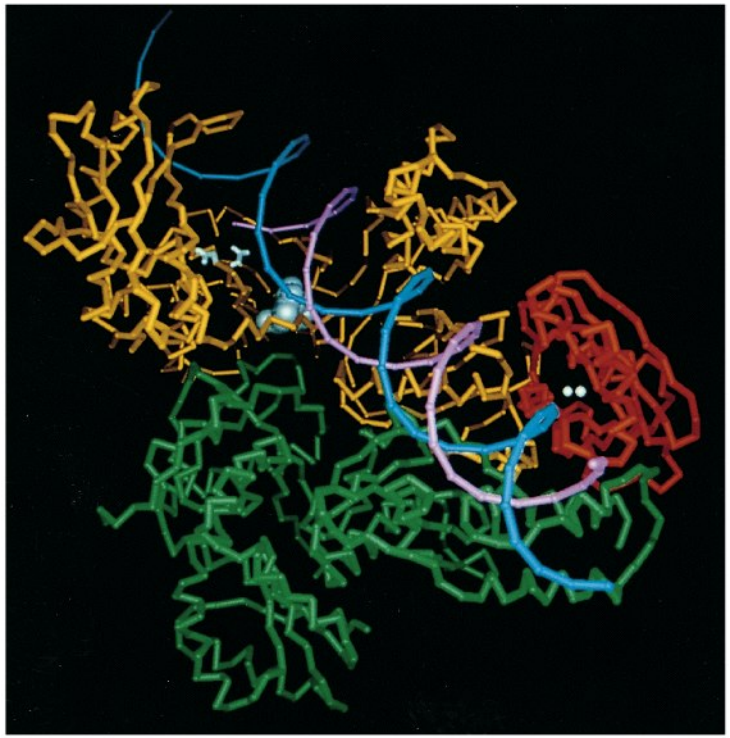
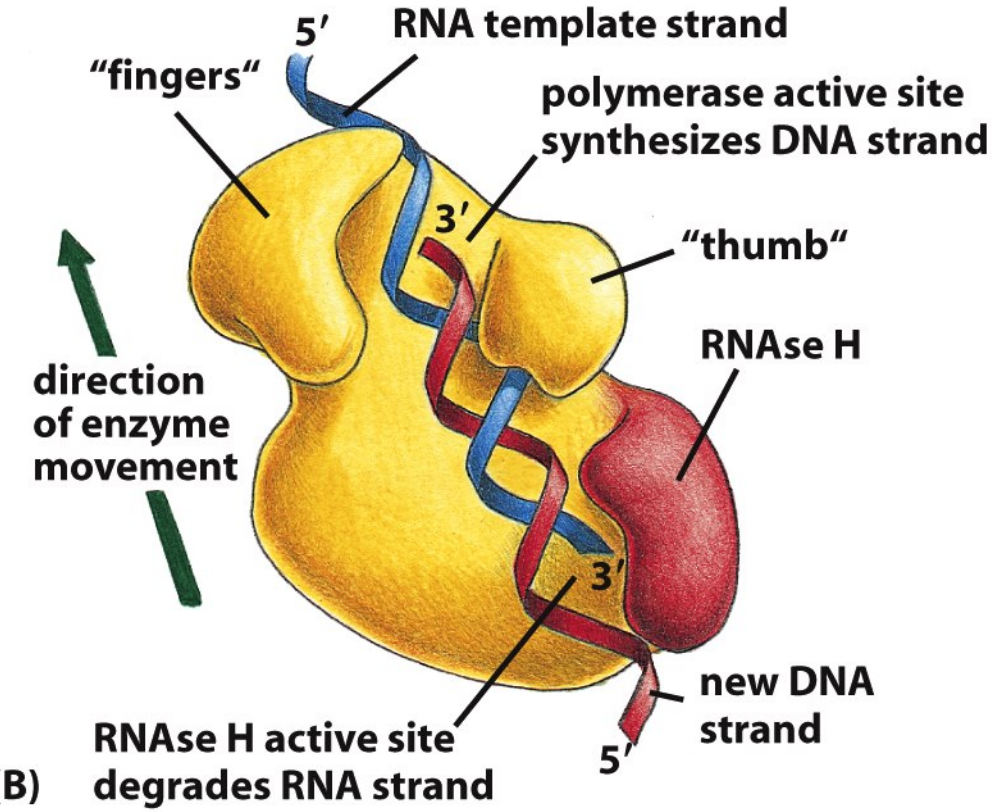



Figure 5-71 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 321



(A)




(B)

Figure 5-72 Molecular Biology of the Cell 5/e (© Garland Science 2008)

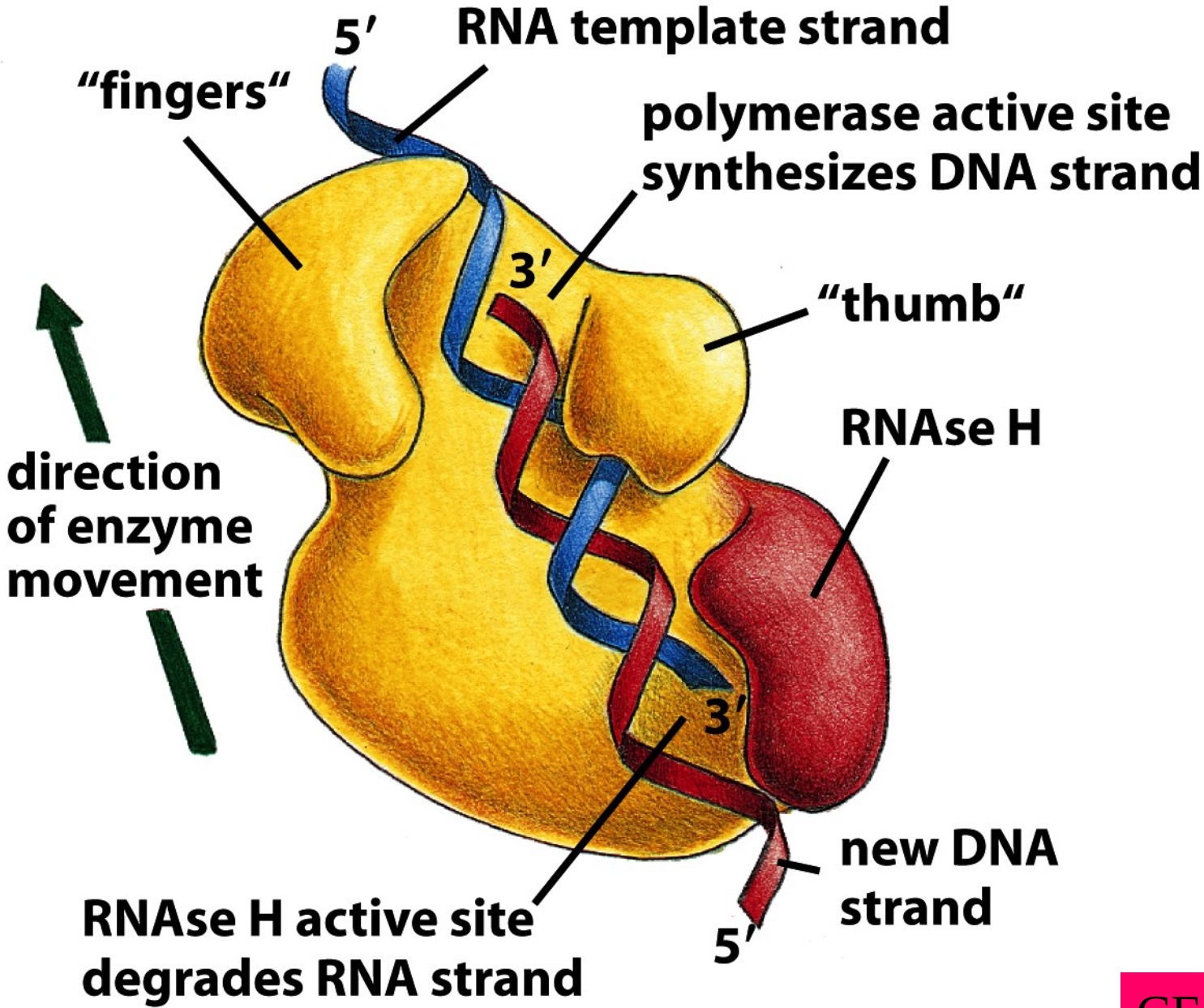



Figure 5-72b Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 321

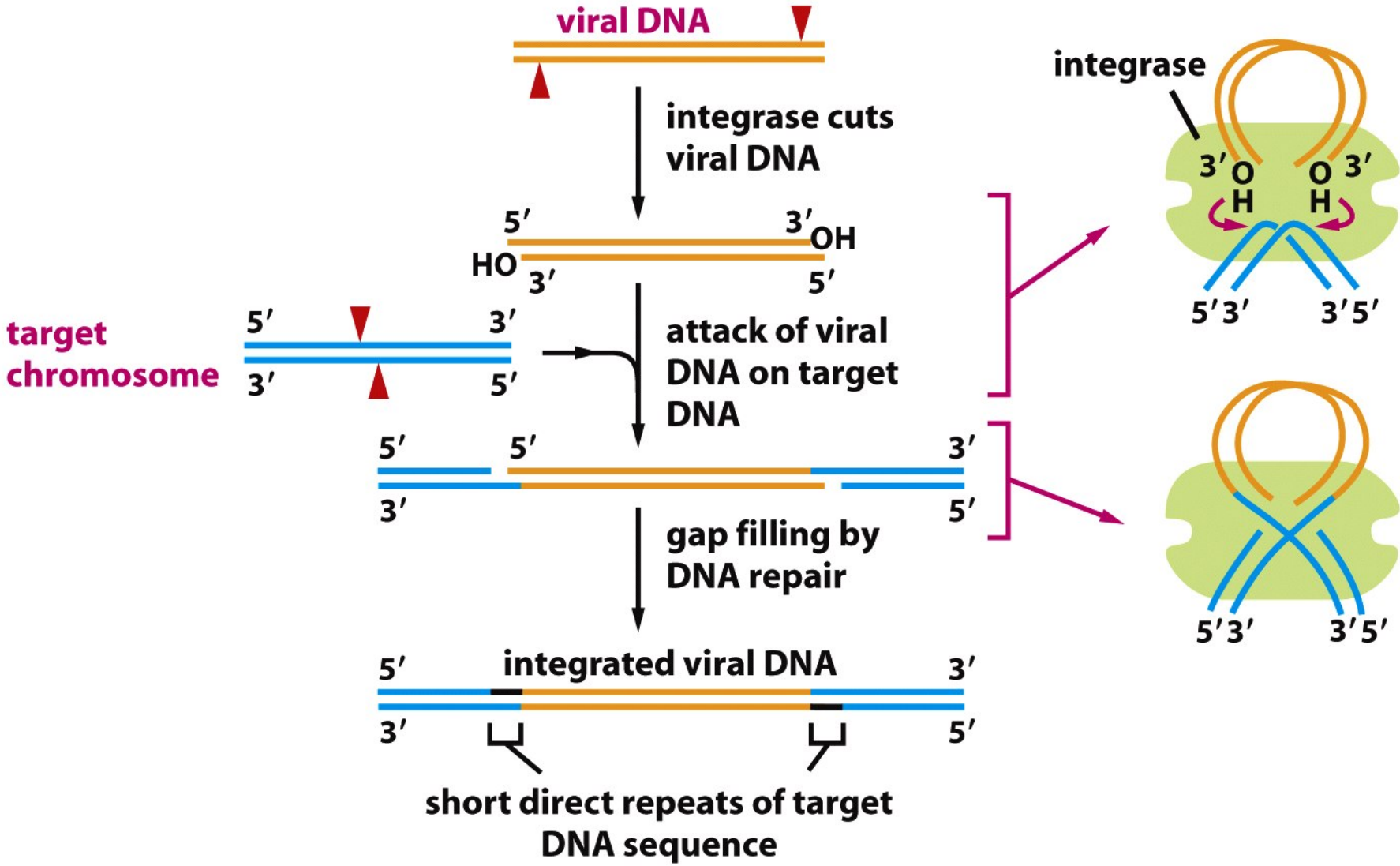



Figure 5-73 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 322

# Retrotransposonin siirtyminen CELL 322

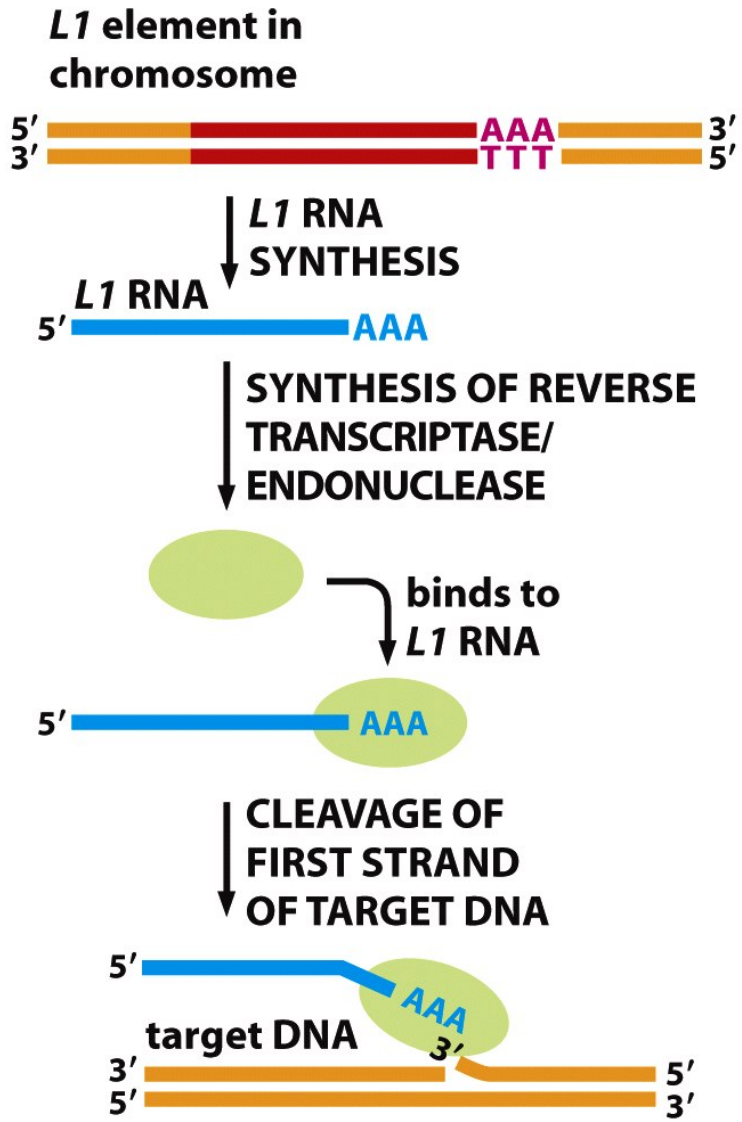



Figure 5-74 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

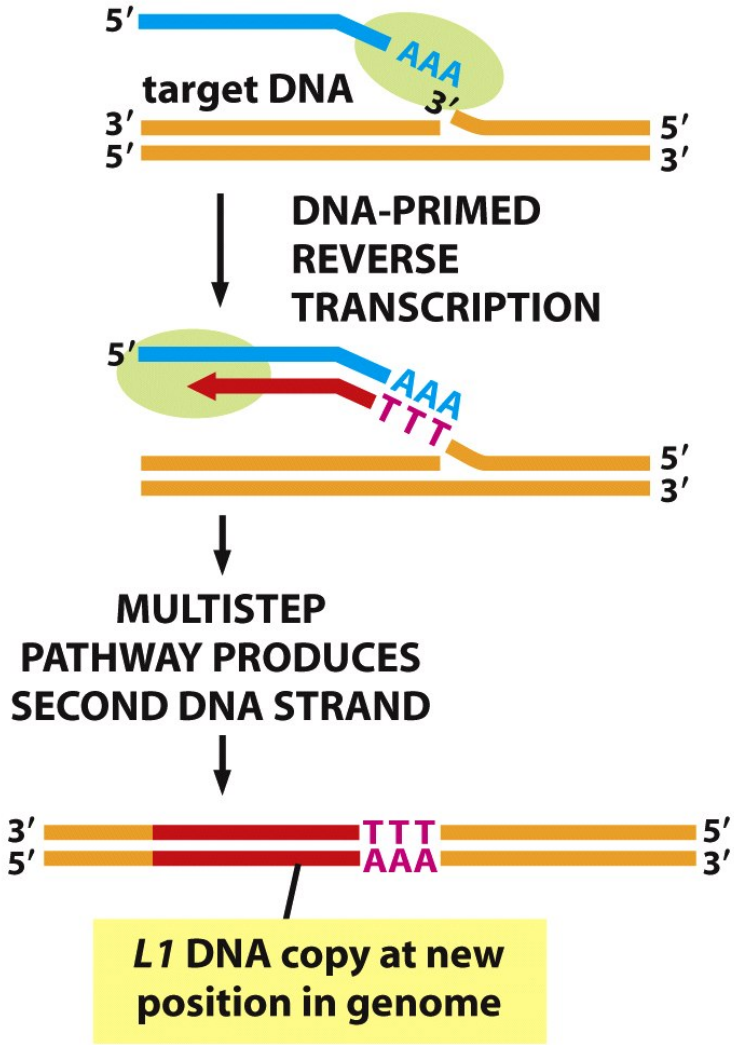



Figure 5-74 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

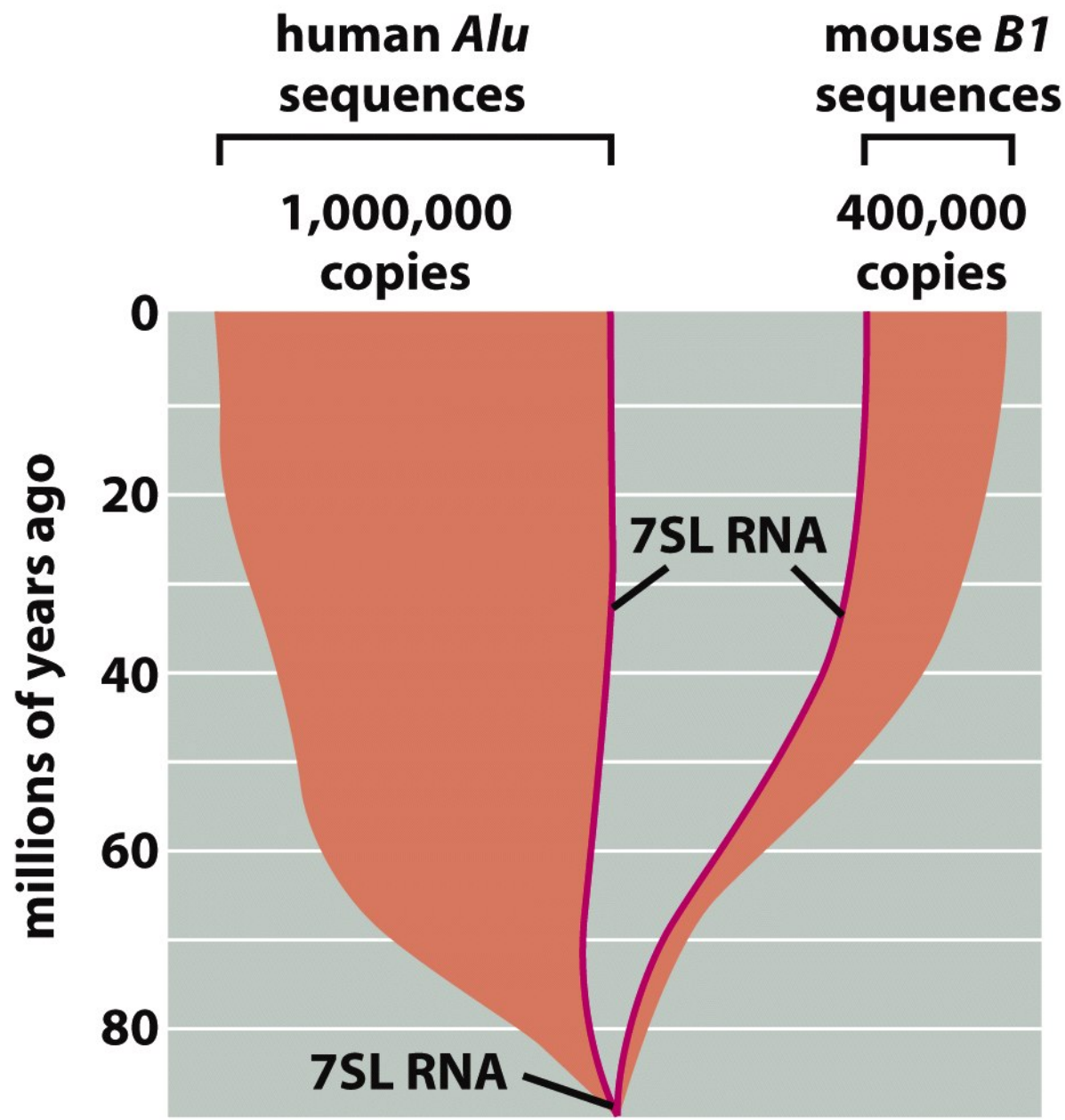



Figure 5-75 Molecular Biology of the Cell 5/e (© Garland Science 2008)

**TABLE 5–3 Three Major Classes of Transposable Elements**

| CLASS DESCRIPTION AND STRUCTURE | GENES IN COMPLETE ELEMENT | MODE OF MOVEMENT | EXAMPLES |
|---|---|---|---|
| **DNA-only transposons** | | | |
| short inverted repeats at each end | encodes transposase | moves as DNA, either excising or following a replicative pathway | P element (*Drosophila*)<br>Ac-Ds (maize)<br>Tn3 and IS1 (*E.coli*)<br>Tam3 (snapdragon) |
| **Retroviral-like retrotransposons** | | | |
| directly repeated long terminal repeats (LTRs) at ends | encodes reverse transcriptase and resembles retrovirus | moves via an RNA intermediate produced by promoter in LTR | Copia (*Drosophila*)<br>Ty1 (yeast)<br>THE-1 (human)<br>Bs1 (maize) |
| **Nonretroviral retrotransposons** | | | |
| Poly A at 3′ e<br>transcript;<br>truncated | | often produced<br>from a neighboring<br>promotor | F element (*Droso*<br>L1 (human)<br>Cin4 (maize) |

These elements range in length from 1000 to about 12,000 nucleotide pairs; each family contains many members, only a few of wh
are listed here. In addition to transposable elements, there are selected viruses that can move in and out of host cell chromosomes;
these viruses are related to the first two classes of transposons.

Nobel-palkinto: Barbara McClintock 1983

# Viruksen (lambda) integraatio

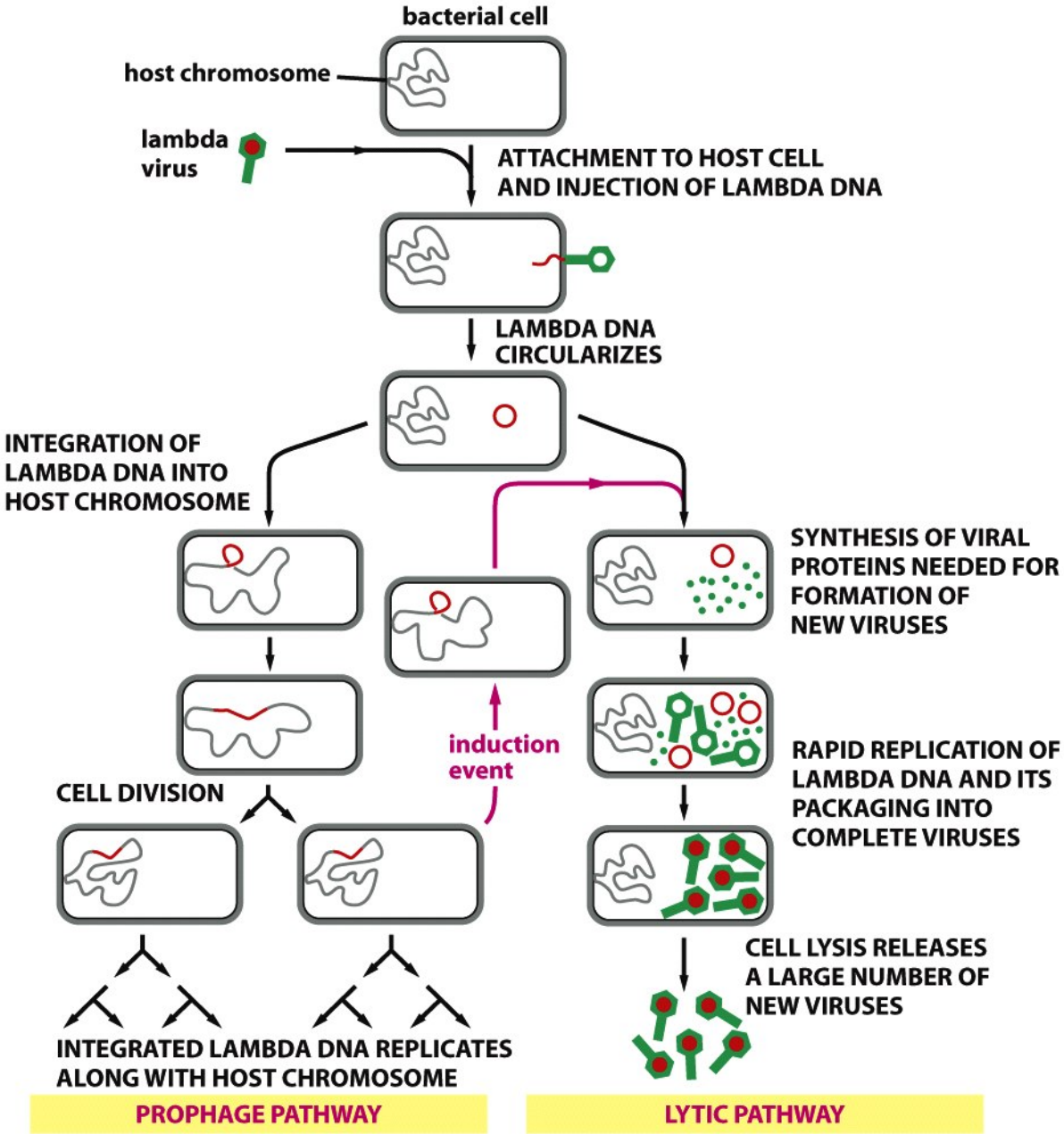



Figure 5-78 Molecular Biology of the Cell 5/e (© Garland Science 2008)

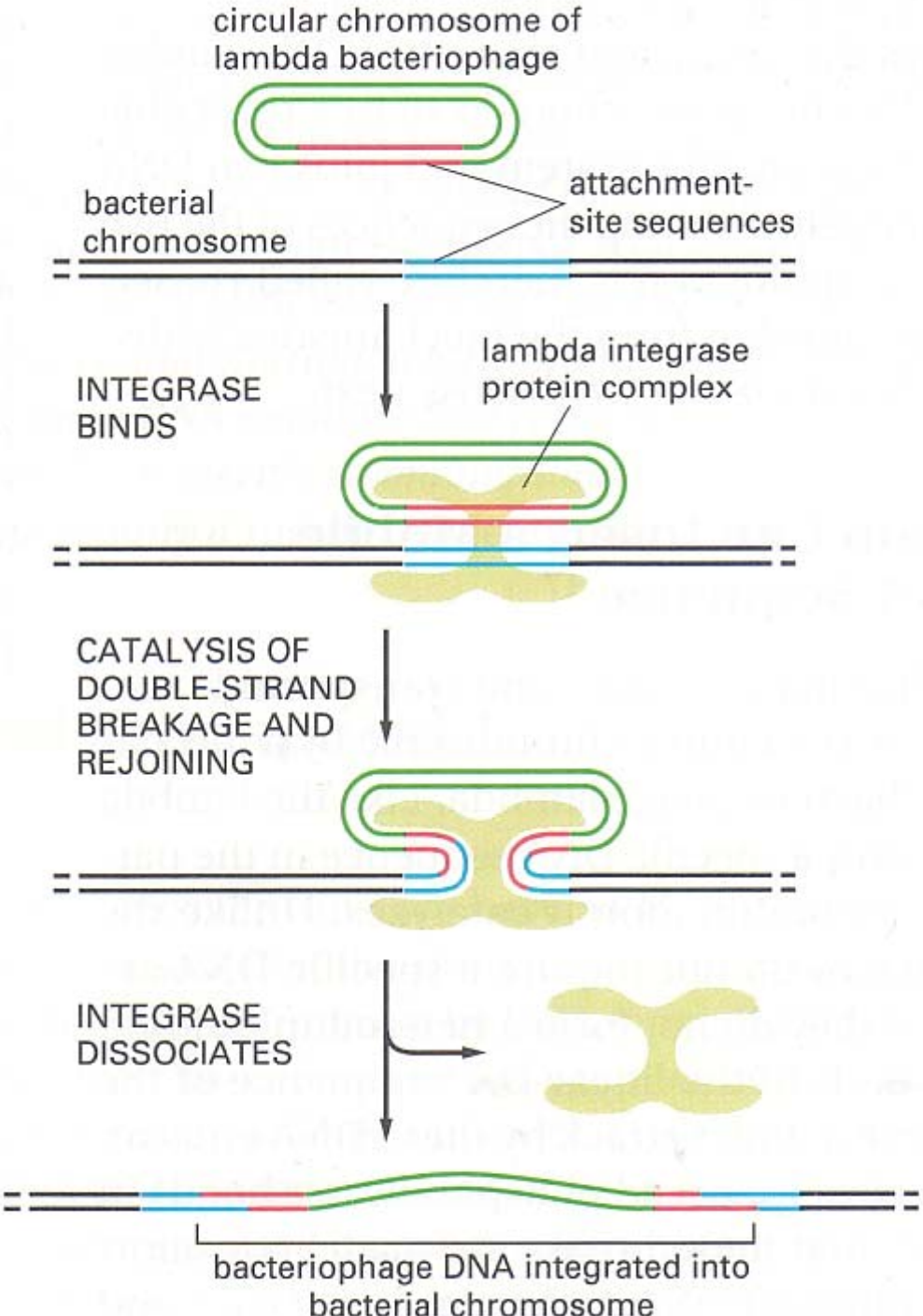



**Figure 6–68 The insertion of bacteriophage lambda DNA into the bacterial chromosome.** In this example of site-specific recombination, the lambda integrase enzyme binds to a specific "attachment site" DNA sequence on each chromosome, where it makes cuts that bracket a short homologous DNA sequence; the integrase thereby switches the partner strands and reseals them so as to form a heteroduplex joint 7 base pairs long. Each of the four strand-breaking and strand-joining reactions required resembles that made by a DNA topoisomerase, inasmuch as the energy of a cleaved phosphodiester bond is stored in a transient covalent linkage between the DNA and the enzyme (see Figure 6–64).

CELL 325

# Knock-out rekombinaatio

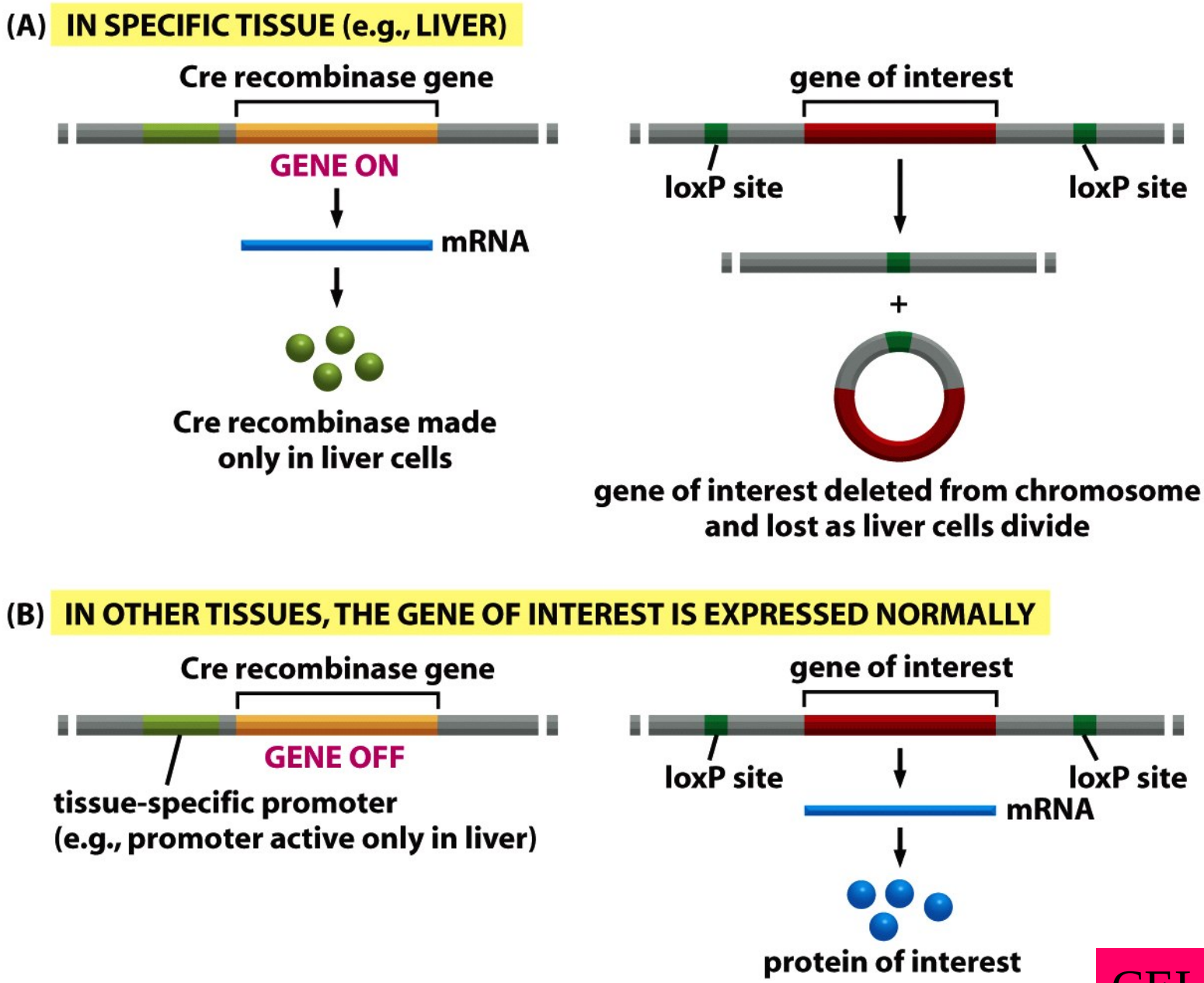



Figure 5-79 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 326

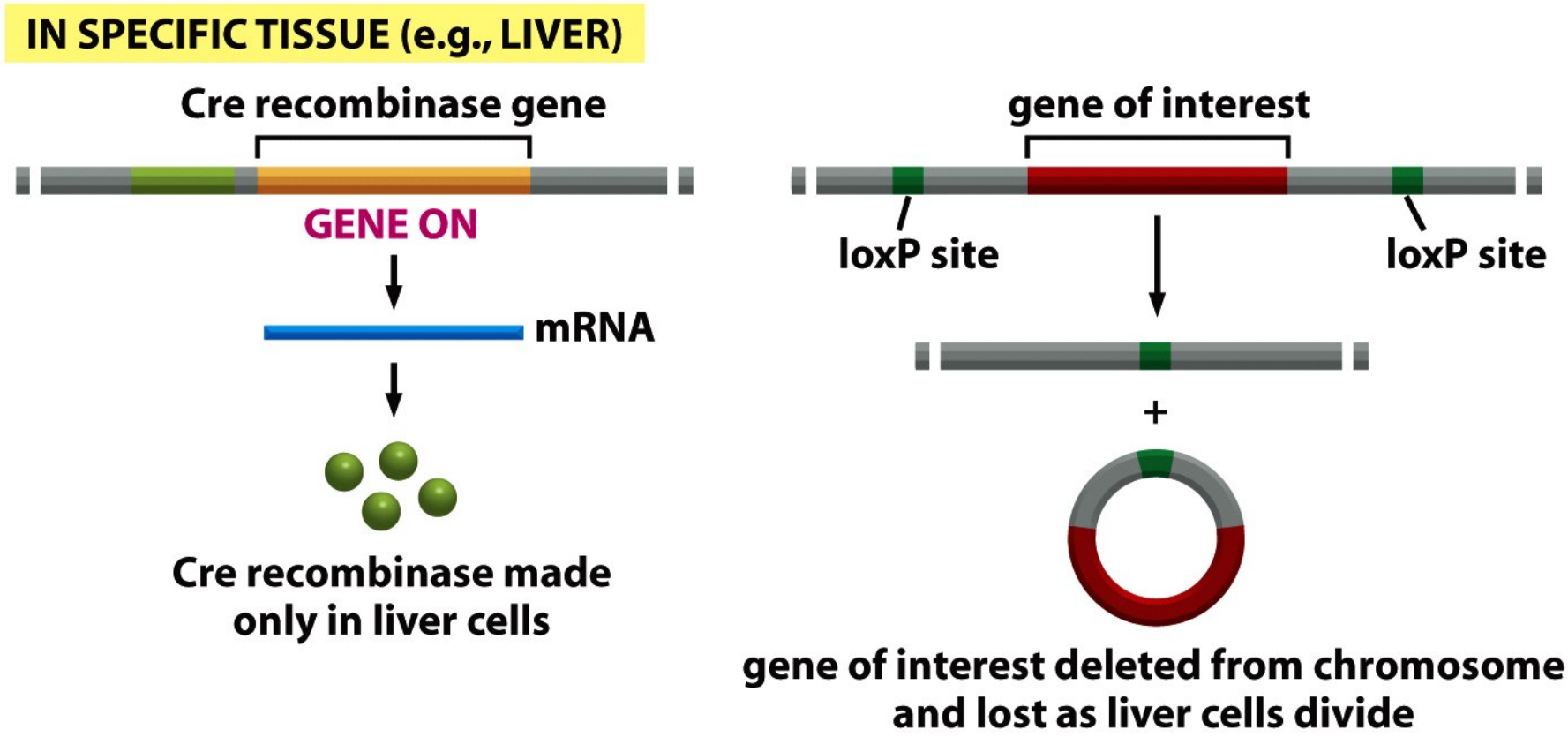



Figure 5-79a Molecular Biology of the Cell 5/e (© Garland Science 2008)

## IN OTHER TISSUES, THE GENE OF INTEREST IS EXPRESSED NORMALLY

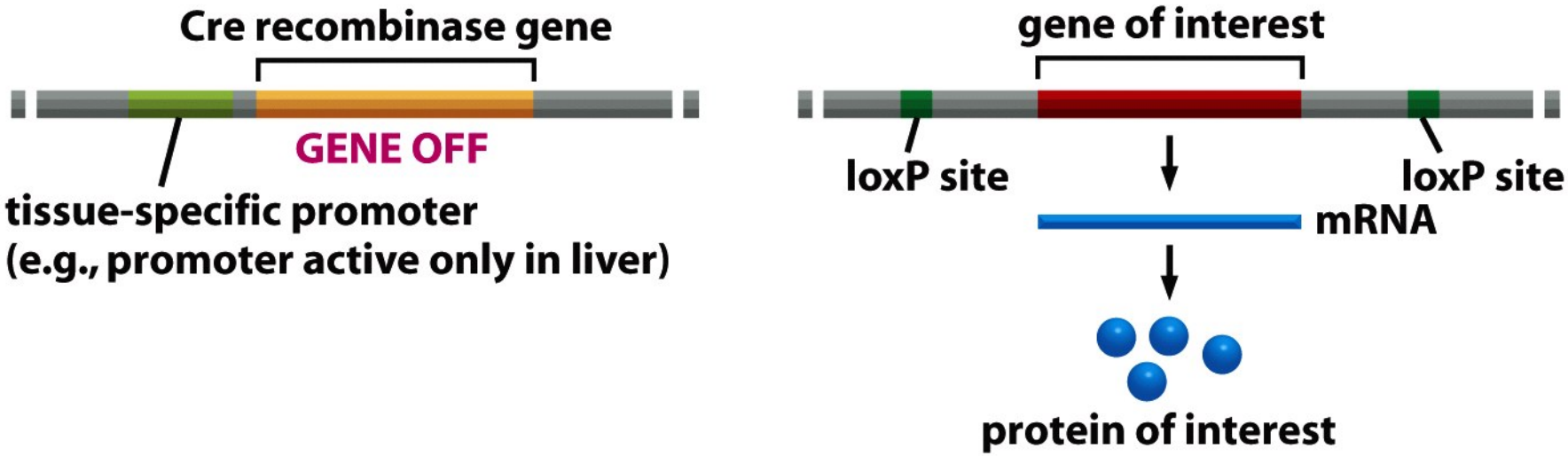



Figure 5-79b Molecular Biology of the Cell 5/e (© Garland Science 2008)

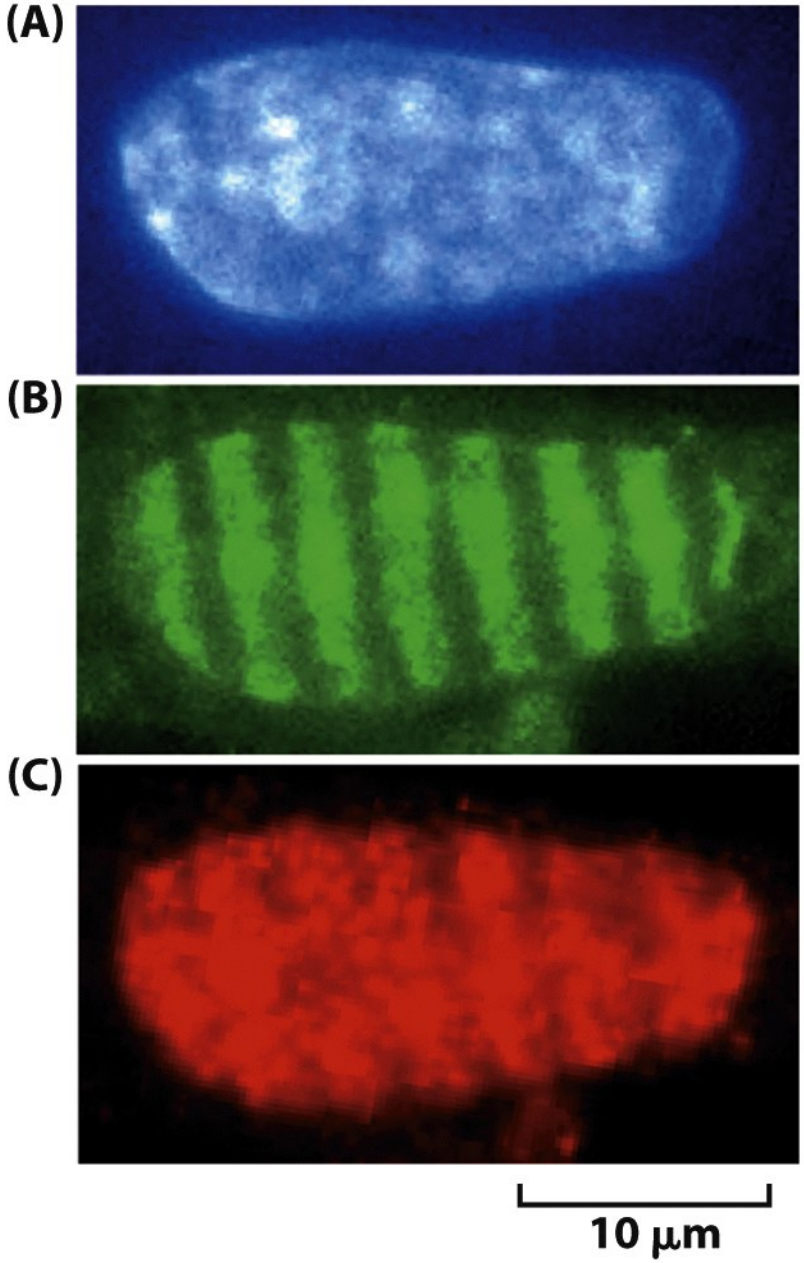



Figure 5-60 Molecular Biology of the Cell 5/e (© Garland Science 2008)