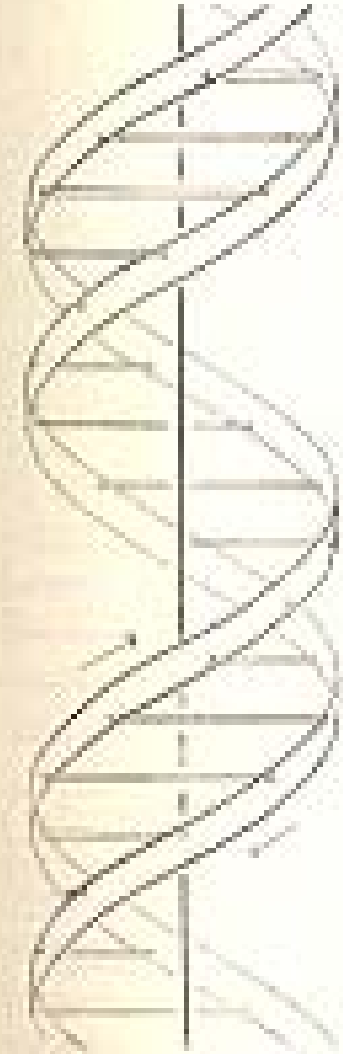


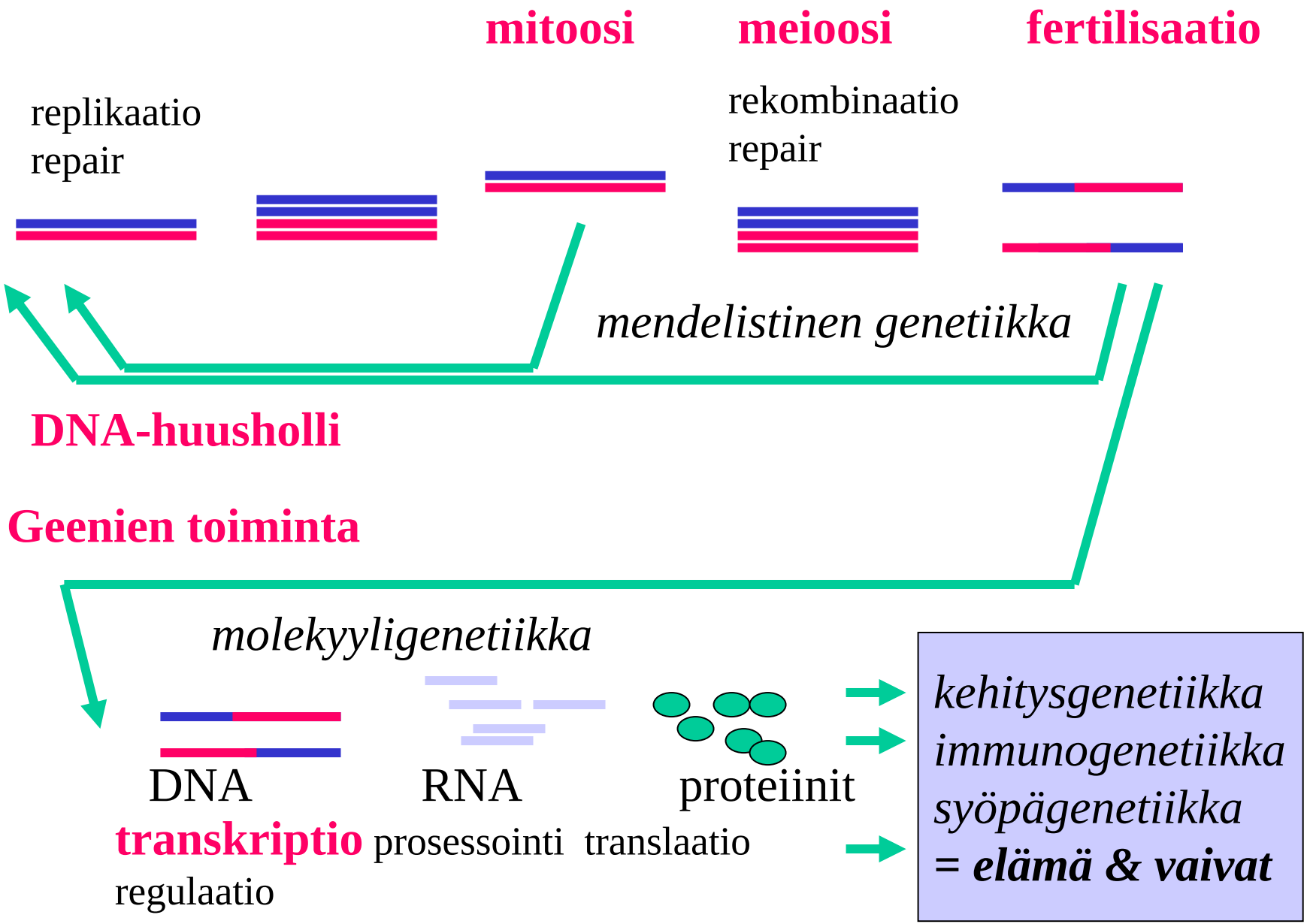
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Genetiikan perusteiden luentojen ensimmäisessä osassa tarkasteltiin transmissiogenetiikkaa eli sitä, kuinka geenit siirtyvät sukupolvesta toiseen

Toisessa osassa ryhdymme tarkastelemaan sitä, mitä geenit ovat, miten ne toimivat ja miten ne tuottavat meille tuttuja elämänilmiöitä

DNA > RNA > Proteiinit

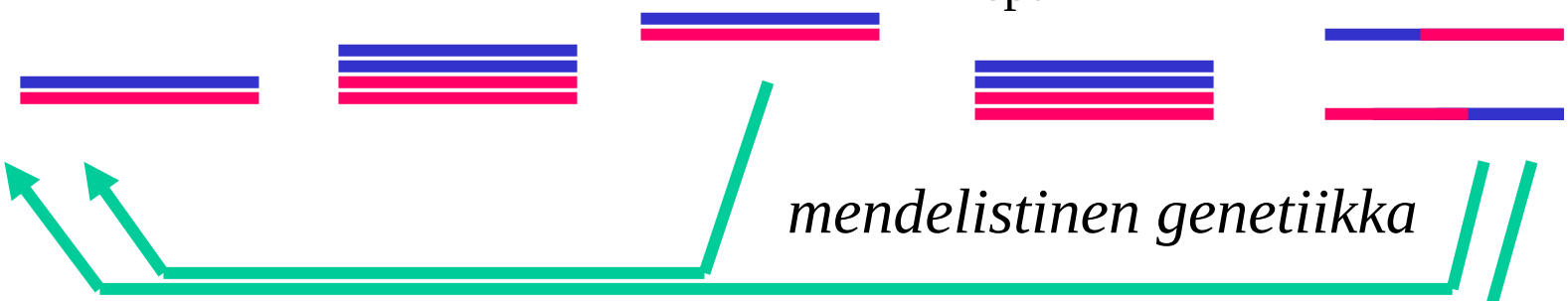
CELL kappale 6


mitoosi
meioosi
fertilisaatio
replikaatio
repair
rekombinaatio
repair
mendelistinen genetiikka
DNA-huusholli
Geenien toiminta
molekyyligenetiikka
DNA
RNA
proteiinit
transkriptio
prosessointi
translaatio
regulaatio
kehitysgenetiikka
immunogenetiikka
syöpägenetiikka
= elämä & vaivat

CELL 309

mitoosi **meioosi** **fertilisaatio**

rekombinaatio
repair



mendelistinen genetiikka

DNA-huusholli

Geenien toiminta

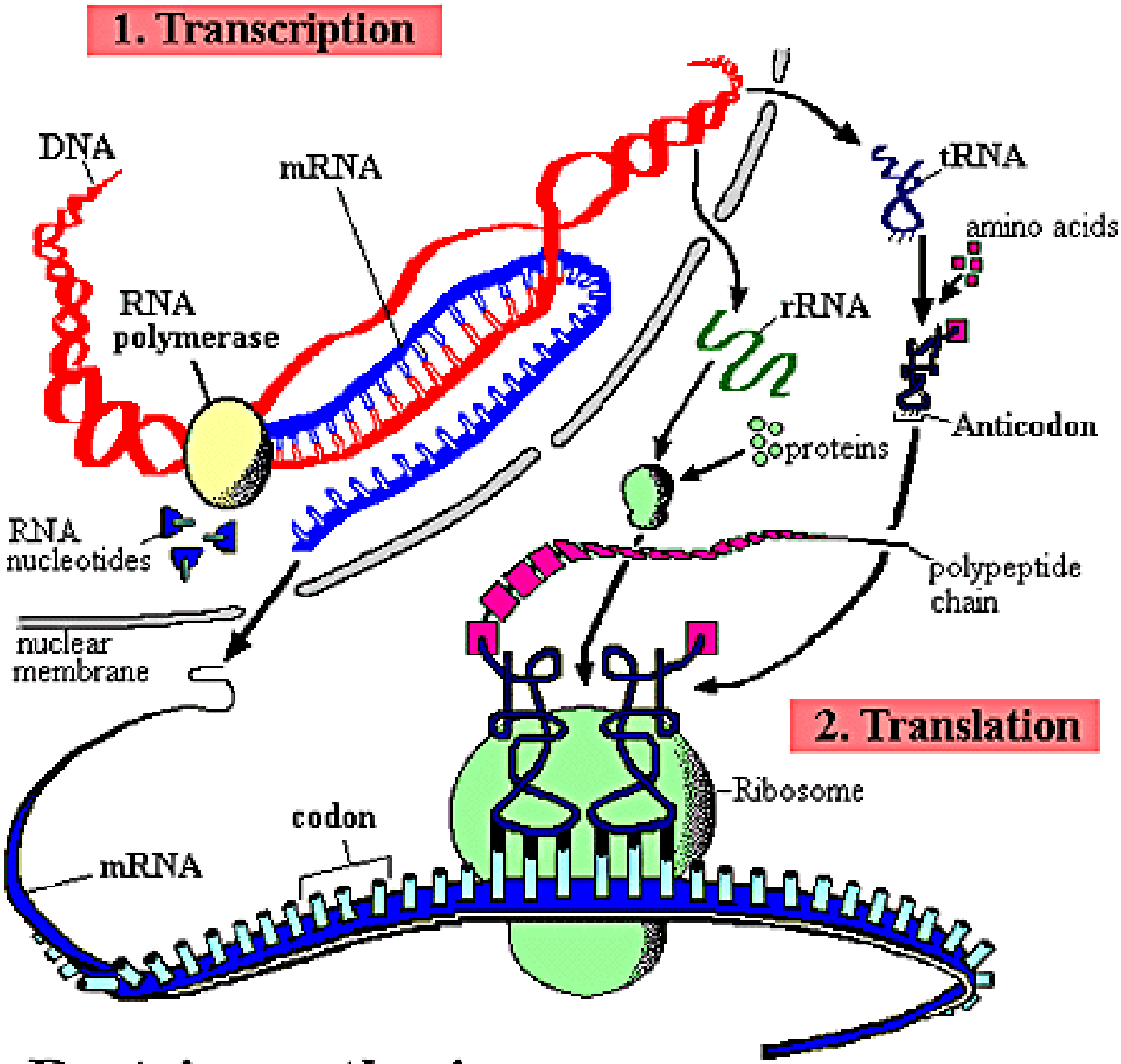
molekyyligenetiikka

DNA RNA proteiinit

transkriptio prosessointi translaatio
regulaatio

kehitysgenetiikka
immunogenetiikka
syöpägenetiikka
ELÄMÄ!

Genetiikan perusteiden miellekartta: tänään alkaa transkriptio



Protein synthesis

RNA:n polymerisaatio eli

(DNA:n) transkriptio (RNA:ksi)

Vain toinen DNA:n juosteista toimii **templaattina**

RNA

RNA:n toimialueet ovat DNA:han verrattuna huikean monipuoliset ja aktiiviset. DNA on pelkkä tietovarasto.

Uskotaan, että ennen kuin RNA "keksi" varastoida tietonsa DNA:han, se joutui yksin edustamaan ”elämää” ja komentelemaan satunnaista proteiinijoukkoa (CELL 402). Informaation kasautuminen ei oikein toiminut, erilaisia reaktioita saatiin aikaan, ehkä hiukan ketjujen pitenemistäkin yms

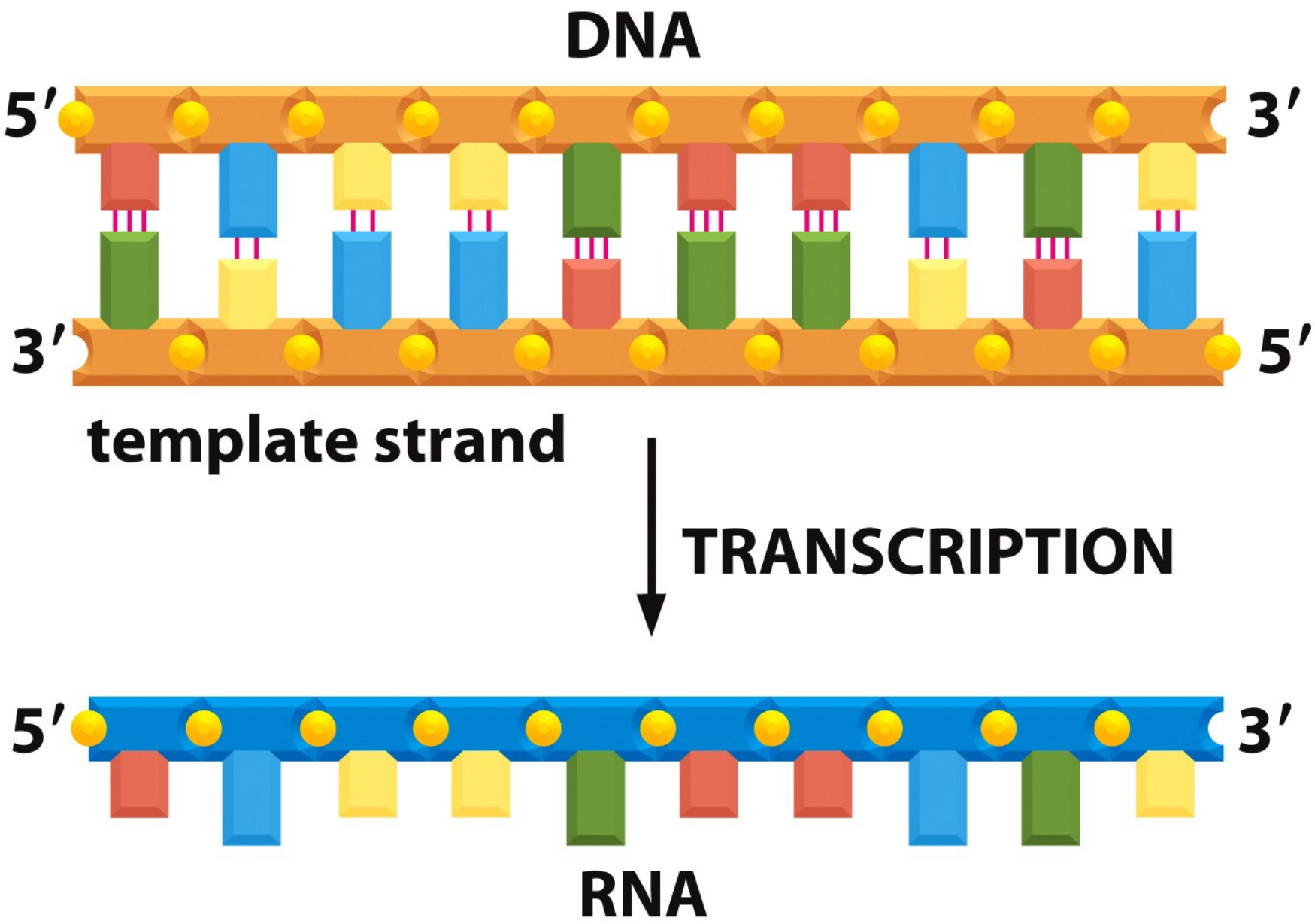


Figure 6-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

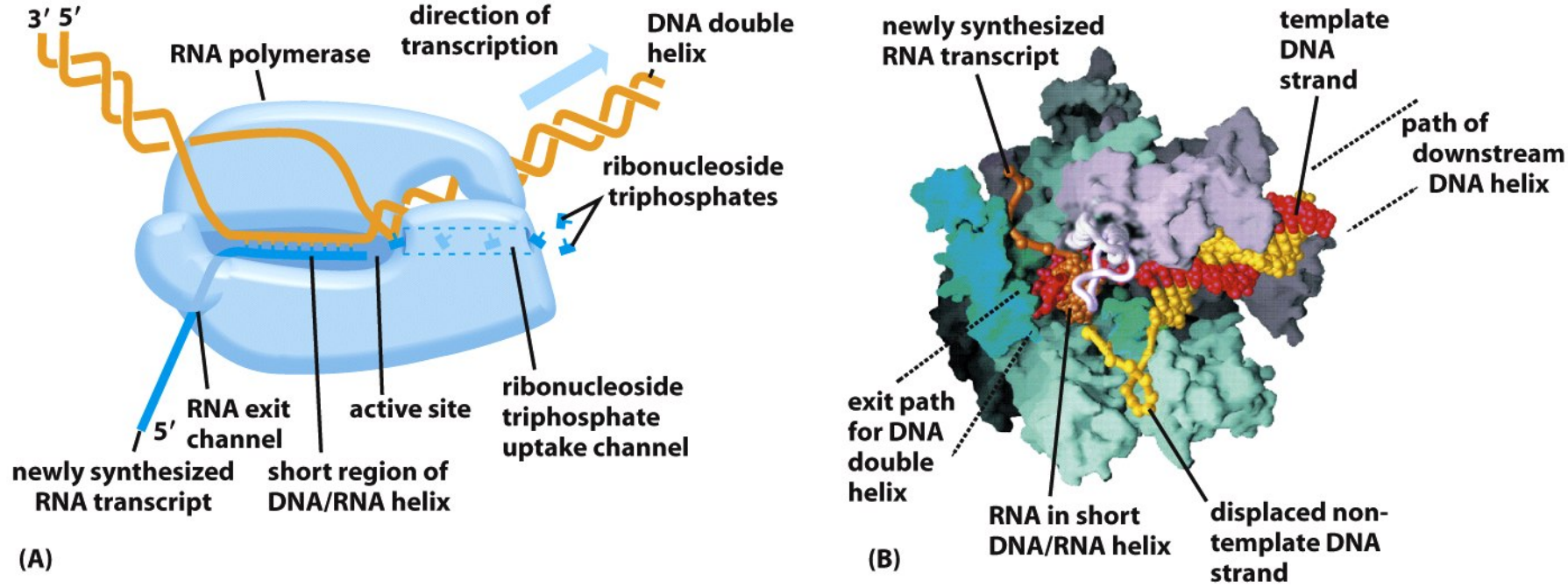


Figure 6-8 Molecular Biology of the Cell 5/e (© Garland Science 2008)

TRANSKRIPTIO -kohdassa sivutaan aivan ohimennen seuraavia asioita, jotka käsitellään syvemmin myöhemmin

– mitä DNA:sta transkriboidaan (koodi, geenin rakenne, DNA:n sisältö **CELL 367-, 550**

– miten transkriptiota säädellään **CELL kappale 7**

– miten mRNA kuljetetaan **CELL 359-** (solubilsa)

TRANSKRIPTIO –kohdassa otetaan esille kaikki erilaiset RNA –laadut (**CELL 336**)

mRNA (lähetti) **CELL 346-**
tRNA (transfer) **CELL 368**
rRNA (ribosomaalinen) **CELL 361-**

Mistä aloitetaan?

- transkription start pointista, usein TATA-boksista (**CELL 311**)

Milloin aloitetaan?

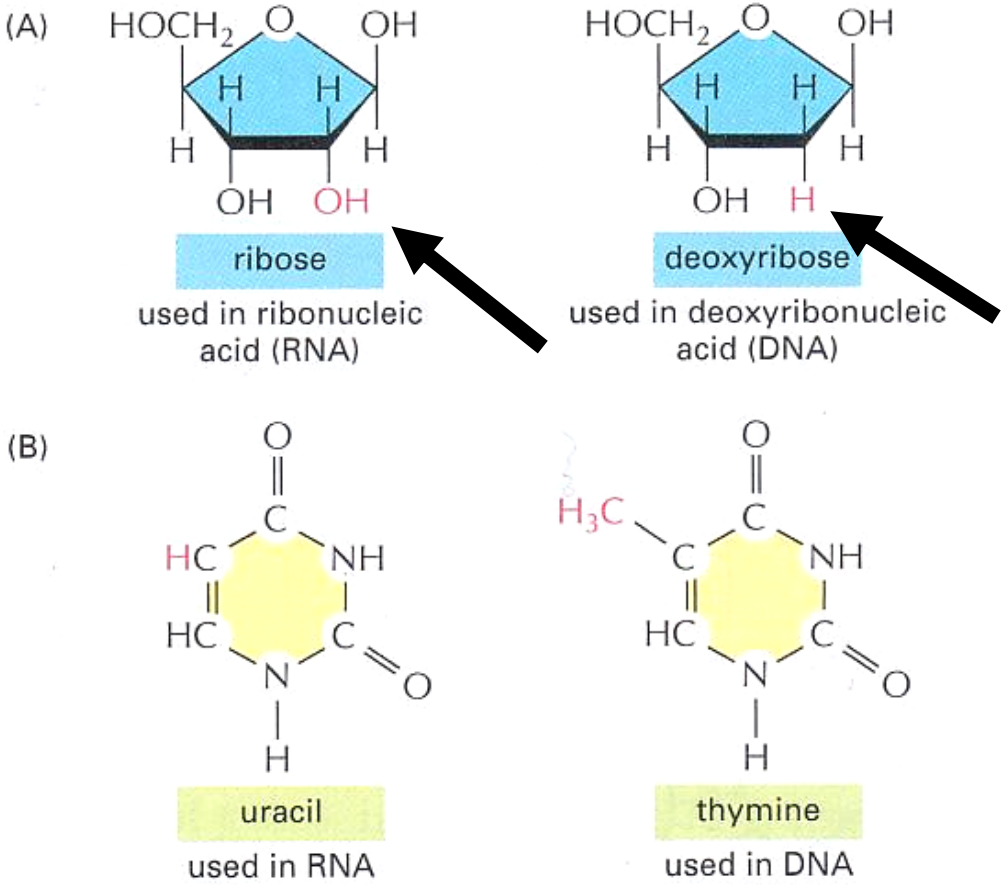
-kun kaikki transkriptiofaktorit ja polymeraasi ovat paikalla (**CELL 311**)

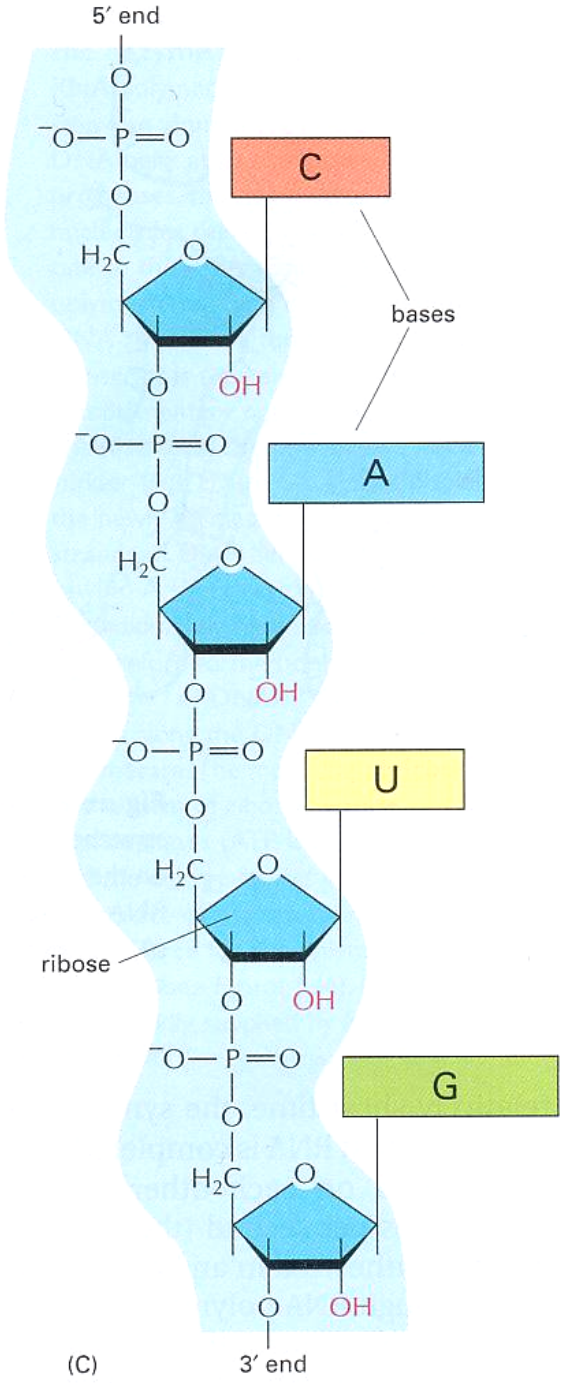
LISÄÄ TÄSTÄ ASIASTA GEENIREGULAATION YHTEYDESSÄ

Ketkä suorittavat?

-no joku kolmesta RNA-polymeraasista (**CELL 310**):

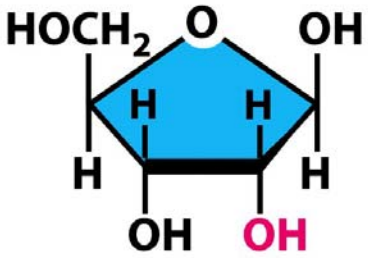
- P-I tekee rRNA (5.8S, 18S, 28S)
- P-II proteiinigeenit, snoRNA ja jotkut snRNA geenit
- P-III tRNA, 5S RNA, jotkut snRNA ja muita pieniä





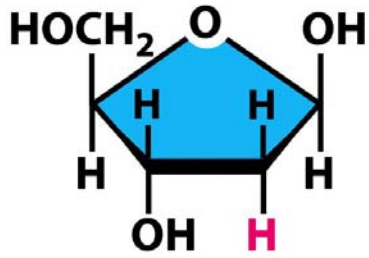
Pienet on erot DNA:n ja RNA:n välillä

CELL 332



ribose

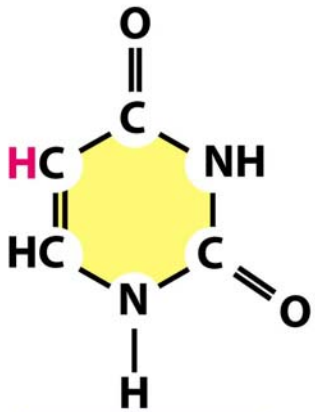
used in ribonucleic acid (RNA)



deoxyribose

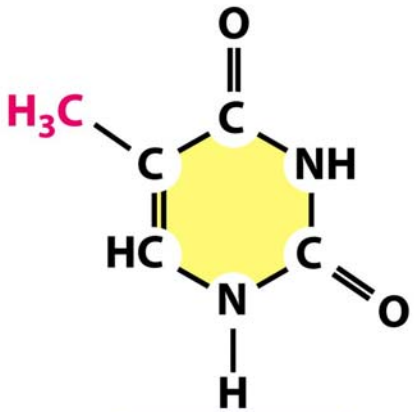
used in deoxyribonucleic acid (DNA)

Figure 6-4a Molecular Biology of the Cell 5/e (© Garland Science 2008)



uracil

used in RNA



thymine

used in DNA

Figure 6-4b Molecular Biology of the Cell 5/e (© Garland Science 2008)

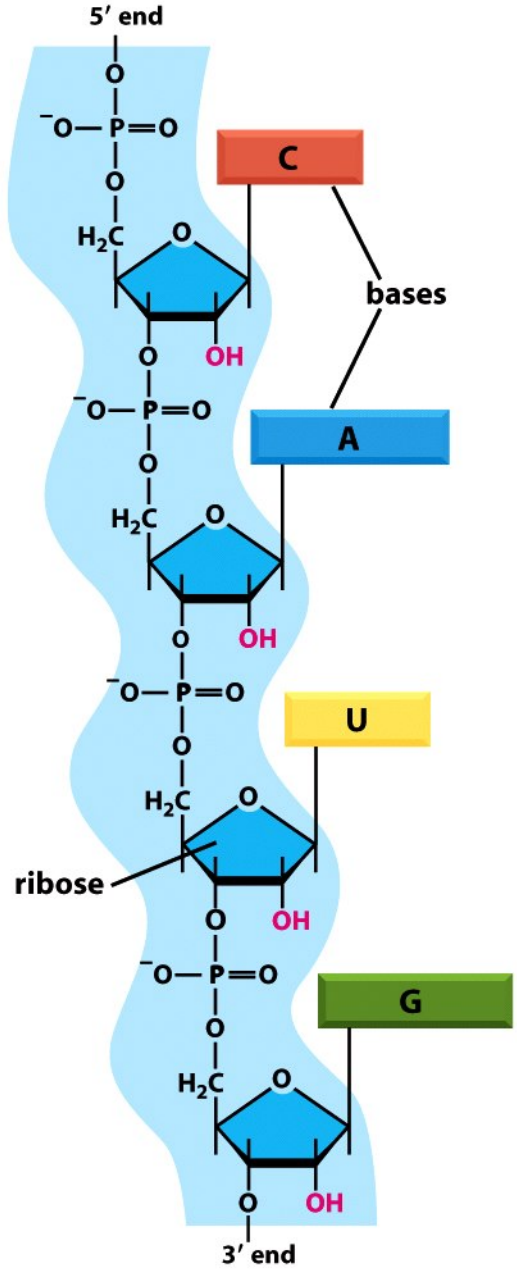
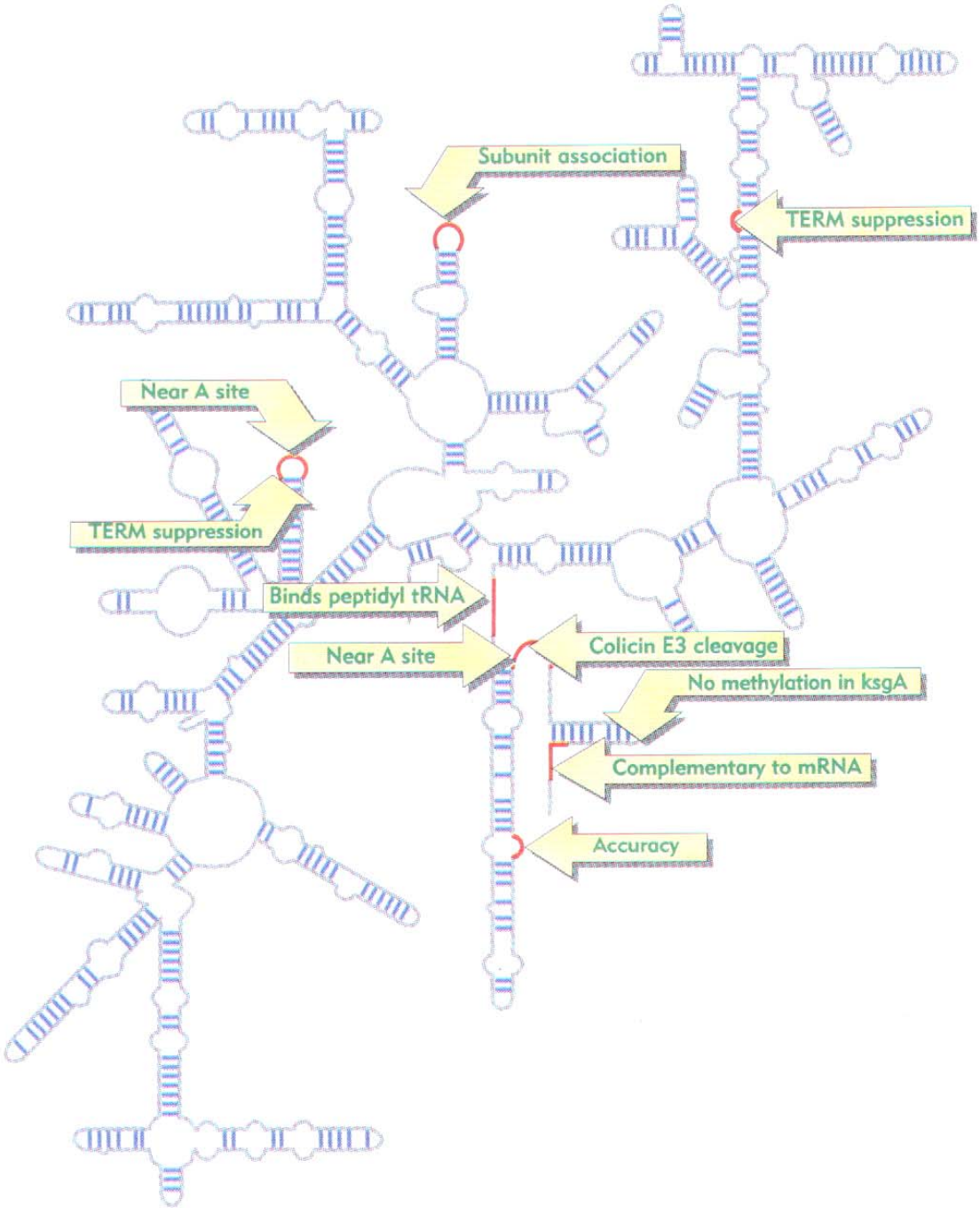


Figure 6-4c Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 332



RNA on yhtä ahnas muodostamaan kaksoiskierrettä kuin DNA, mutta se joutuu tekemään sen yleensä itsensä kanssa, niin kuin tässä rRNA

Uudet, vastalöydetyt RNA-laadut toimivat tekemällä kaksoiskierteitä muiden, esimerkiksi mRNA-molekyylien kanssa

CELL 345

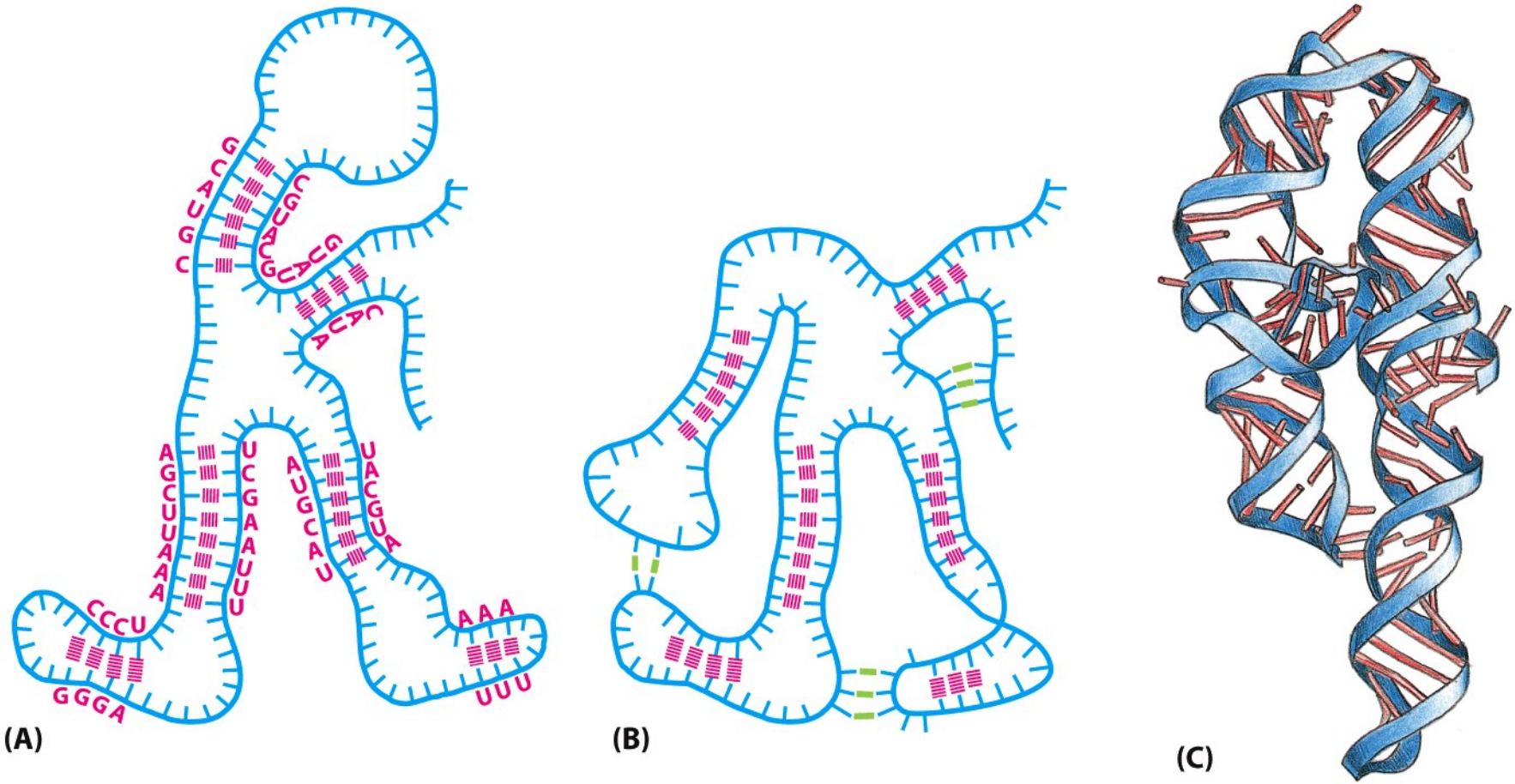
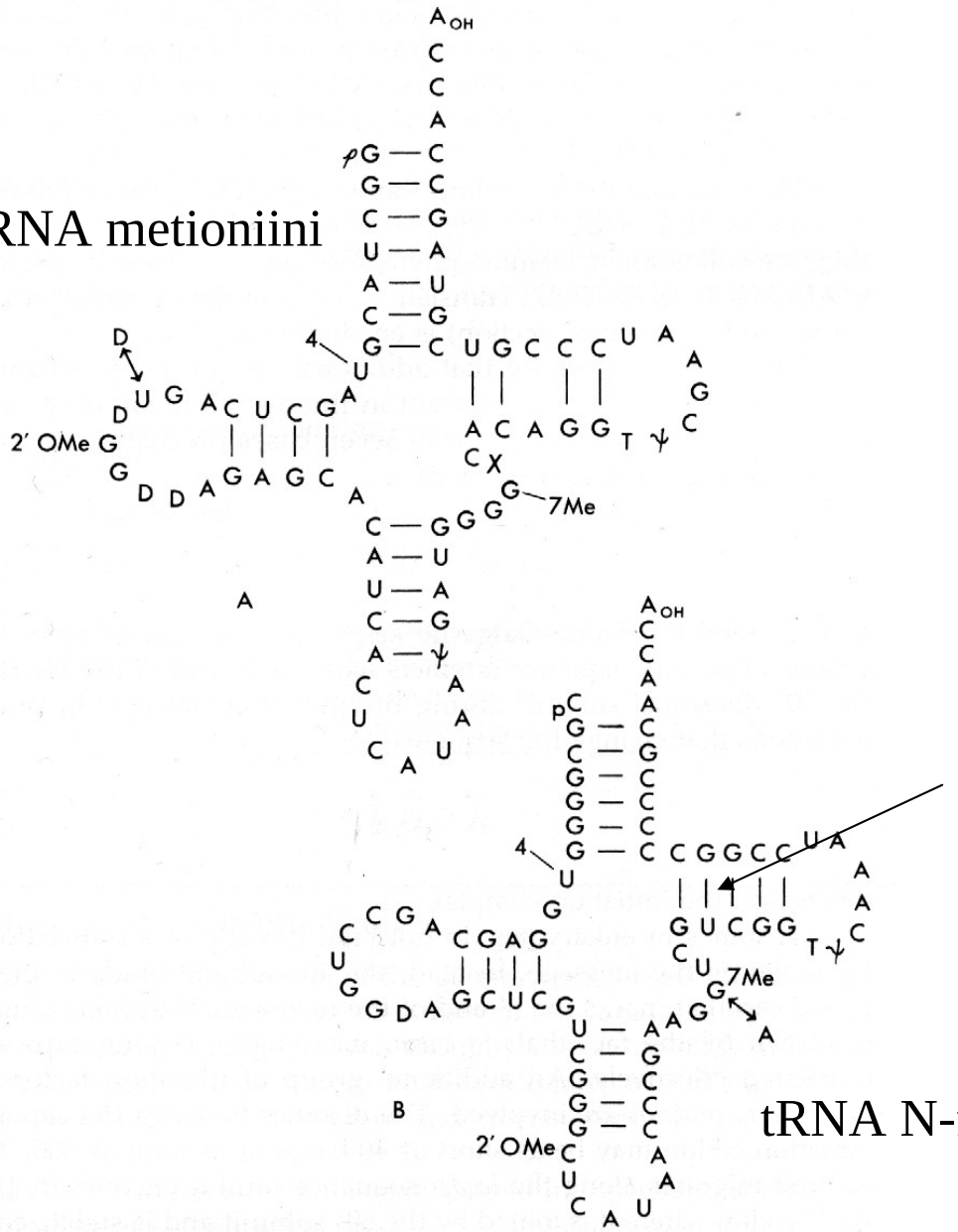


Figure 6-6 Molecular Biology of the Cell 5/e (© Garland Science 2008)

tRNA metioniini



tRNA-molekyyleissä on sen U:n lisäksi useita muitakin muuttuneita emäksiä, jotka muokataan transkription jälkeen, esimerkiksi

- N,N-dimethyl G
- Dihydro U
- 4-thiouridine
- Inosine
- psi pseudouridyyli
- 7MeG 7-metyyliguanyylih

-RNA:ssa GU –pariutuminen on luvallista

tRNA N-formylmetioniini eli aloitus!

met AUG

CELL 339

FIGURE 10–4. *Ribonucleotide sequences of (A) $tRNA_m$, and (B) $tRNA_f$. Unusual ribonucleotides are symbolized as follows: D, dihydrouridylic acid; ψ, pseudouridylic acid; T, thymidylic acid; 7MeG, 7-methylguanylic acid; 2MeC, 2 O-methylcytidylic acid; 2 OMeG, 2 O-methylguanylic acid; 4U, 4-thiouridylic acid.* (From S. K. Dube, et al. 1968, and S. Corey, et al. 1968, used by permission.)

Table 6–1 Principal Types of RNAs Produced in Cells

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
scaRNAs	small cajal RNAs, used to modify snoRNAs and snRNAs
miRNAs	microRNAs, regulate gene expression typically by blocking translation of selective mRNAs
siRNAs	small interfering RNAs, turn off gene expression by directing degradation of selective mRNAs and the establishment of compact chromatin structures
Other noncoding RNAs	function in diverse cell processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

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

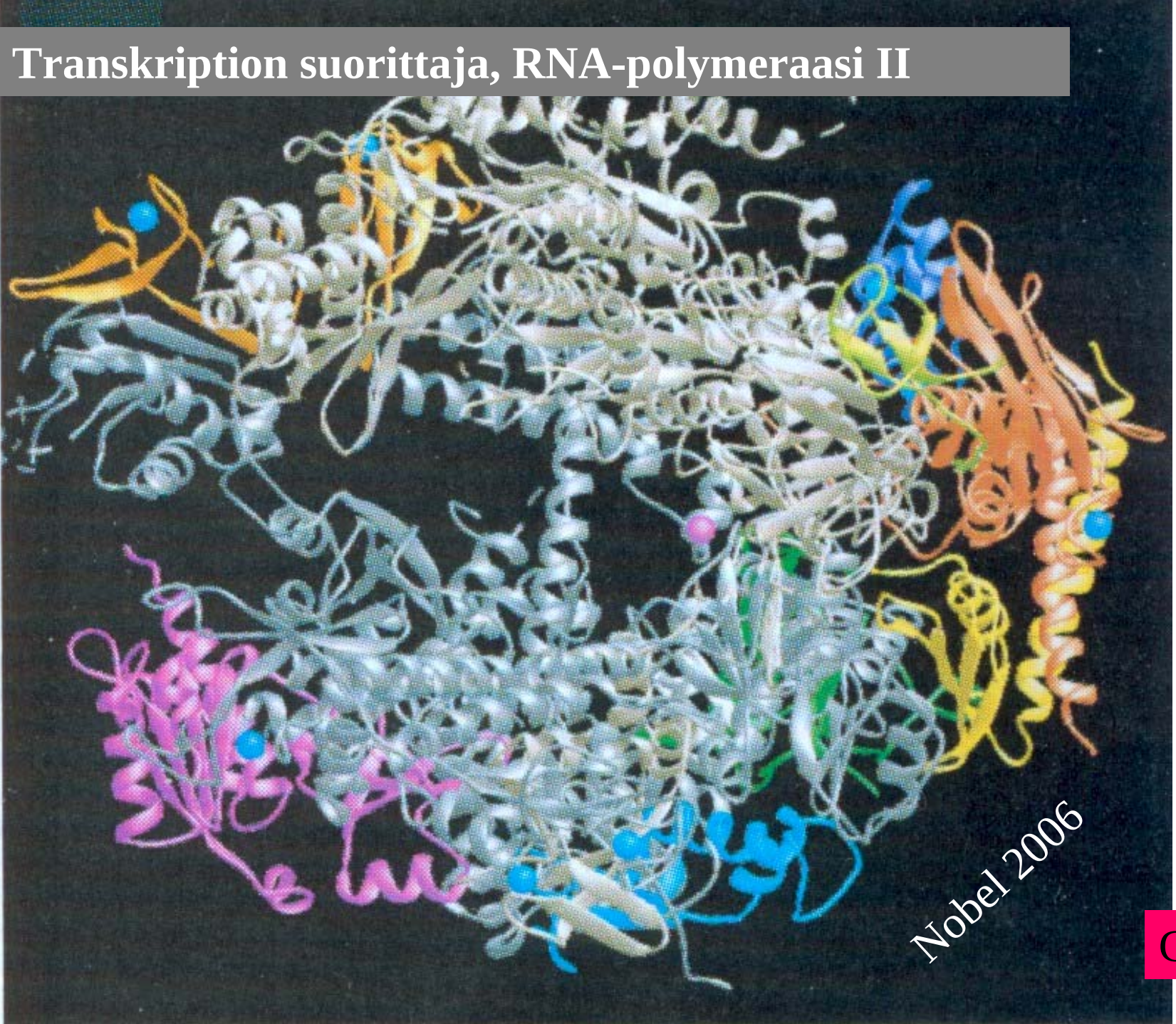
Table 6–3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Table 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transkription suorittaja, RNA-polymeraasi II



Nobel 2006

CELL 340

NOBEL PRIZE: CHEMISTRY

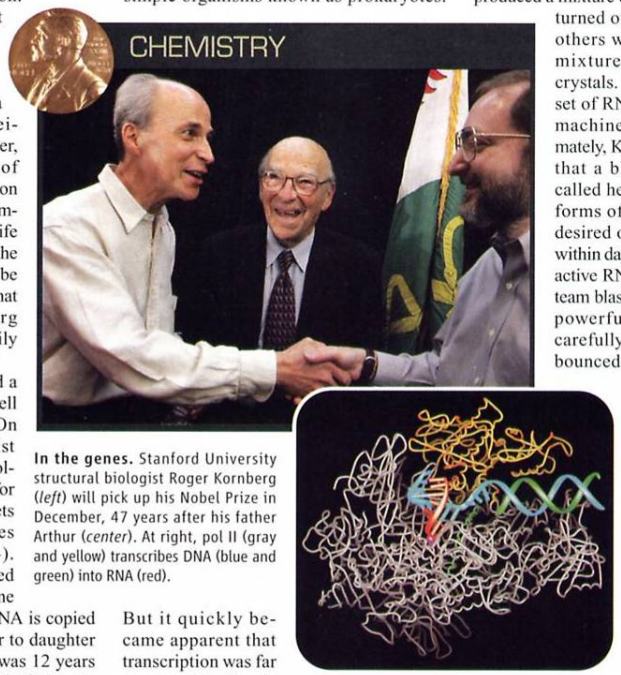
Solo Winner Detailed Path From DNA to RNA

DNA to RNA to proteins. Biology's central dogma—explaining how the secrets carried in the genes are animated—was limned decades ago. But "it doesn't say anything about how it's actually done," says E. Peter Geiduschek, a molecular biologist at the University of California, San Diego. Through decades of painstaking work, Roger Kornberg, a biochemist and structural biologist at the Stanford University School of Medicine in Palo Alto, California, revealed in atomic detail the first step in this process, how DNA in cells is converted into messenger RNA, a process known as transcription. Last week, that achievement earned Kornberg a rare honor: sole possession of the 2006 Nobel Prize in chemistry.

Kornberg's work has been a "terrific contribution," Geiduschek says. Adds Peter Fraser, who heads the Laboratory of Chromatin and Gene Expression at the Babraham Institute in Cambridge, U.K., "If the secret of life could be likened to a machine, the process of transcription would be a central cog in the machinery that drives all others. Kornberg has given us an extraordinarily detailed view of this machine."

The announcement capped a banner week for Stanford as well as Kornberg's own family. On 2 October, Stanford geneticist Andrew Fire shared the physiology or medicine Nobel Prize for his part in revealing that snippets of RNA can inactivate genes (*Science*, 6 October, p. 34). Kornberg's father Arthur shared the 1959 physiology or medicine prize for helping show how DNA is copied and passed down from mother to daughter cells. The younger Kornberg was 12 years old when he accompanied his father to Stockholm. "I have felt for some time that he richly deserved it," says the senior Kornberg—an emeritus professor at Stanford—of his son's work. However, he quips, "I'm disappointed it was so long in coming." The Kornbergs are the sixth parent and child to win the Nobel Prize. Who knows, but the family could be in for further scientific accolades. One of Roger's two brothers, Tom, is a developmental biologist at the University of California, San Francisco. Ken, meanwhile is an architect specializing in part on designing research buildings. Roger Kornberg, who says he was "simply stunned" when he received the news, is slated to collect the Nobel and $1.37 million at a December ceremony in Stockholm.

When Kornberg began studying transcription in the 1960s, the notion of revealing the process on an atomic scale was "daunting," he says. By then, researchers had found the process by which the enzyme RNA polymerase transcribes genetic information in bacteria and other simple organisms known as prokaryotes.



In the genes. Stanford University structural biologist Roger Kornberg (*left*) will pick up his Nobel Prize in December, 47 years after his father Arthur (*center*). At right, pol II (gray and yellow) transcribes DNA (blue and green) into RNA (red).

But it quickly became apparent that transcription was far more complex in eukaryotes, higher organisms that include all plants and animals. To get a handle on this complexity, in the late 1980s, Kornberg's lab purified a eukaryotic transcription complex from yeast that included RNA polymerase II (pol II)—the primary transcription enzyme—and five associated proteins called general transcription factors. To their surprise, this complex didn't respond to other proteins known to activate specific genes. That discovery led them to another key molecular player known as "mediator"—a complex of some 20 proteins that relays signals from the proteins that turn on specific genes to pol II.

Kornberg wanted to use x-ray crystallography to visualize just how pol II and its partner proteins work. But that required coming up with millions of identical copies of the protein complexes so they could pack together in an ordered crystal, much like the arrangement of oranges on a store shelf. Other groups had found a way to stop pol II in the act of transcribing DNA to RNA. But that produced a mixture of RNAs, some of which turned out to be active whereas others were inactive, and that mixture wouldn't form good crystals. Separating out just one set of RNAs in the transcription machinery took 6 years. Ultimately, Kornberg's lab discovered that a blood-clotting protein called heparin binds to inactive forms of the RNA, leaving the desired ones behind. "Literally within days, we had crystals of the active RNA," Kornberg says. His team blasted those crystals with a powerful beam of x-rays and carefully mapped out how they bounced off each of the atoms. That allowed the team to construct the first-ever images of pol II in action in exquisite detail (*Science*, 20 April 2001, p. 411). Since then, Kornberg's team has produced more than a dozen related images that have revealed everything from how pol II selects the right RNA bases to how it recognizes proteins that turn on expression of specific genes.

Kornberg says one of the lab's central goals today is to produce more detailed x-ray structures of the mediator complex. "We have already got crystals of about one-third of the mediator, [from] which we believe a structure will be delivered soon," Kornberg says. That result will itself be a prize that biochemists will treasure for years to come.

–ROBERT F. SERVICE

CREDITS (TOP TO BOTTOM): JUSTIN SULLIVAN/GETTY IMAGES; ADAPTED FROM A. L. GNATT *ET AL.*, *SCIENCE* 292 (2001)

Roger Kornberg

Arthur-pappa sai nobelinsa 1959

Linkki

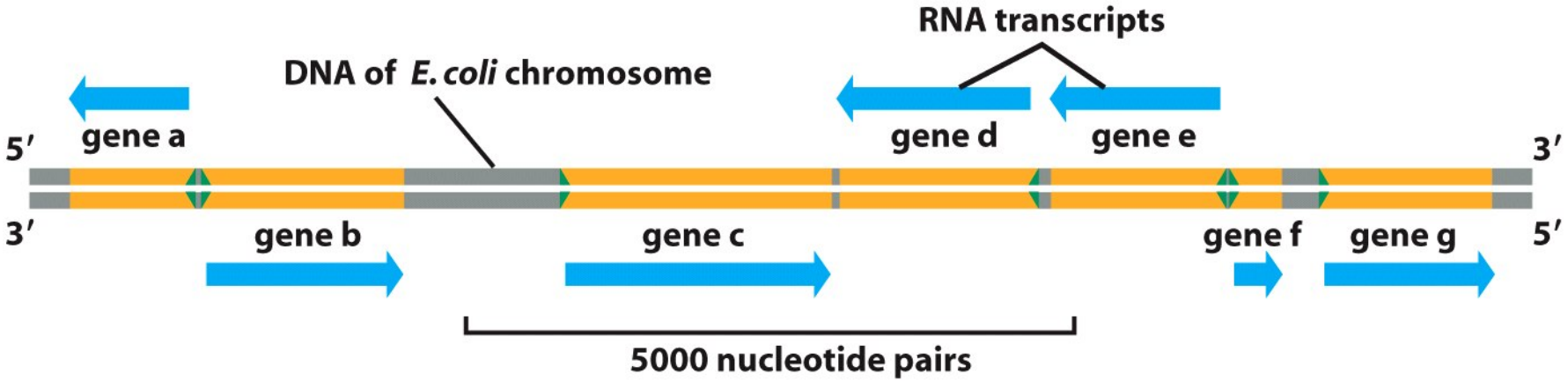
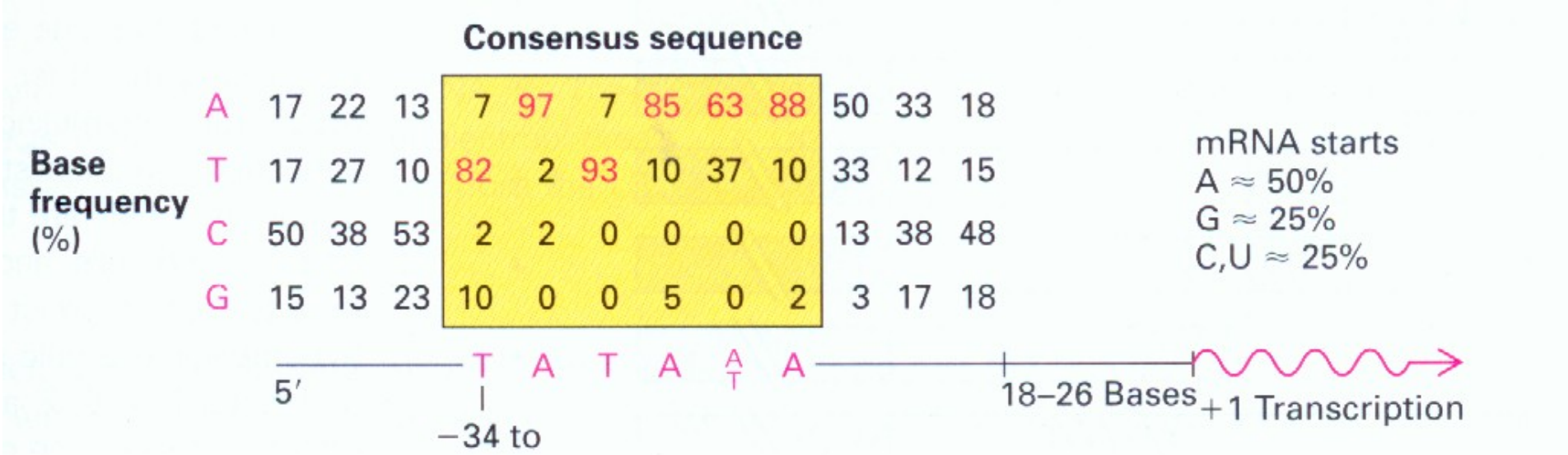


Figure 6-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Geenien lukusuunta vaihtelee, vain toinen DNA:n kaksoisjuosteista toimii templaattina

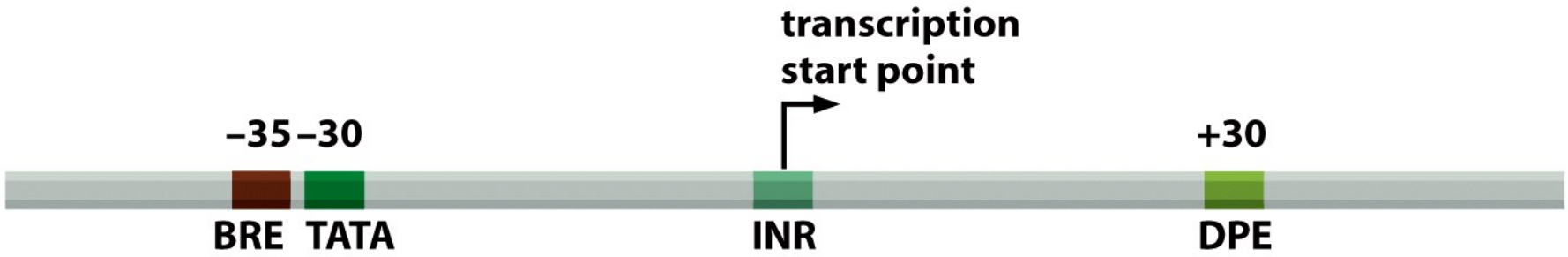
Joissakin tiukoissa genomeissa voidaan samaa kohtaa lukea kahteen suuntaan, mutta ne on silti eri geenejä

Diploidilla luetaan (yleensä) molempia kromosomeja



Eukaryoottien **promoottorin** konsensus-sekvenssi (**TATA-boksi**)

Transkriptio aloitetaan DNA:han ”merkitystä” kohdasta, joilla on usein paljon yhteistä (yhteisen polymeraasi II pitää osata aloittaa, ja sen pitää osua oikeaan lukukehykseen triplettikoodissa)

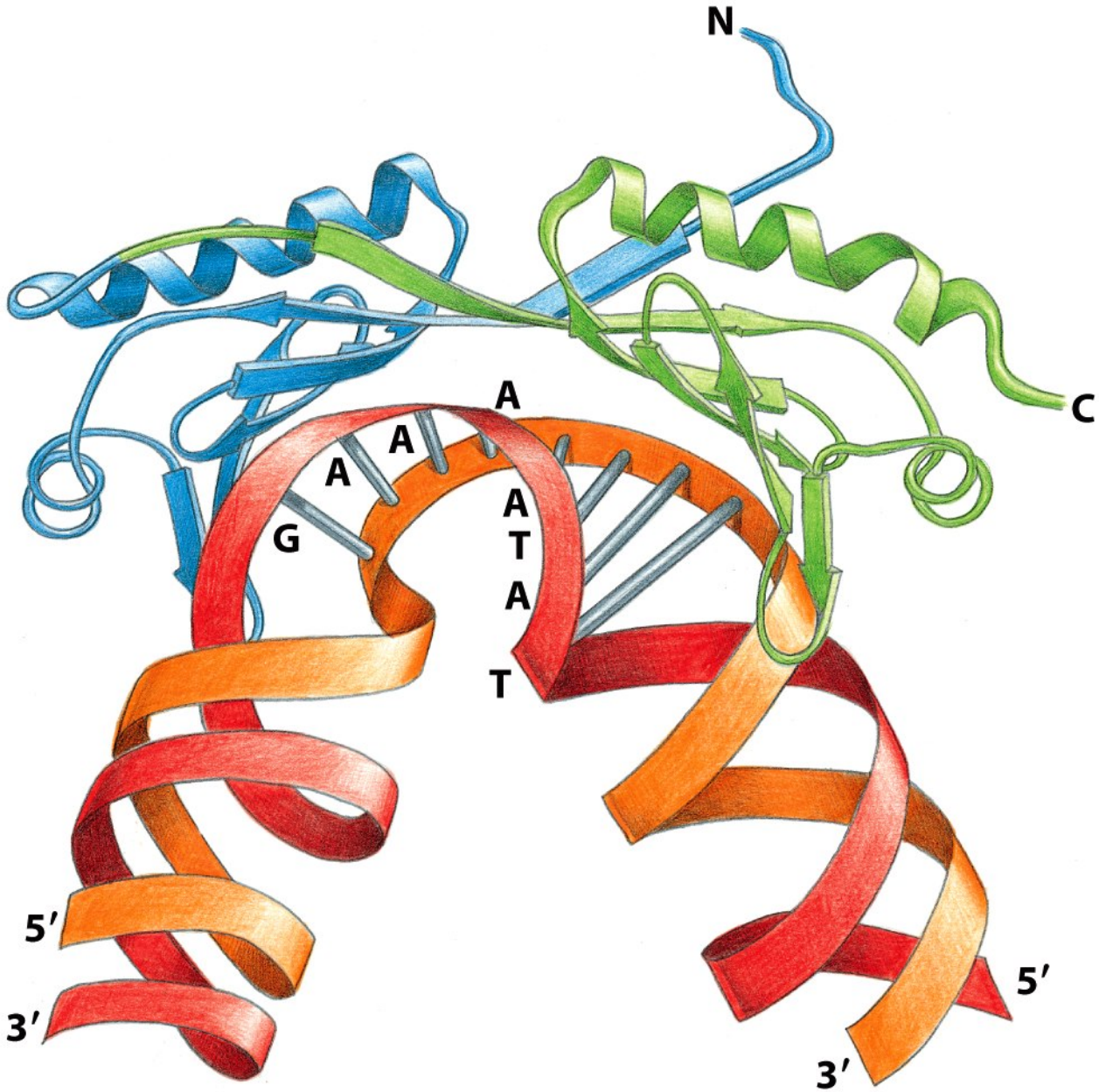


element	consensus sequence	general transcription factor
BRE	G/C G/C G/A C G C C	TFIIB
TATA	T A T A A/T A A/T	TBP
INR	C/T C/T A N T/A C/T C/T	TFIID
DPE	A/G G A/T C G T G	TFIID

Figure 6-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Muita aloitusaihelmia, sama tehtävä

CELL 342



TATA-binding protein

Figure 6-18 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 342

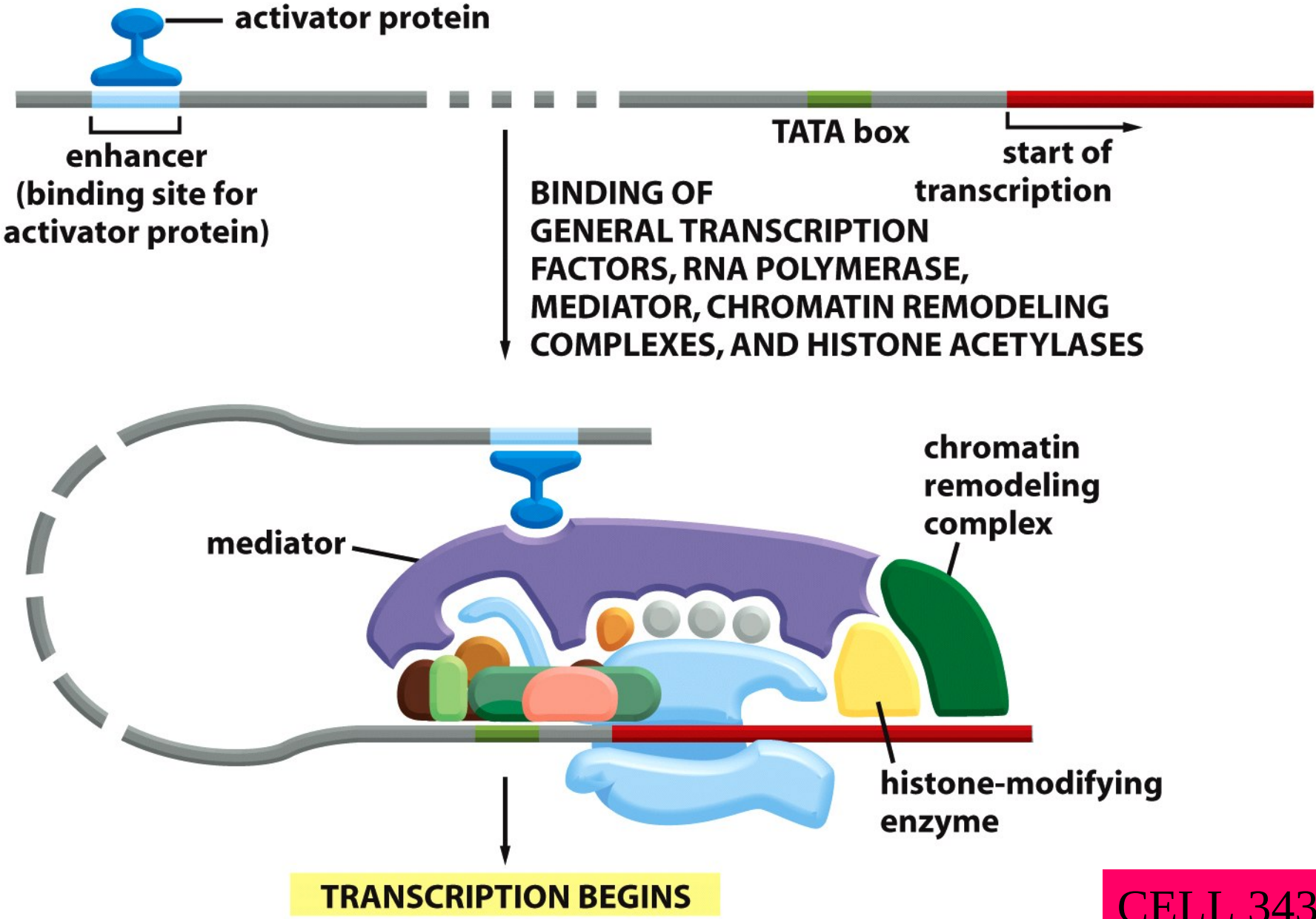


Figure 6-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 343

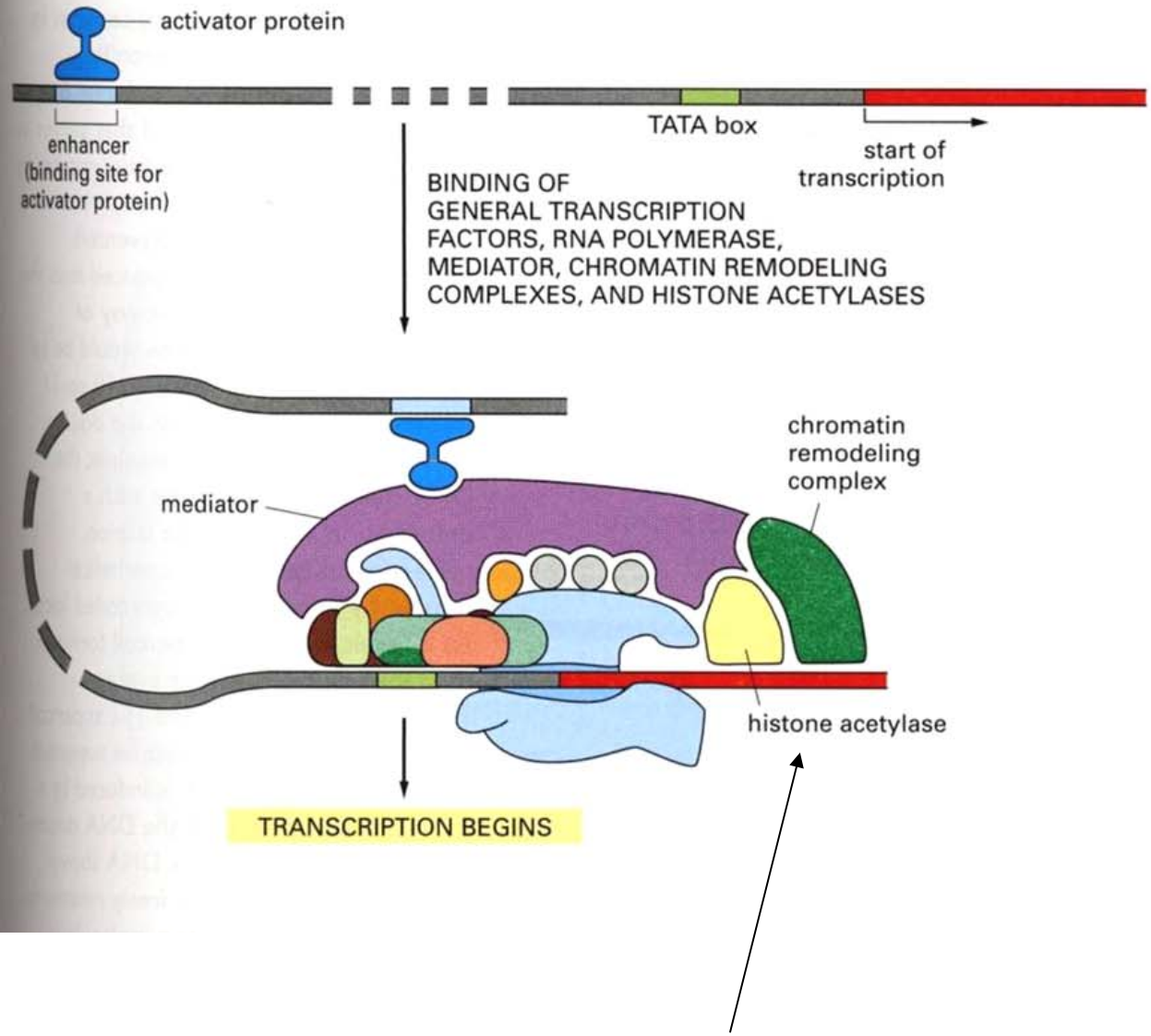
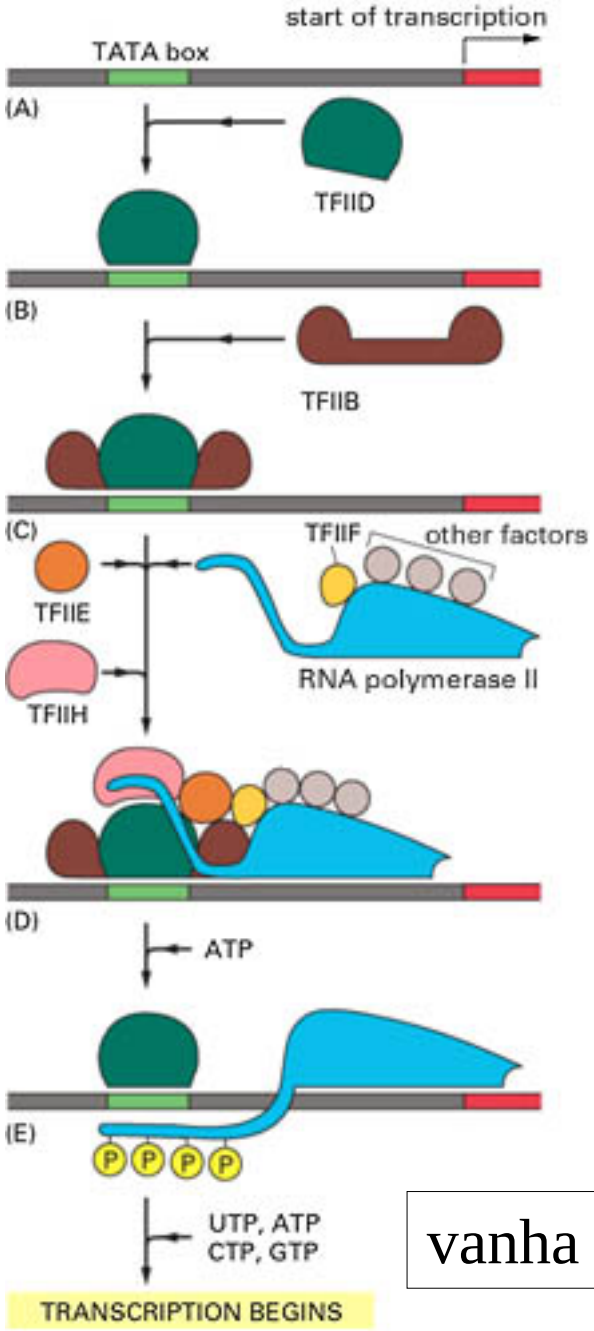


Figure 6–19 Transcription initiation by RNA polymerase II in a eucaryotic cell. Transcription initiation *in vivo* requires the presence of transcriptional activator proteins. As described in Chapter 7, these proteins bind to specific short sequences in DNA. Although only one is shown here, a typical eucaryotic gene has many activator proteins, which together determine its rate and pattern of transcription. Sometimes acting from a distance of several thousand nucleotide pairs (indicated by the dashed DNA molecule), these gene regulatory proteins help RNA polymerase, the general factors, and the mediator all to assemble at the promoter. In addition, activators attract ATP-dependent chromatin-remodeling complexes and histone acetylases.

As discussed in Chapter 4, the "default" state of chromatin is probably the 30-nm filament, and this is likely to be a form of DNA upon which transcription is initiated. For simplicity, it is not shown in the figure.

pieni ero

CELL 343



Transkription hallinta edellyttää monia vaiheita ja työkaluja

Mitä luetaan kullakin hetkellä?
Kaikki geenit eivät suinkaan ole "päällä" koko ajan

TF = transcription factor

vanha versio

CELL 341

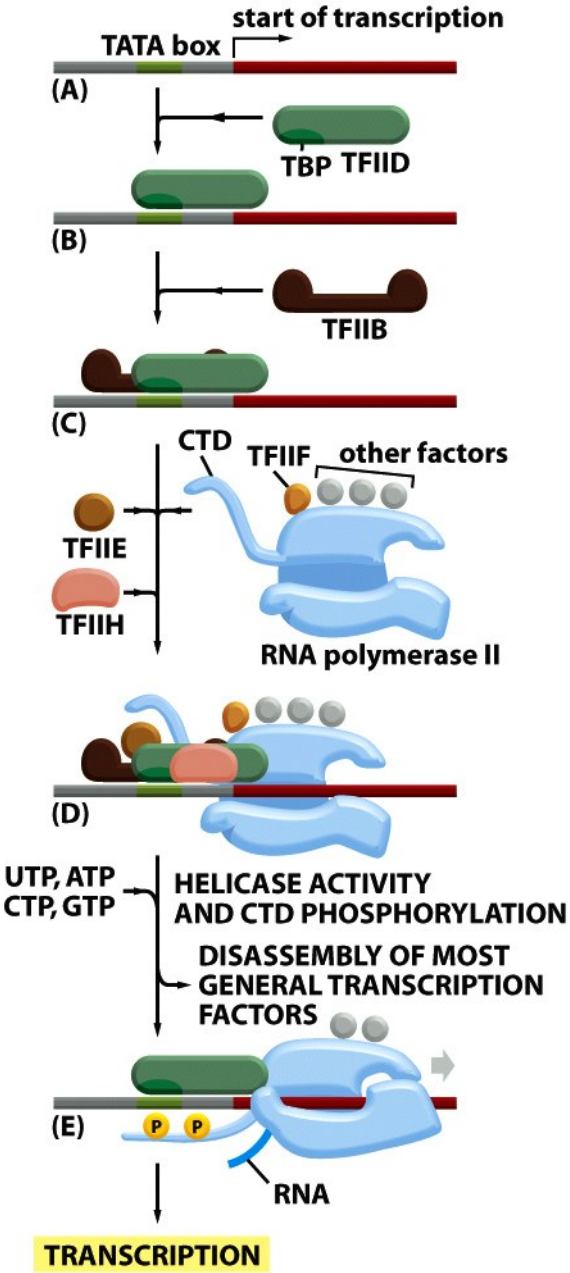


Figure 6-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transkription hallinta edellyttää monia vaiheita ja työkaluja

Mitä luetaan kullakin hetkellä?
Kaikki geenit eivät suinkaan ole "päällä" koko ajan

TF = transcription factor

CELL 341

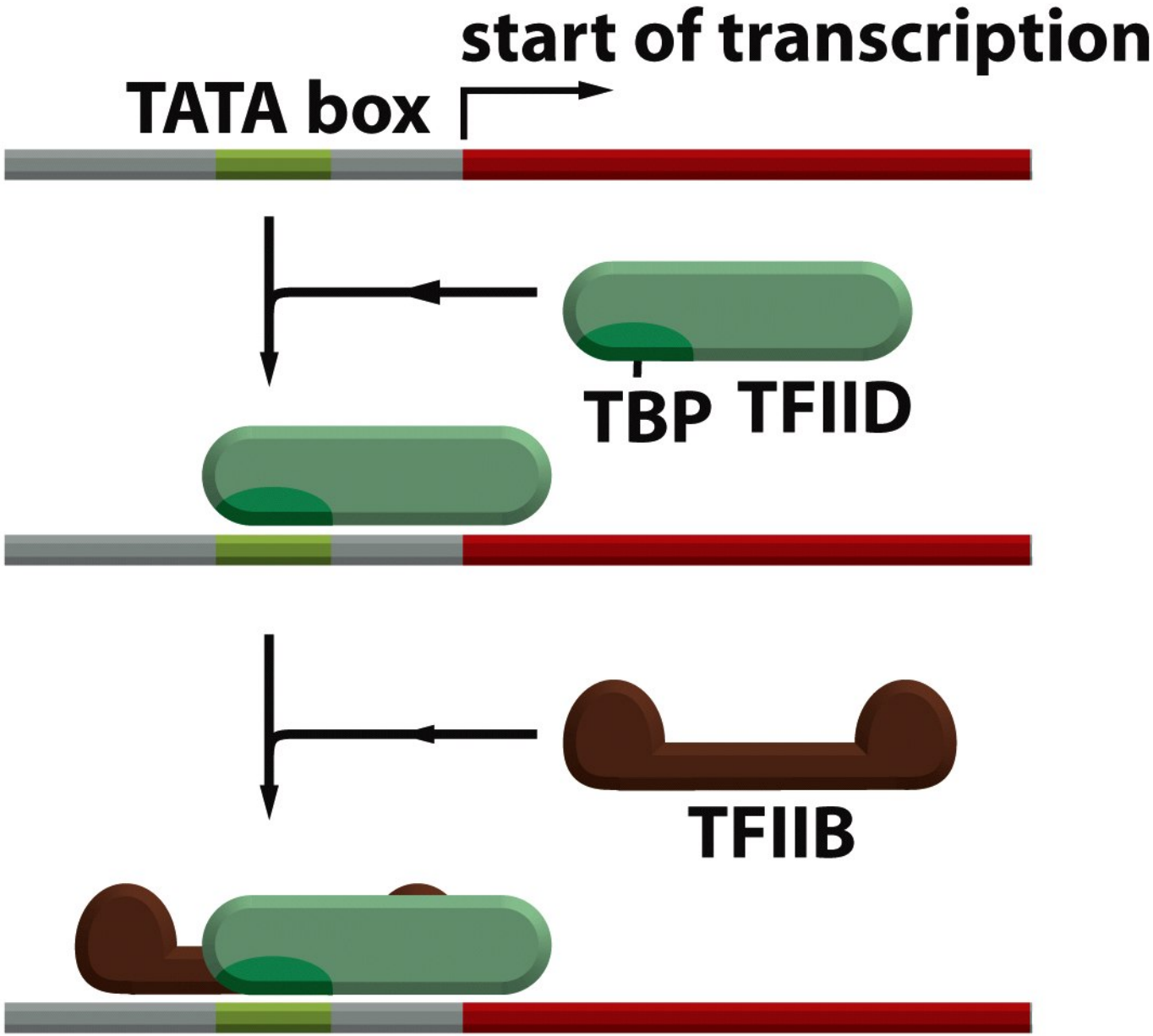


Figure 6-16 part 1 of 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

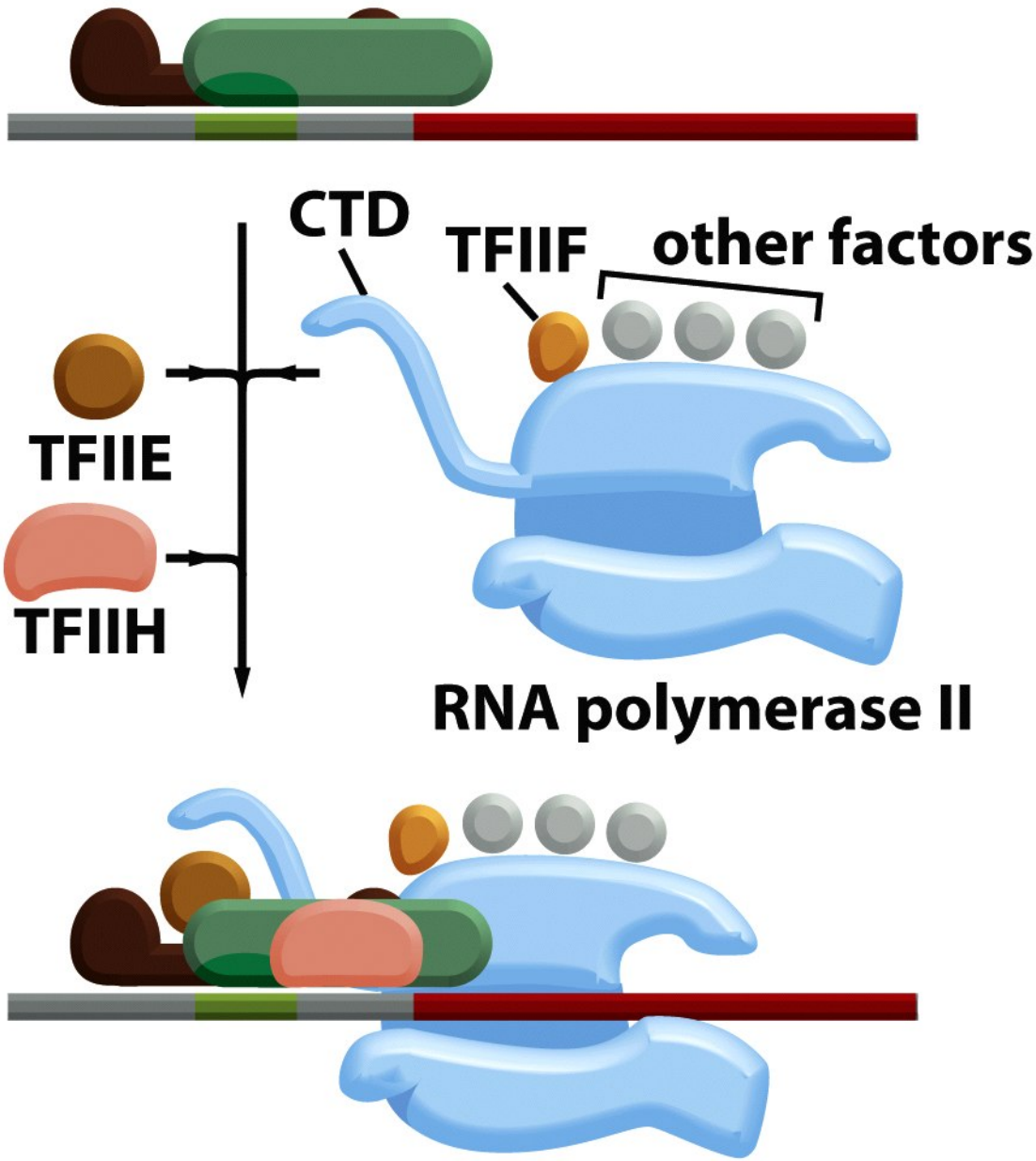


Figure 6-16 part 2 of 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

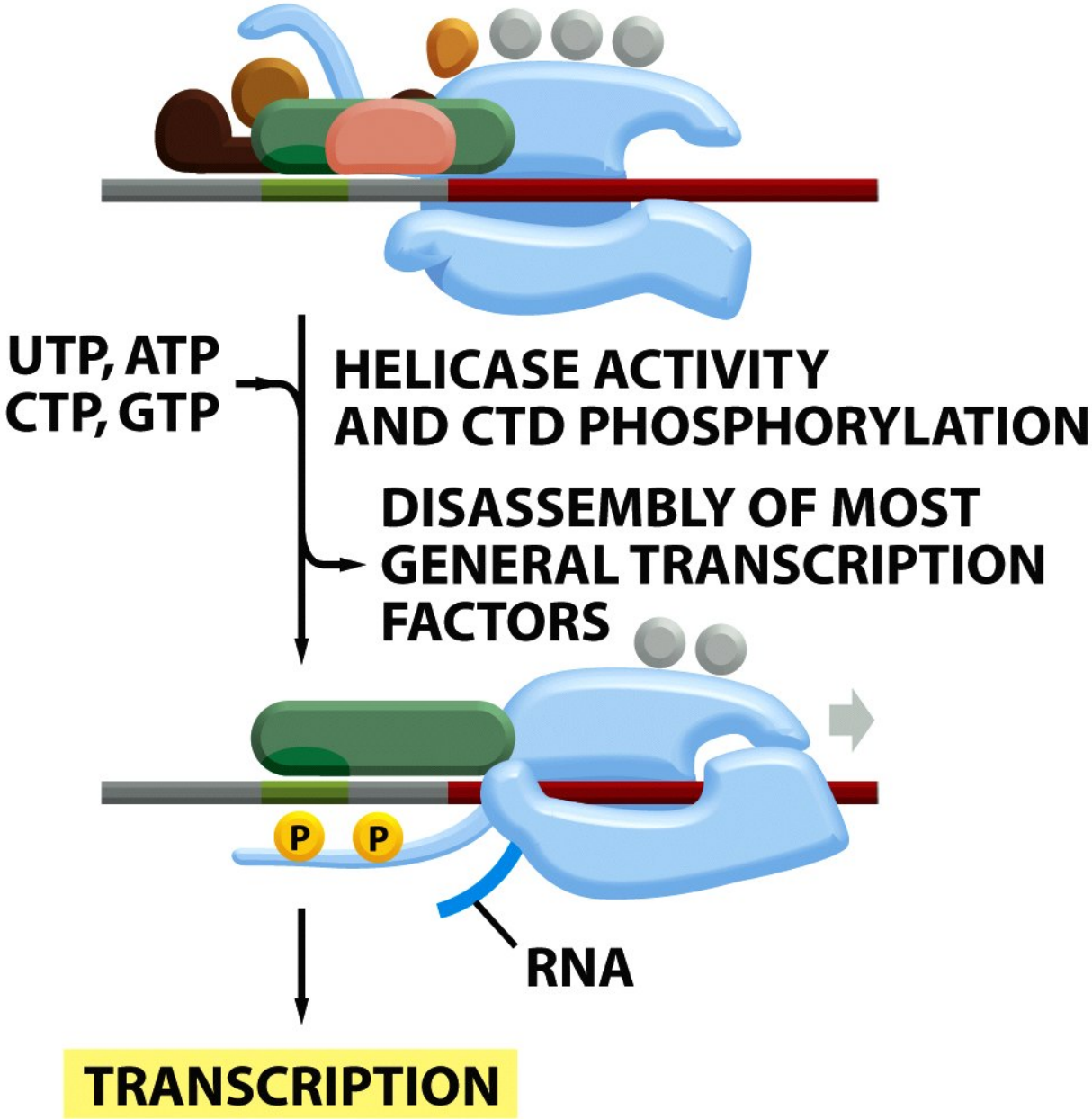


Figure 6-16 part 3 of 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

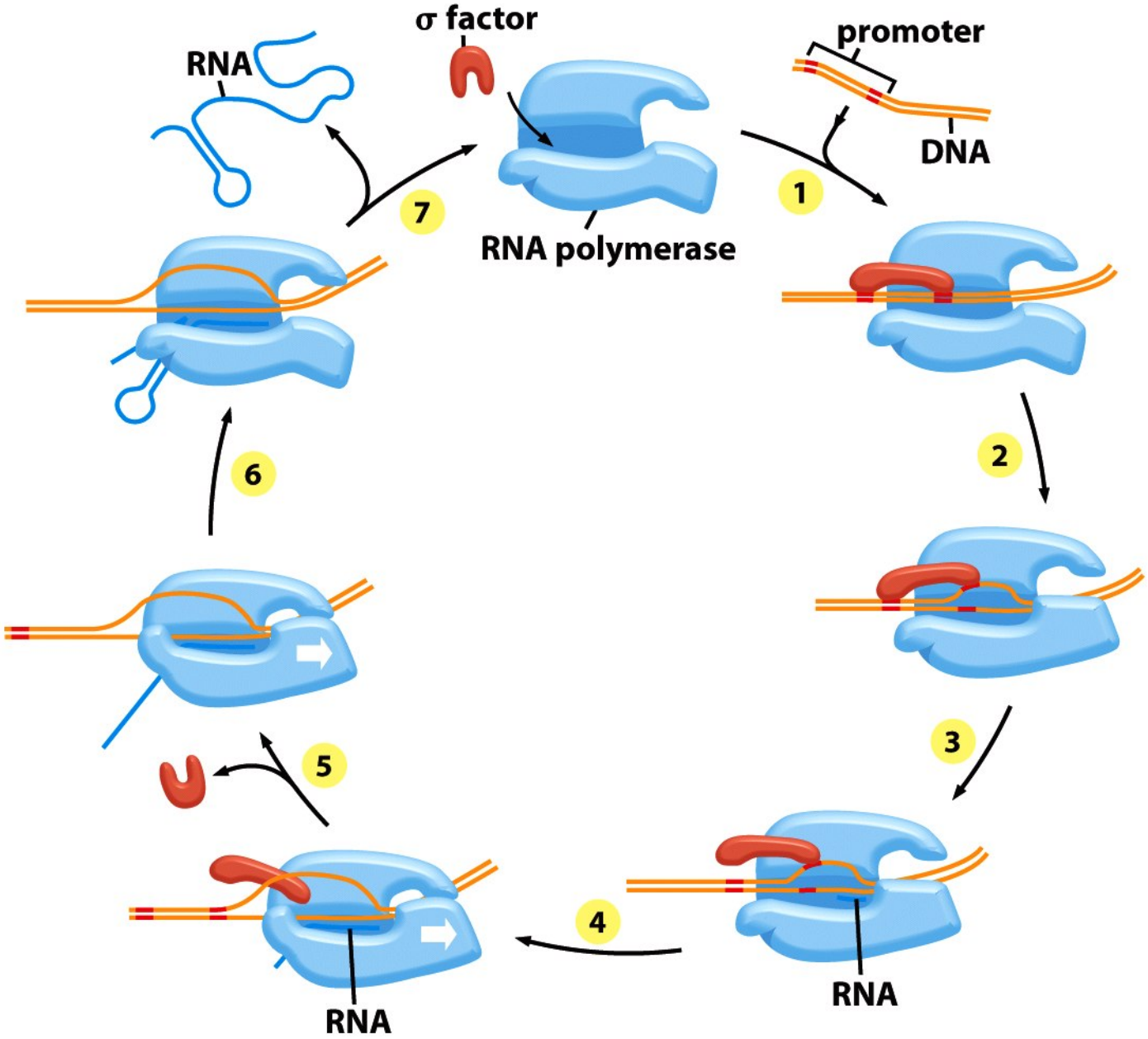


Figure 6-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

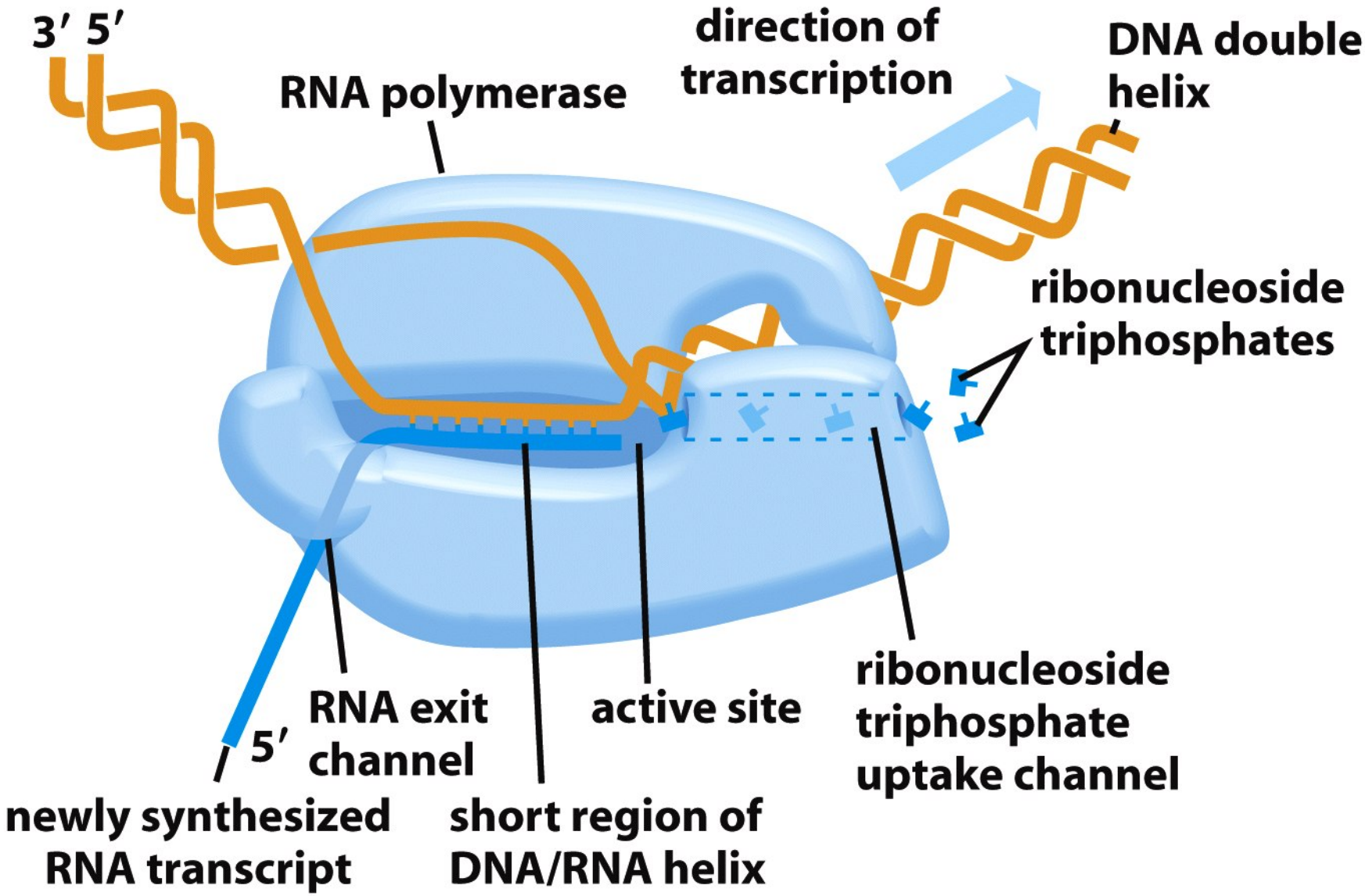


Figure 6-8a Molecular Biology of the Cell 5/e (© Garland Science 2008)

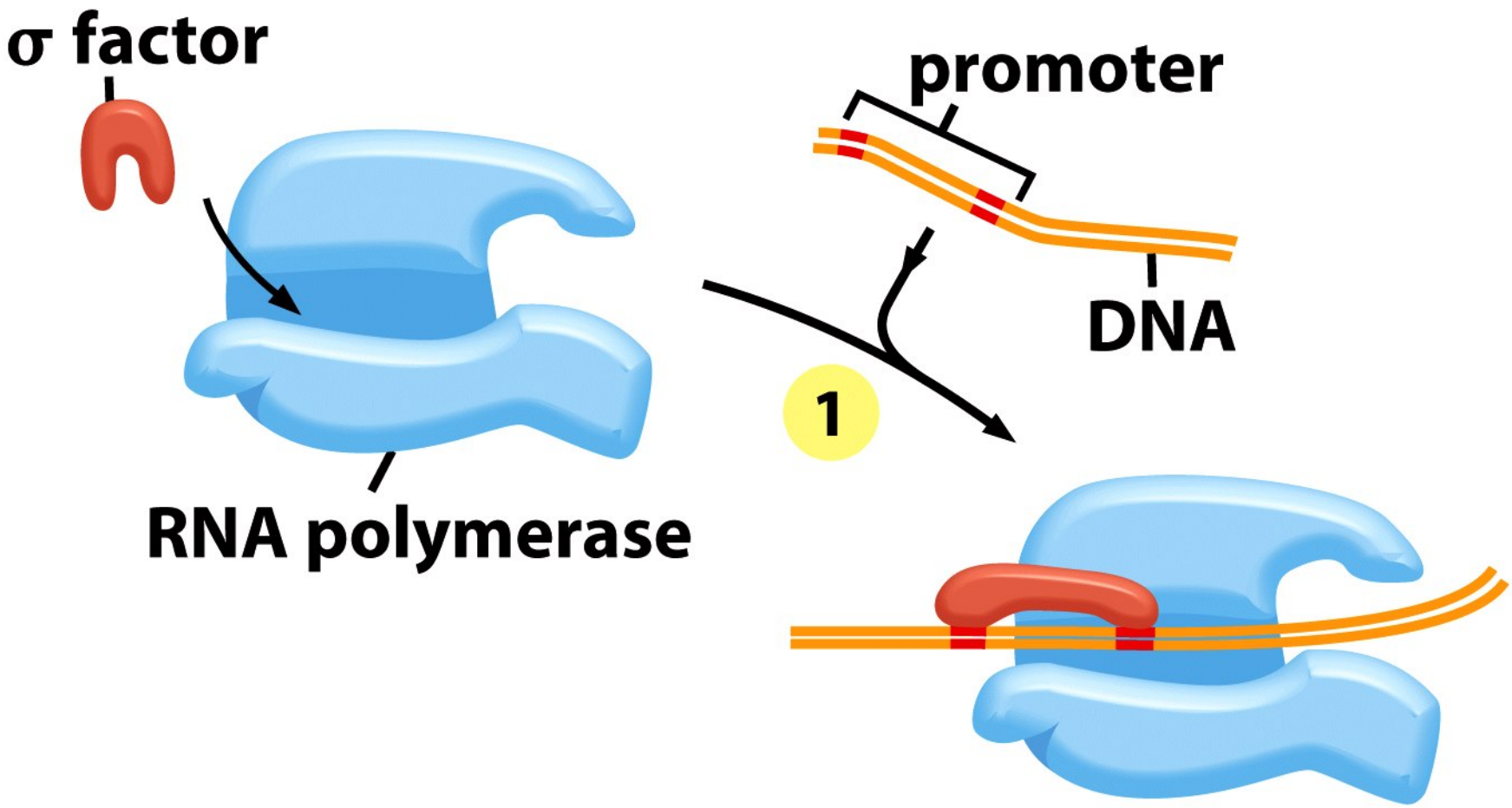


Figure 6-11 part 1 of 7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

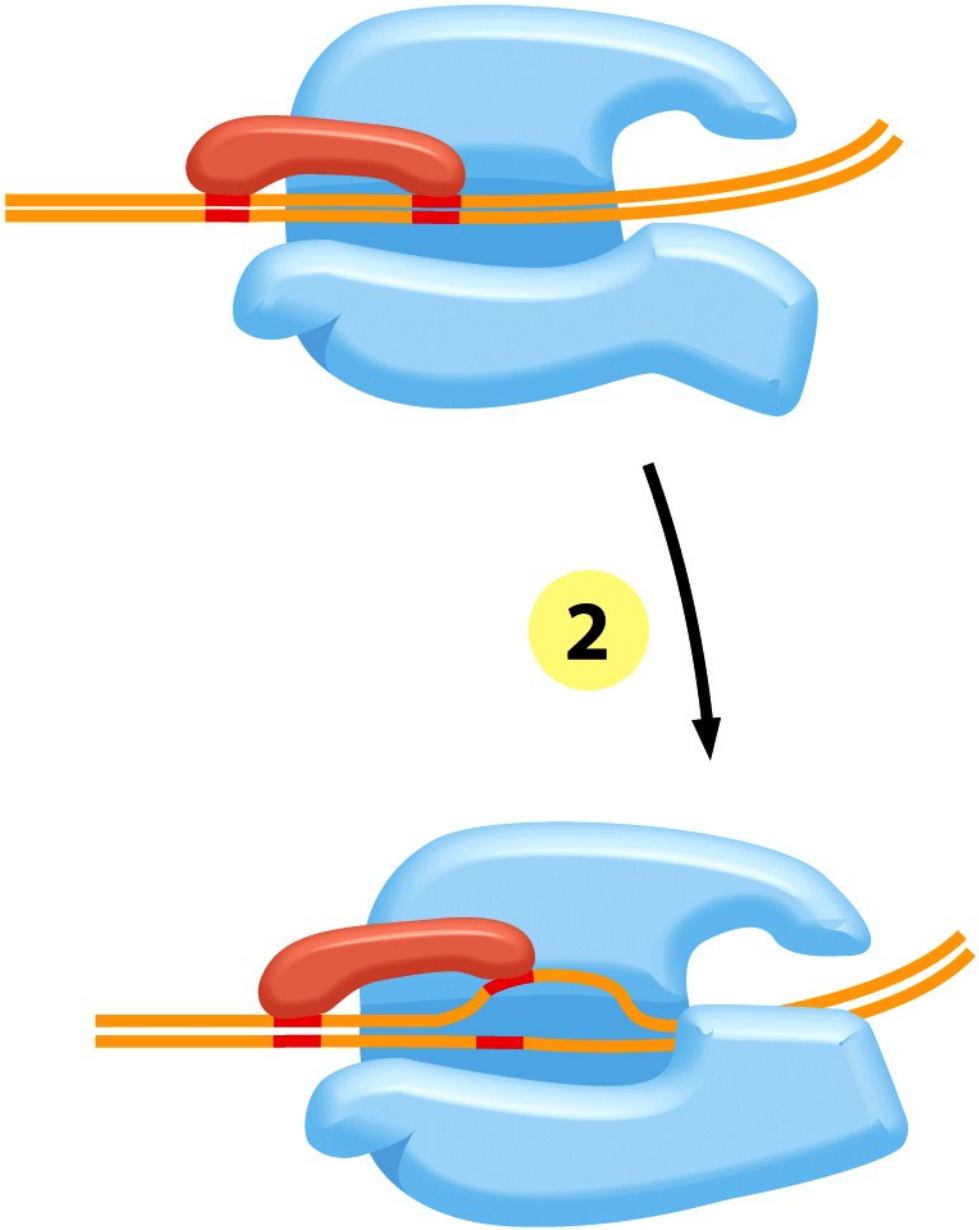


Figure 6-11 part 2 of 7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

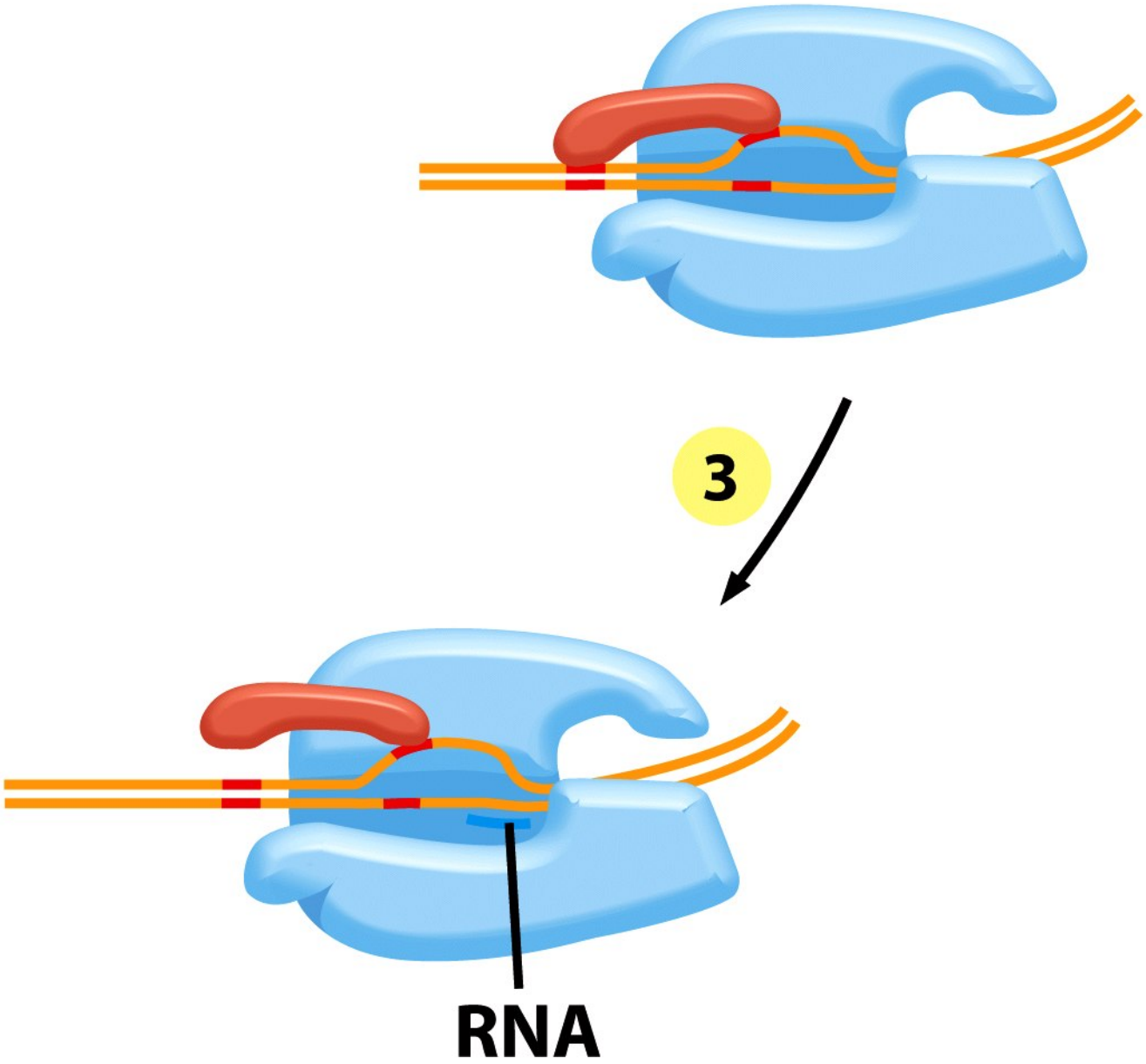


Figure 6-11 part 3 of 7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

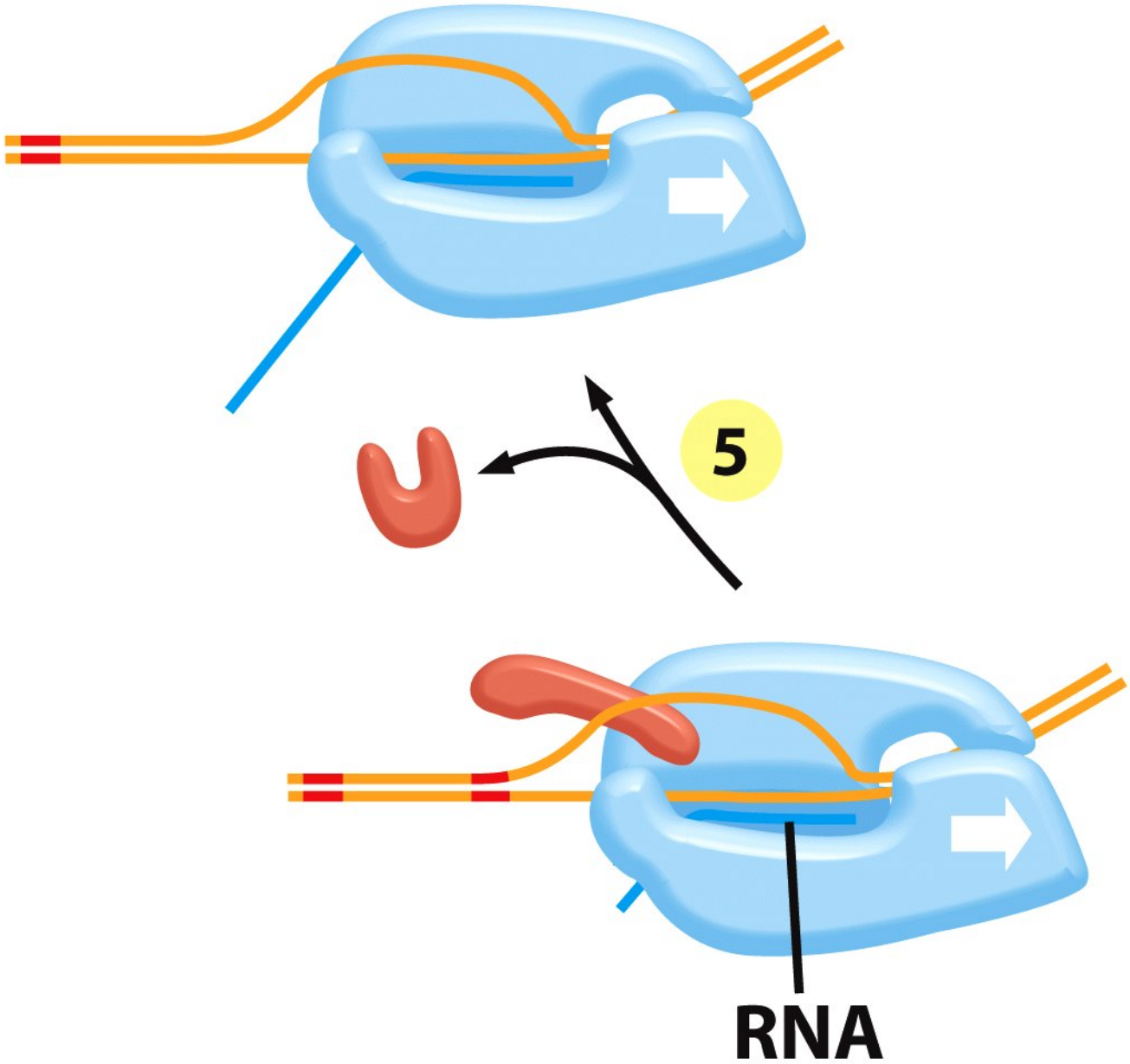


Figure 6-11 part 5 of 7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

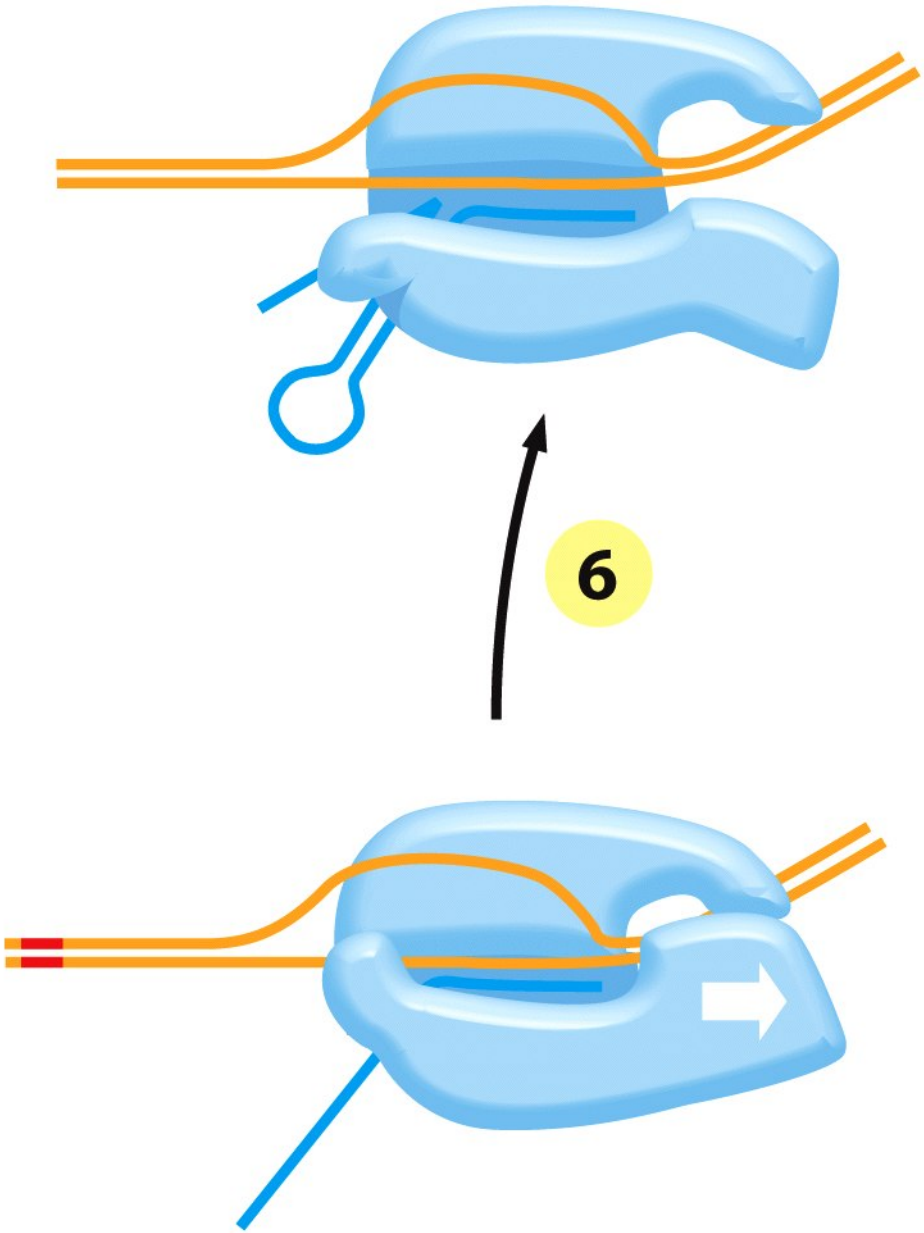


Figure 6-11 part 6 of 7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

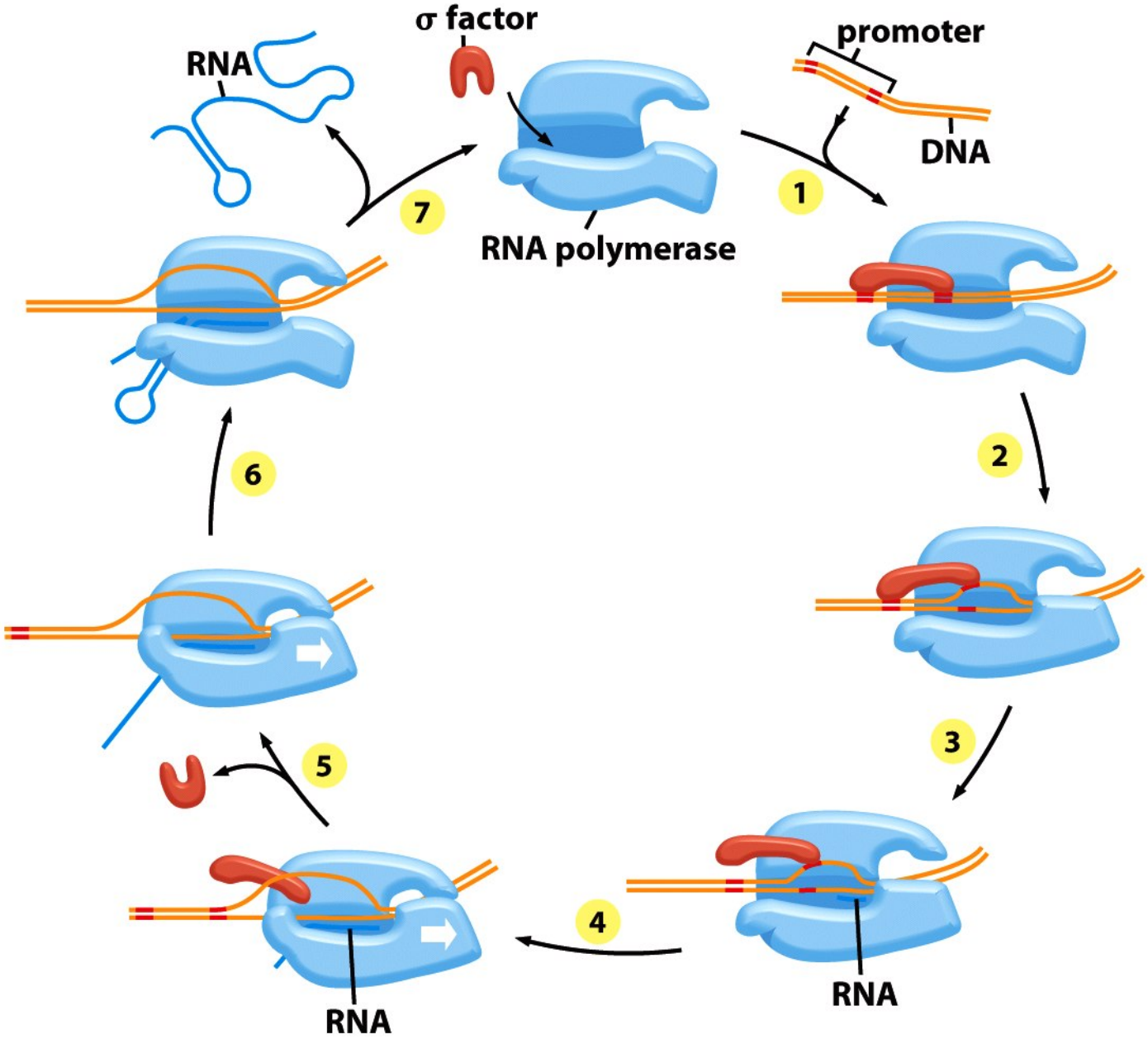
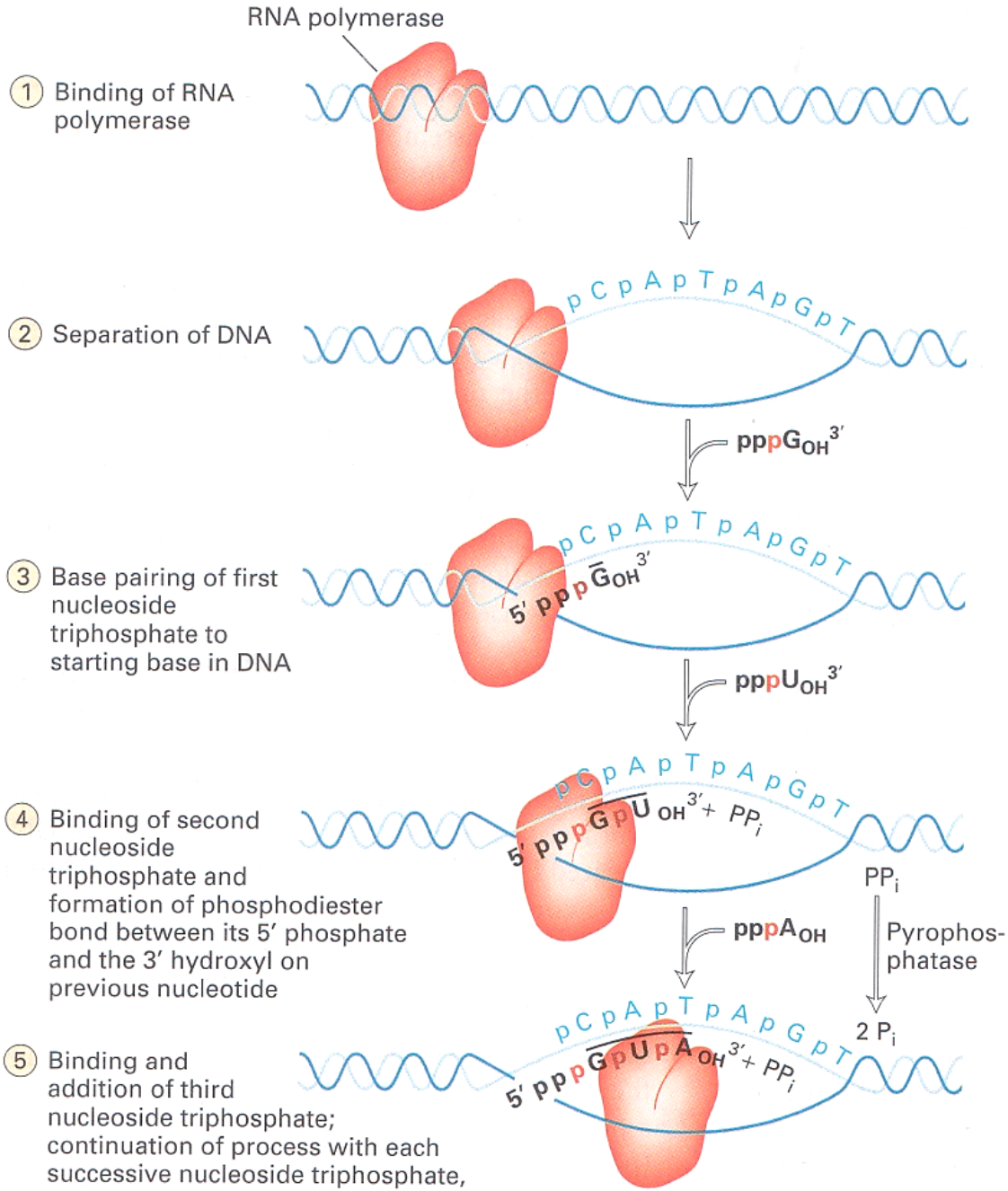
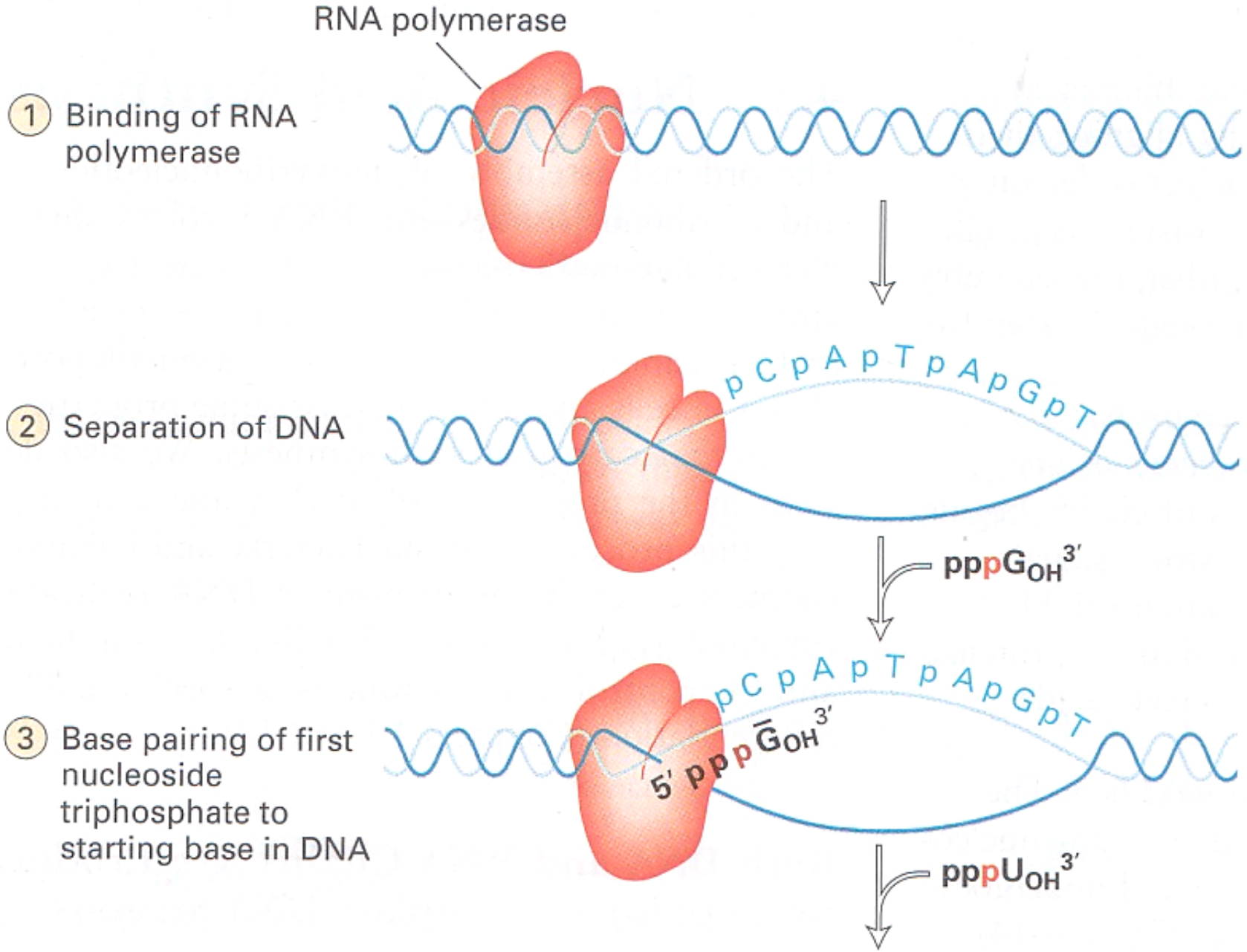


Figure 6-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)


RNA polymerase
1 Binding of RNA polymerase
2 Separation of DNA
pCpApTpApGpT
pppGOH3′
3 Base pairing of first nucleoside triphosphate to starting base in DNA
5′ pppGOH3′
pppUOH3′
4 Binding of second nucleoside triphosphate and formation of phosphodiester bond between its 5′ phosphate and the 3′ hydroxyl on previous nucleotide
5′ pppGpUOH3′ + PPi
PPi
pppAOH
Pyrophos-phatase
2 Pi
5 Binding and addition of third nucleoside triphosphate; continuation of process with each successive nucleoside triphosphate, as RNA polymerase moves along template DNA
5′ pppGpUpAOH3′ + PPi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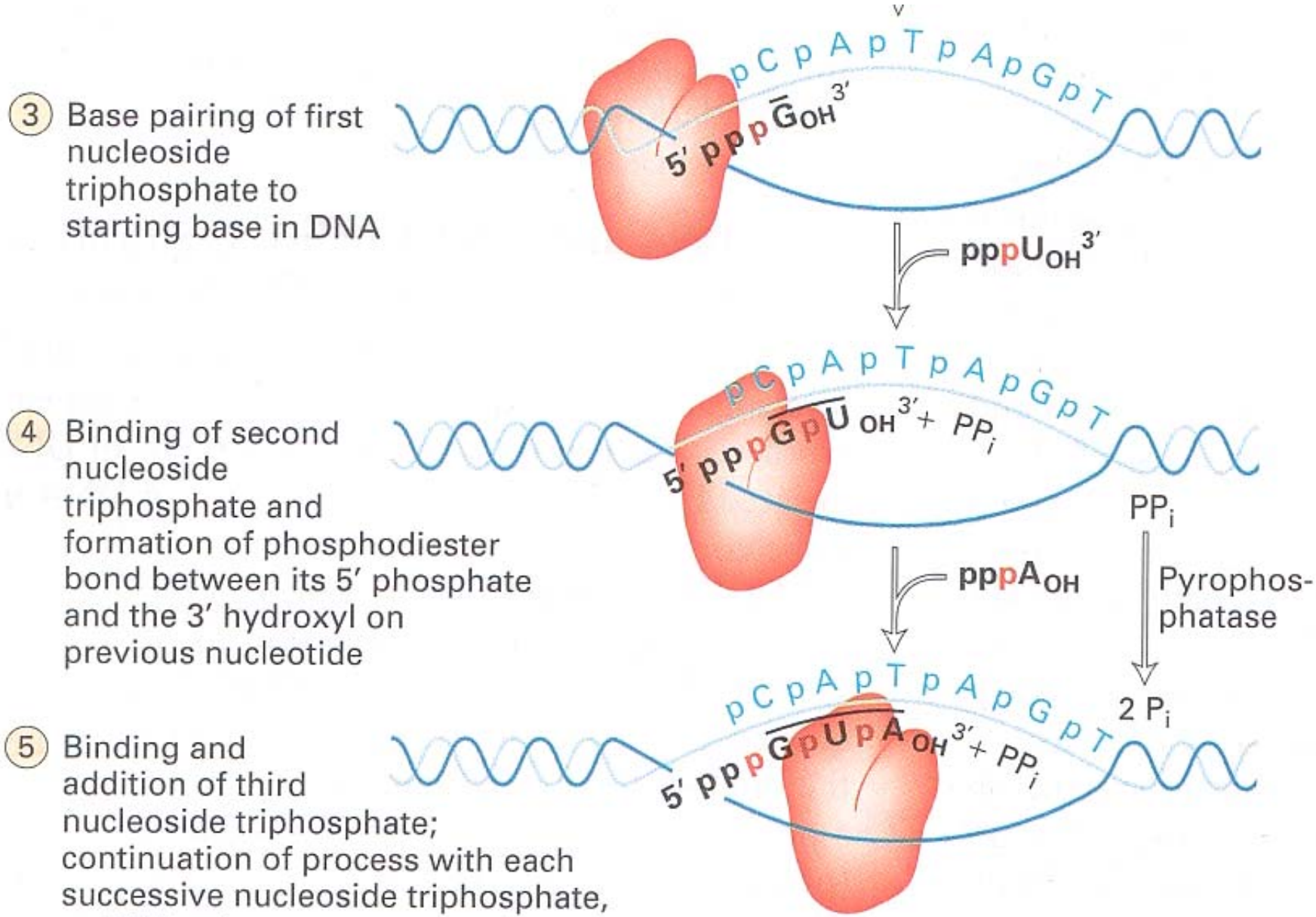
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Pyrophos-phatase
2 Pi
5 Binding and addition of third nucleoside triphosphate; continuation of process with each successive nucleoside triphosphate, as RNA polymerase moves along template DNA
5' pppGpUpA OH 3' + PPi

Myös RNA voi polymeroitua vain 3’ päähän

Transkription jälkeen *eukaryootin* mRNA:ta aina prosessoidaan:

- mG-cap eli pipo (CELL 346-7)

- poly-A häntä (CELL 346, 357)

- splicing (CELL 347---)

- mRNA viedään sitten ulos tumasta (CELL 359)

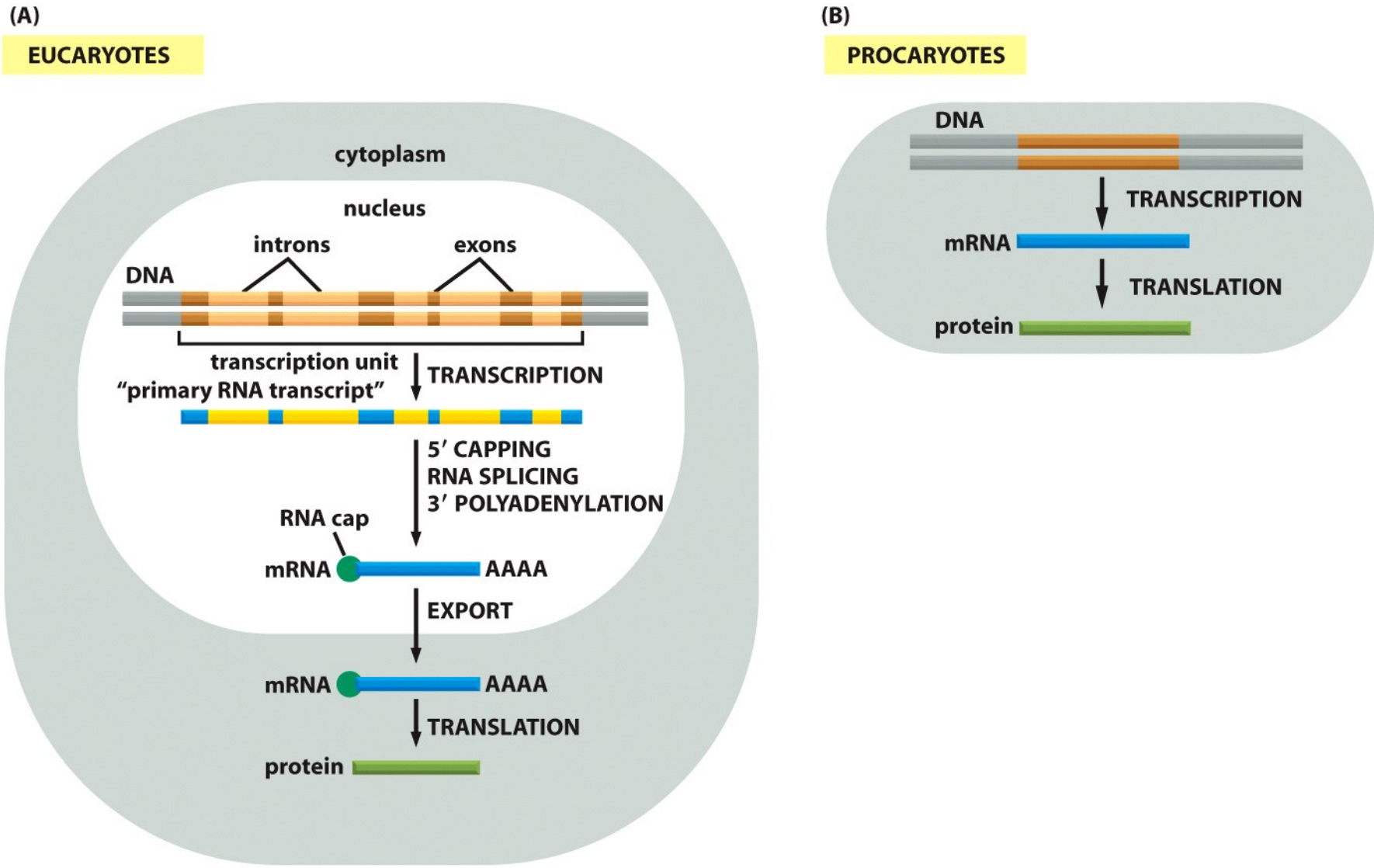


Figure 6-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 345

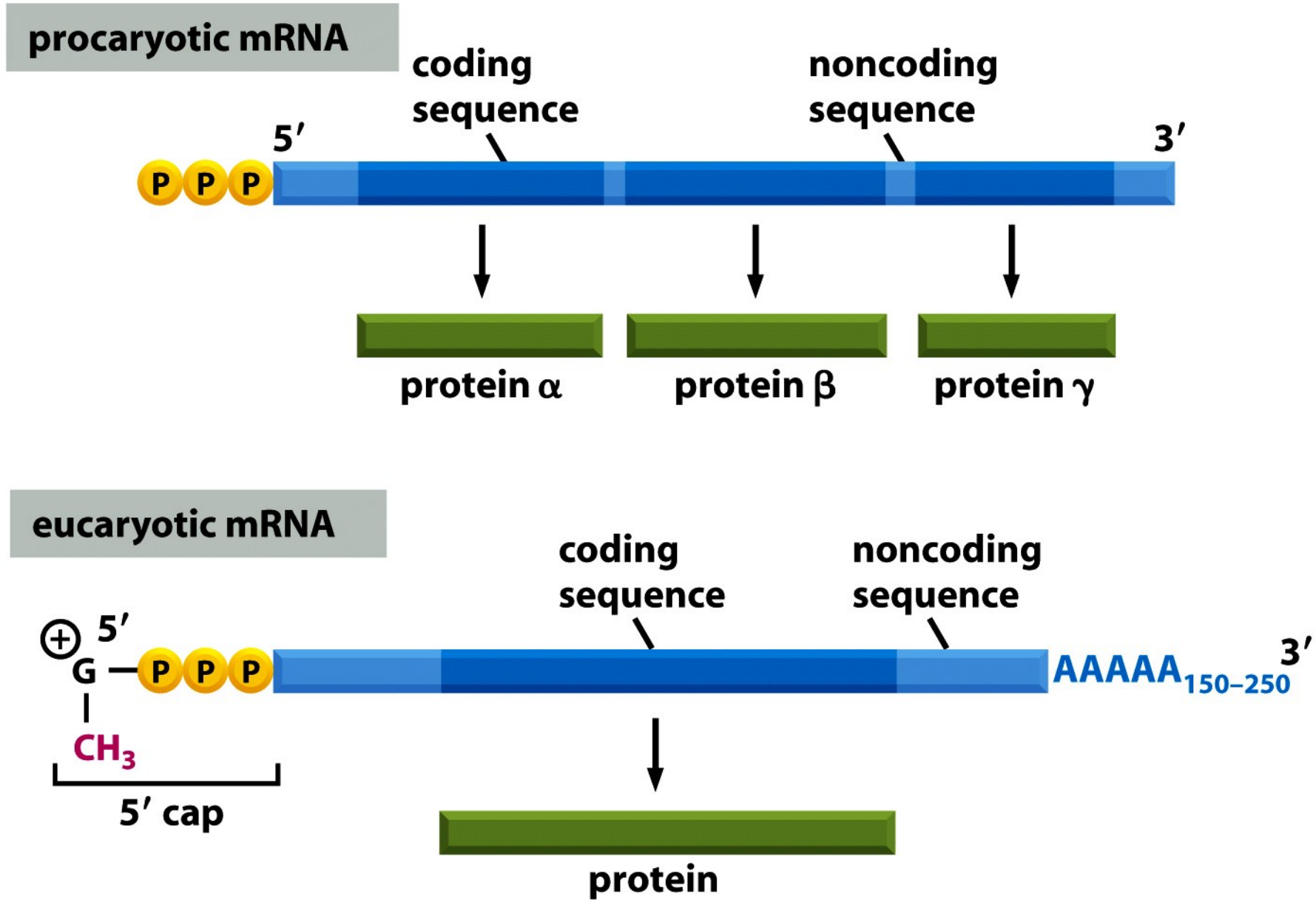


Figure 6-22a Molecular Biology of the Cell 5/e (© Garland Science 2008)

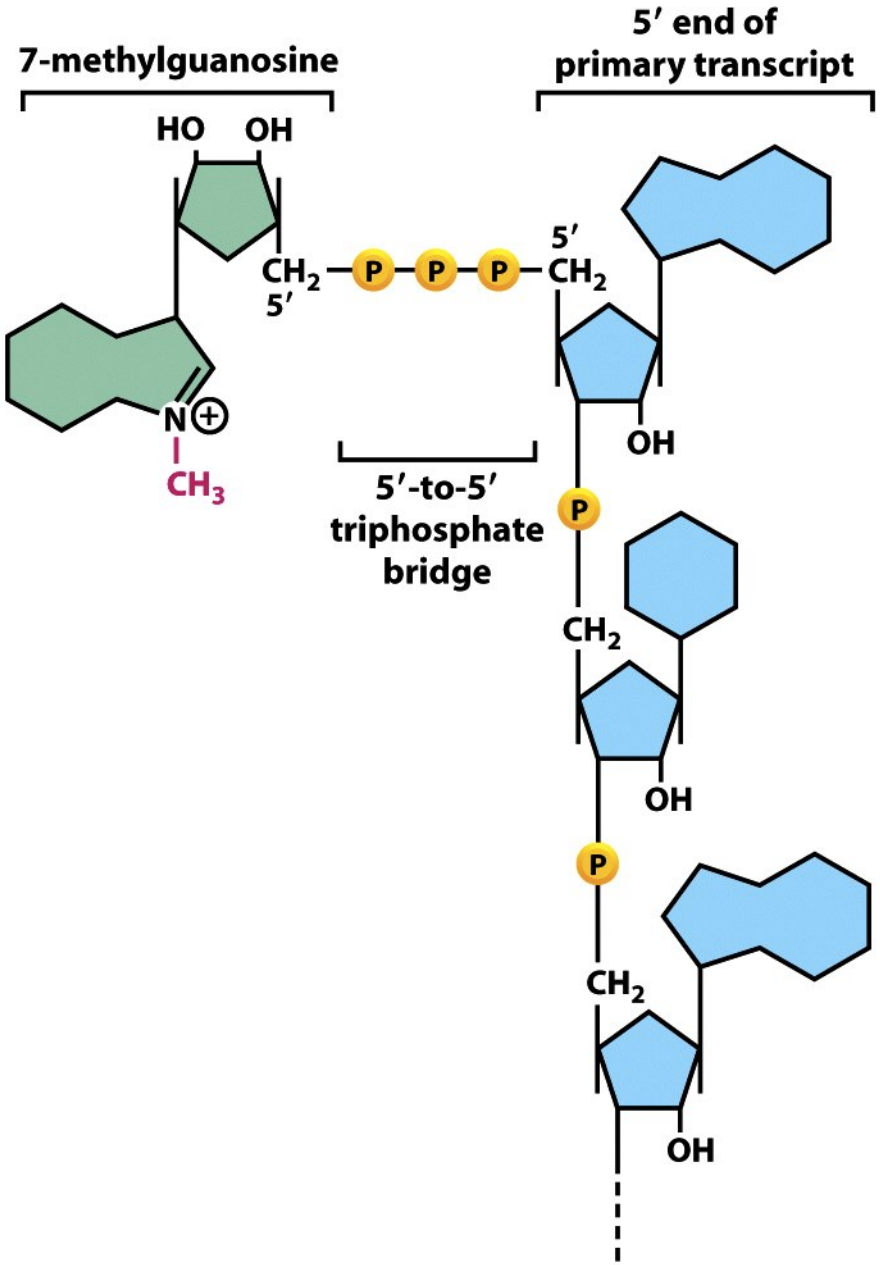


Figure 6-22b Molecular Biology of the Cell 5/e (© Garland Science 2008)

mRNA:n 5' -päähän lisätään **cap** (pipo), m^7G

Huomatkaa, että liitos on aivan epätavallinen: siinä on 3 fosfaattia, mitä muuten ei tapahdu koskaan!

Pipo on merkkinä siitä, että tässä nyt on tahallaan tuotettu ja toimintaan valmis RNA-molekyyli eikä mikä tahansa jäte tai tunkeilija. Ilman sitä RNA syötäisiin pois

CELL 346

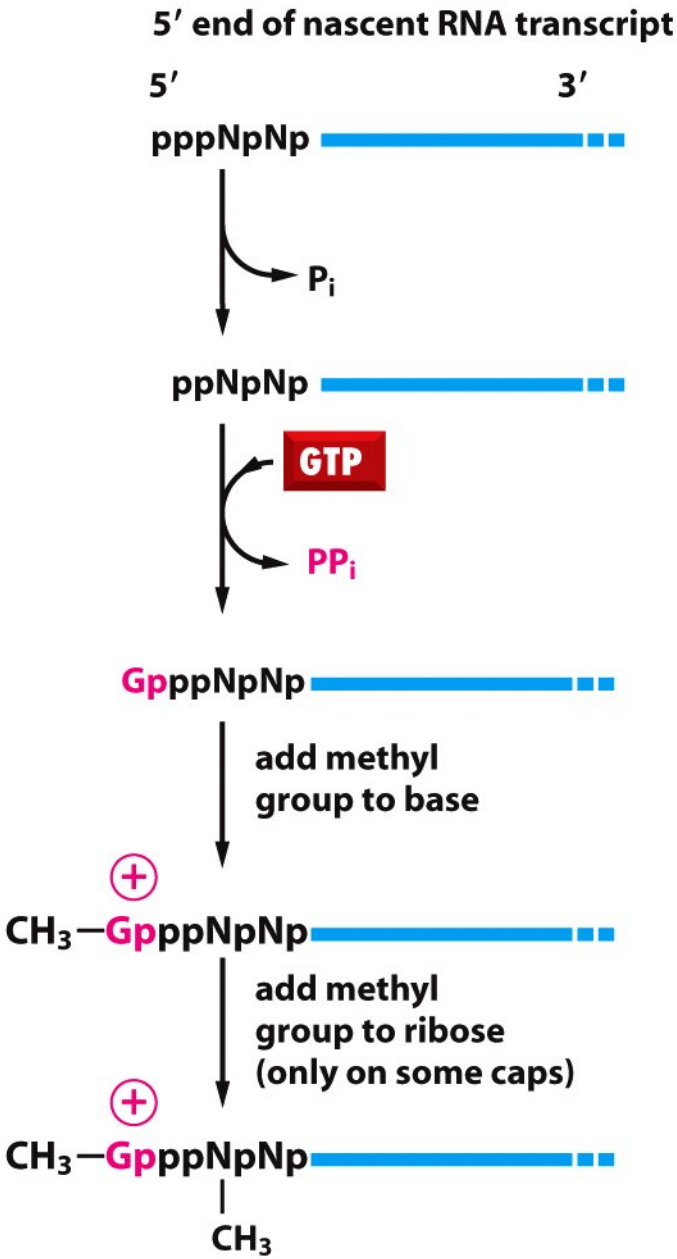


Figure 6-24 Molecular Biology of the Cell 5/e (© Garland Science 2008)

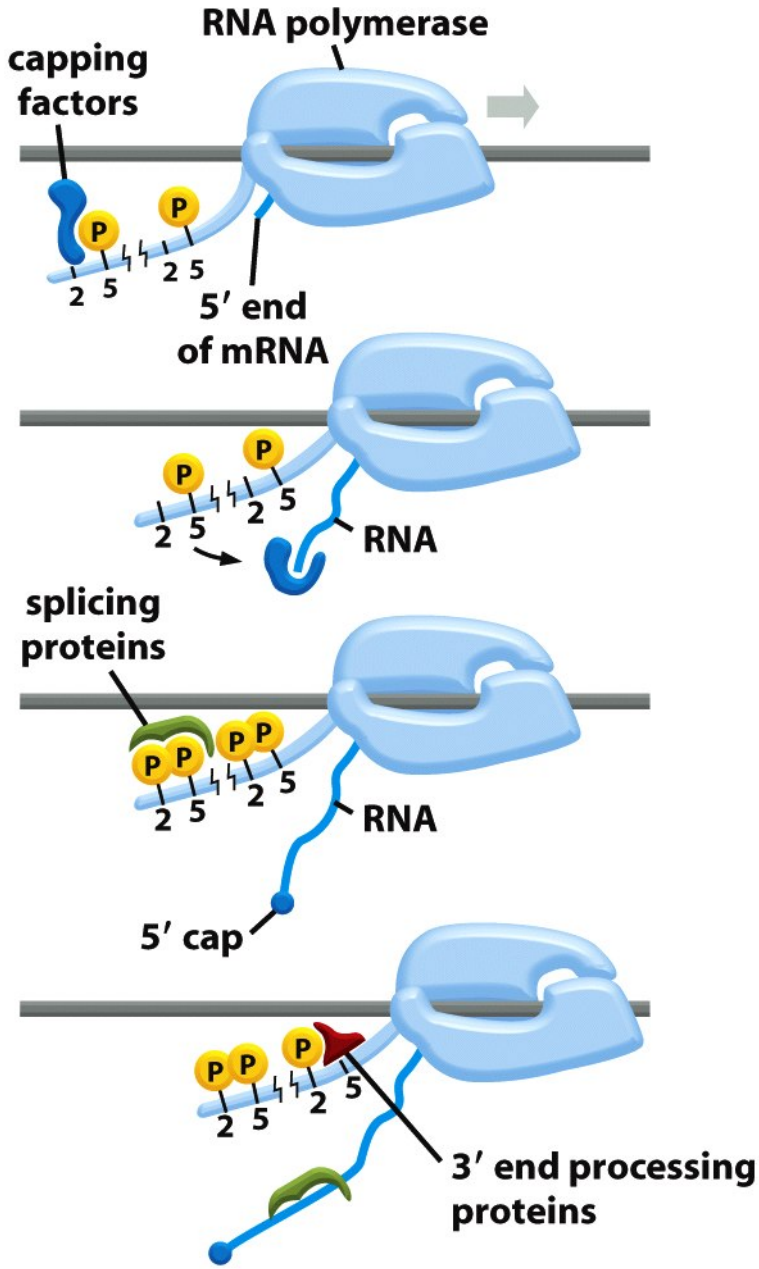
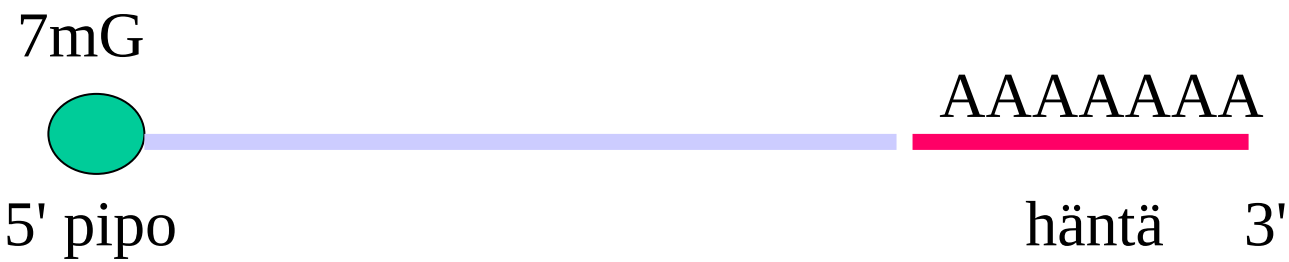


Figure 6-23 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Lähetti-RNA:n toiseen päähän lisätään häntä, eli pitkä jakso Adeniiniemäksiä: **polyA tail**



Häntää ei ole koodattu DNA:han, signaali kyllä

CELL 327

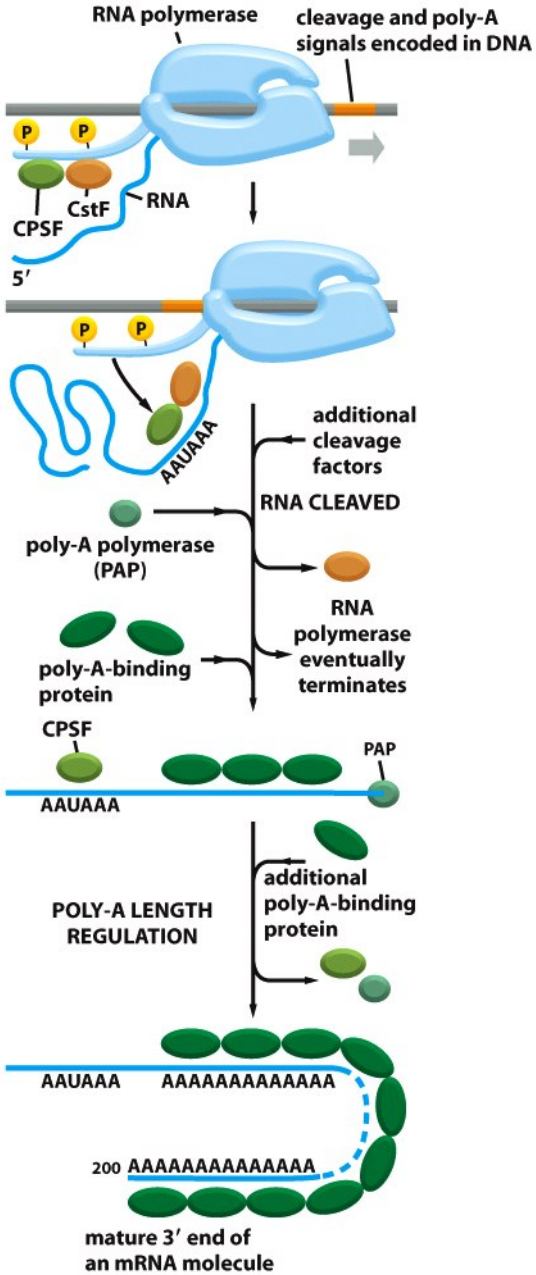


Figure 6-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 357

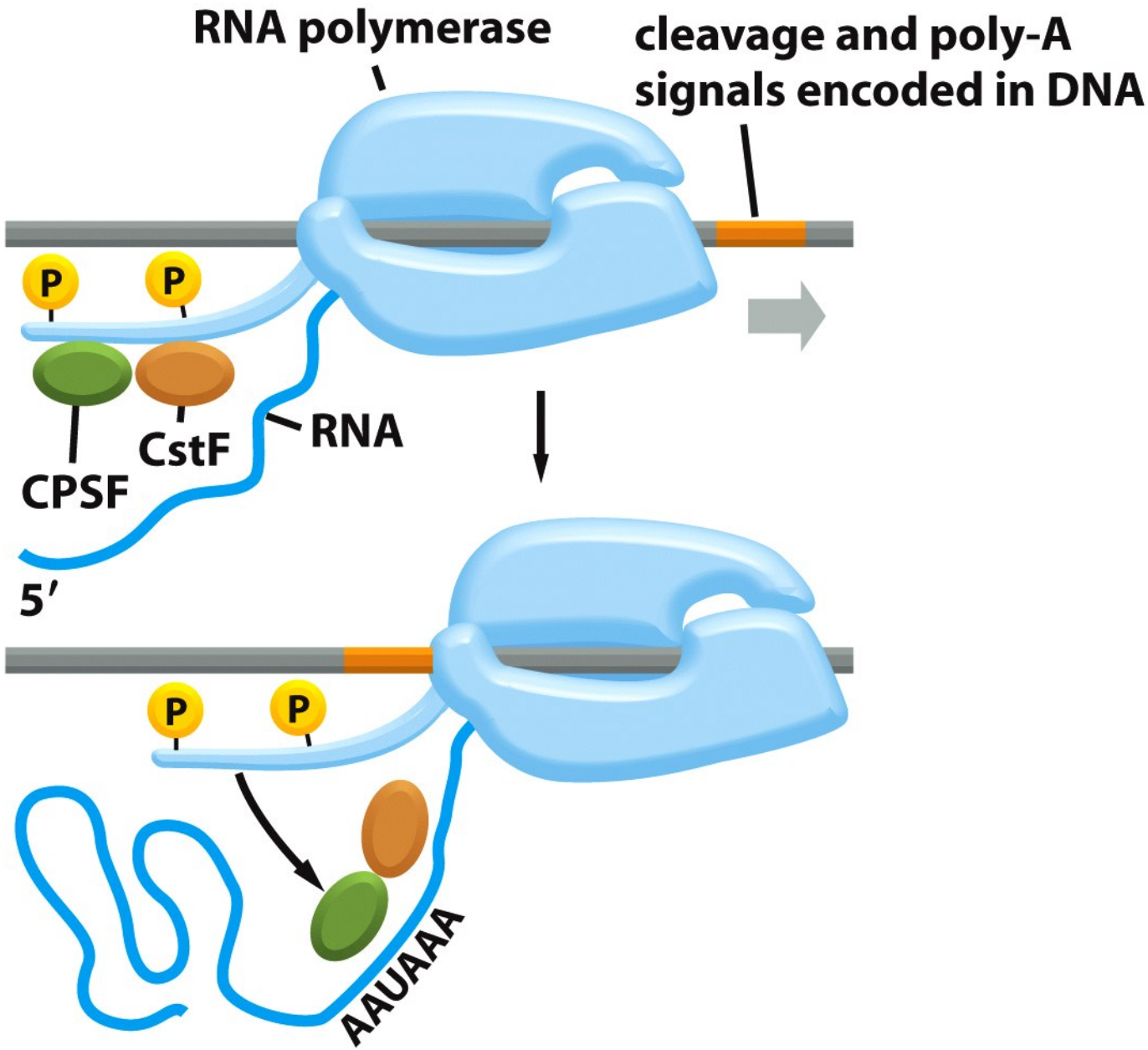


Figure 6-38 part 1 fo 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

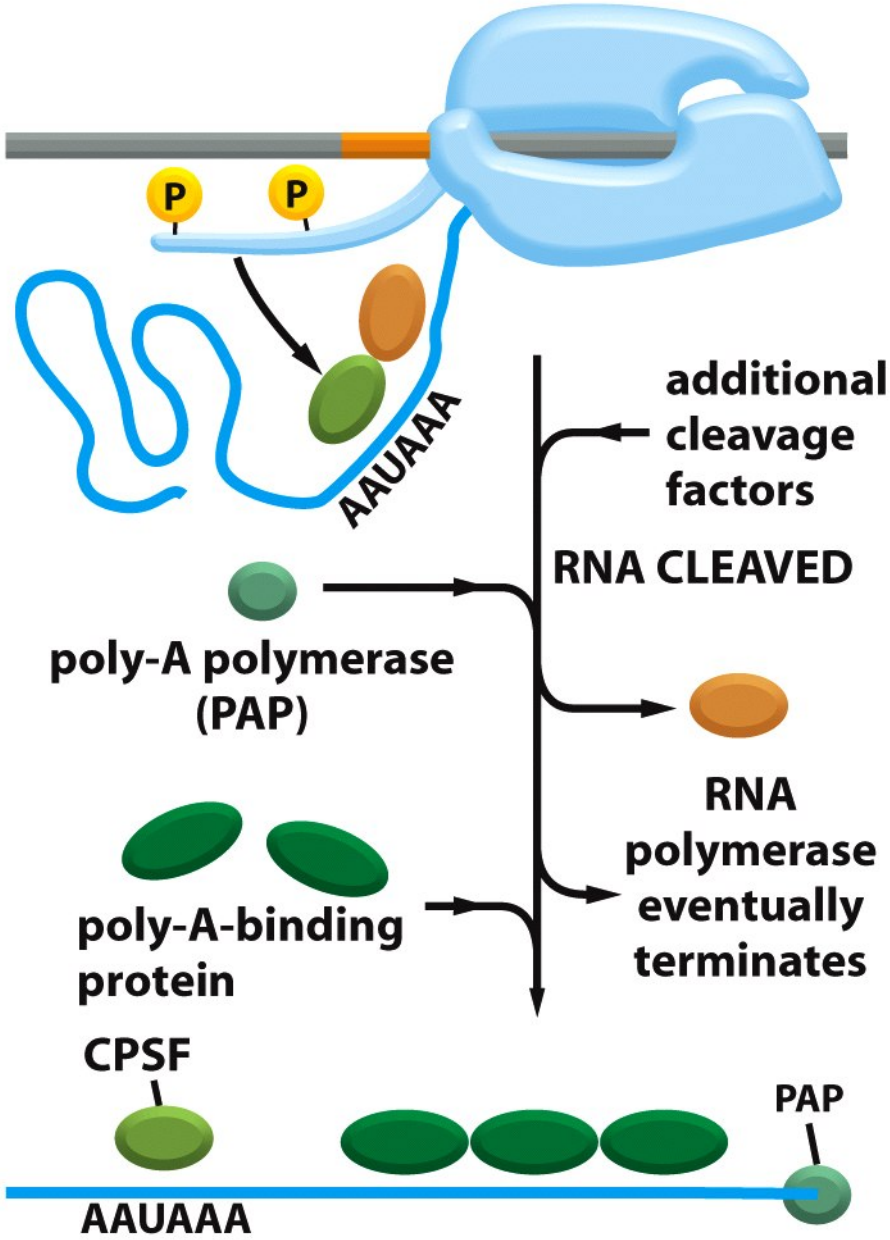


Figure 6-38 part 2 fo 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

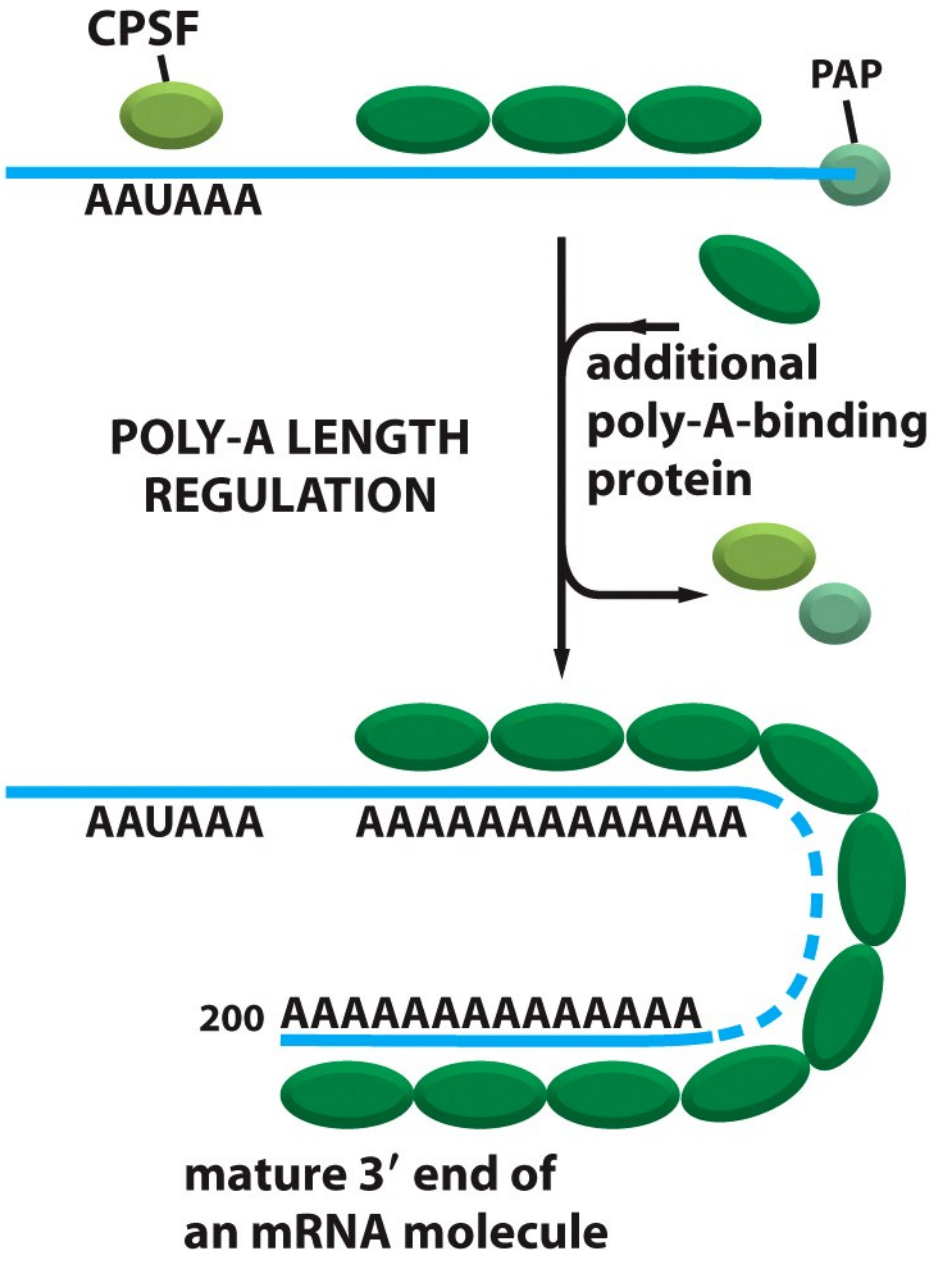
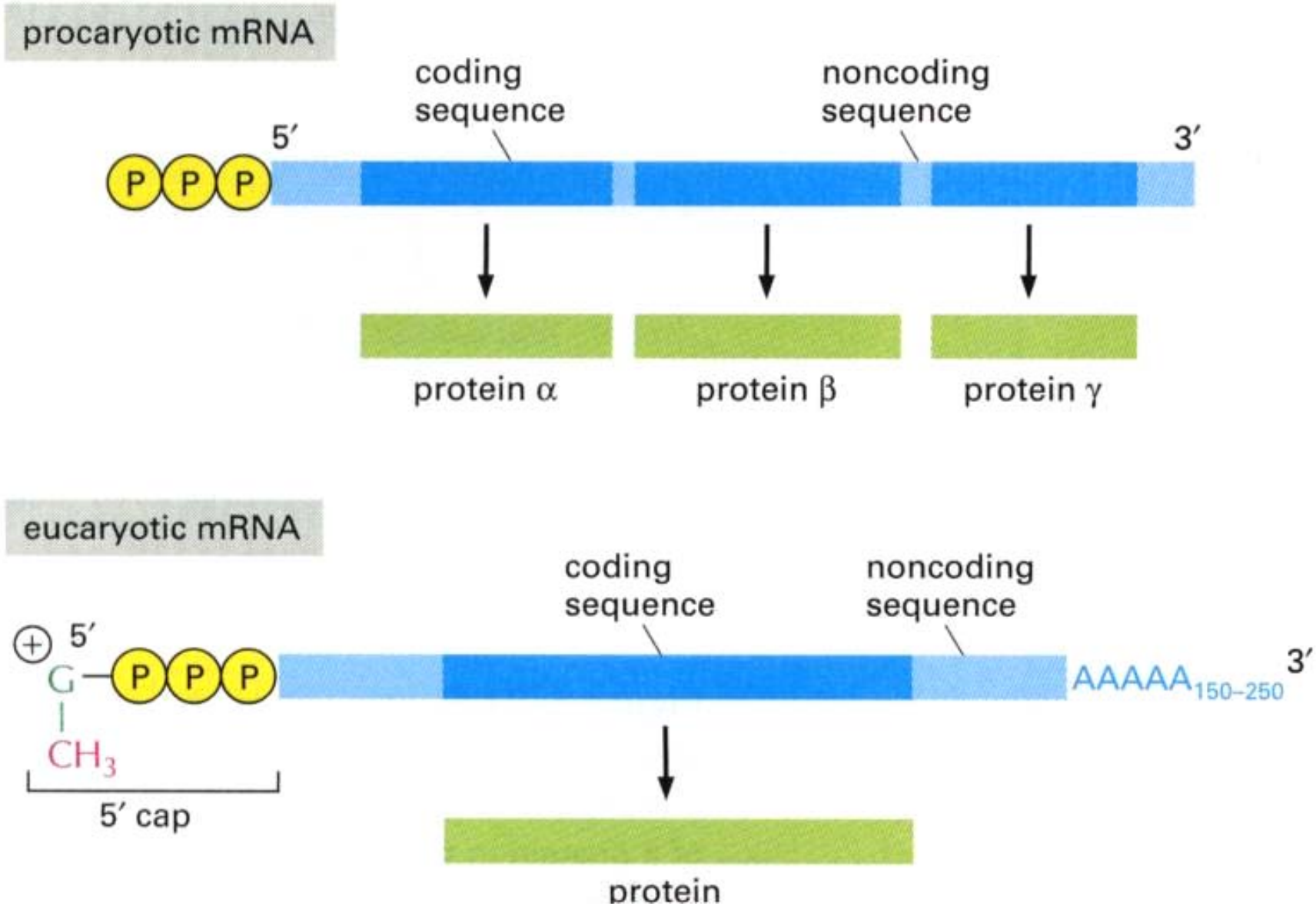


Figure 6-38 part 3 fo 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Pro- ja eukaryootin mRNA on aika paljon erilainen

Kuljetus

mRNA tuotetaan eukaryootilla tumassa ja translaatio tapahtuu aina ”soluliman” puolella, joten mRNA on kuljetettava ulos

Tumakotelon huokosen liikenteeseen tutustuttiin jo solubilsan kurssilla

Solubilsan slidet ovat verkossa, tarvitsette kyllä kertausta

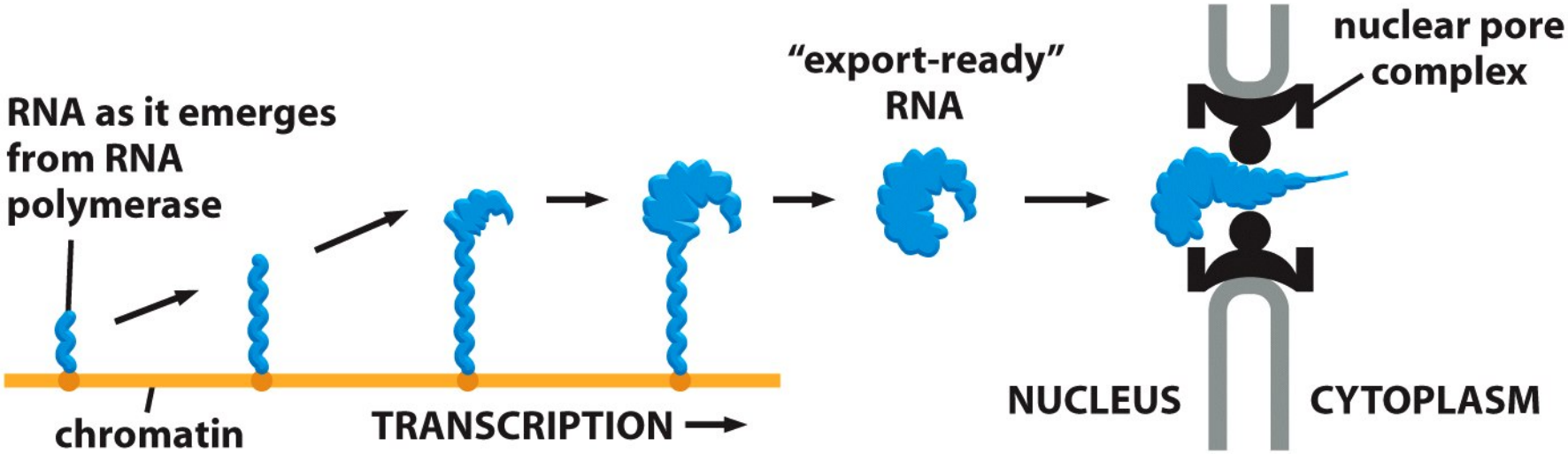


Figure 6-39a Molecular Biology of the Cell 5/e (© Garland Science 2008)

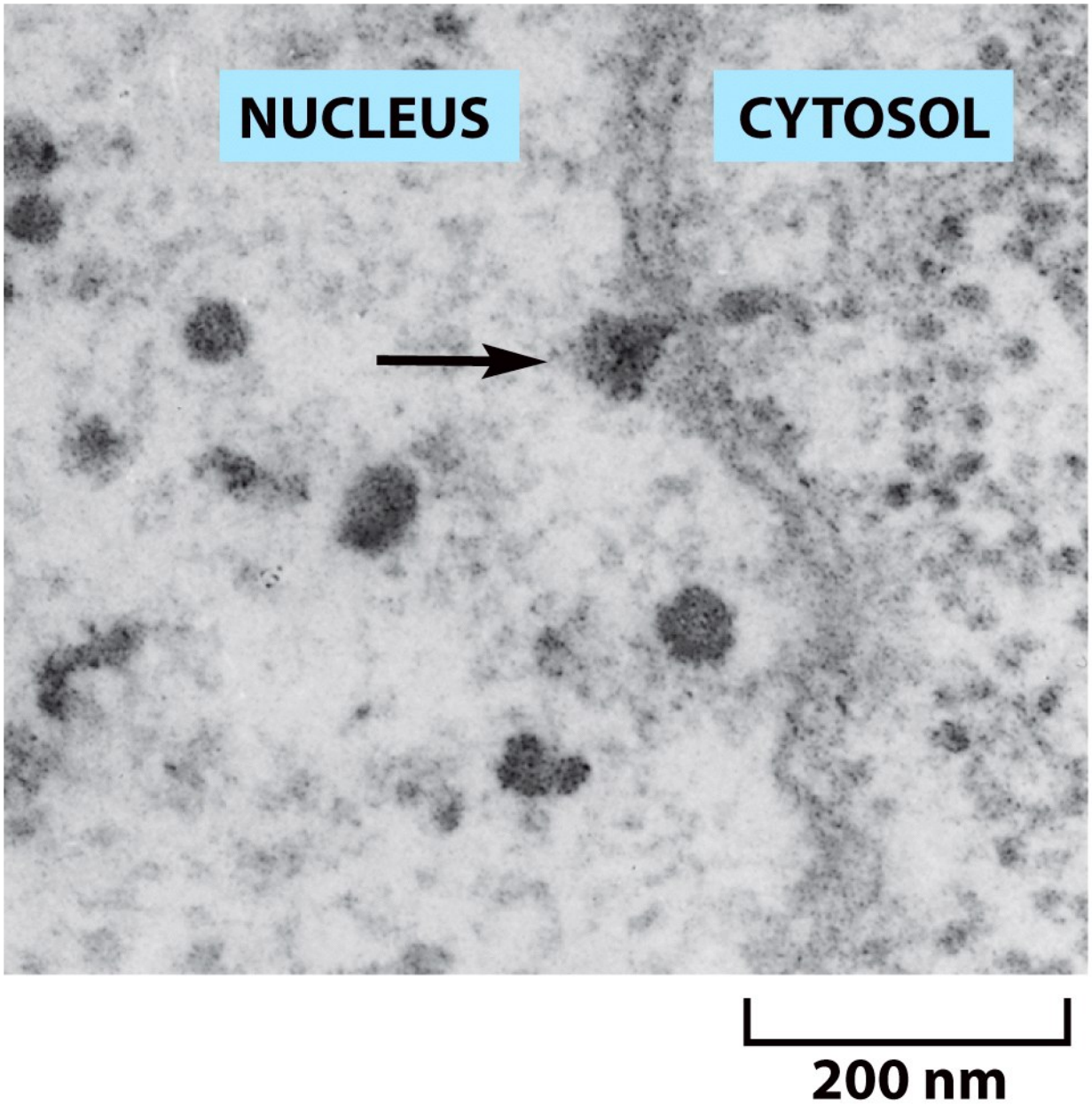


Figure 6-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 359

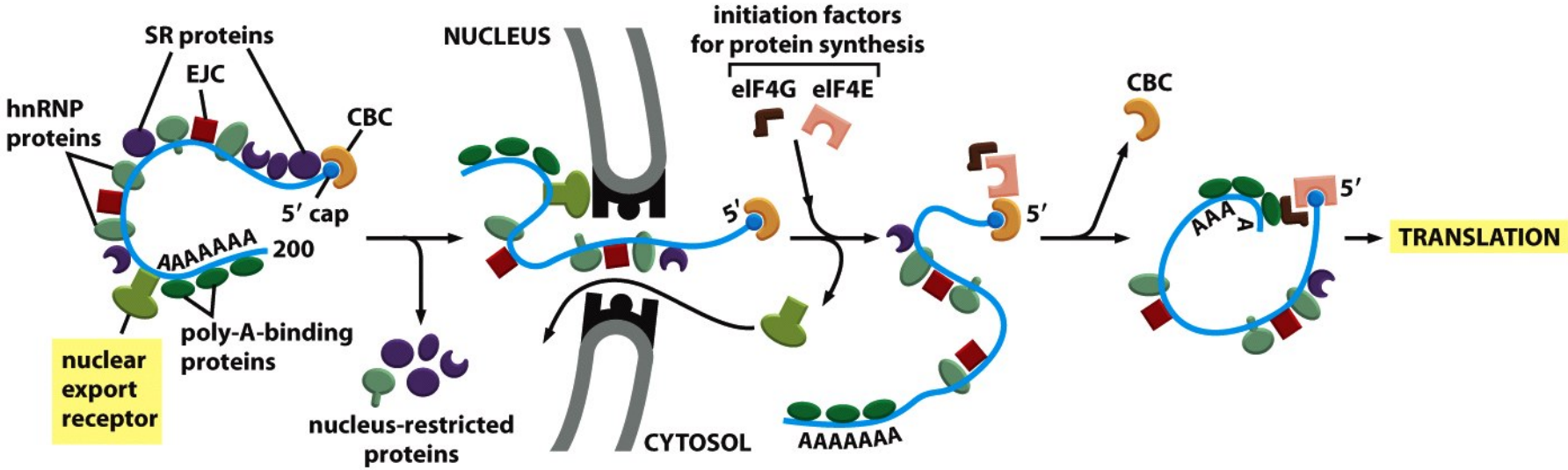
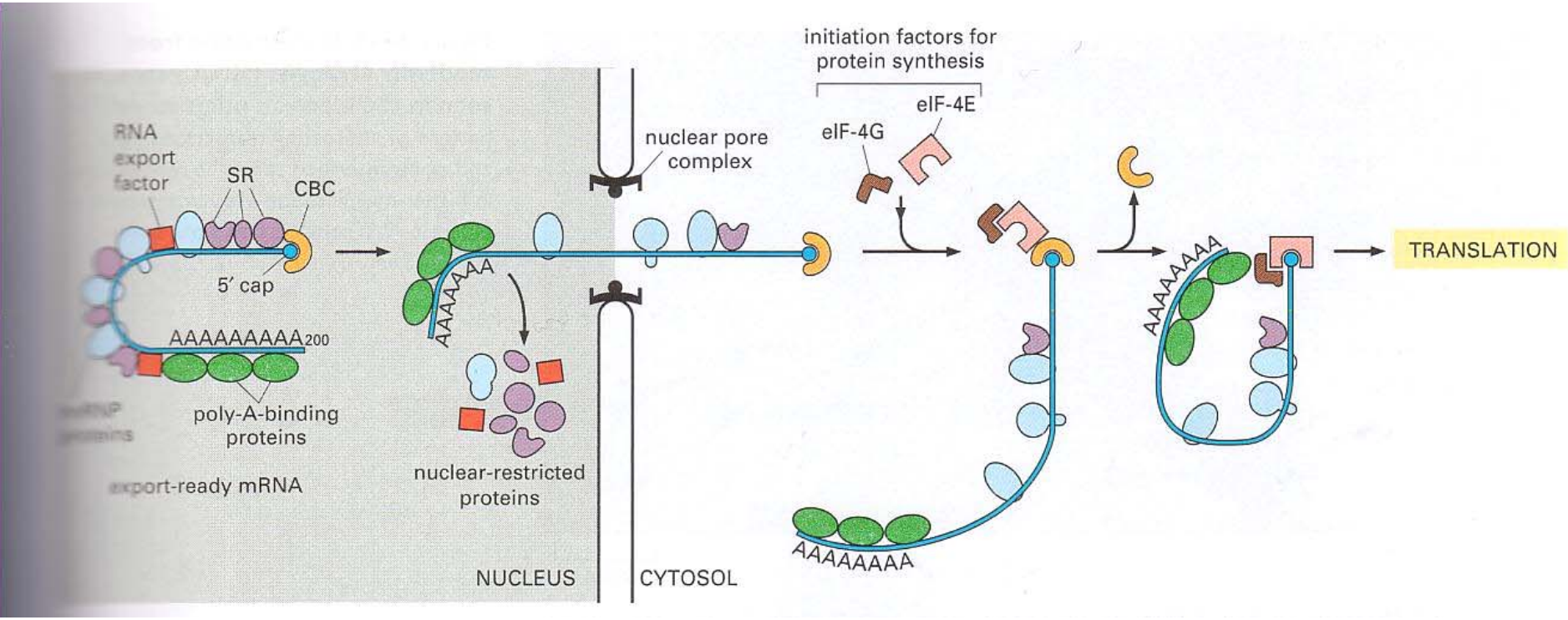


Figure 6-40 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 359

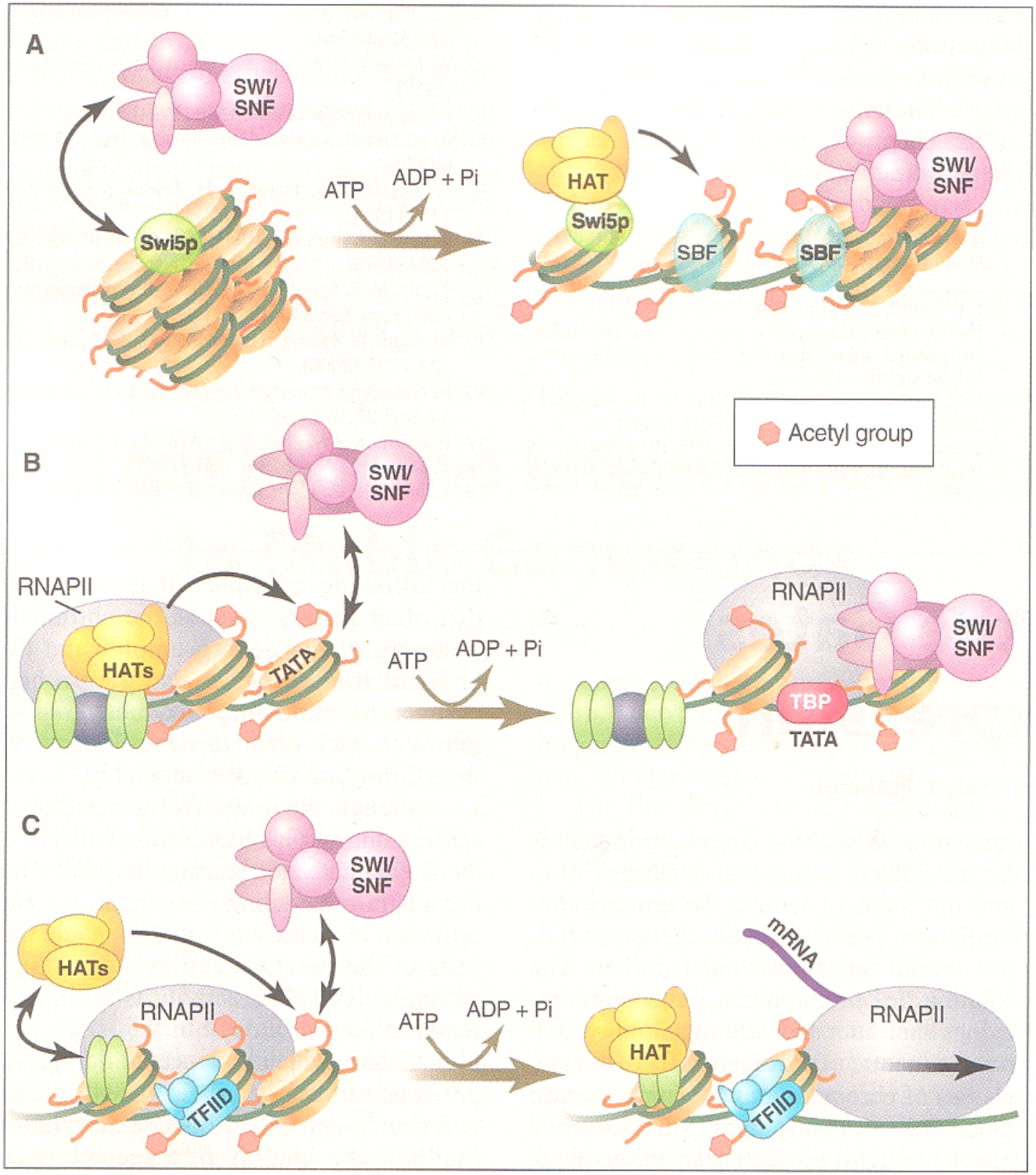

initiation factors for protein synthesis
eIF-4E
eIF-4G
RNA export factor
SR
CBC
nuclear pore complex
5′ cap
AAAAAAAAA200
poly-A-binding proteins
export-ready mRNA
AAAAAAA
nuclear-restricted proteins
NUCLEUS
CYTOSOL
AAAAAAAA
AAAAAAAA
TRANSLATION

CELL 359

Kaikissa kuvissa DNA on transkription aikana kaksiulotteisena tekstiversiona, vaikka se todellisuudessa on histonipillereiden ympärillä. Seuraavissa kuvissa avaruudelliseen geometria-ajatteluun pystyvät tieteilijät ankarasti pohtivat näitä asioita

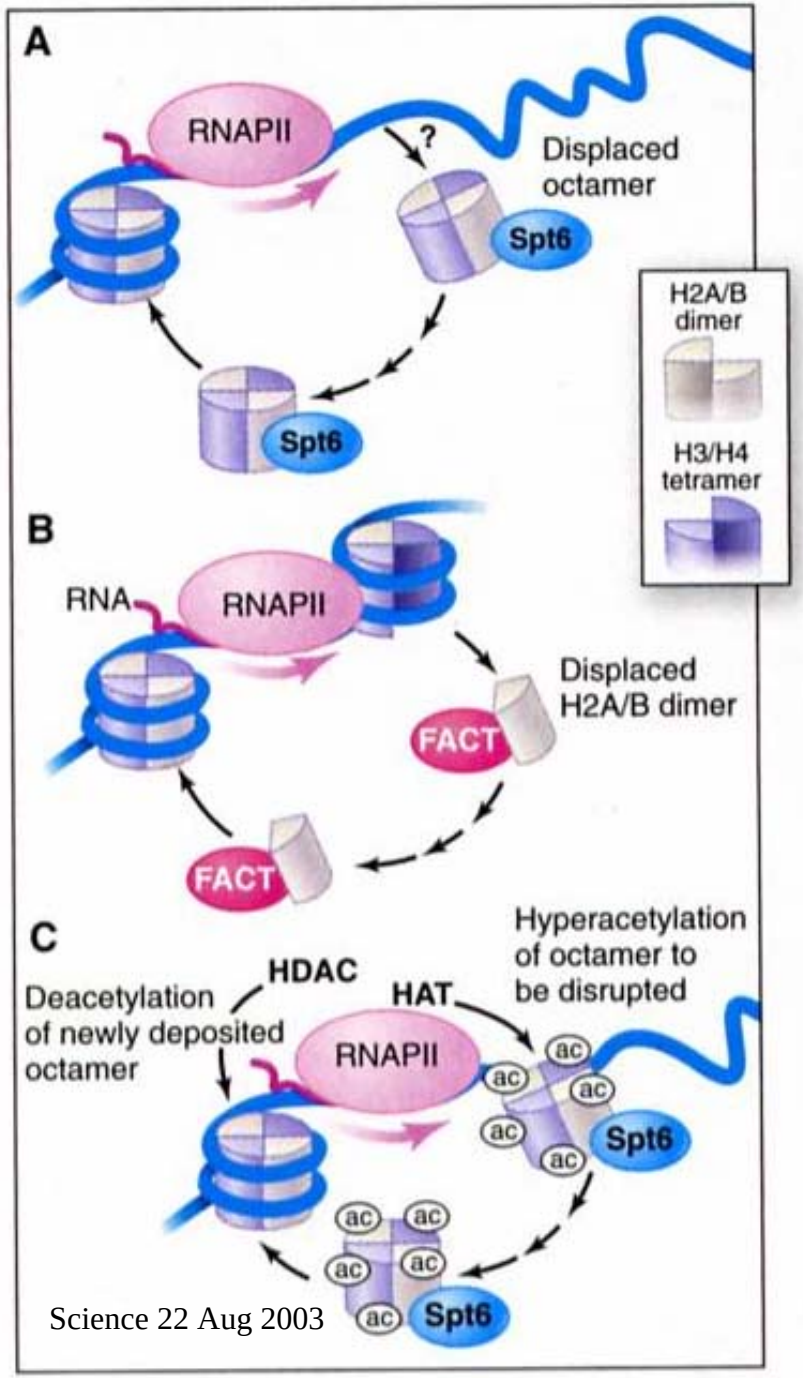
Supercoiling-ilmiö liittyy tähän ongelmavyyhteen

Science 8 March 2002



Todellisuudessa transkriptio tapahtuu 30 nm juosteessa, joten nukleosomien ohittaminen on ainakin tutkijoille päänvaiva, joskin solu taitaa selvitä siitä muina miehinä, sen kummemmin ajattelematta, vai mitä? Myös kierteet pohdituttavat, kuten **CELL 314** osoittaa

Science 8 March 2002



Transkription aikana **nukleosomit** on joko purettava tai siirrettävä, jotta RNA polymeraasi II voisi tehdä hommaansa. Se lukee kaikki proteiinia koodaavat geenit, snoRNA-geenit ja jotkut snRNA-geeneistä

RNA-polymeraasi III lukee geenit niin että nukleosomit väistyvät, sanoo sama lehti. Se alaa ovat tRNA, 5SrRNA, jotkut snRNA ja muut pienet RNAt

CELL 310-313

DNA with free end

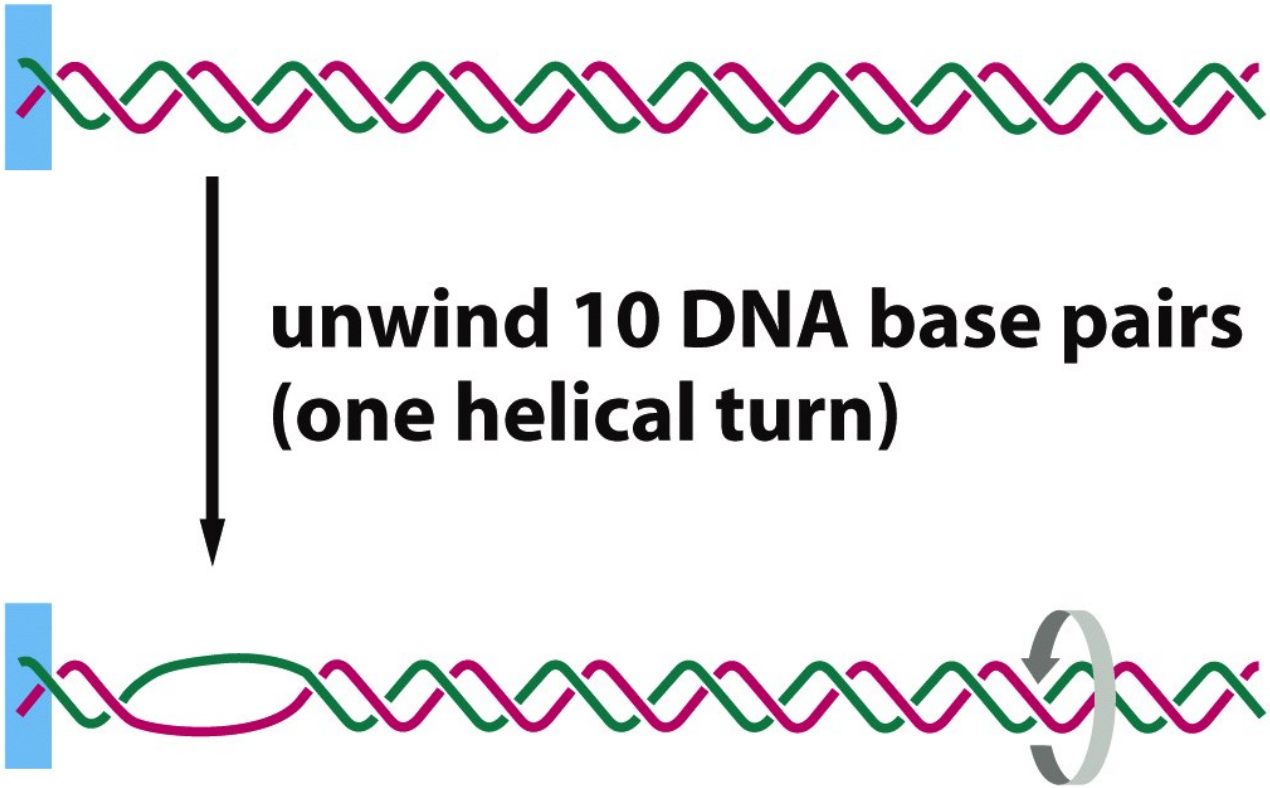


Figure 6-20a Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNA with fixed ends

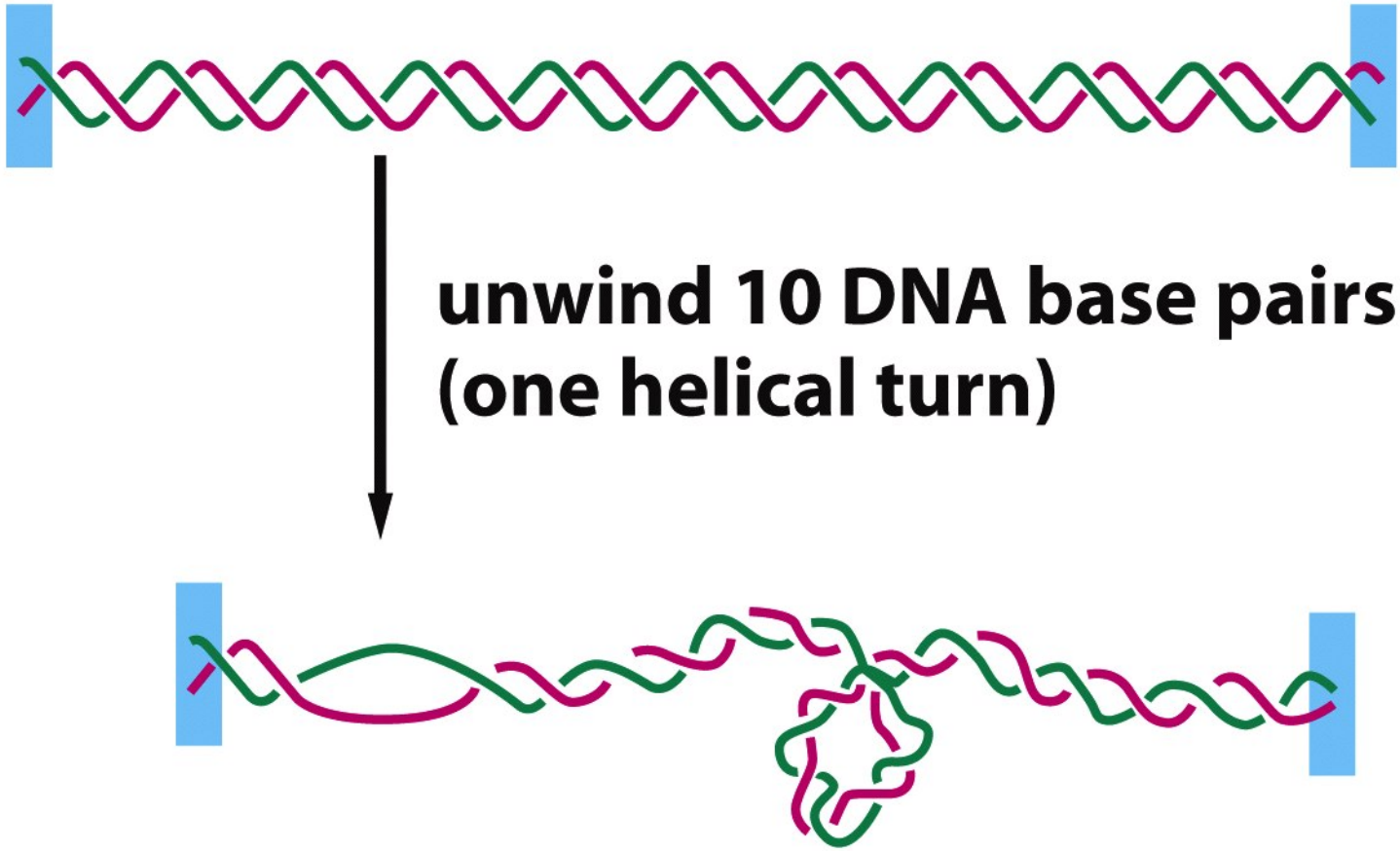


Figure 6-20b Molecular Biology of the Cell 5/e (© Garland Science 2008)

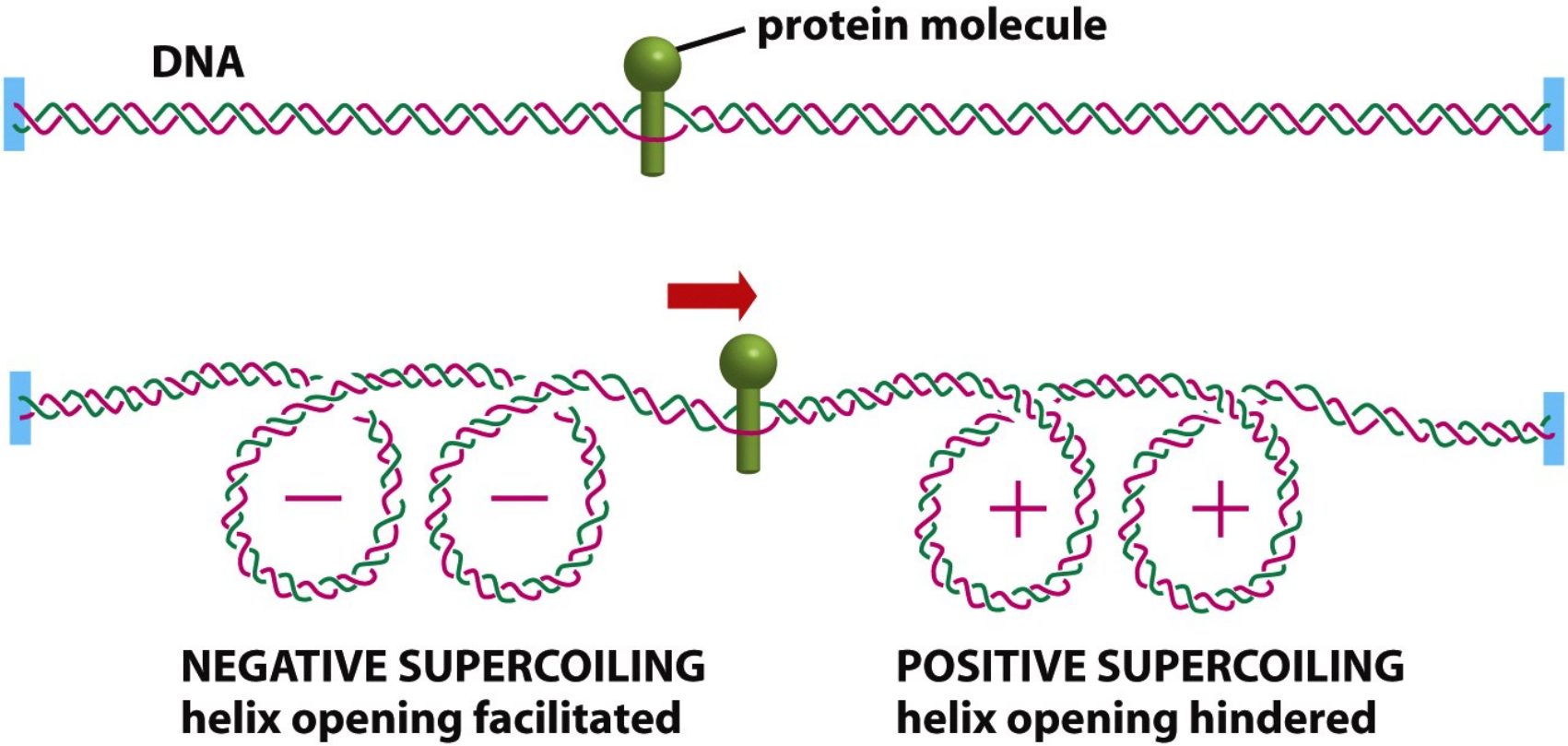


Figure 6-20c Molecular Biology of the Cell 5/e (© Garland Science 2008)