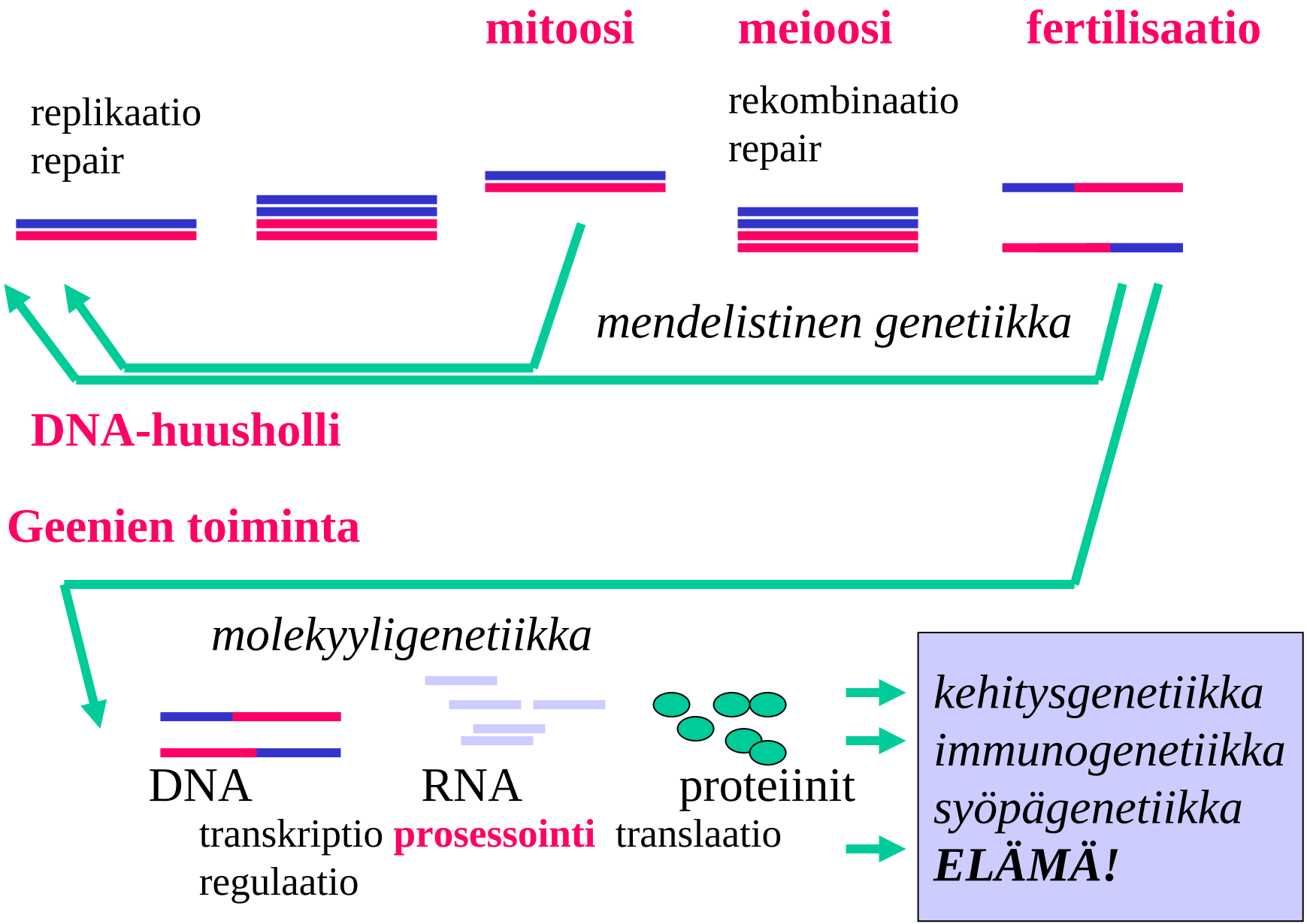
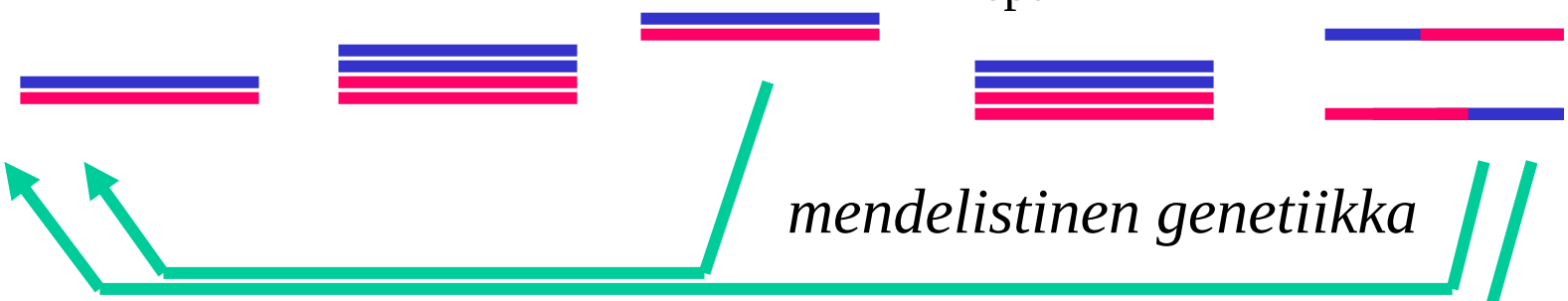



Genetiikan perusteiden miellekartta

CELL 319

**mitoosi** **meioosi** **fertilisaatio**

rekombinaatio
repair



*mendelistinen genetiikka*

**DNA-huusholli**

**Geenien toiminta**

*molekyyligenetiikka*


*kehitysgenetiikka*
*immunogenetiikka*
*syöpägenetiikka*
***ELÄMÄ!***

Genetiikan perusteiden miellekartta: RNA:n muokkaus: silputus

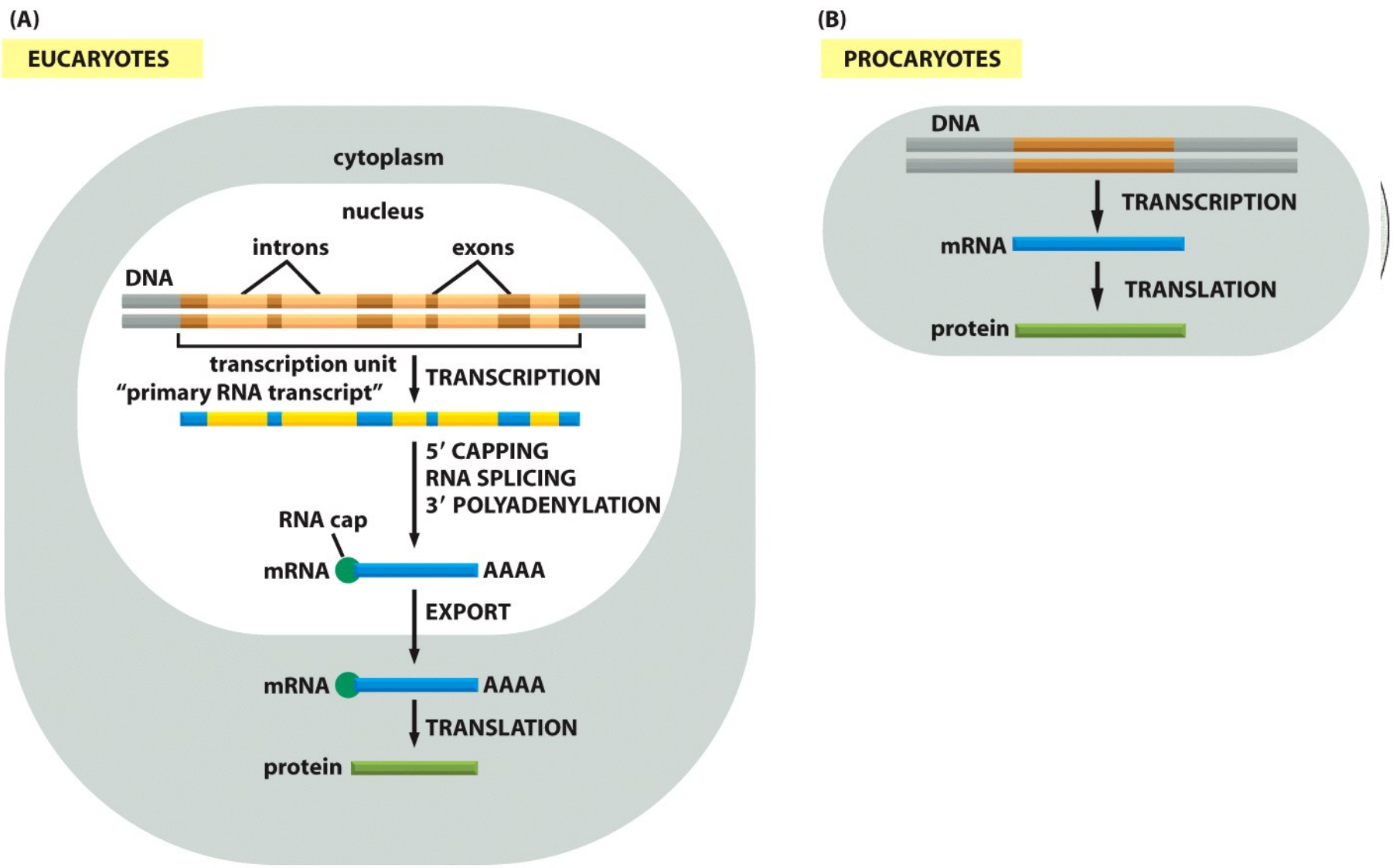
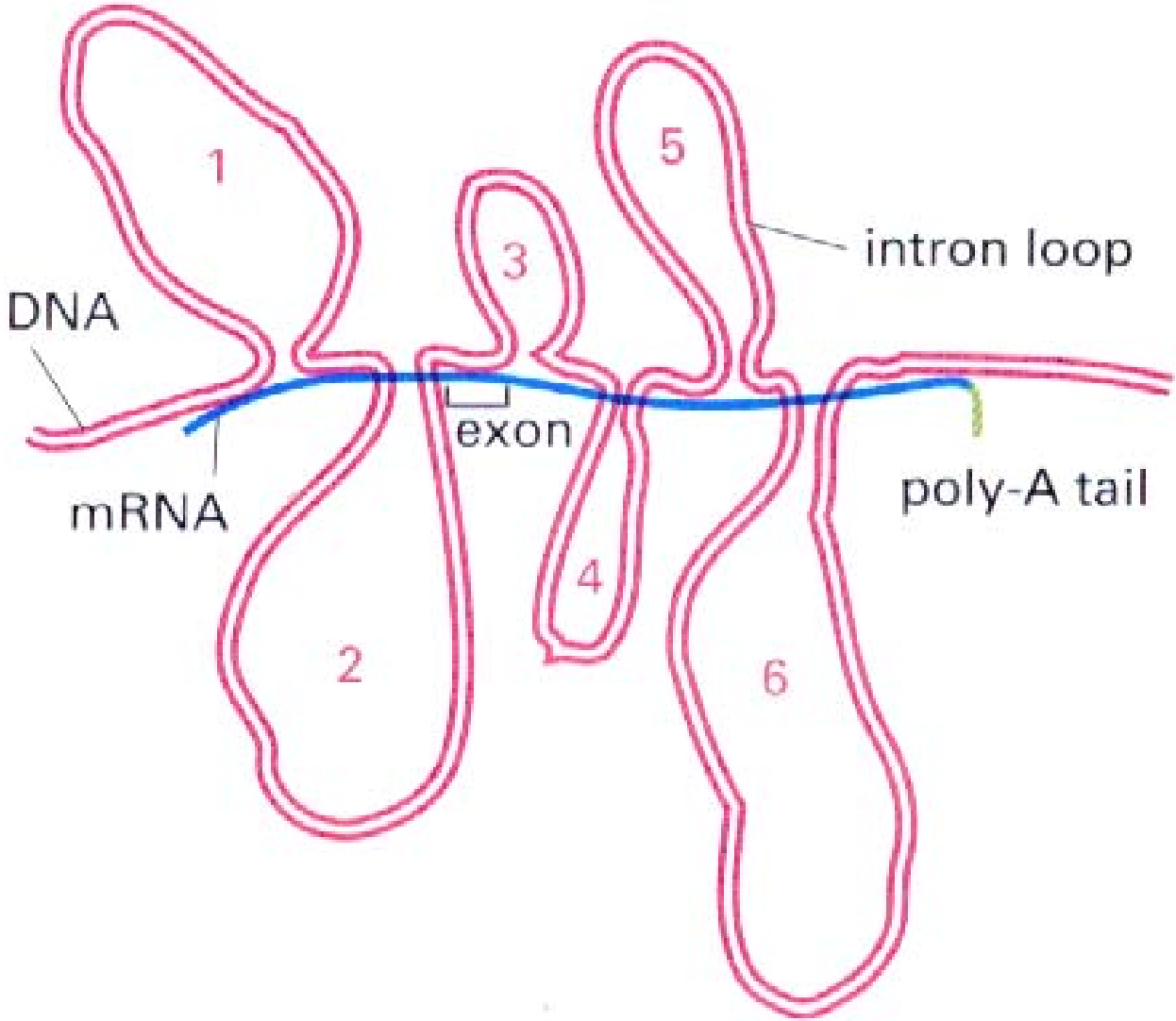



Figure 6-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

# Silputus eli splicing

**Eksonit**

**Intronit**




Varhainen demo silputuksesta

RNA-DNA -*hybridisaatio*. Sopivissa oloissa RNA sitoutuu omaan templaattiinsa ahnaammin kuin vastinjuoste (DNA denaturoitu ja hyydytetty)

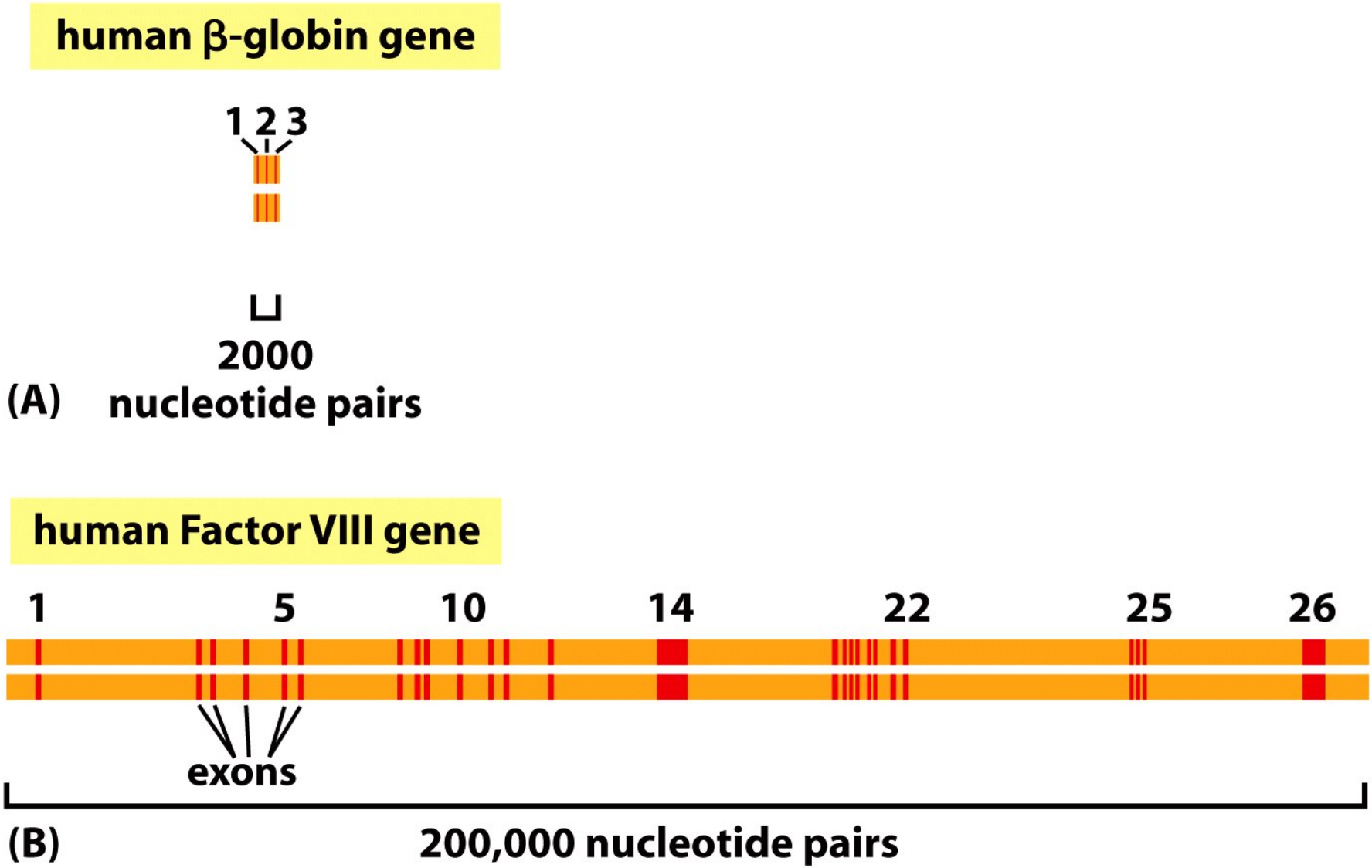



Figure 6-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

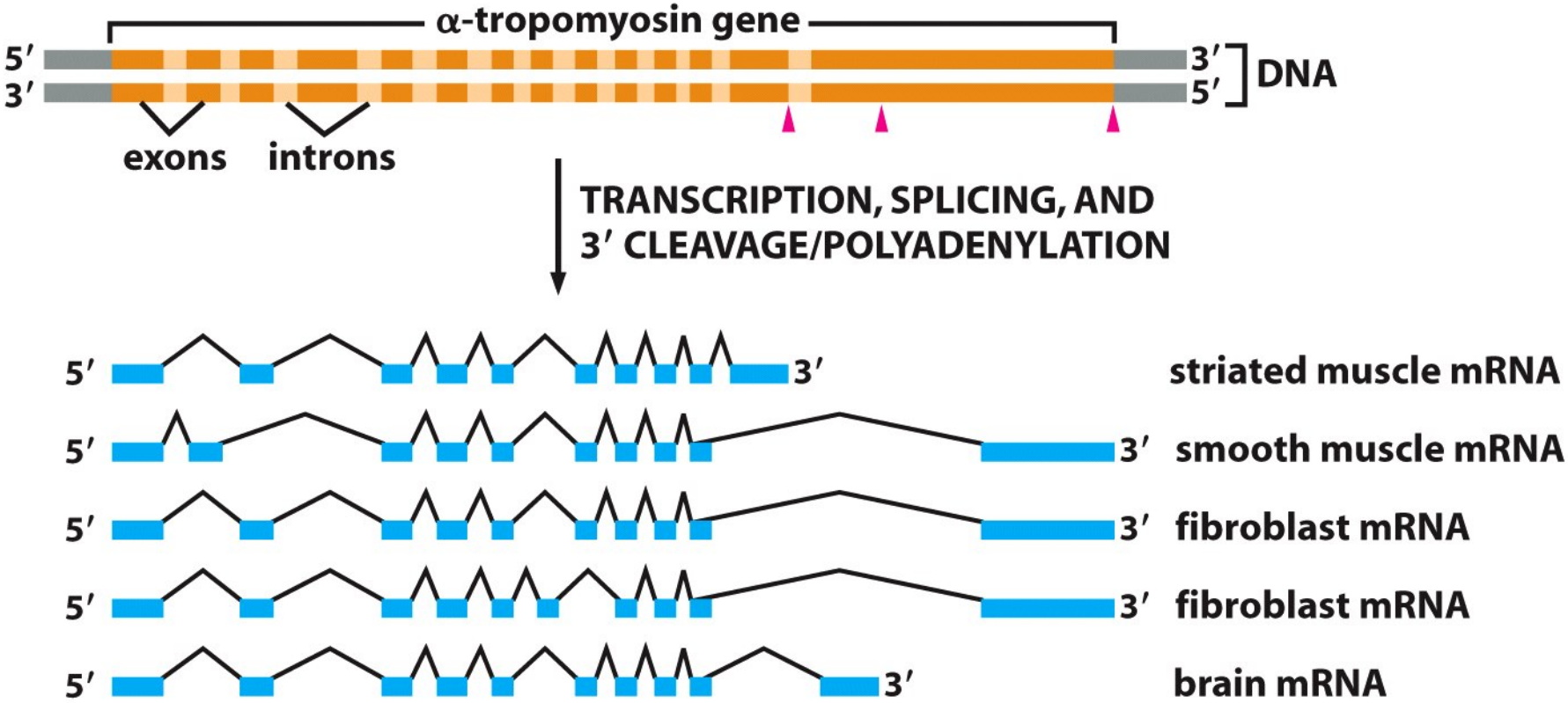



Figure 6-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Rotan tropomyosiinigeenin tuotteita saadaan *alternative splicingilla* eli vaihtoehtoisella silputuksella

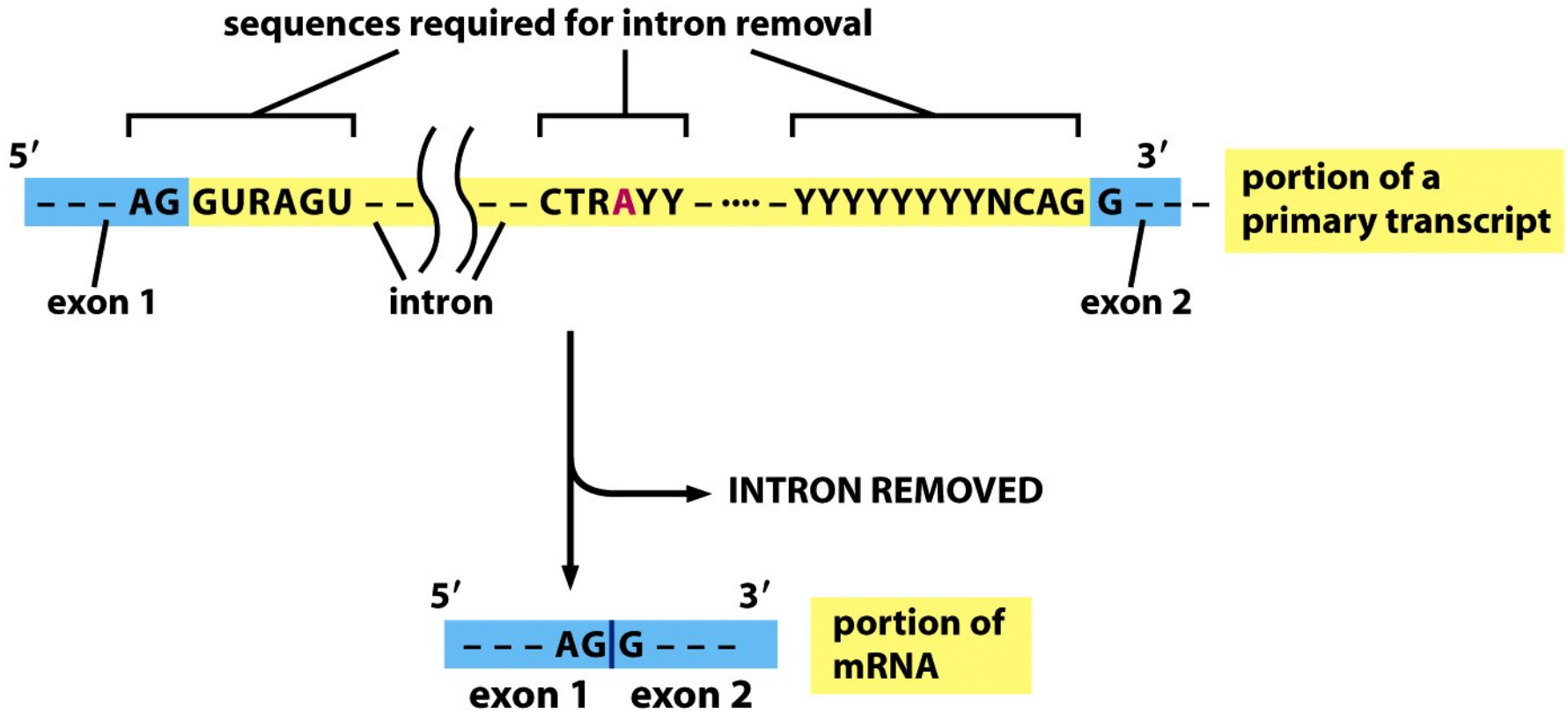
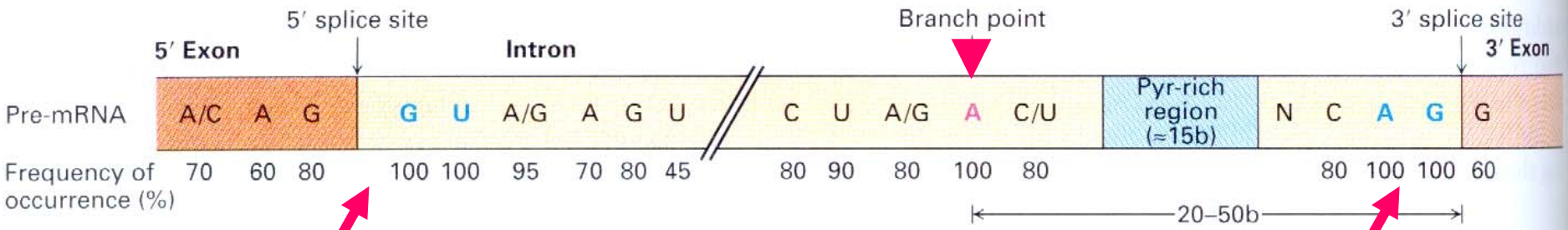



Figure 6-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Intronin tuntomerkit: solunhan täytyy tietää, missä koodi loppuu ja missä se alkaa uudestaan

CELL 349



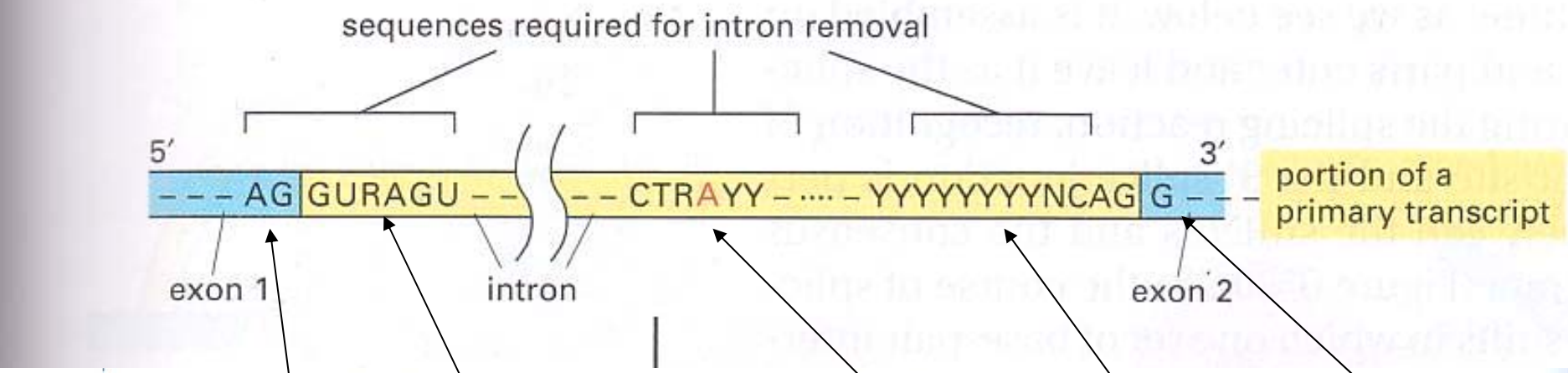

▲ **FIGURE 11-14 Consensus sequences around 5′ and 3′ splice sites in vertebrate pre-mRNAs.** The only nearly invariant bases are the (5′)GU and (3′)AG of the intron, although the flanking bases indicated are found at frequencies higher than expected based on a random distribution. A pyrimidine-rich region (light blue) near the 3′ end of the intron is found in most cases. The branch-point adenosine, also invariant, usually is 20–50 bases from the 3′ splice site. The central region of the intron, which may range from 40 bases to 50 kilobases in length, generally is unnecessary for splicing to occur. [See R. A. Padgett et al., 1986, *Ann. Rev. Biochem.* **55**:1119; E. B. Keller and W. A. Noon, 1984, *Proc. Nat'l. Acad. Sci. USA* **81**:7417.]

100% GU

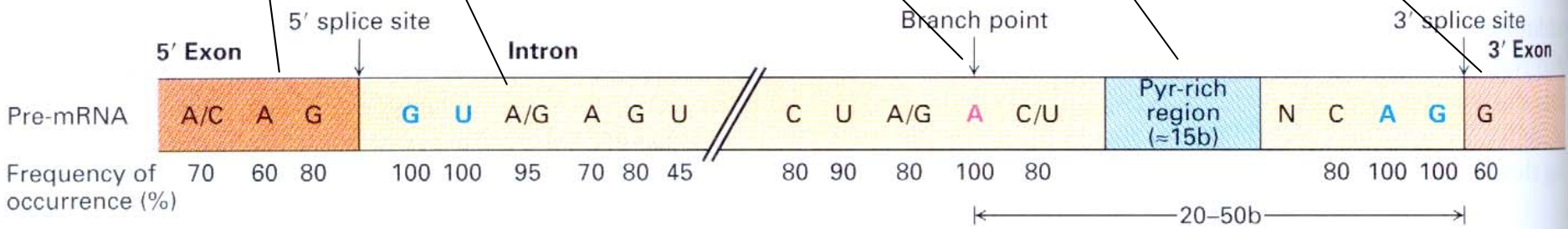
100 % AG

Intronit on merkitty poistamista varten

**GU-AG -intronien poiston suorittaa U12-ryhmä**






**▲ FIGURE 11-14 Consensus sequences around 5′ and 3′ splice sites in vertebrate pre-mRNAs.** The only nearly invariant bases are the (5′)GU and (3′)AG of the intron, although the flanking bases indicated are found at frequencies higher than expected based on a random distribution. A pyrimidine-rich region (light blue) near the 3′ end of the intron is found in most cases. The branch-point adenosine, also invariant, usually is 20–50 bases from the 3′ splice site. The central region of the intron, which may range from 40 bases to 50 kilobases in length, generally is unnecessary for splicing to occur. [See R. A. Padgett et al., 1986, *Ann. Rev. Biochem.* **55:**1119; E. B. Keller and W. A. Noon, 1984, *Proc. Nat'l. Acad. Sci. USA* **81:**7417.]

Eri kirjat näyttävät olevan samaa mieltä, vaikka eri väreillä

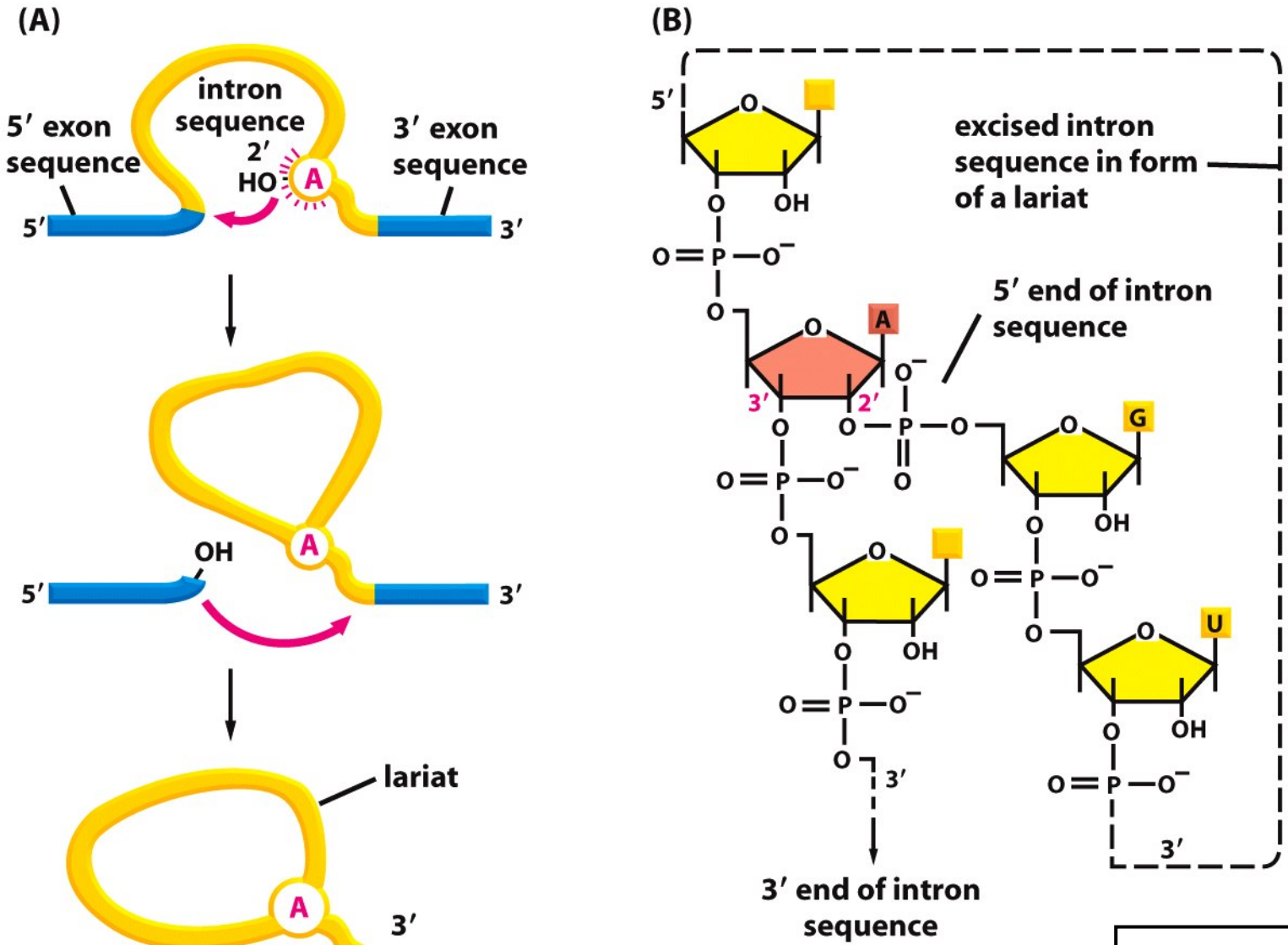



Figure 6-26 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Silputusreaktio

CELL 348

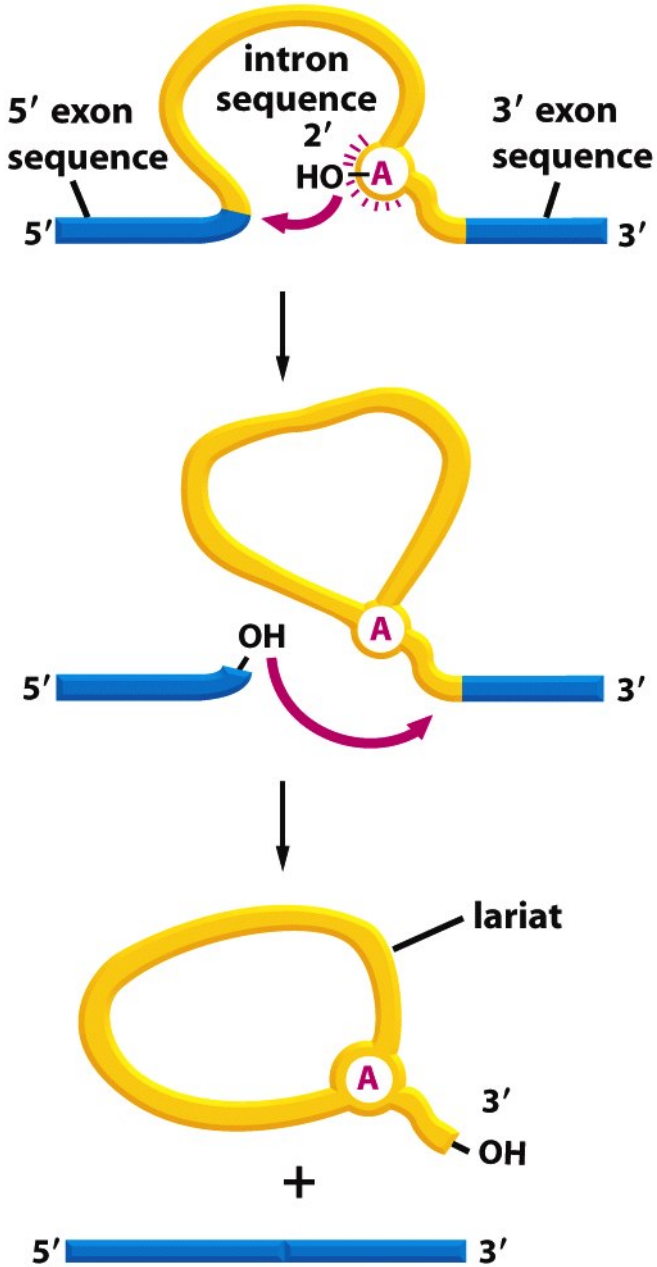



Figure 6-26a Molecular Biology of the Cell 5/e (© Garland Science 2008)

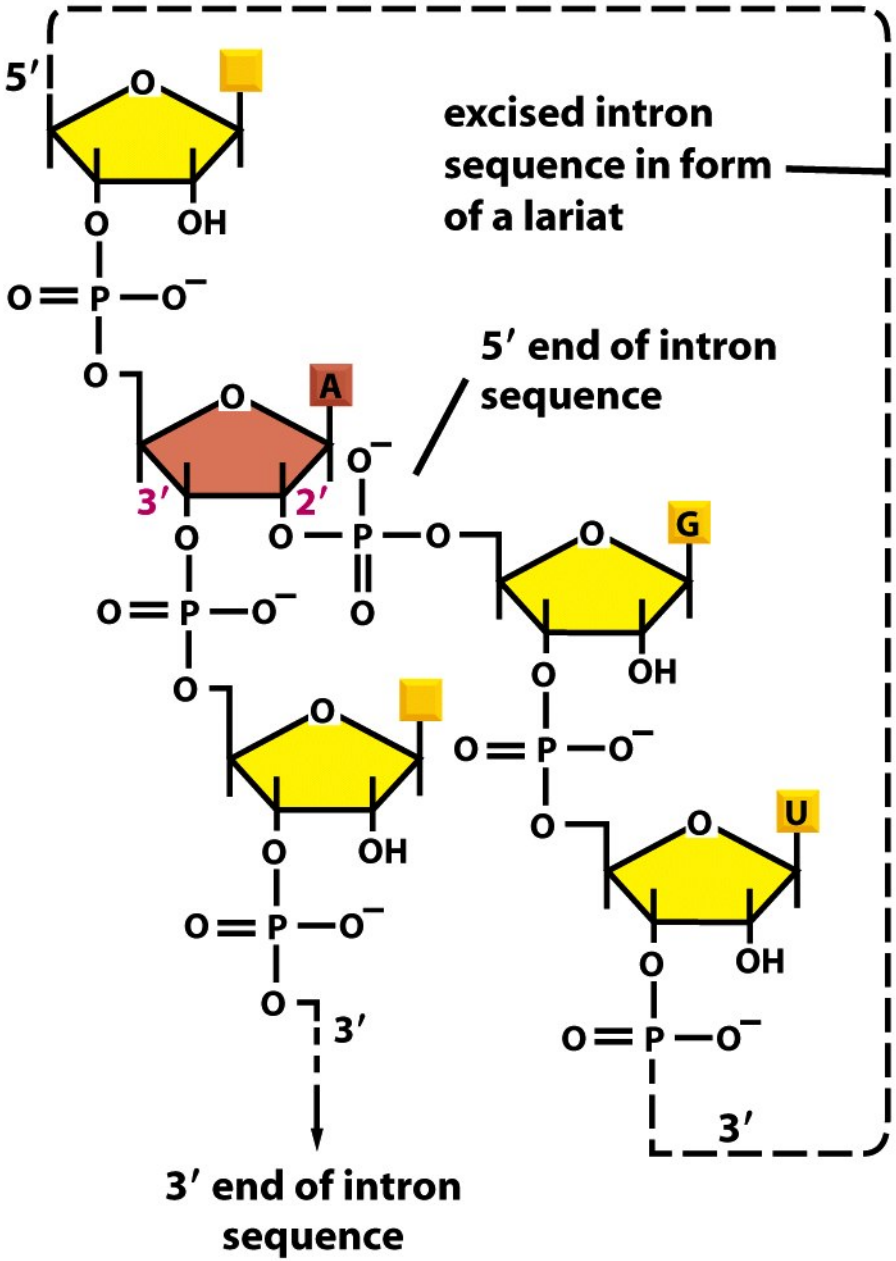
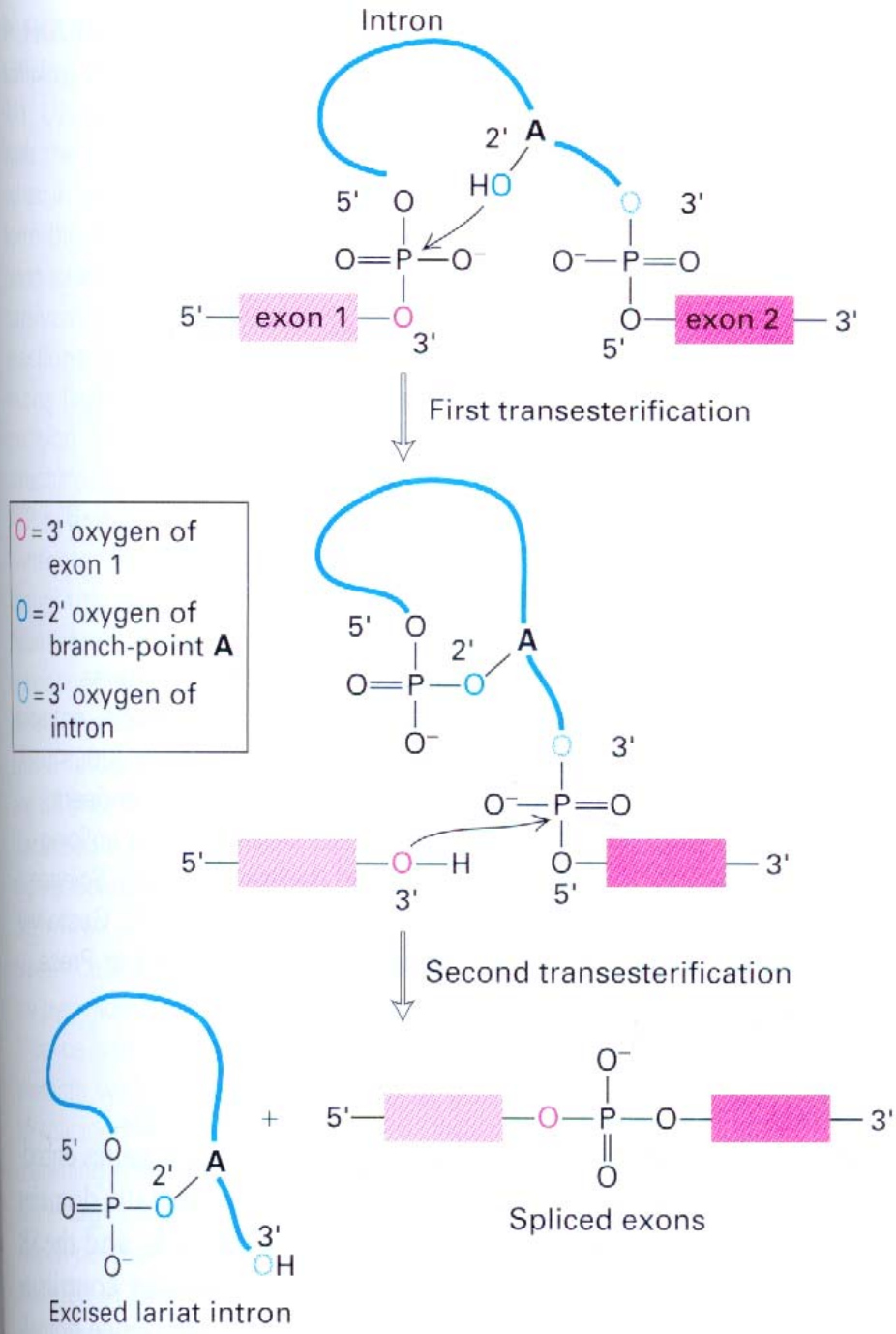
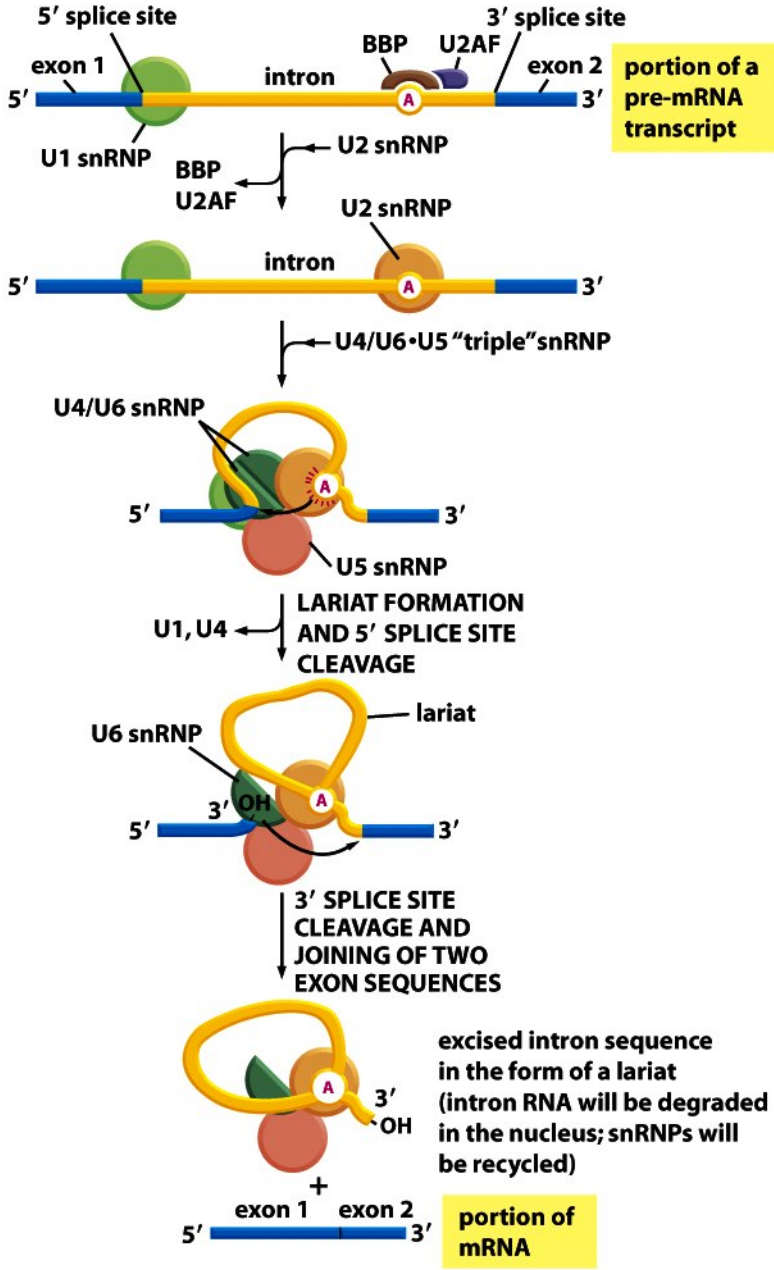



Figure 6-26b Molecular Biology of the Cell 5/e (© Garland Science 2008)


Intron
2' A
HO
5' O
O=P—O⁻
O⁻—P=O
3'
5'— exon 1 —O 3'
O— exon 2 —3'
5'
First transesterification
O = 3' oxygen of exon 1
O = 2' oxygen of branch-point A
O = 3' oxygen of intron
5' O
2' A
O=P—O
O⁻
3'
O⁻—P=O
5'— O—H
3'
O— —3'
5'
Second transesterification
5' O
2' A
O=P—O
O⁻
3'
OH
Excised lariat intron
+
O⁻
5'— —O—P—O— —3'
O
Spliced exons




The U1 snRNP forms base pairs with the 5′ splice junction (see Figure 6–30A) and the BBP (branch-point binding protein) and U2AF (U2 auxilliary factor) recognize the branch-point site.

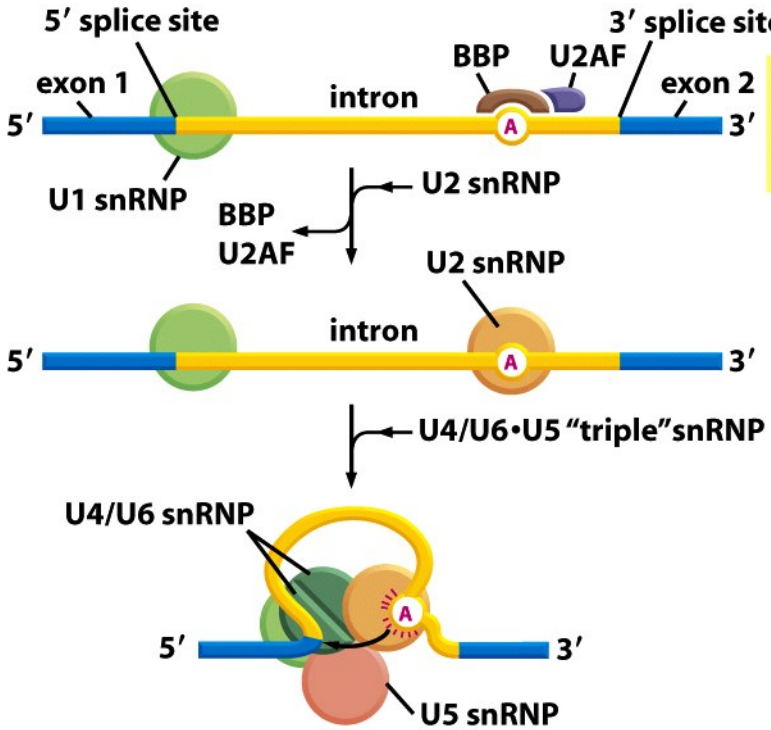
The U2 snRNP displaces BBP and U2AF and forms base pairs with the branch-point site consensus sequence (see Figure 6–30B).

The U4/U6•U5 "triple" snRNP enters the reaction. In this triple snRNP, the U4 and U6 snRNAs are held firmly together by base-pair interactions. Subsequent rearrangements create the active site of the spliceosome and position the appropriate portions of the pre-mRNA substrate for the first phosphoryl-transferase reaction.

Several more RNA–RNA rearrangements occur that break apart the U4/U6 base pairs and allow the U6 snRNP to displace U1 at the 5′ splice junction (see Figure 6–30A) to form the active site for the second phosphoryl-transferase reaction, which completes the splice.

Figure 6-29 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 350



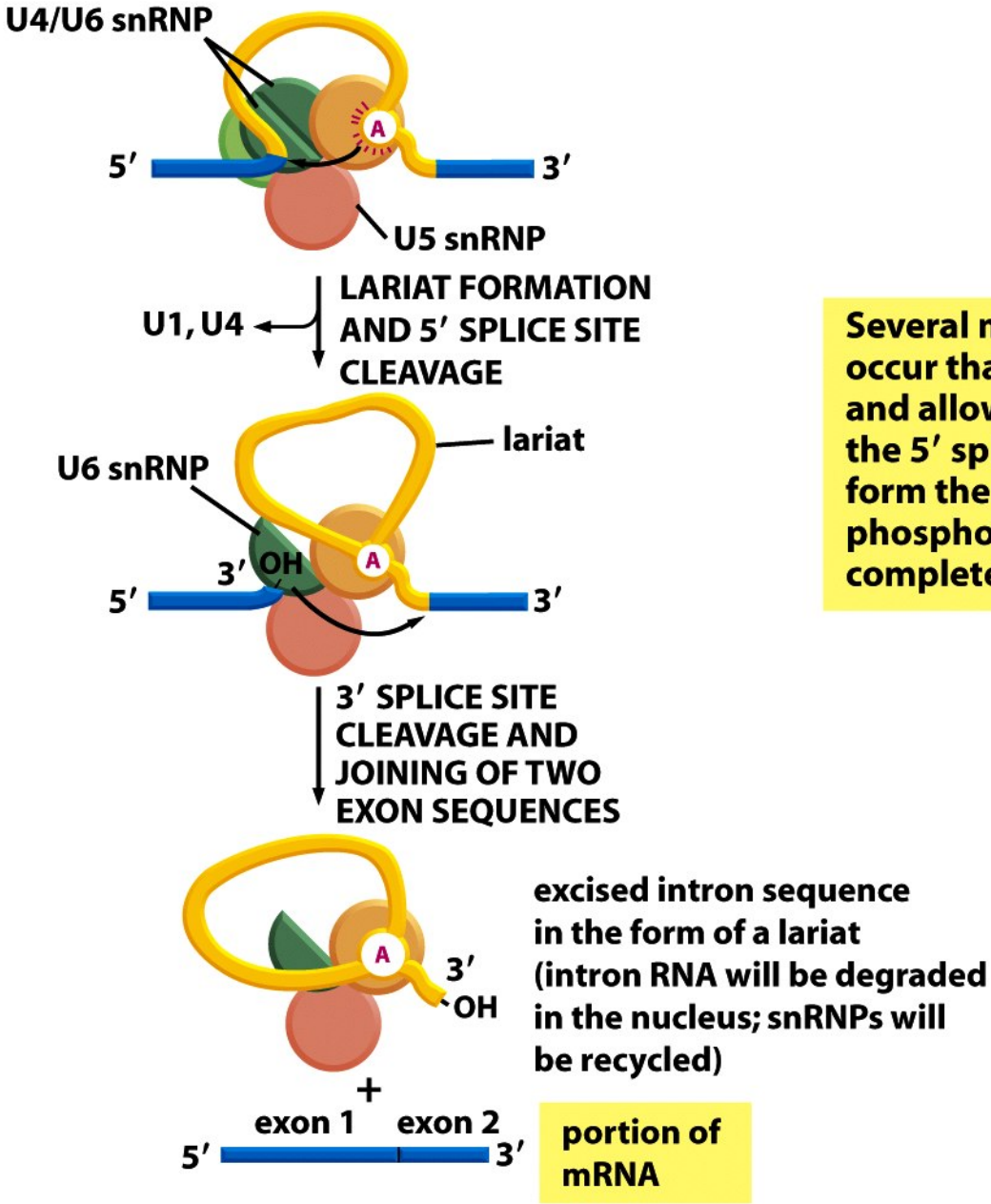

portion of a pre-mRNA transcript

The U1 snRNP forms base pairs with the 5′ splice junction (see Figure 6–30A) and the BBP (branch-point binding protein) and U2AF (U2 auxilliary factor) recognize the branch-point site.

The U2 snRNP displaces BBP and U2AF and forms base pairs with the branch-point site consensus sequence (see Figure 6–30B).

The U4/U6•U5 "triple" snRNP enters the reaction. In this triple snRNP, the U4 and U6 snRNAs are held firmly together by base-pair interactions. Subsequent rearrangements create the active site of the spliceosome and position the appropriate portions of the pre-mRNA substrate for the first phosphoryl-transferase reaction.

Figure 6-29 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)




Several more RNA–RNA rearrangements occur that break apart the U4/U6 base pairs and allow the U6 snRNP to displace U1 at the 5′ splice junction (see Figure 6–30A) to form the active site for the second phosphoryl-transferase reaction, which completes the splice.

Figure 6-29 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

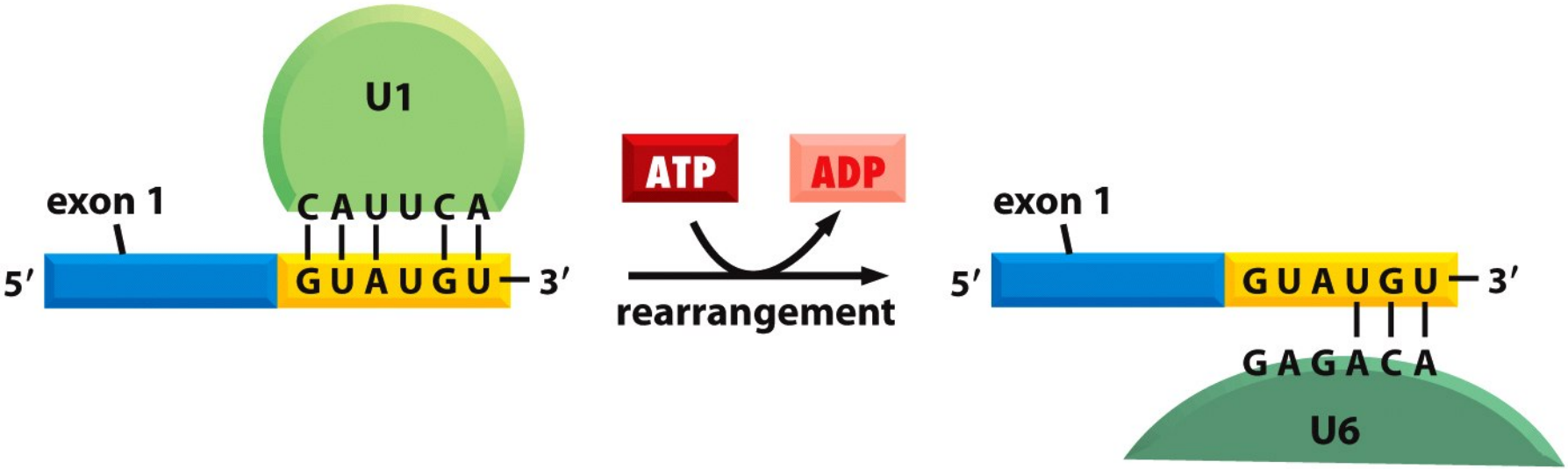



Figure 6-30a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Hiivan prosessia, jossa näytetään kuinka spliceosomien RNA tunnnistaa mRNA:n silputuskohdat.

CELL 351

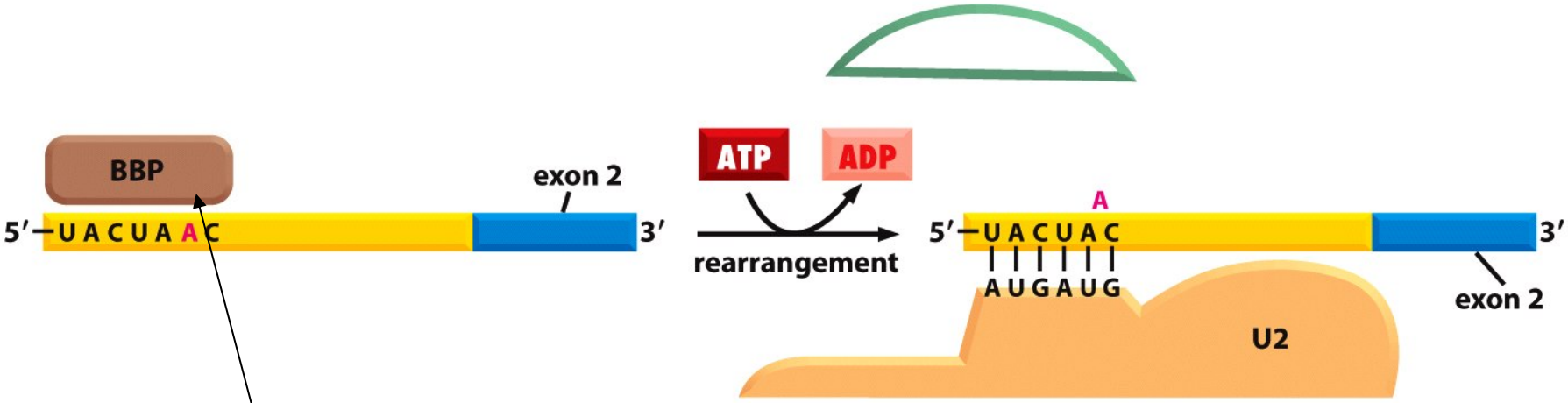



Figure 6-30b Molecular Biology of the Cell 5/e (© Garland Science 2008)

branch-point binding protein

CELL 351

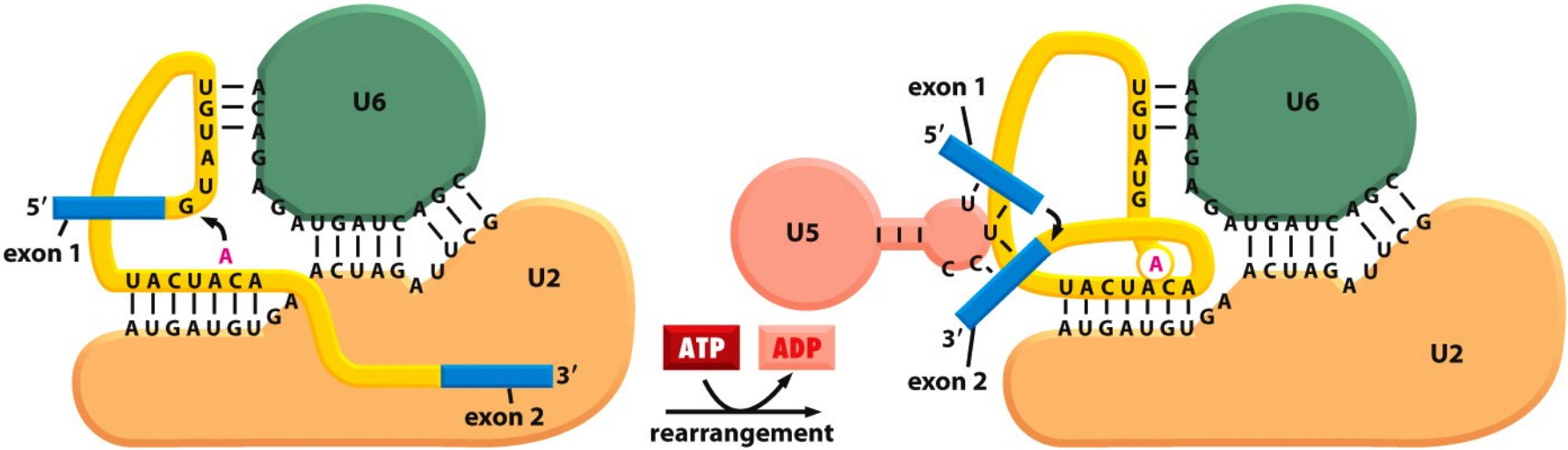
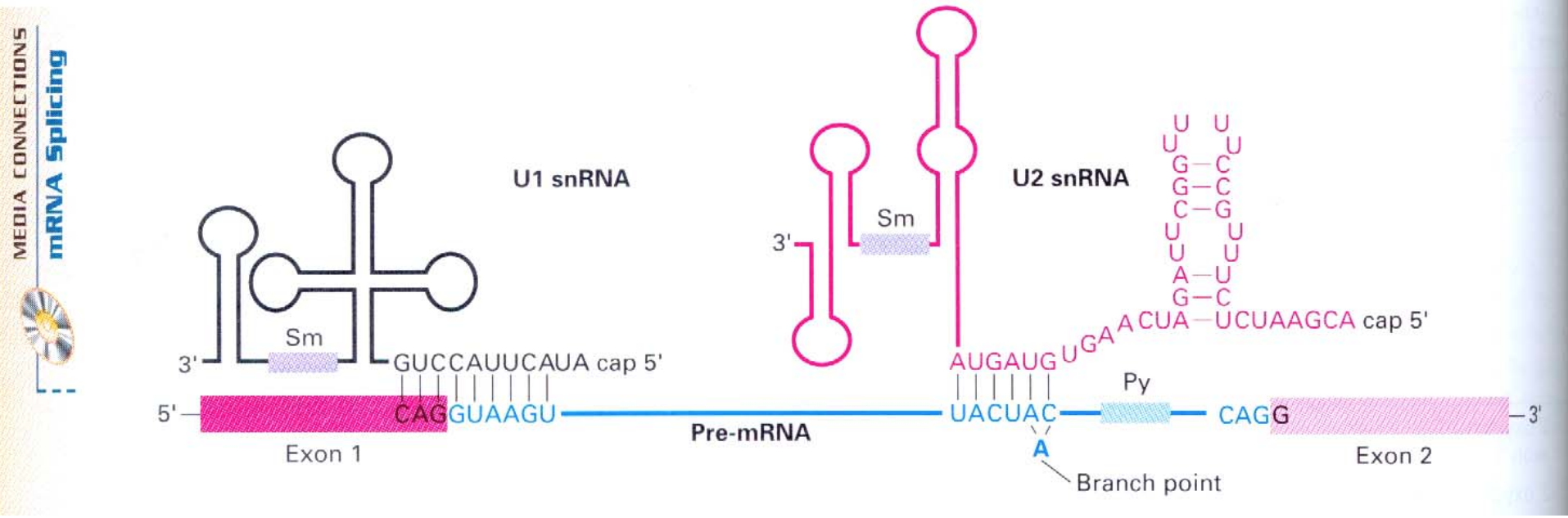


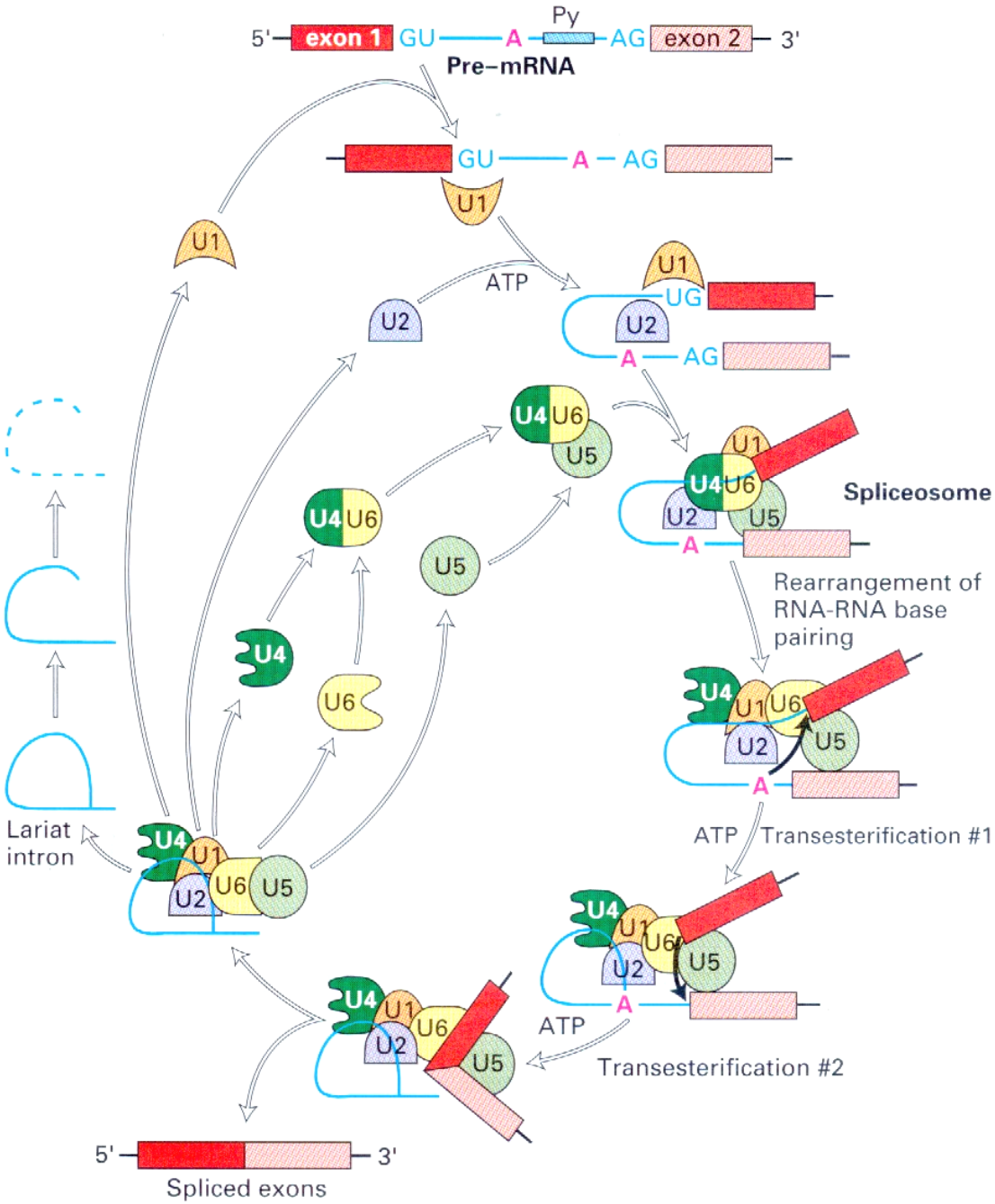

Figure 6-30c Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 351




Sama aihe, eri kirja. Pienet nukleaariset RNA:t **U1** ja **U2** tunnistavat intronin merkkikohdat muodostamalla kaksoisjuostetta eli hybridisoitumalla sen kanssa. Tässä proteiiniosia ei ole piirretty ollenkaan

CELL 351-


5'
exon 1
GU
A
Py
AG
exon 2
3'
Pre-mRNA
U1
U2
ATP
U4
U6
U5
Spliceosome
Rearrangement of RNA-RNA base pairing
Lariat intron
Transesterification #1
Transesterification #2
Spliced exons

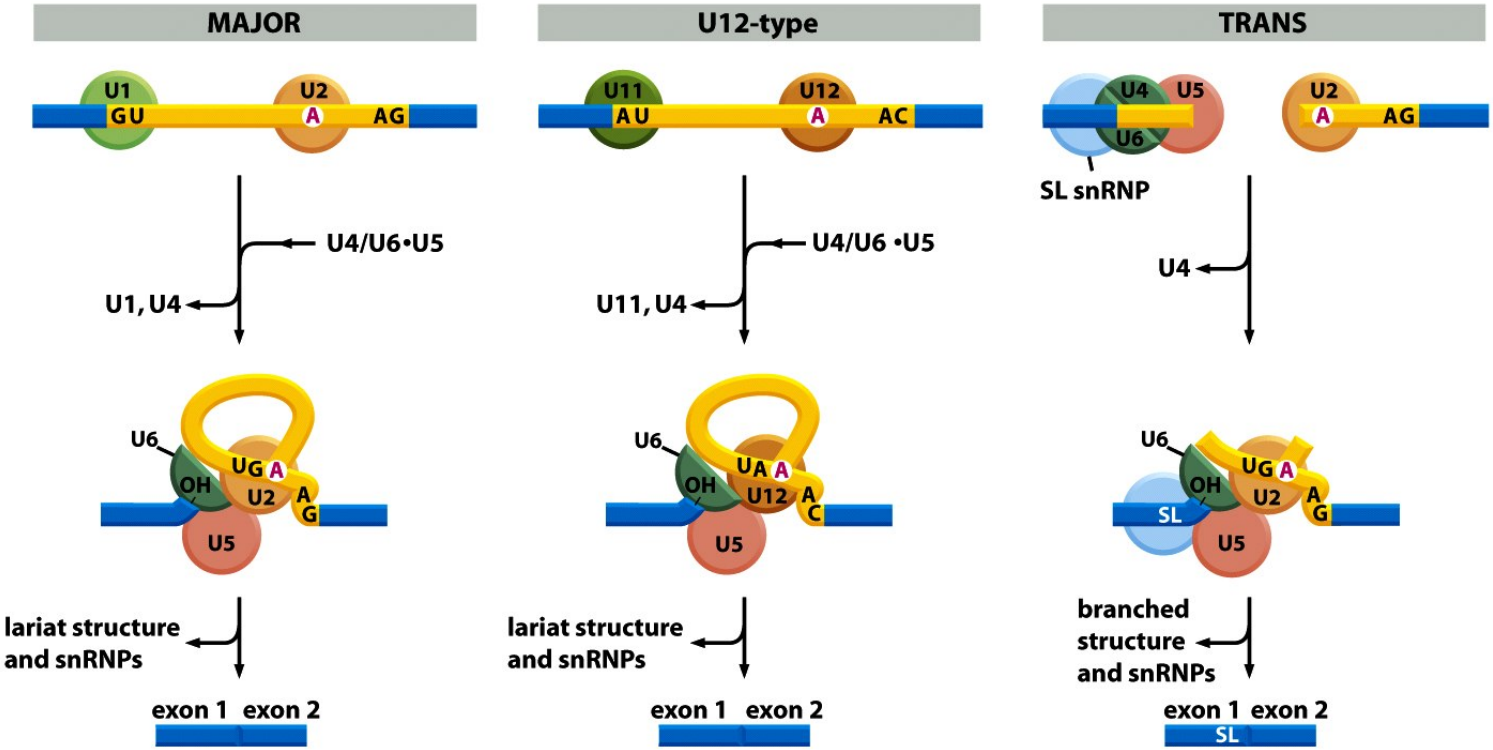



Figure 6-34a Molecular Biology of the Cell 5/e (© Garland Science 2008)

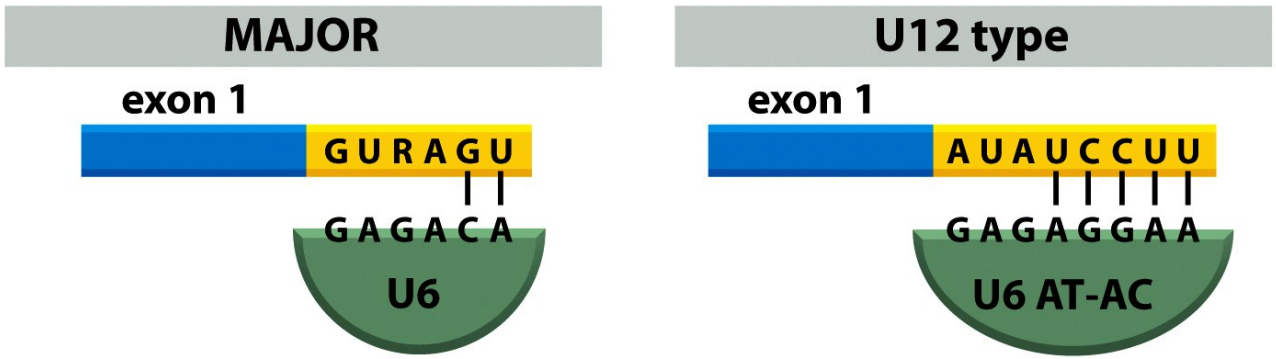
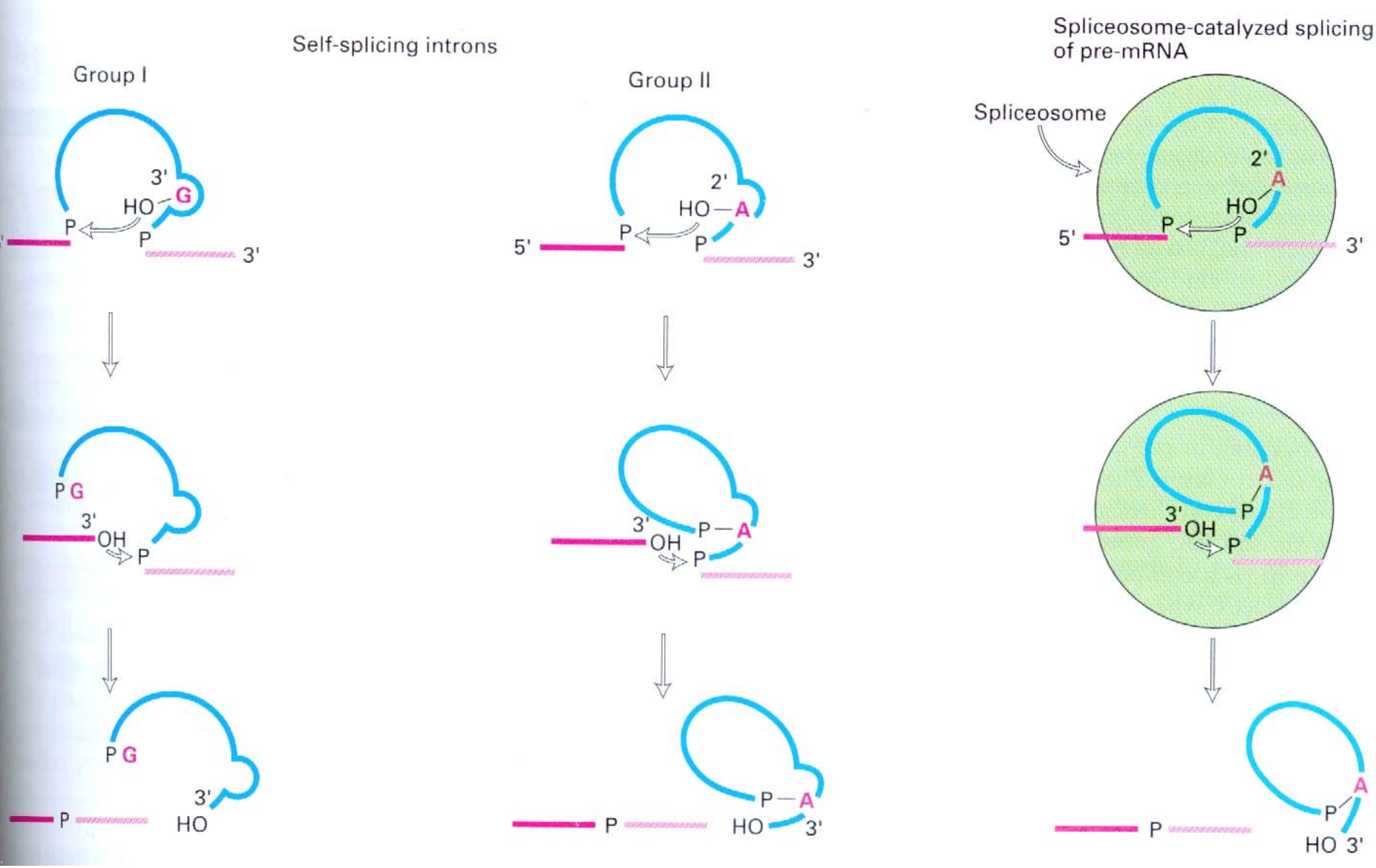



Figure 6-34b Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 354




On myös itsensä-silppuavia introneita

CELL 356

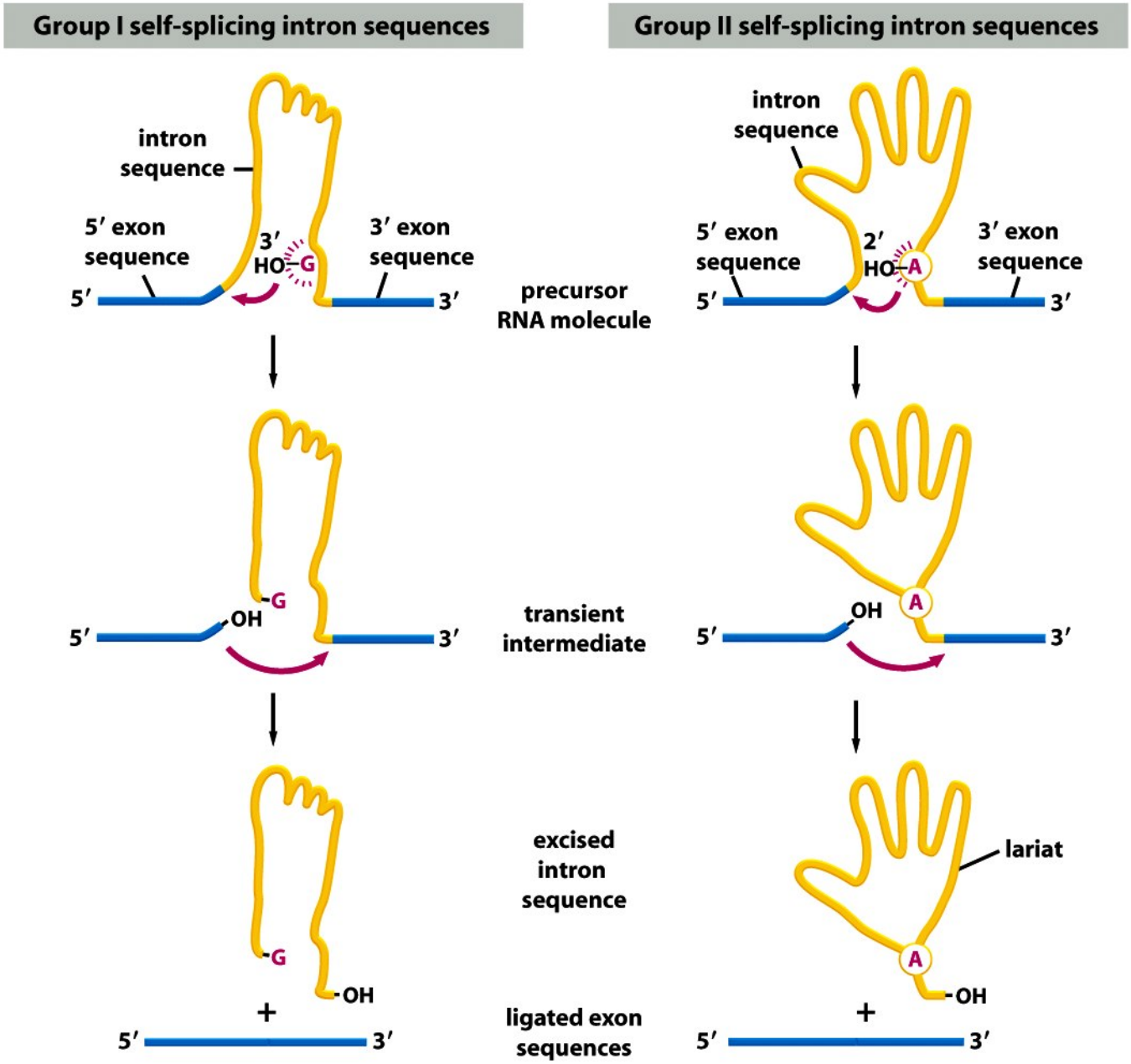
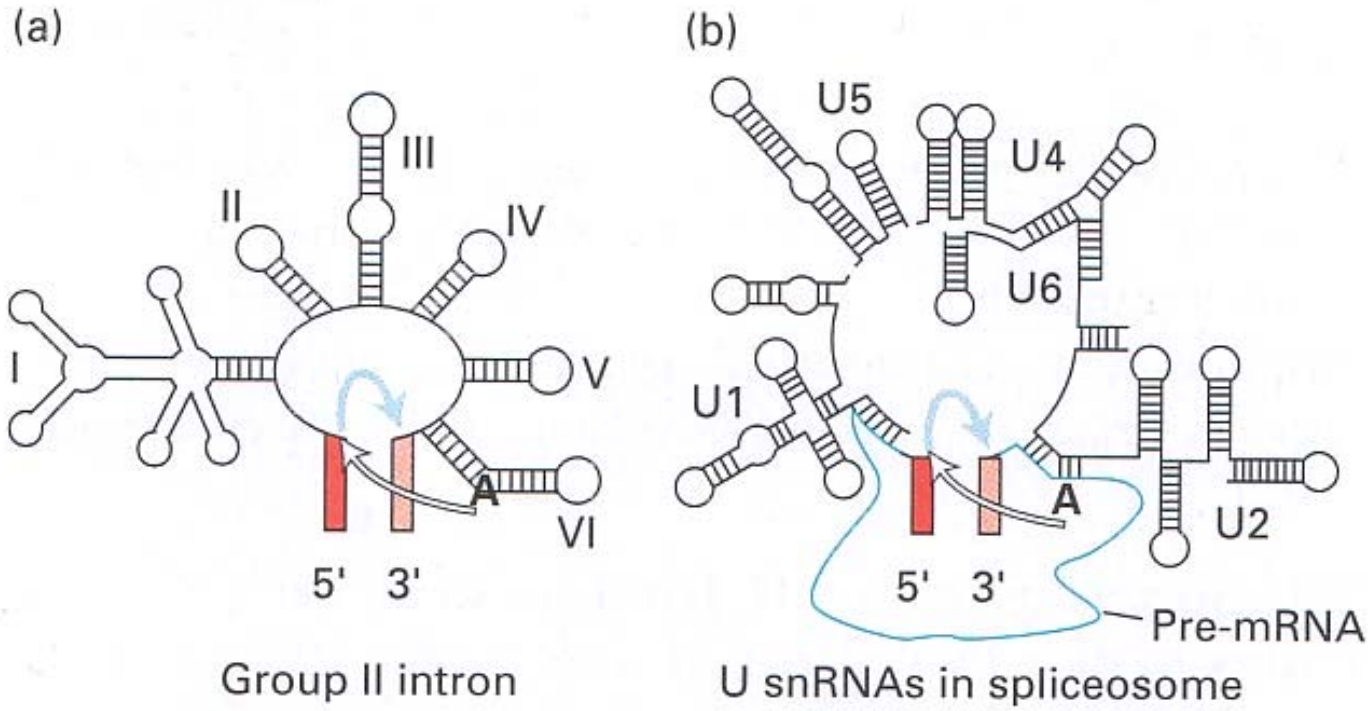



Figure 6-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 356




**▲ FIGURE 11-20 Schematic diagrams comparing the secondary structures of group II self-splicing introns (a) and U snRNAs present in the spliceosome (b).** The first transesterification reaction is indicated by black arrows; the second reaction, by blue arrows. The branch-point A is boldfaced. The similarity in these structures suggests that the spliceosomal snRNAs evolved from group II introns, with the trans-acting snRNAs being functionally analogous to the corresponding domains in group II introns. [Adapted from P. A. Sharp, 1991, *Science* **254**:663.]

# Silputuksen merkitys?

**Splicing Nature 2010**

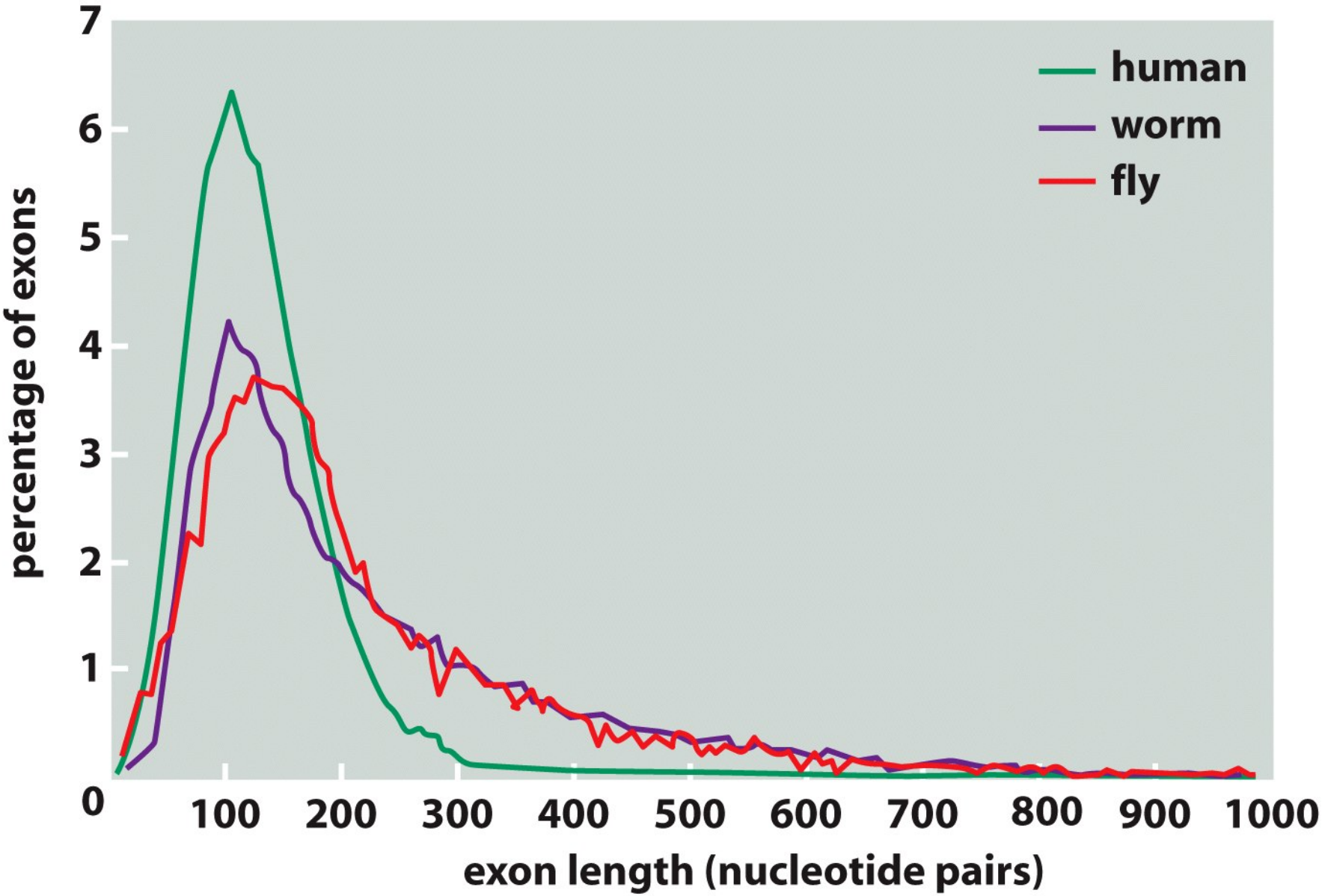



Figure 6-32a Molecular Biology of the Cell 5/e (© Garland Science 2008)

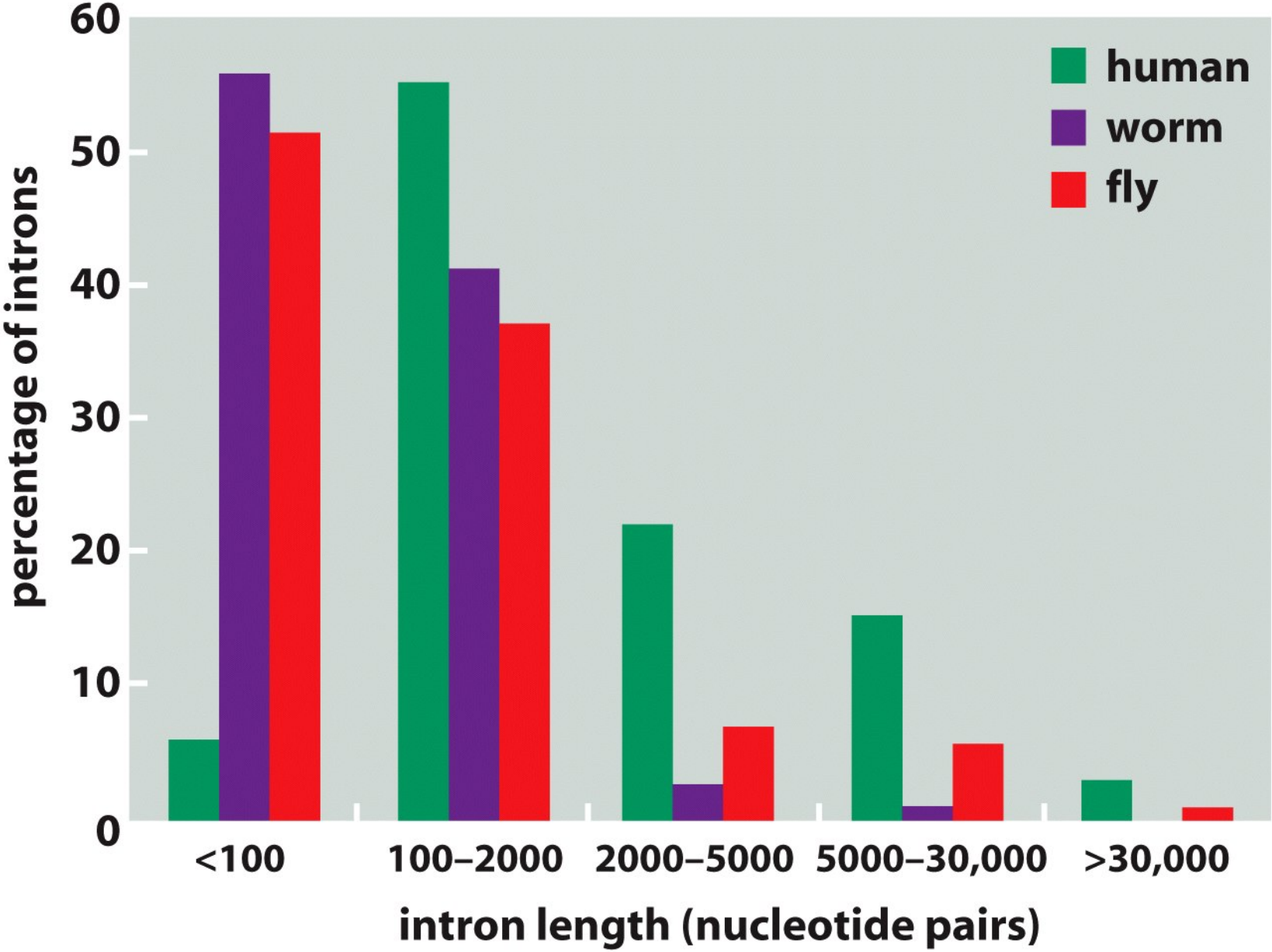



Figure 6-32b Molecular Biology of the Cell 5/e (© Garland Science 2008)

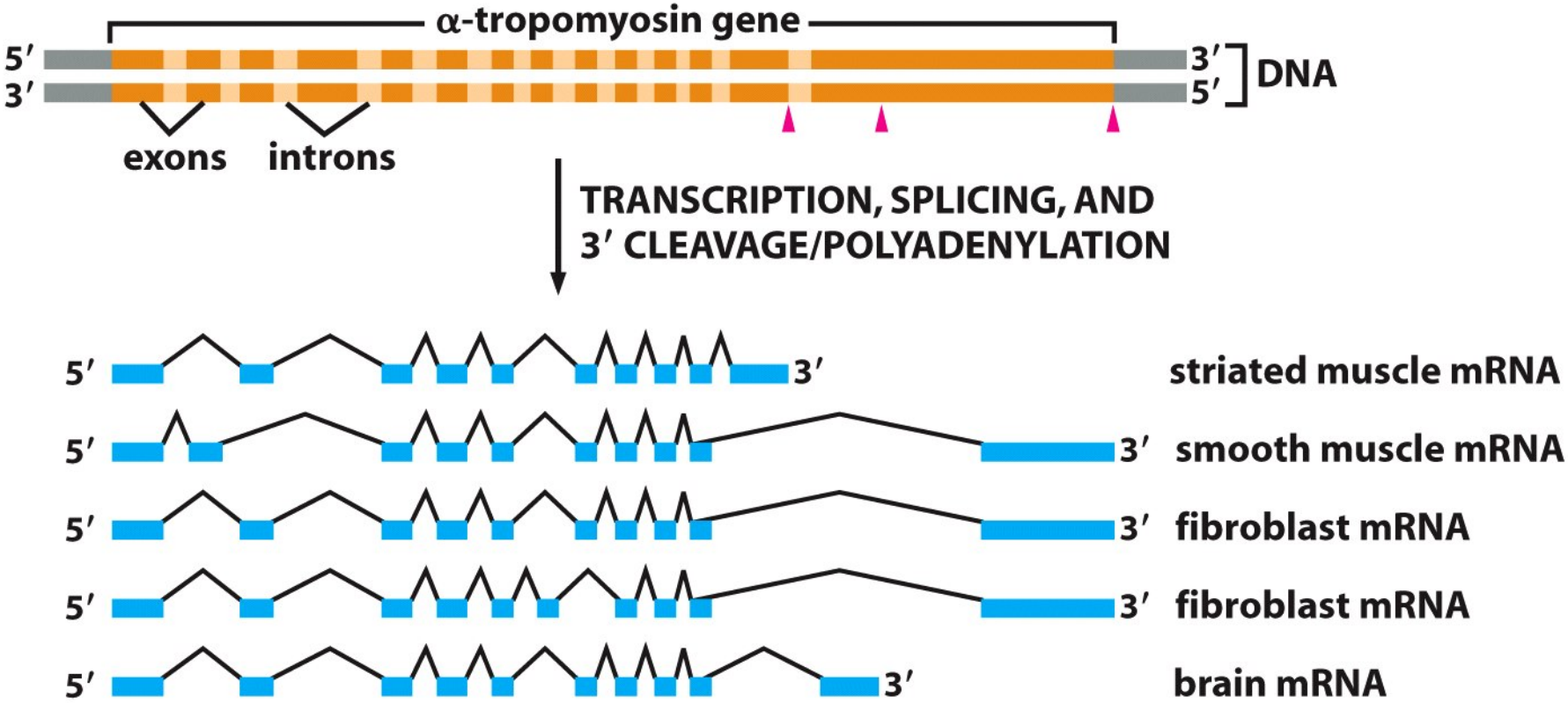



Figure 6-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Rotan tropomyosiinigeenin tuotteita saadaan *alternative splicingilla* eli vaihtoehtoisella silputuksella

CELL 349

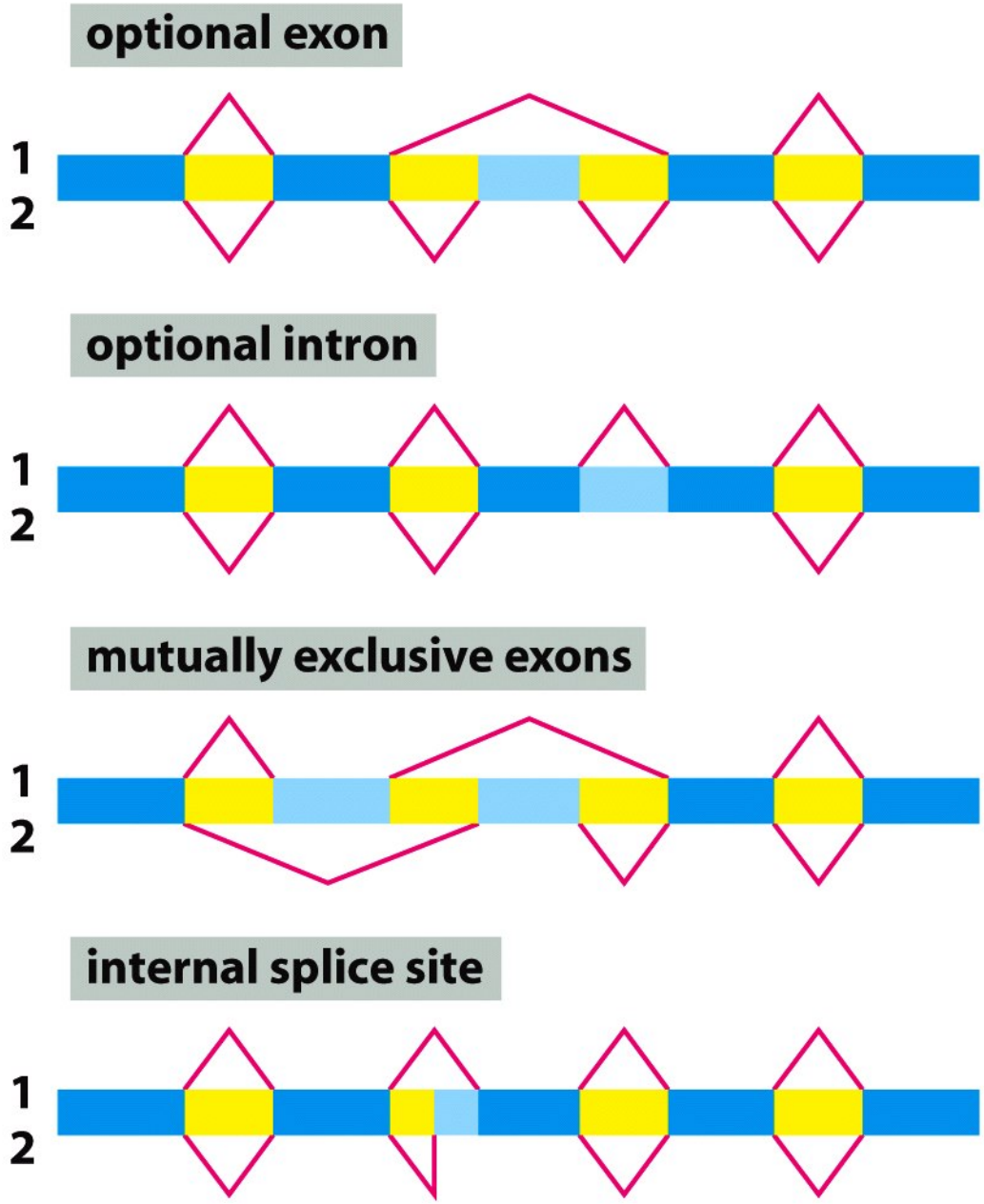



Figure 7-94 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 479

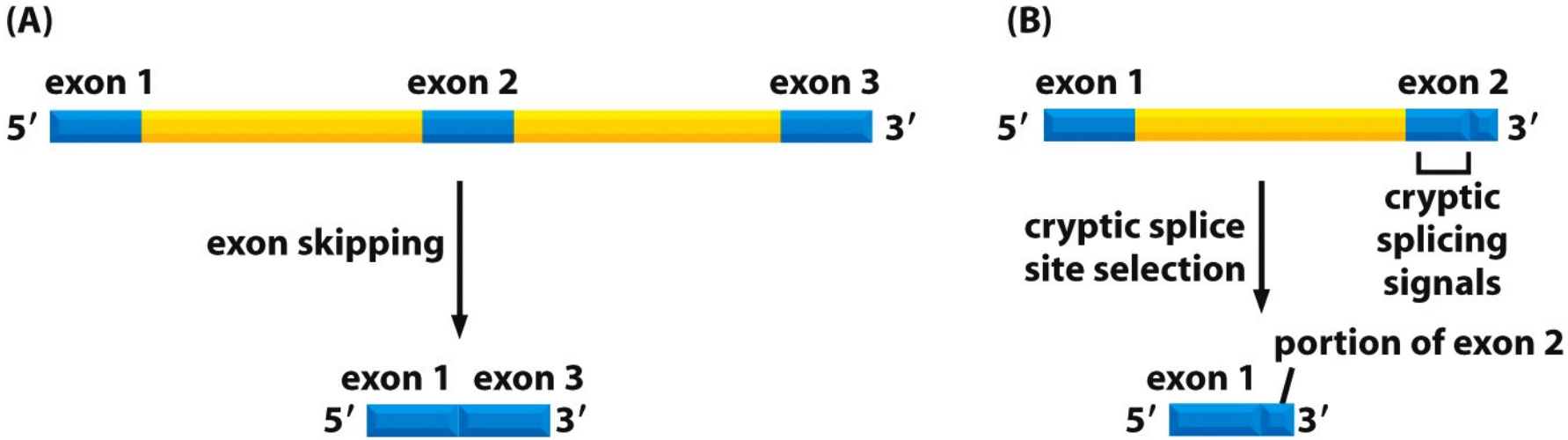



Figure 6-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Silputusvirheitä: (A) exon skippping ja (B) cryptic splice site selection

CELL 352

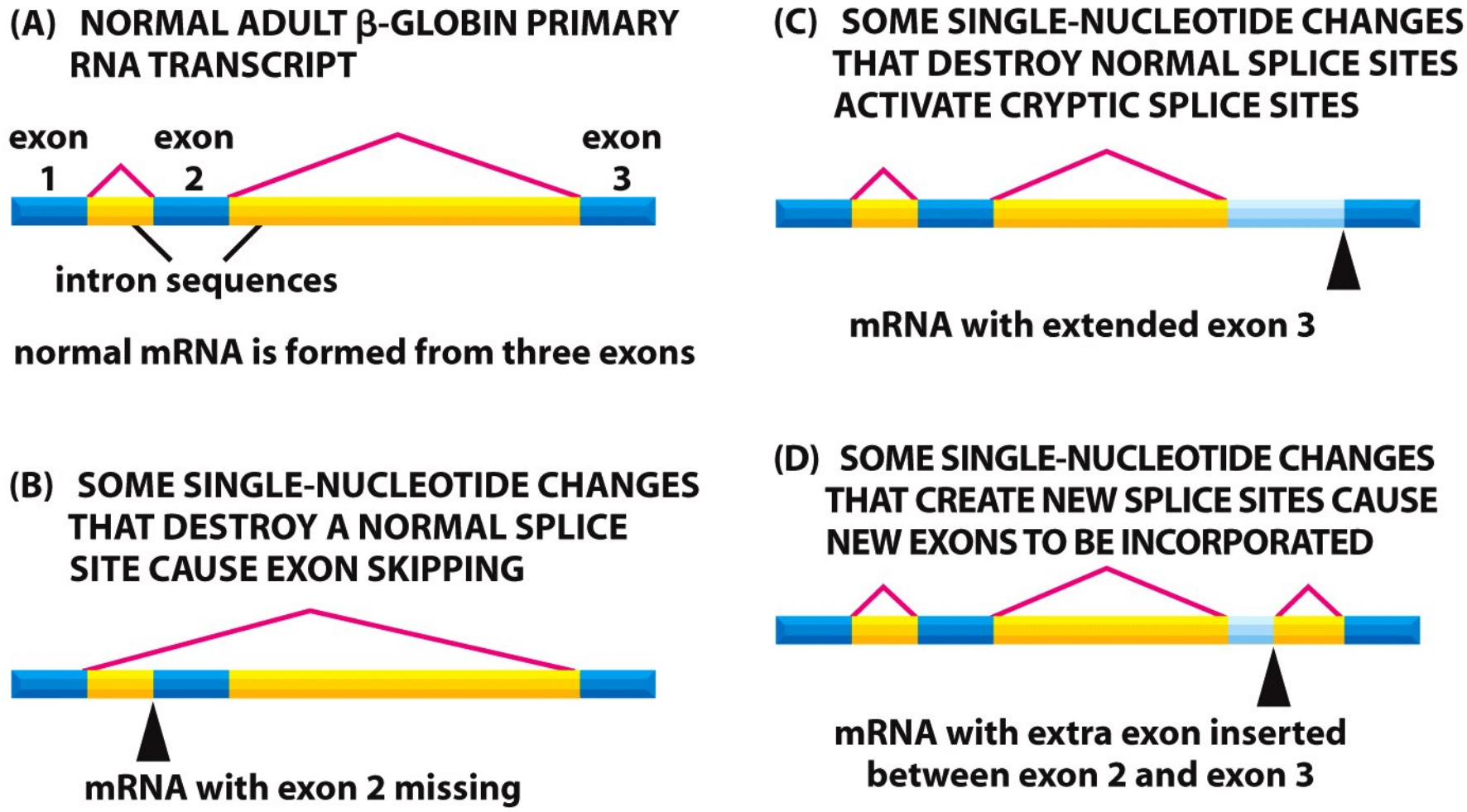



Figure 6-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Beta-thalassemia, ihmisen sairaus: yhden geenin splicing-erehdyksiä, jotka johtuvat pistemutaatioista

CELL 355

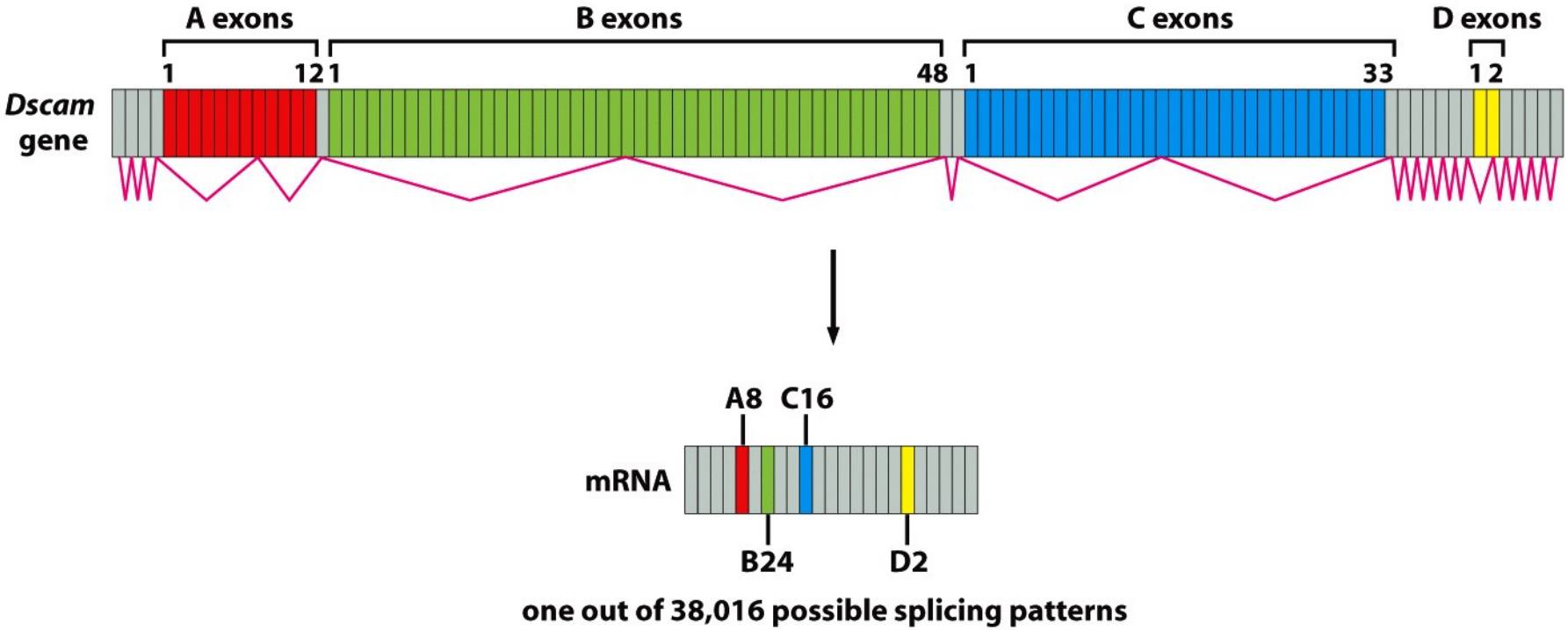



Figure 7-95 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 483

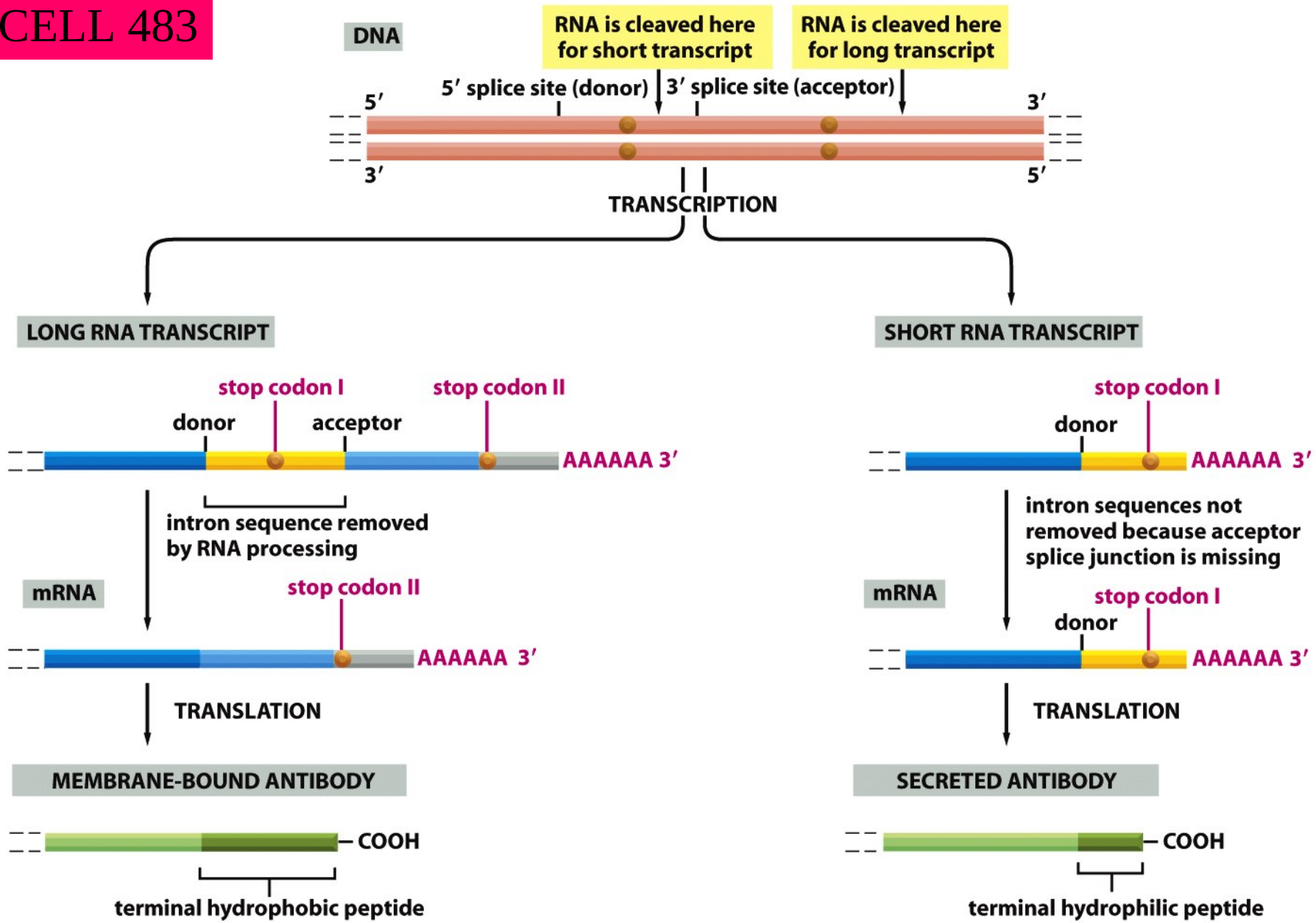



Figure 7-99 Molecular Biology of the Cell 5/e (© Garland Science 2008)

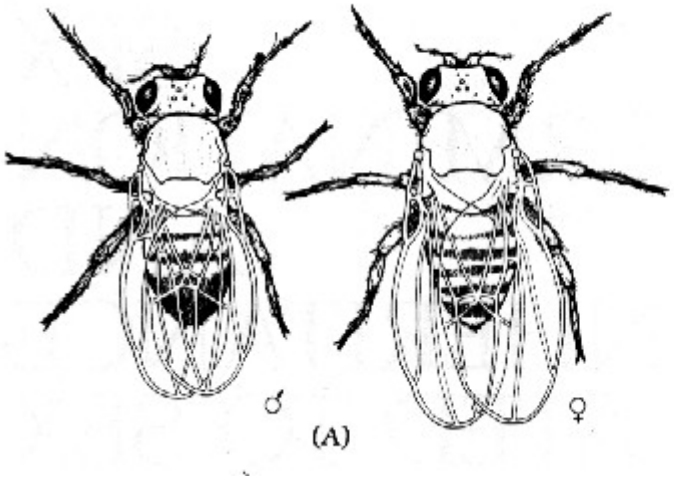
Kaikista **järkyttävin** esimerkki vaihtoehtoisesta silputuksesta on *Drosophilan* sukupuolenmääräytymisestä.

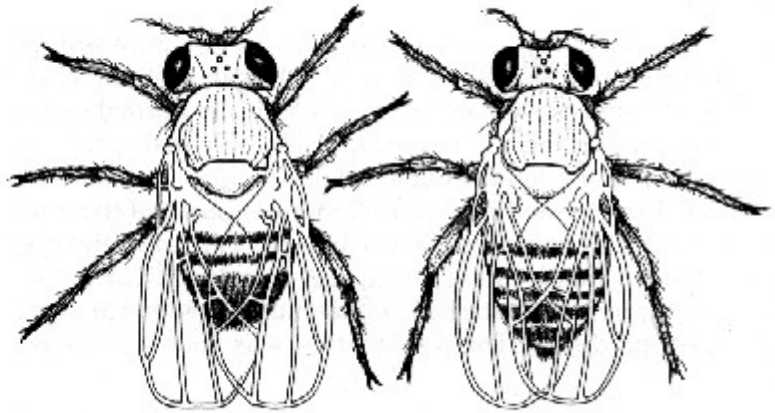
Ennen riitti, kun tiesi, että sukupuolen määrää autosomien ja X-kromosomi lukumääräinen suhde

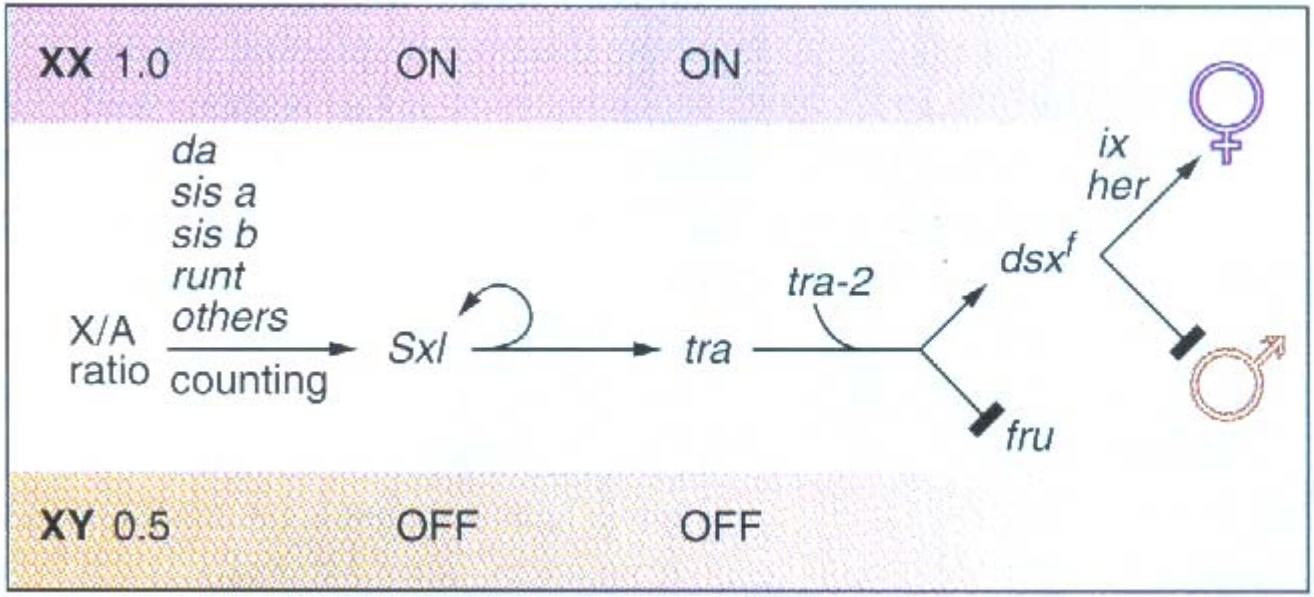
Katsotaanpa, riittääkö tänään enää mikään

MCB 423-425

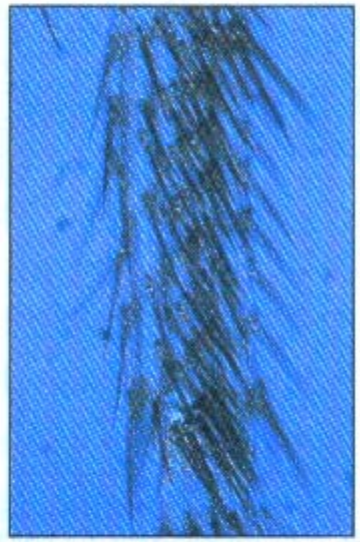
Bridges sekoitteli kromosomeja ja sai selville, että sukupuolen määrää X-kromosomien ja autosomiannosten lukusuhde: XX:AA = 1 > naaras, XY:AA = 0.5 > koiras.  Tämä tieto oli valmis 1921.






**How flies do it.** In females, activation of *Sxl* leads to production of a feminizing Dsx variant. Males, without active *Sxl*, make a different variant. Flies overexpressing the male *Dsx* have male sex combs along the entire leg *(right)*. A normal female leg is at far right.

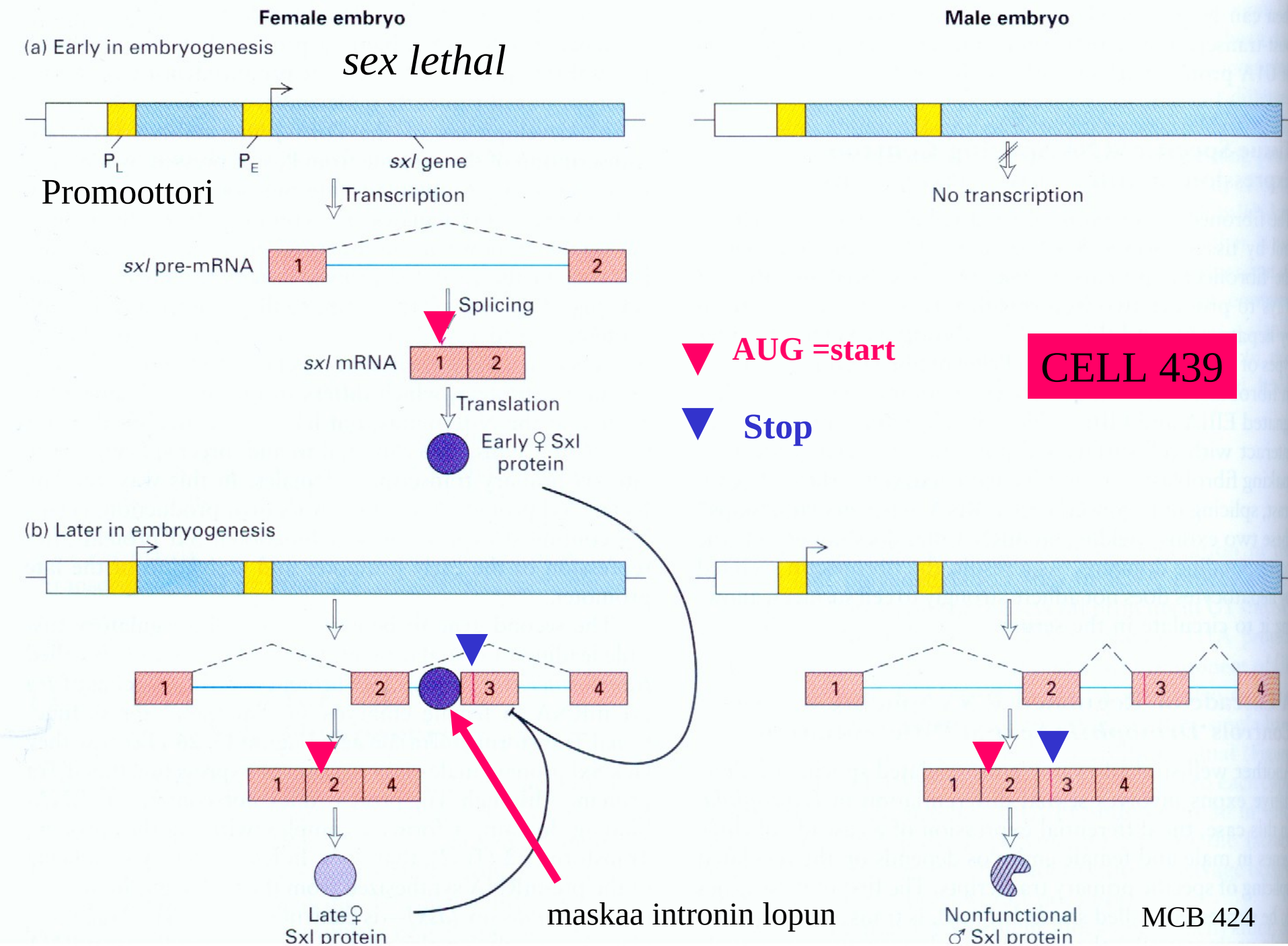


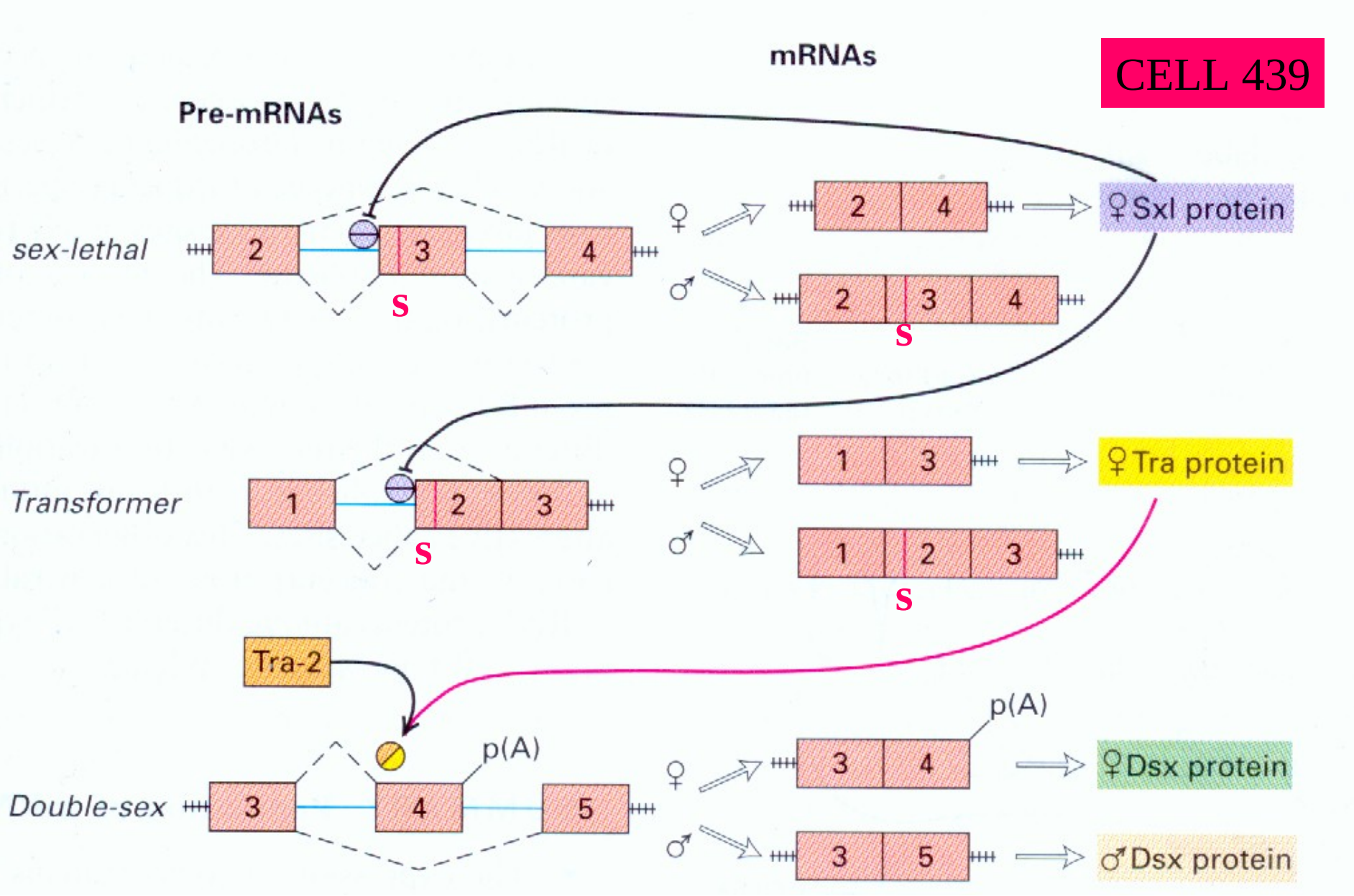
...netimes goes to each other, let alone ...etic males to to those in mammals. ...ely feminized "What's mind-boggling is ...on male char- how few real similarities

...mination. He fou... dying females wer... males. By itself, t... work by Cline's ... Lucchesi of Atl... among others, s... named *Sex-lethal*,

supposed to damp... genes that increa... sion in males. Tha... activate *Sxl* kill fe...

MCB 424-425


Female embryo
Male embryo
(a) Early in embryogenesis
sex lethal
$P_L$
$P_E$
sxl gene
Promoottori
Transcription
No transcription
sxl pre-mRNA
1
2
Splicing
sxl mRNA
1
2
Translation
Early ♀ Sxl protein
AUG =start
Stop
CELL 439
(b) Later in embryogenesis
1
2
3
4
1
2
4
Late ♀ Sxl protein
maskaa intronin lopun
1
2
3
4
1
2
3
4
Nonfunctional ♂ Sxl protein
MCB 424




Reguloitua splicingia edelleen, ja lopulta saadaan aikaan koiras ja naaras (kärpänen) MCB 425

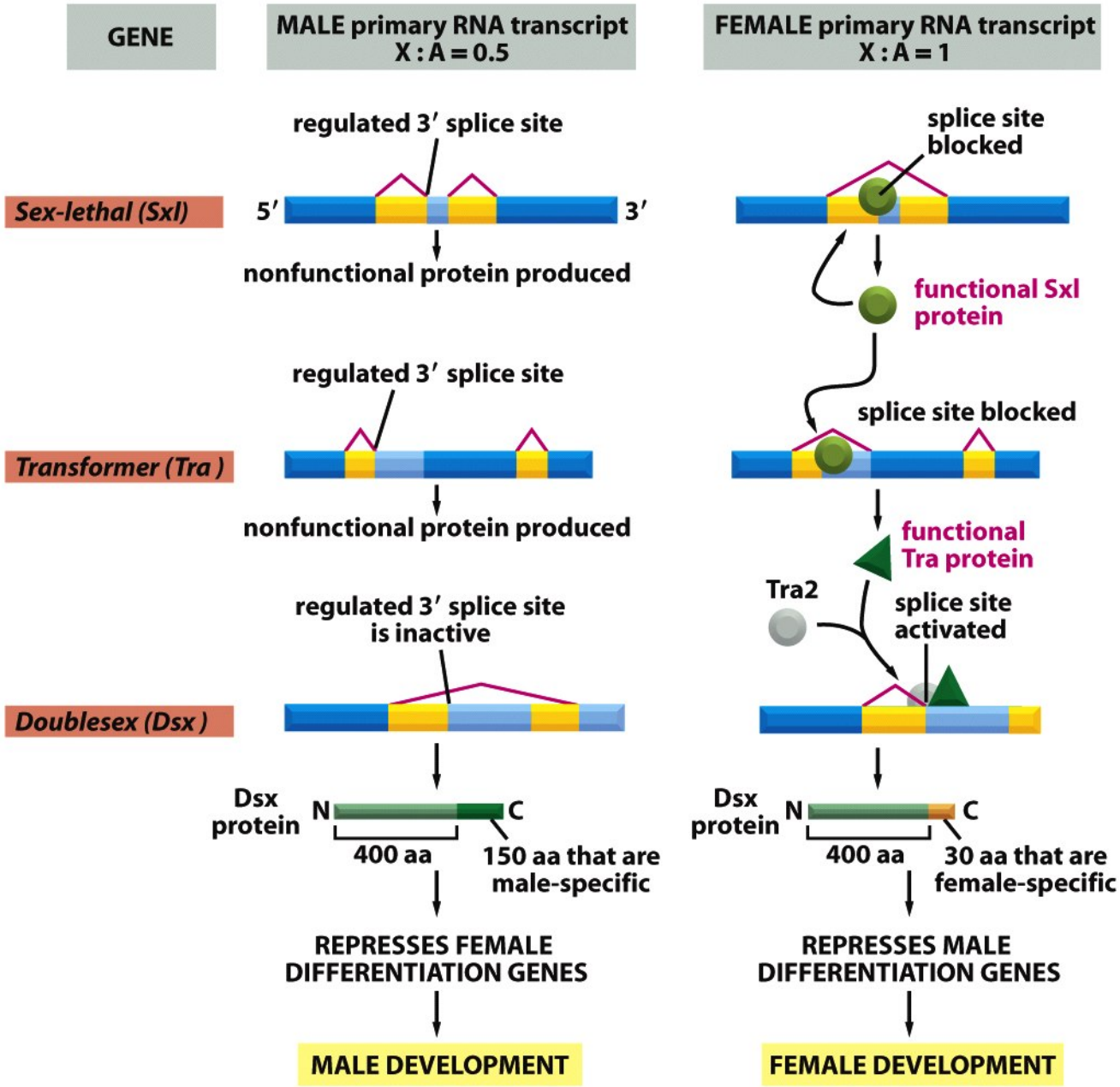
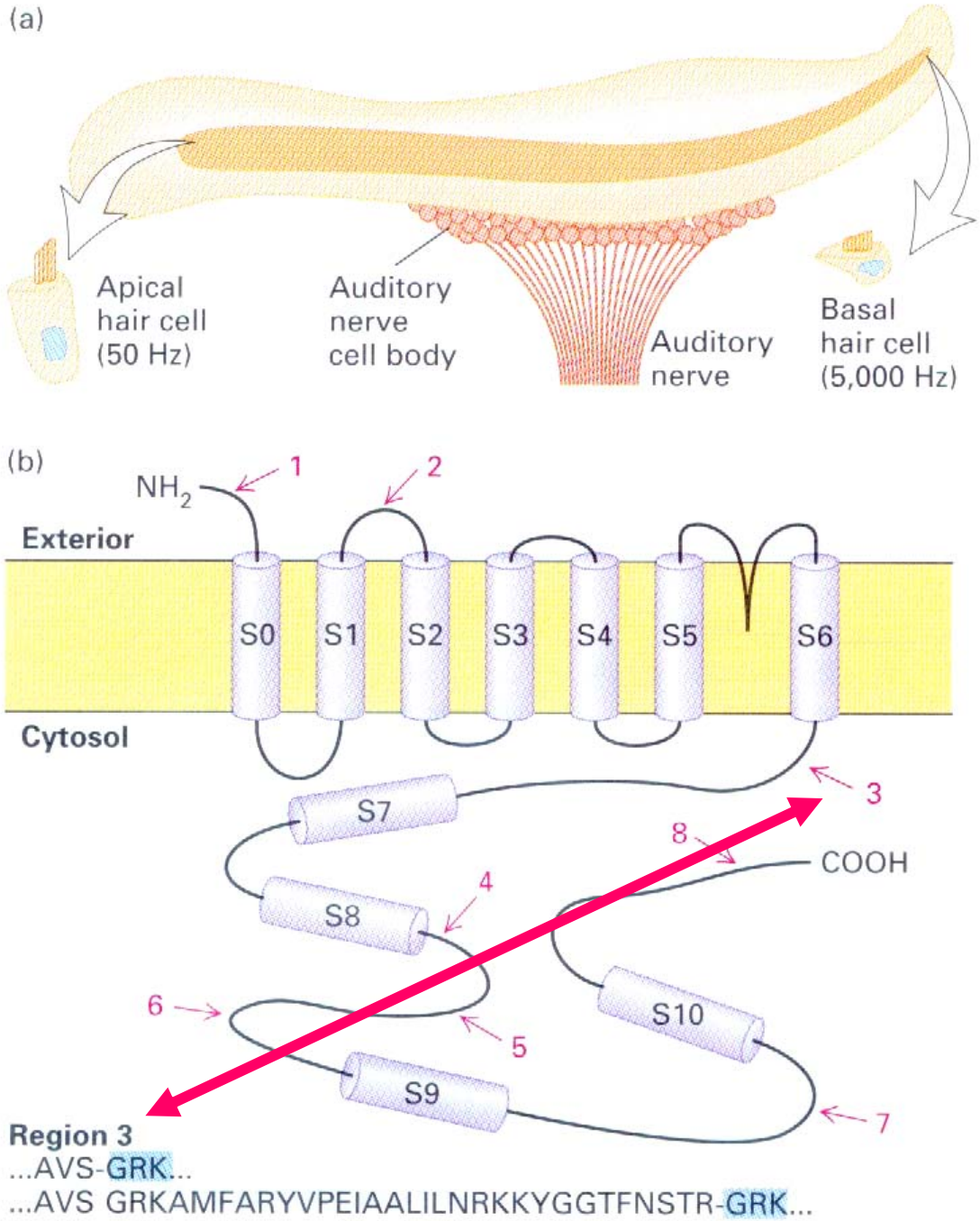



Figure 7-98 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 482




*slo* mRNA:n vaihtoehtoinen splicing virittää korvan!!!!

Transmembraani-proteiineista tehdään erikokoisia versioita korkeitten ja matalien värähtelyjen asteikon havaitsemiseen

Punaisilla numeroilla merkityissä paikoissa on splicing-vaihtoehtoja

MCB/6 340

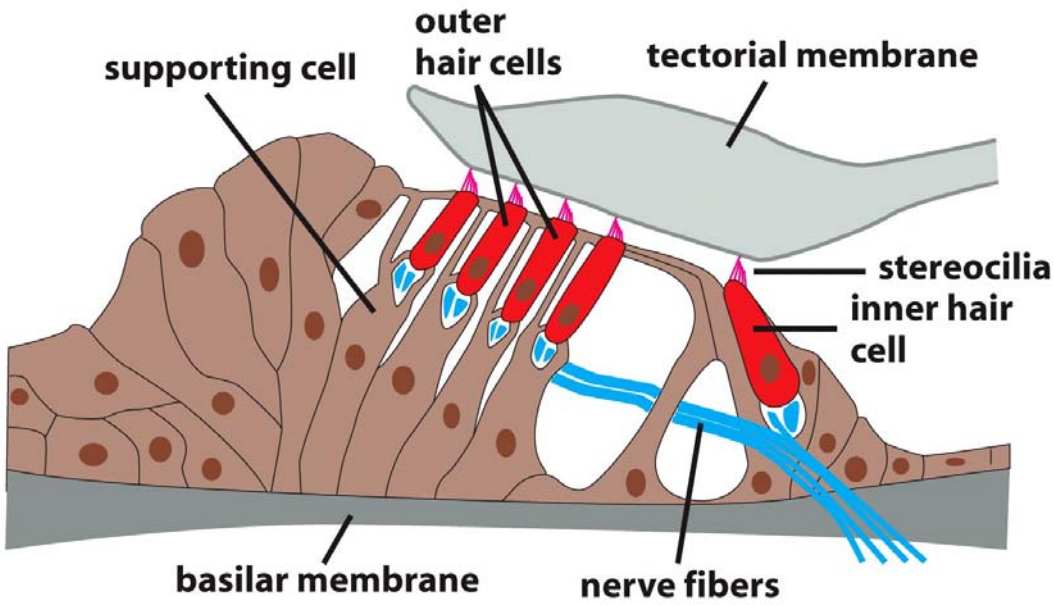



Figure 23-13a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Kuuloaistimen rakenne ja toiminta CELLin kappaleessa 23

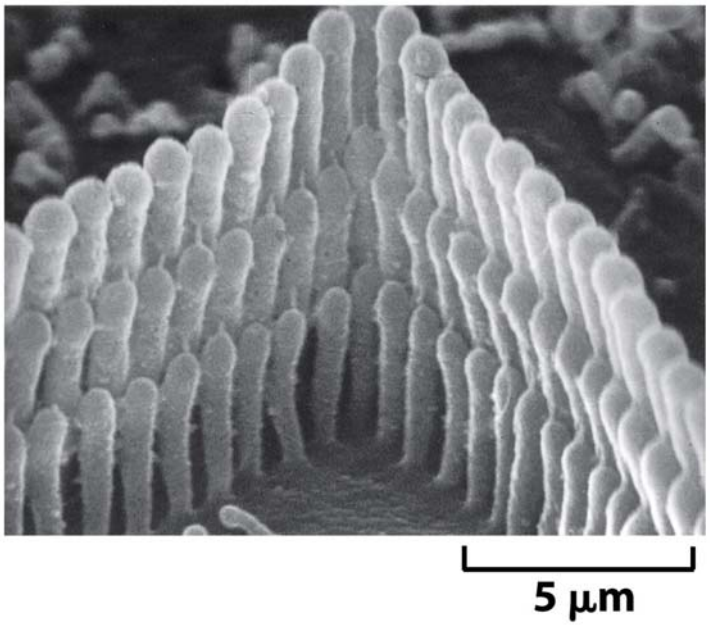



Figure 23-13b Molecular Biology of the Cell 5/e (© Garland Science 2008)

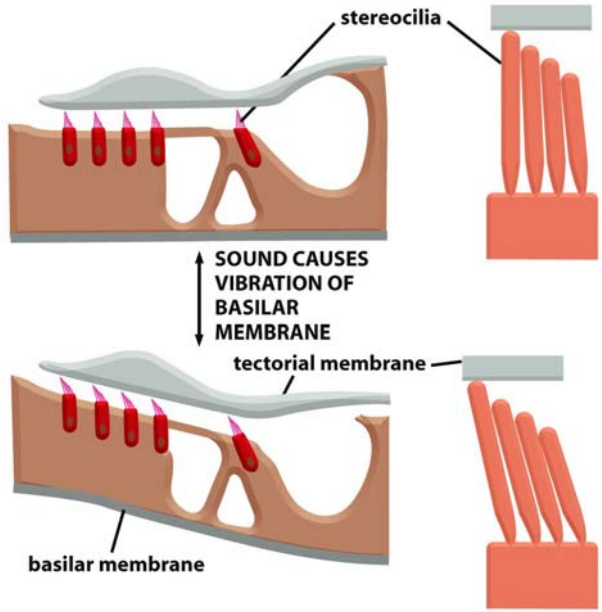



Figure 23-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Roberts

Sharp

Split genes nobeloitiin 1993

http://www.nobel.se/medicine/laureates/1993/index.html

Voit myös käydä katsomassa pitemmän elokuvan Cold Spring Harbor Laboratoriossa : konsepti 24

**1.1% genomin DNA:sta eksoneissa, 24 % introneissa**

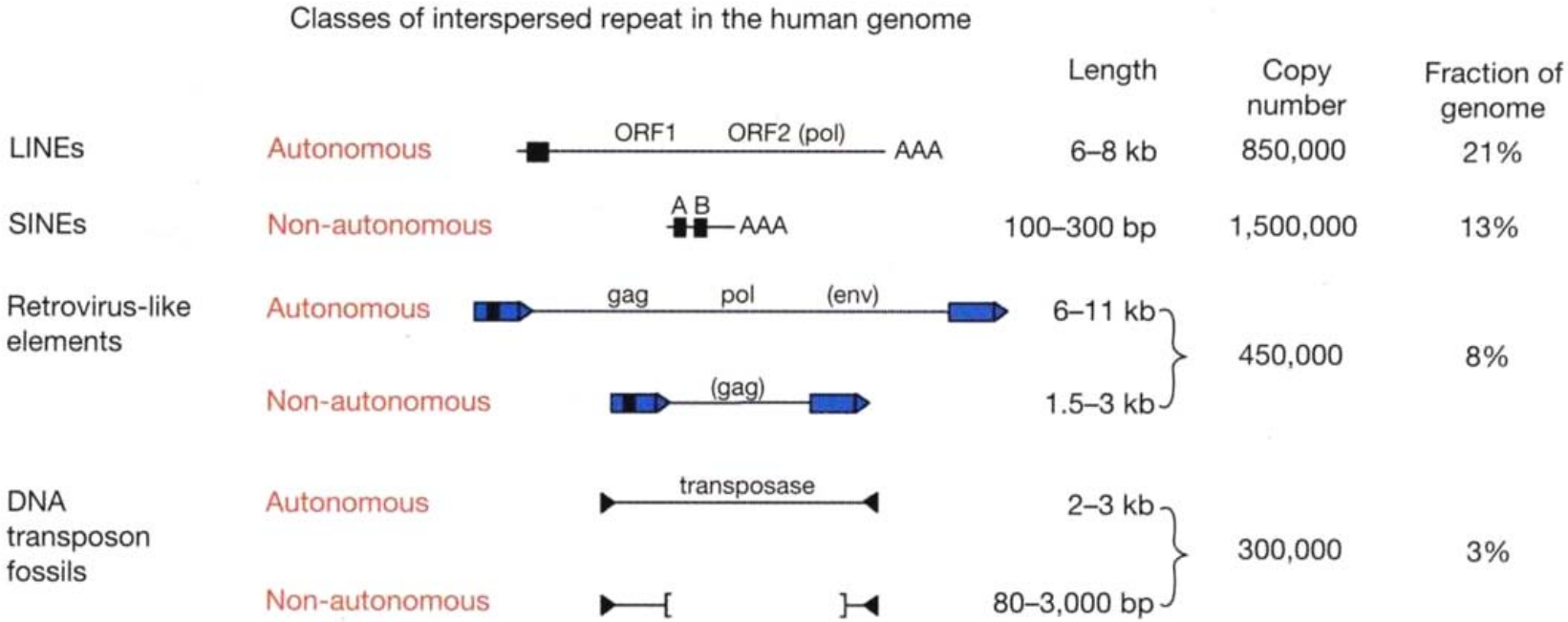



**Figure 17** Almost all transposable elements in mammals fall into one of four classes. See text for details.

**1.1% genomin DNA:sta eksoneissa, 24 % intronеissa, ja lopusta osa näissä turhissa? toistoissa (tässä 45 %)**