26 RNA Metabolism

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Principles of BIOCHEMISTRY

Eighth Edition

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P1 Principle 1 (1 of 5)

RNA is synthesized by RNA polymerases using DNA templates and ribonucleoside 5′-triphosphates. RNA is made in the 5′→3′ direction and complementary to the template DNA strand. Transcription is highly regulated and initiates by recruitment of the transcription machinery to gene promoters. Although bacterial and eukaryotic polymerases share many conserved features, the transcriptional machinery and its regulation are much more complex in eukaryotes.

RNA Is Synthesized by RNA Polymerases

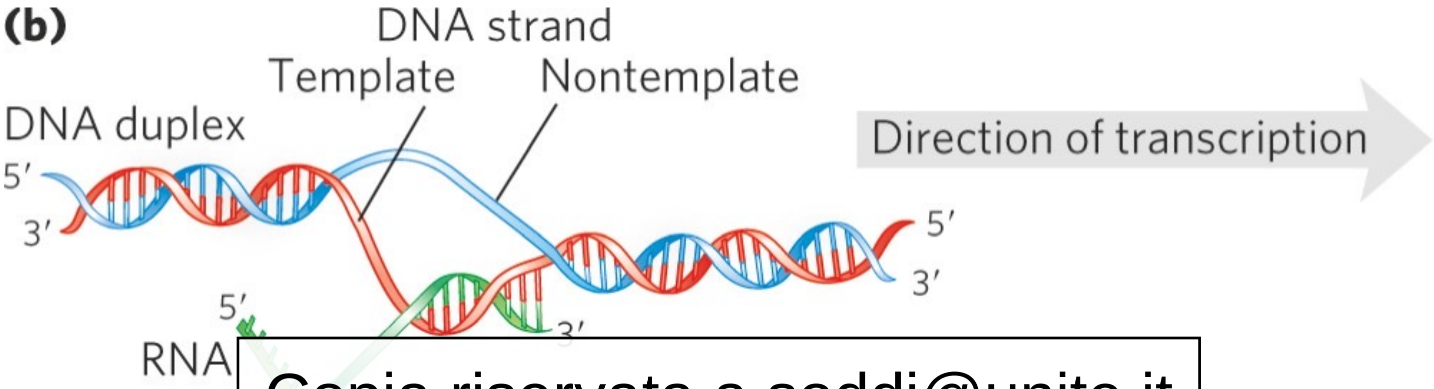
- **DNA-dependent RNA polymerase** = catalyzes transcription
  - requires a DNA template, four ribonucleoside 5′-triphosphates (ATP, GTP, UTP, and CTP), and Mg2$^+$

- RNA polymerase adds ribonucleotide units to the 3′-OH end, building RNA in the 5′→3′ direction

Initiation and Elongation of Transcription

- initiation occurs when RNA polymerase binds at promoter sequences
  - promoters are not required

- during elongation, the growing RNA strand temporarily base-pairs with the DNA template to form a short hybrid RNA-DNA double helix



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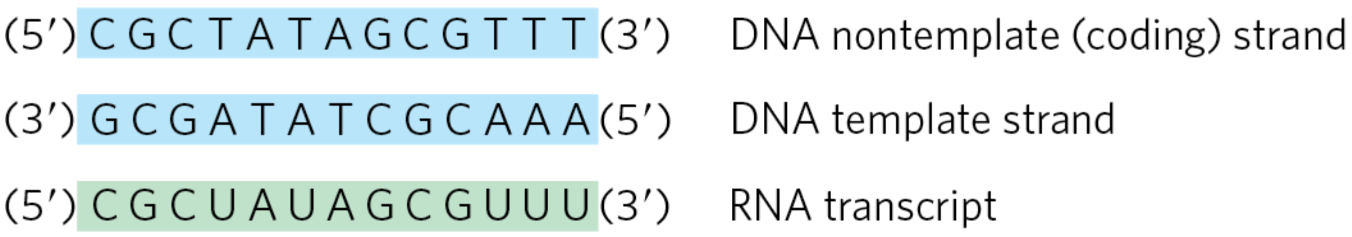
The Template and Nontemplate Strand

- **template strand** = DNA strand that serves as template for RNA synthesis

- **nontemplate strand (coding strand)** = DNA strand that is identical in base sequence to the transcribed RNA, with U in RNA in place of T in DNA



(5′) CGCTATAGCGTTT (3′) DNA nontemplate (coding) strand

(3′) GCGATATCGCAAA (5′) DNA template strand

(5′) CGCUAUAGCGUUU (3′) RNA transcript

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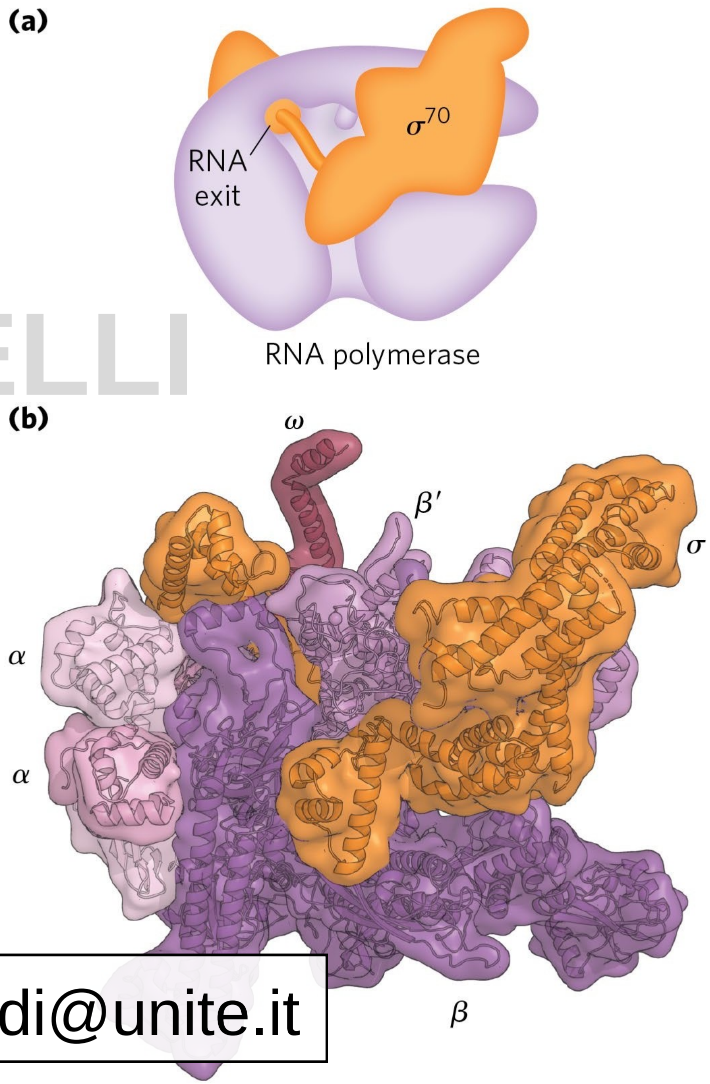
Six Subunits Constitute the RNA Polymerase Holoenzyme

- the σ subunit directs the enzyme to specific DNA binding sites
  - has variants designated by molecular weight
  - the most common subunit is σ^{70}
- RNA polymerases lack a separate proofreading 3′→5′ exonuclease active site
  - leads to a higher error rate



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P1 Principle 1 (3 of 5)

RNA is synthesized by RNA polymerases using DNA templates and ribonucleoside 5triphosphates. RNA is made in the 5→3 direction and complementary to the template DNA strand. Transcription is highly regulated and initiates by recruitment of the transcription machinery to gene promoters. Although bacterial and eukaryotic polymerases share many conserved features, the transcriptional machinery and its regulation are much more complex in eukaryotes.

RNA Synthesis Begins at Promoters

- **promoters** = specific sequences in DNA that RNA polymerase binds to
  - direct that transcription of adjacent segments of DNA (genes)
  - sequences are variable

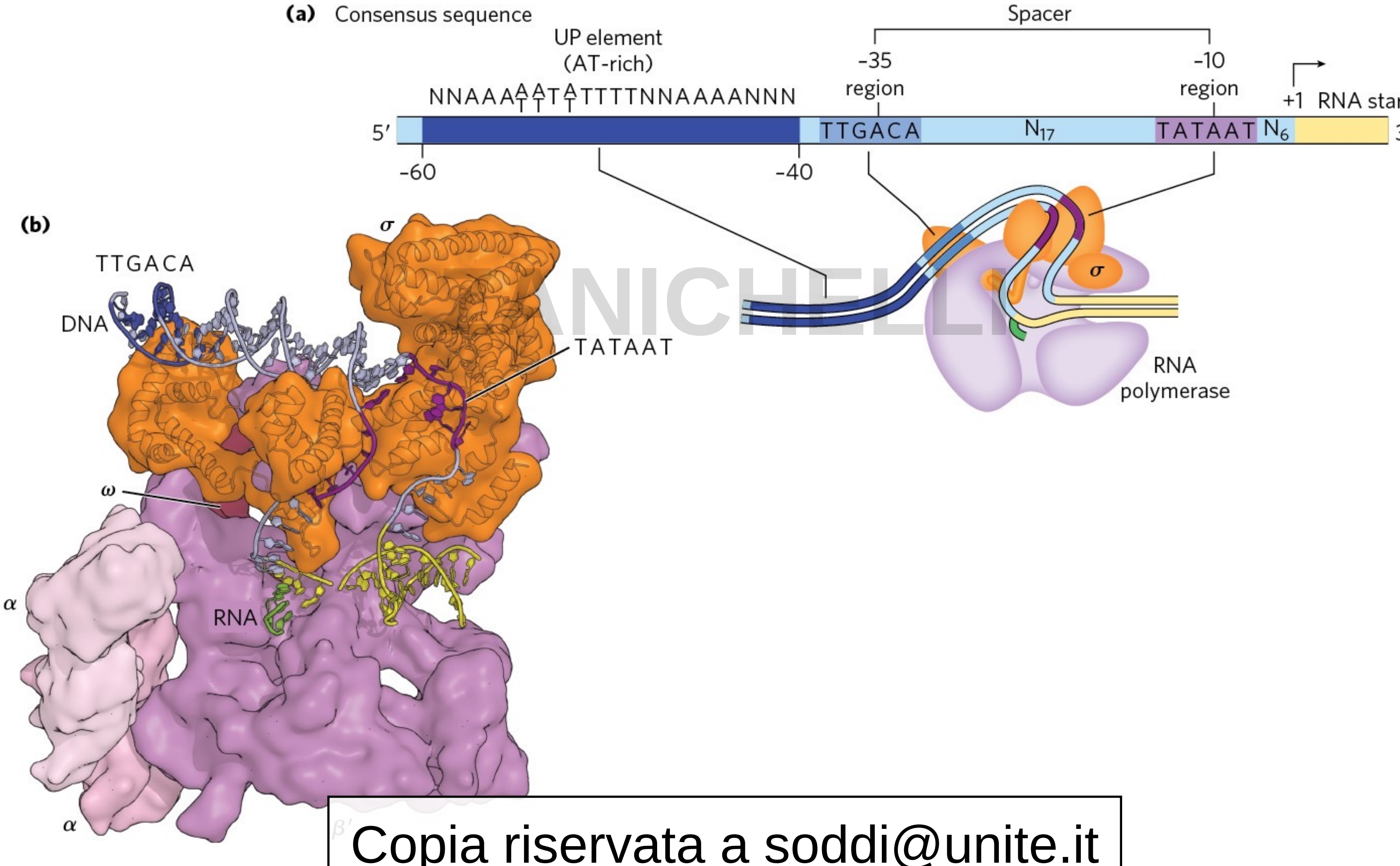
- promoter regions extend between positions -70 and +30

Consensus Sequences and the UP Element

- **consensus sequence** = formed by certain nucleotides that are particularly common at a given position
  - (5′)TATAAT(3′) at the -10 region
  - (5′)TTGACA(3′) at the -35 region

- UP (upstream promoter) element = a third AT-rich recognition element that occurs between positions -40 and -60 in the promoters of certain highly expressed genes
  - bound by the *α* subunit of RNA polymerase

Promoter Recognition by σ^{70} RNA Polymerase Holoenzyme



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Initiation by *E. coli* RNA Polymerase

- the polymerase binds to the promoter
  - directed by its bound σ factor

- a **closed complex** (bound DNA remains double-stranded) forms, followed by an **open complex** (bound DNA is partially unwound near the −10 sequence)

- initiation causes a conformational change that converts the complex to the elongation form and moves the complex away from the promoter (promoter clearance)

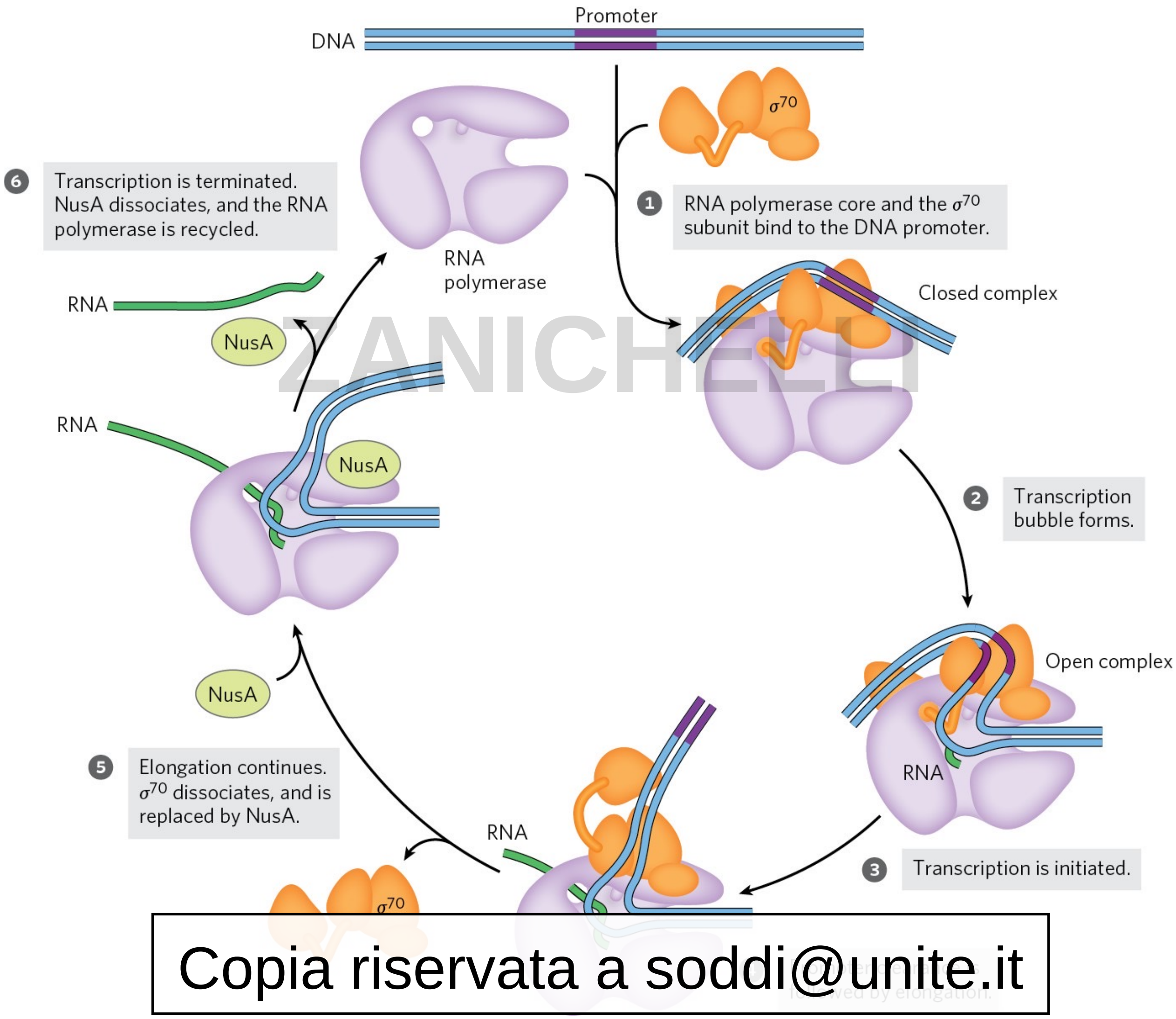
Elongation by *E. coli* RNA Polymerase

- the *σ* subunit dissociates at random as the polymerase enters elongation

- the NusA protein competes with the *σ* subunit to bind the polymerase

- after transcription:
  - NusA dissociates from the enzyme
  - RNA polymerase dissociates from the DNA

Initiation and Elongation by *E. coli* RNA Polymerase



1. RNA polymerase core and the σ^{70} subunit bind to the DNA promoter.

2. Transcription bubble forms.

3. Transcription is initiated.

5. Elongation continues. σ^{70} dissociates, and is replaced by NusA.

6. Transcription is terminated. NusA dissociates, and the RNA polymerase is recycled.

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The σ^{32} Subunit

- the σ^{32} subunit = specific for the heat shock promoters
  - RNA polymerase binds these promoters only when σ^{70} is replaced with σ^{32}

- the products of heat shock genes are made at higher levels when the cell is exposed to environmental stress

- using different σ subunits allows the cell to coordinate the expression of sets of genes

P1 Principle 1 (4 of 5)

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Transcription Is Regulated at Several Levels

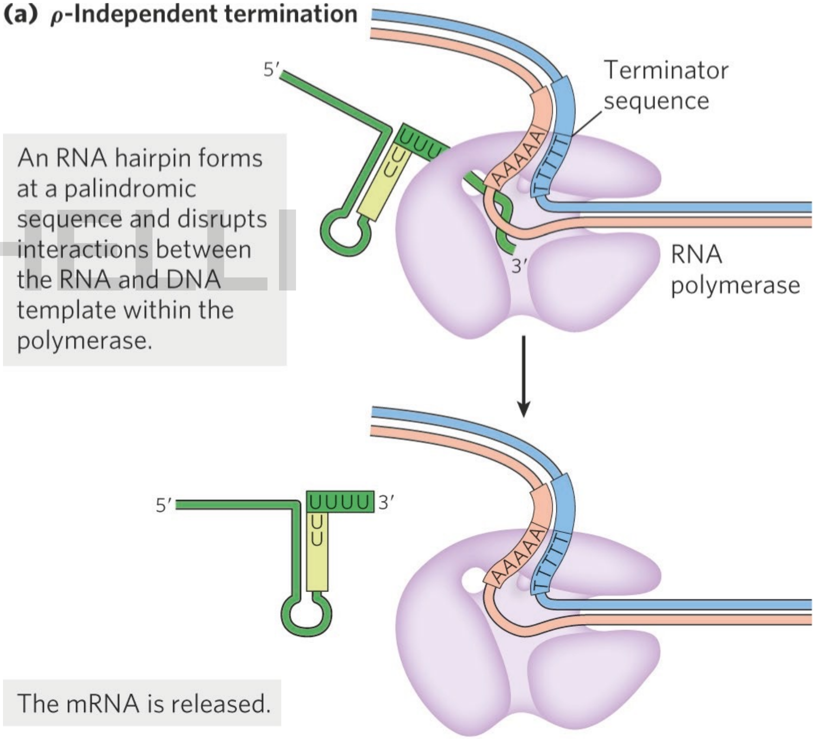
- regulation can occur at any step of transcription

- as the first committed steps in transcription, binding of RNA polymerase to the promoter and initiation of transcription are closely regulated

- protein binding can activate or repress transcription
  - **cAMP receptor protein (CRP)** activates transcription
  - **repressors** block RNA synthesis at specific genes

Specific Sequences Signal Termination of RNA Synthesis

- *ρ*-independent terminators:
  - have a self-complementary region that forms a hairpin
  - have a conserved string of three A residues that are transcribed into U residues near the 3′ end of the hairpin



An RNA hairpin forms at a palindromic sequence and disrupts interactions between the RNA and DNA template within the polymerase.

The mRNA is released.

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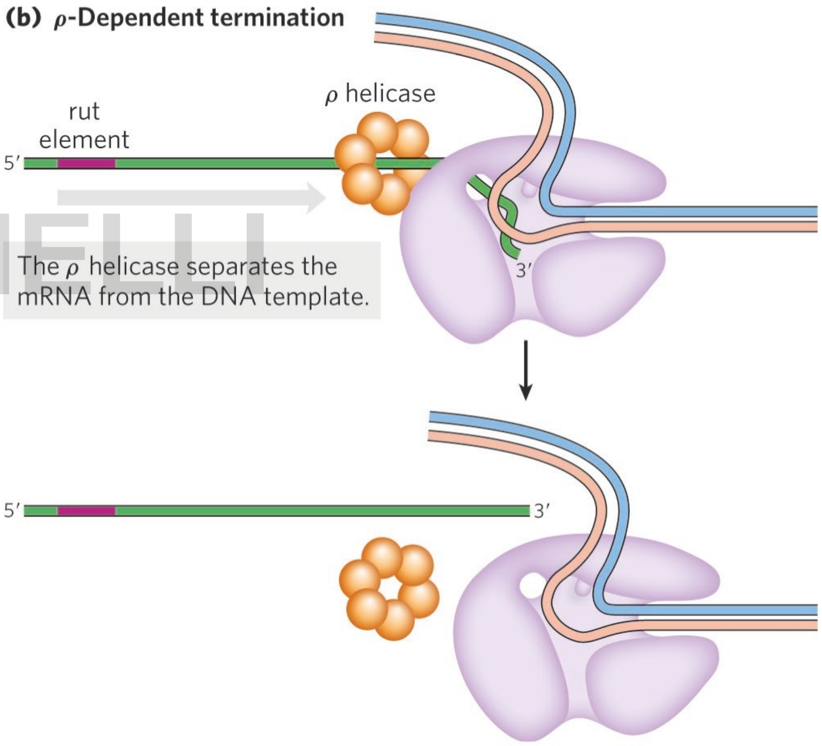
ρ-Dependent Terminators

- *ρ* (rho) = protein factor that has an ATP-dependent RNA-DNA helicase activity

- *ρ*-dependent terminators = class of terminators that rely on the protein factor *ρ* (rho)
  - have a CA-rich sequence called a *rut* (*r*ho *ut*ilization) element
  - *ρ* promotes release of t



The ρ helicase separates the mRNA from the DNA template.

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Eukaryotic Cells Have Three Kinds of Nuclear RNA Polymerases

- eukaryotes have three nuclear RNA polymerases: I, II, and III
  - each has a specific function and is recruited to a specific promoter sequence

Table 26-1 Eukaryotic Nuclear RNA Polymerases

RNA polymerase	Types of RNA synthesized
I	Pre-ribosomal RNA
II	mRNA ncRNA
III	tRNA 5S rRNA ncRNA

Mitochondria and Chloroplast RNA Polymerases

- eukaryotic mitochondria and chloroplasts have their own RNA polymerases

- RNA polymerases in these organelles are similar to bacterial RNA polymerases

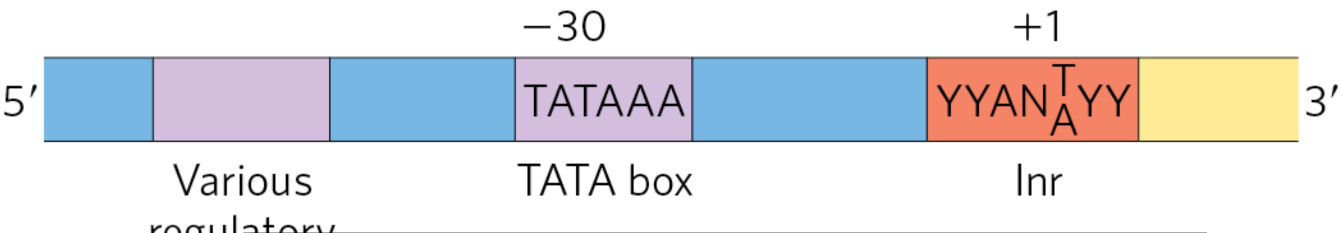
RNA Polymerase I (Pol I)

- RNA polymerase I (Pol I) = responsible for the synthesis of a transcript called pre-ribosomal RNA (or pre-rRNA)
- pre-rRNA contains the precursor for the 18S, 5.8S, and 28S rRNAs

RNA Polymerase II (Pol II)

- RNA polymerase II (Pol II) = responsible for the synthesis of mRNAs and many ncRNAs
  - can recognize 1000s of promoters

- some Pol II promoters have sequences in common:
  - a TATA box consensus sequence (TATA(A/T)A(A/T)(A/G)) near -30
  - an Inr sequence (initiator) at +1



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RNA Polymerase III (Pol III)

- RNA polymerase III (Pol III) = responsible for the synthesis of tRNAs, the 5S rRNA, and other ncRNAs

- Pol III promoters are well characterized

P1 Principle 1 (5 of 5)

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RNA Polymerase II Requires Many Other Protein Factors for Its Activity

- Pol II is more complex than its bacterial counterpart, but structure, function, and mechanism are conserved
  - RBP1 subunit exhibits a high degree of homology to the bacterial β′ subunit
  - RBP2 subunit is structurally similar to the bacterial *β* subunit
  - RBP3 and RBP11 subunits show some structural homology to the two bacterial *α* subunits

The Carboxyl-Terminal Domain (CTD)

- **carboxyl-terminal domain (CTD)** = a long carboxyl-terminal tail in RBP1 that consists of many repeats of a consensus heptad amino acid sequence, -YSPTSPS-
  - yeast enzyme contains 26 repeats
  - human and mouse enzymes contain 52 repeats
  - separated from the main body of the enzyme by an intrinsically disordered linker sequence

Transcription Factors

- **transcription factors** = an array of proteins that work with Pol II form the active transcription complex

- **general transcription factors** = those required at every Pol II promoter

Proteins Required for Initiation of Transcription at Pol II Promoters of Eukaryotes

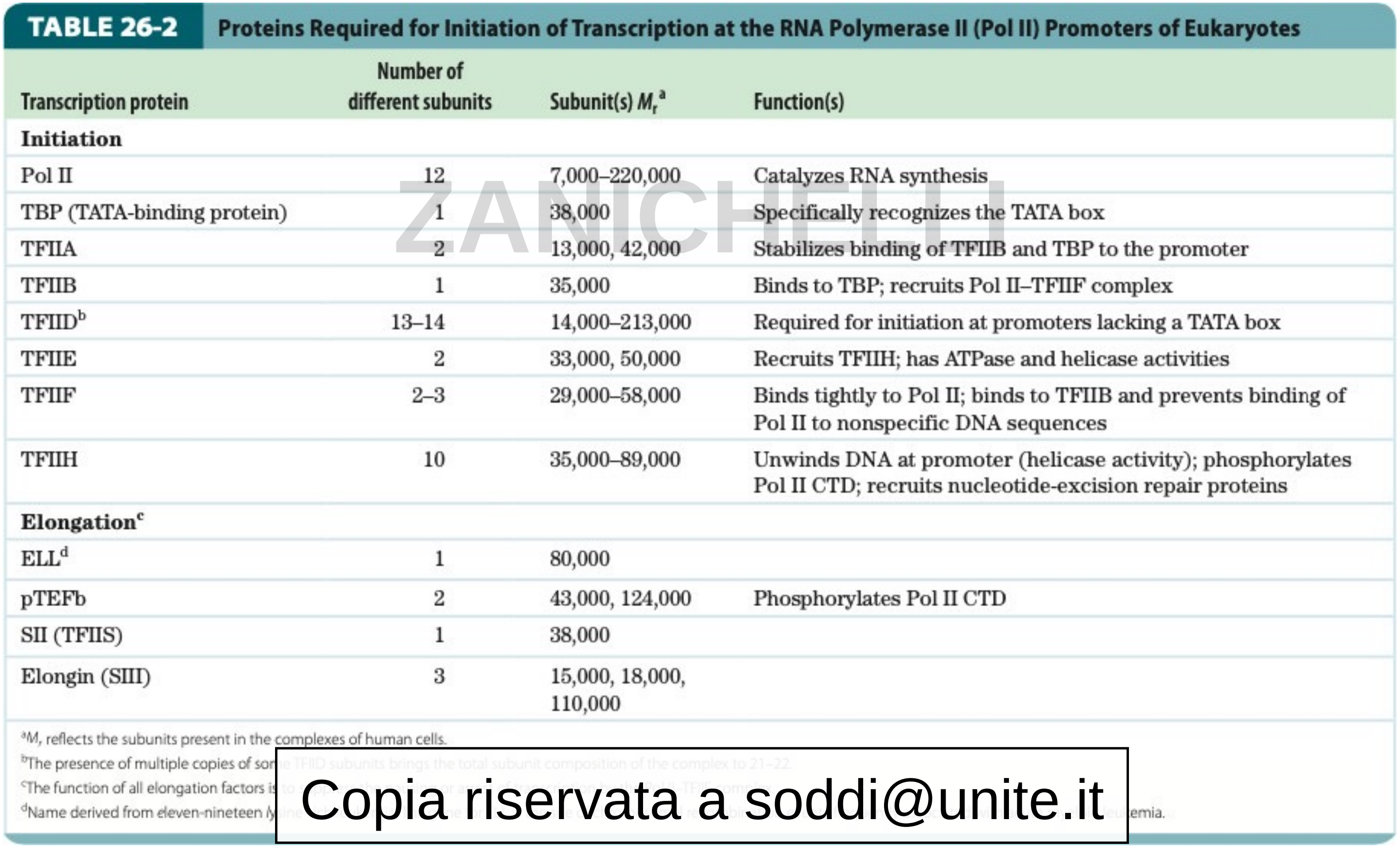


TABLE 26-2 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	Number of different subunits	Subunit(s) M_r[a]	Function(s)
Initiation			
Pol II	12	7,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	2	13,000, 42,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIID[b]	13–14	14,000–213,000	Required for initiation at promoters lacking a TATA box
TFIIE	2	33,000, 50,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2–3	29,000–58,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	10	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II CTD; recruits nucleotide-excision repair proteins
Elongation[c]			
ELL[d]	1	80,000	
pTEFb	2	43,000, 124,000	Phosphorylates Pol II CTD
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

[a] M_r reflects the subunits present in the complexes of human cells.

[b] The presence of multiple copies of some TFIID subunits brings the total subunit composition of the complex to 21–22.

[c] The function of all elongation factors is to suppress the pausing or arrest of transcription by the Pol II–TFIIF complex.

[d] Name derived from eleven-nineteen lysine [illegible] leukemia.

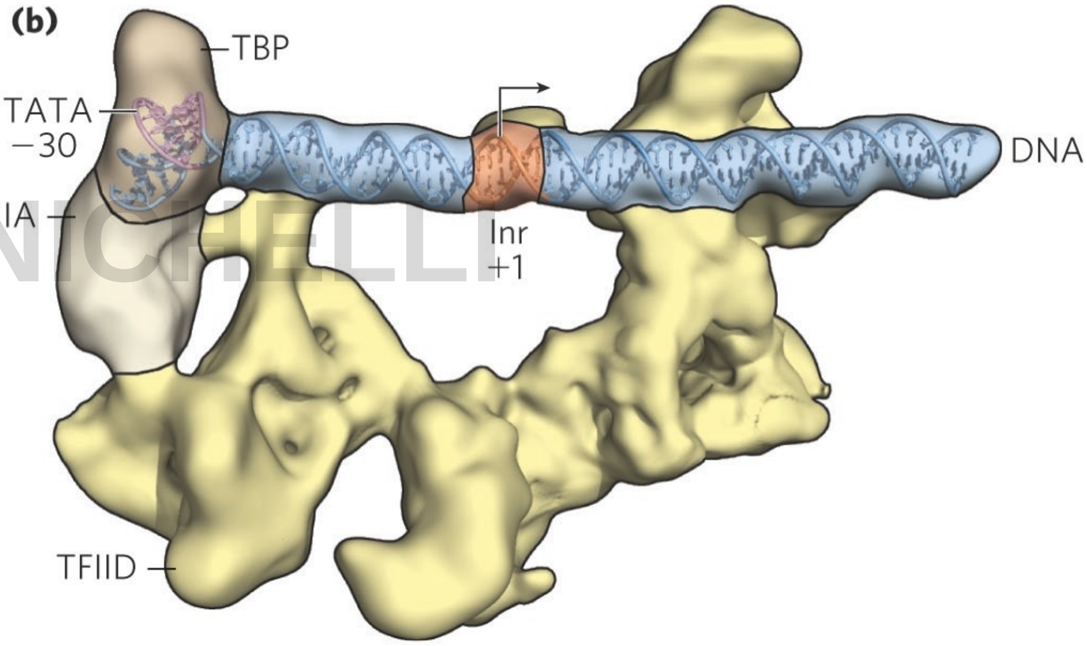
Assembly of RNA Polymerase and Transcription Factors at a Promoter

- formation of a closed complex begins when TATA-binding protein (TBP) binds to the TATA box

- **preinitiation complex (PIC)** = a closed complex
  - contains TBP, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and Pol II

TFIID Positions TBP and Pol II on the Promoter

- TFIID binds the promoter DNA in an elongated complex that is anchored by TBP-DNA interactions on one end
- the **Inr sequence** is straddled on both ends by TFIID subunits



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TFIIH Promotes Unwinding of the DNA Near the RNA Start Site

- TFIIH has DNA helicase activity that promotes the unwinding of DNA to create an open complex
  - requires ATP

RNA Strand Initiation and Promoter Clearance

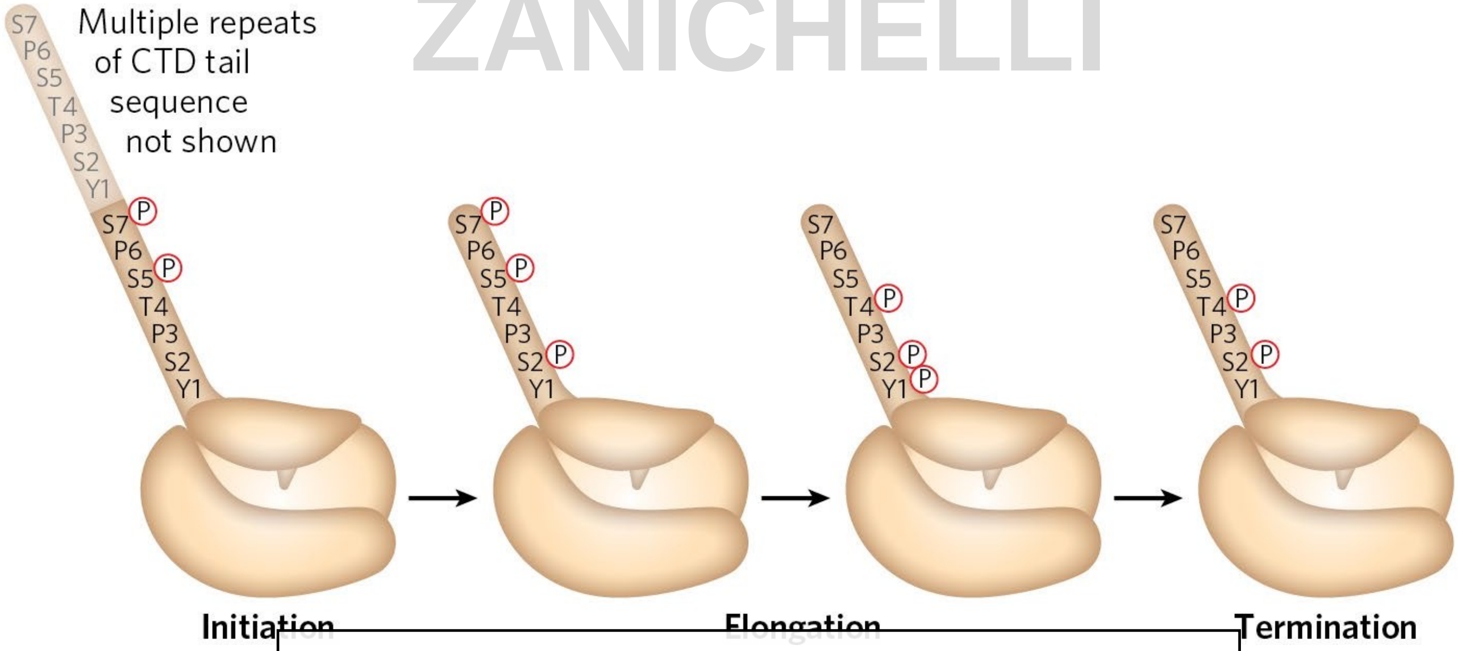
- TFIIH has kinase activity that phosphorylates Pol II at many places in the CTD
  - other kinases also phosphorylate the CTD

- CTD phosphorylation causes a conformational change in the overall complex, initiating transcription

- Pol II enters elongation after TFIIE and TFIIH are released
  - occurs after synthesis of 60 to 70 nucleotides of RNA

Phosphorylation of the CTD of RNA Polymerase II

- the phosphorylation state of the CTD changes throughout transcription



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Elongation, Termination, and Release

- during elongation, elongation factors bound to RNA Pol II enhance processivity and coordinate posttranslational modifications
  - some are bound to phosphorylated CTD

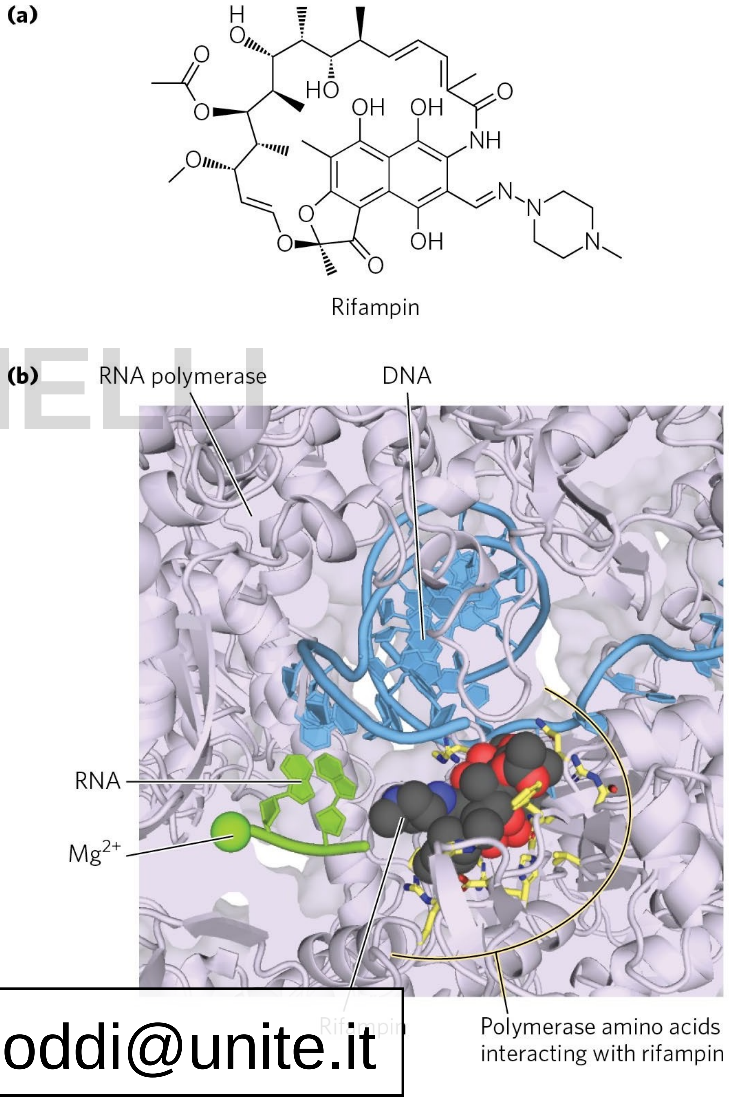
- during termination, Pol II CTD is dephosphorylated and transcription machinery is recycled

RNA Polymerases Are Drug Targets

- **actinomycin D** = an antibiotic that inhibits RNA elongation in bacteria and eukaryotes
  - intercalates into the double-helical DNA to deform the DNA duplex and prevent polymerase movement

Rifampin

- **rifampin** = antibiotic that inhibits bacterial RNA synthesis by preventing the promoter clearance step of transcription
  - important for the treatment of tuberculosis (TB)
  - binds near the active site of RNA polymerase and prevents extension of the RNA product



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The Death Cap Mushroom Produces *α*-Amanitin

- *α*-amanitin = compound produced by the death cap mushroom *Amanita phalloides*
  - disrupts transcription in animal cells by blocking Pol II and, at higher concentrations, Pol III

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RNA Splicing

- **primary** or **precursor transcript** = a newly synthesized RNA molecule

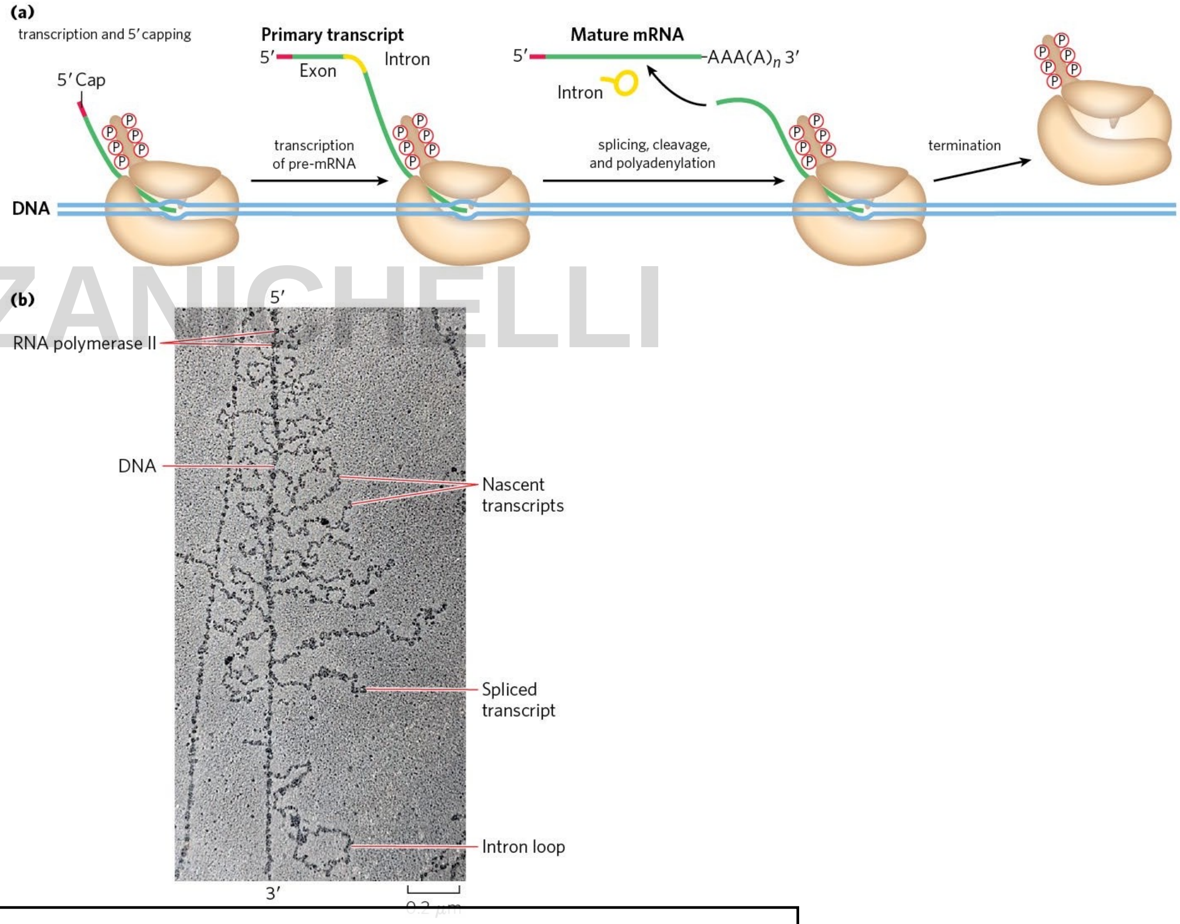
- **introns** = noncoding tracts that break up the coding region of the mRNA transcript
  - human genes average eight introns per gene

- **exons** = coding segments of the mRNA transcript

- **RNA splicing** = the process by which introns are removed from pre-mRNA and exons are spliced together to form a continuous sequence that specifies a functional polypeptide

Processing of the Primary Transcript during mRNA Maturation

- the protein complexes that carry out mRNA-processing reactions are organized in association with each other and with the phosphorylated CTD of Pol II



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Both Introns and Exons Are Transcribed from DNA into RNA

- most genes in vertebrates contain introns
  - exceptions include genes that encode histones

- some yeast and a few bacterial and archaeal genes contain introns

- in eukaryotic mRNAs:
  - exons are <1,000 bp long
  - introns are 50-700,000 bp long (median = 1,800)

- the human genome has 200,000+ introns spread across ~20

RNA Catalyzes the Splicing of Introns

- four classes of introns:
  - group I and II are self-splicing
  - spliceosome introns are removed by a large RNP called the spliceosome
  - protein-catalyzed introns are removed by enzymes

Table 26-3 Mechanisms of RNA Splicing

Mechanism	Components	Features	Cellular locations
Group I Intron	Catalytic RNA	Self-splicing using a guanine-derived cofactor	Found in nuclear, mitochondrial, and chloroplast genes that encode mRNAs, rRNAs, or tRNAs. Can be found in bacteria.
Group II Intron	Catalytic RNA; maturase and reverse transcriptase proteins	Self-splicing using a nucleophile within the intron to form a lariat	Primarily found in mitochondrial and chloroplast genes of fungi, algae, and plants. Can be found in bacteria.
Spliceosome	Catalytic snRNAs; dozens of protein splicing factors	Requires a large RNP for processing using a nucleophile within the intron to form a lariat	Found in nuclear genes of eukaryotes. Capable of alternative splicing to create multiple products from a given transcript.
Protein-catalyzed	Protein enzyme	Uses a splicing endonuclease and ligase	Found in tRNAs and a few mRNAs

Group I and Group II Introns

- group I introns are found in rRNAs, mRNAs, and tRNAs of nuclear, mitochondrial, and chloroplast genes
- group II introns are found in the mRNAs of mitochondrial or chloroplast genes in fungi, algae, and plants
- both are also found in bacteria

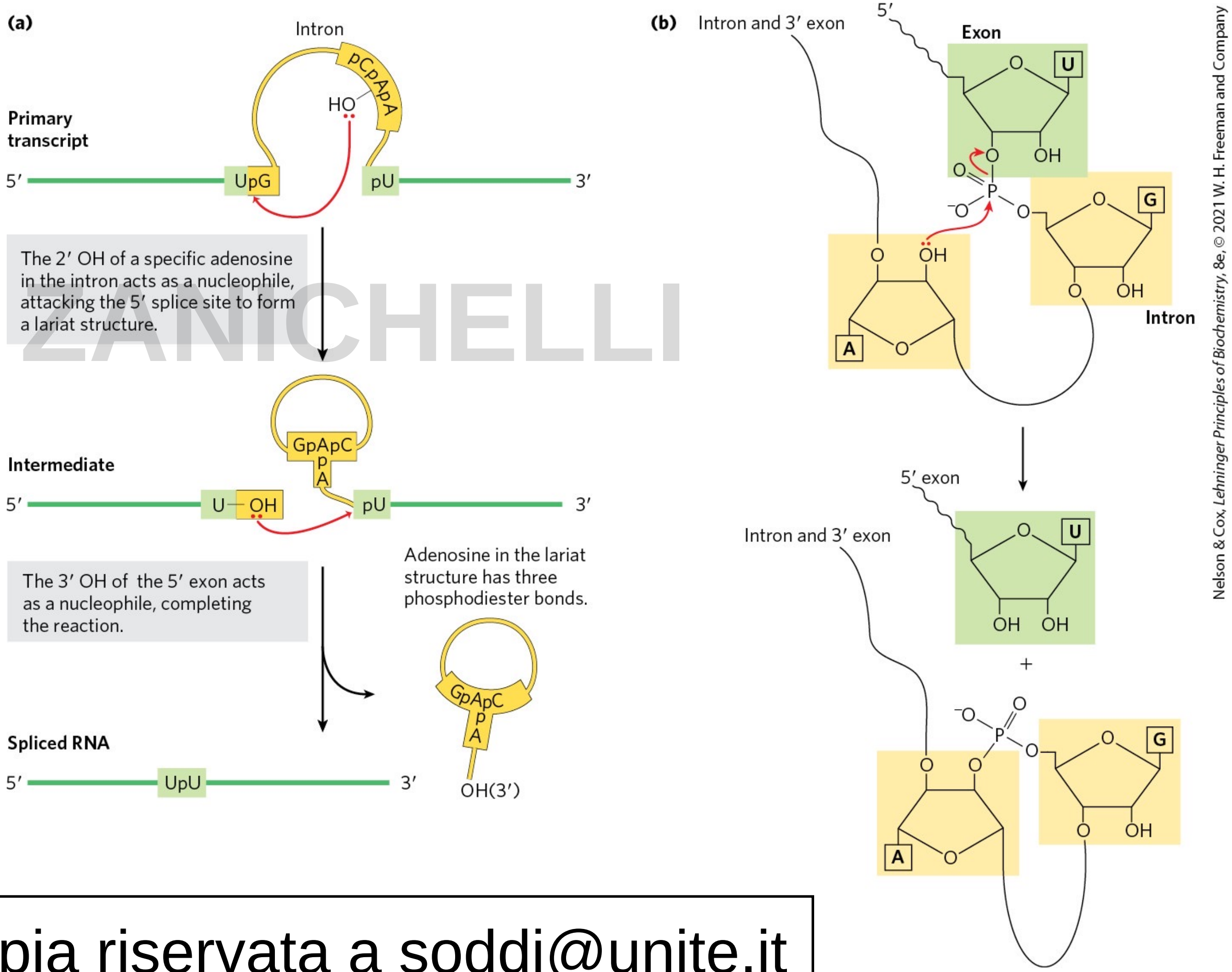
The Splicing Mechanisms of Group I and II Introns

- an –OH group makes a nucleophilic attack on a phosphorus, forming a new phosphodiester bond
  - in group I splicing, the 3′-OH group of guanosine of a required guanine nucleoside or nucleotide cofactor
  - in group II splicing, the 2′-OH group of an A residue within the intron

- the 3′-OH of the displaced exon acts as a nucleophile in a similar reaction at the 3′ end of the intron

Splicing Mechanism of Group II Introns

- a branched lariat structure forms as an intermediate



The 2′ OH of a specific adenosine in the intron acts as a nucleophile, attacking the 5′ splice site to form a lariat structure.

The 3′ OH of the 5′ exon acts as a nucleophile, completing the reaction.

Adenosine in the lariat structure has three phosphodiester bonds.

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In Eukaryotes, the Spliceosome Carries out Nuclear pre-mRNA Splicing

- spliceosome introns are the most common intron in eukaryotes
  - GU at the 5′ end and AG at the 3′ end mark the sites of splicing

- some splicing apparatus components are tethered to the CTD of RNA polymerase II
  - indicates splicing is tightly coordinated with transcription

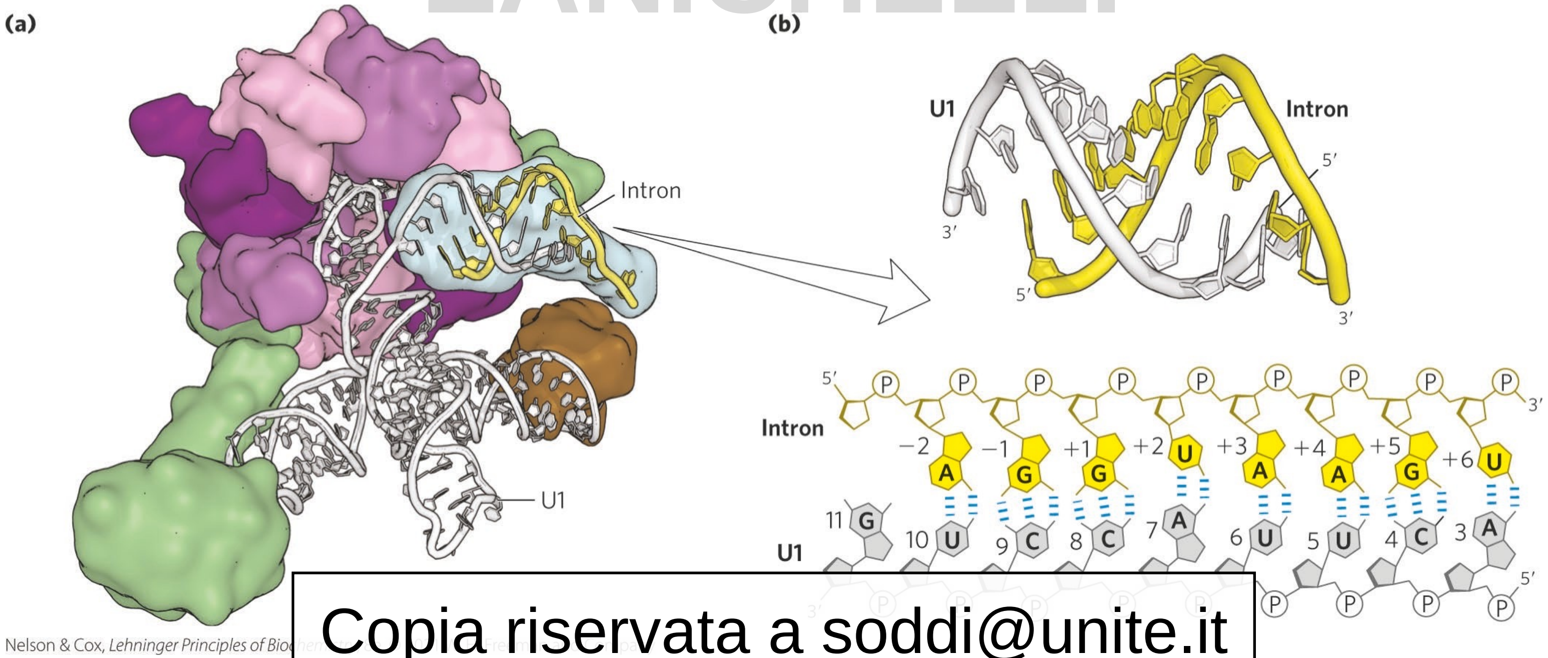
The Spliceosome and snRNAs

- **spliceosome** = a large complex made up of multiple specialized RNP complexes called *s*mall *n*uclear *r*ibo*n*ucleo*p*roteins (snRNPs) and dozens of other proteins

- **small nuclear RNAs (snRNAs)** = 100-200 nucleotide RNAs that make up snRNPs
  - U1, U2, U4, U5, and U6 are abundant in the nuclei

Binding of U1 and U2 snRNA

- U1 snRNA base pairs with the 5′ splice site
- U2 snRNP base pairs with the 3′ end and identifies the A residue that becomes the nucleophile



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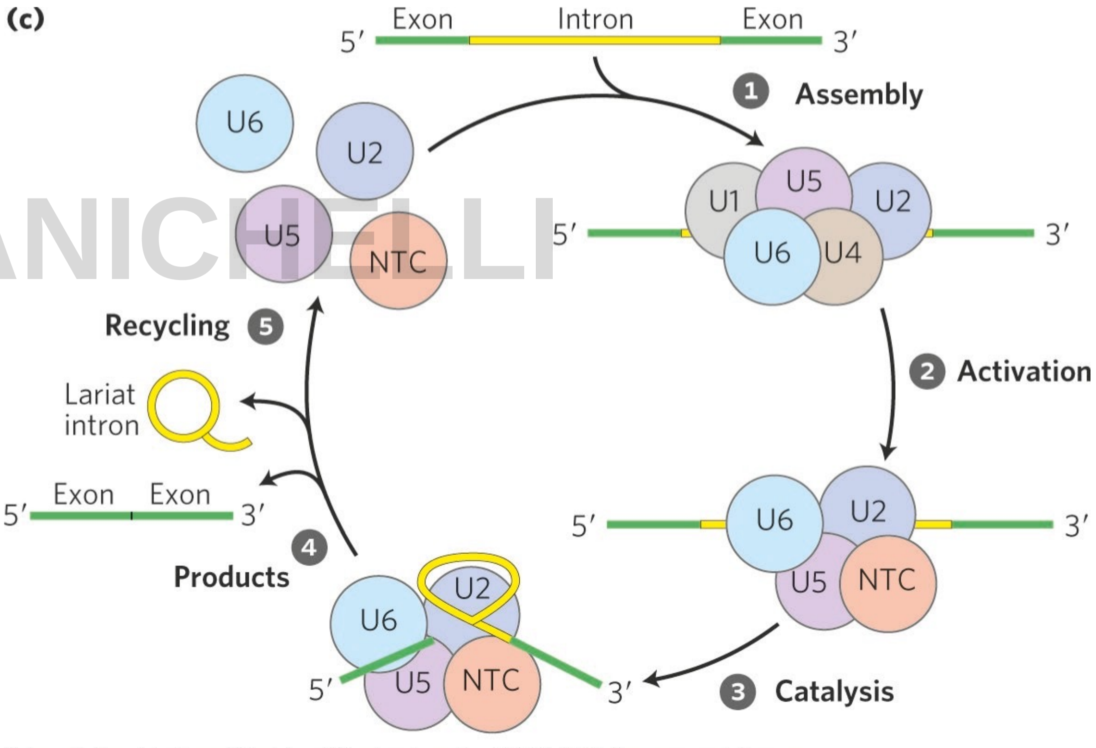
Spliceosome Assembly

- addition the tri-snRNP (a complex of U4, U5, and U6 snRNPs) forms the spliceosome
- ATP is required for assembly
- spliceosomes are single turnover enzymes



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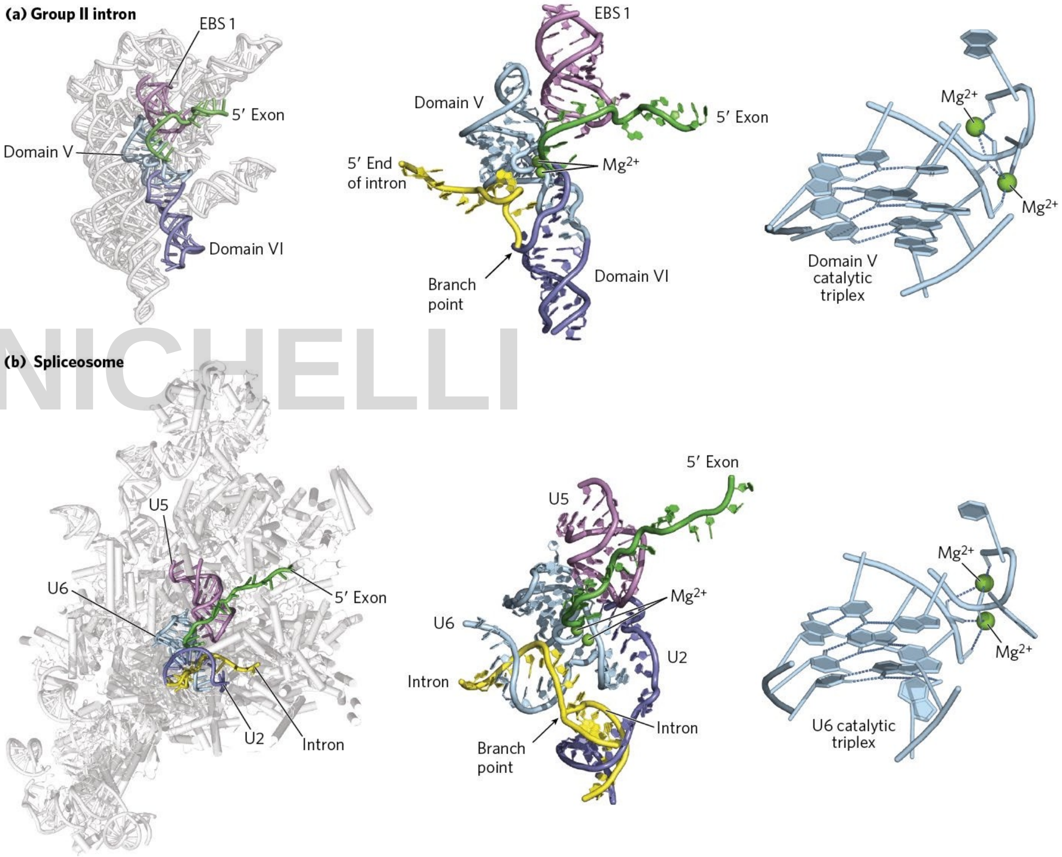
RNA Active Site Conservation

- the chemical events of splicing are identical in mechanism to that of group II introns



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Proteins Catalyze Splicing of tRNAs

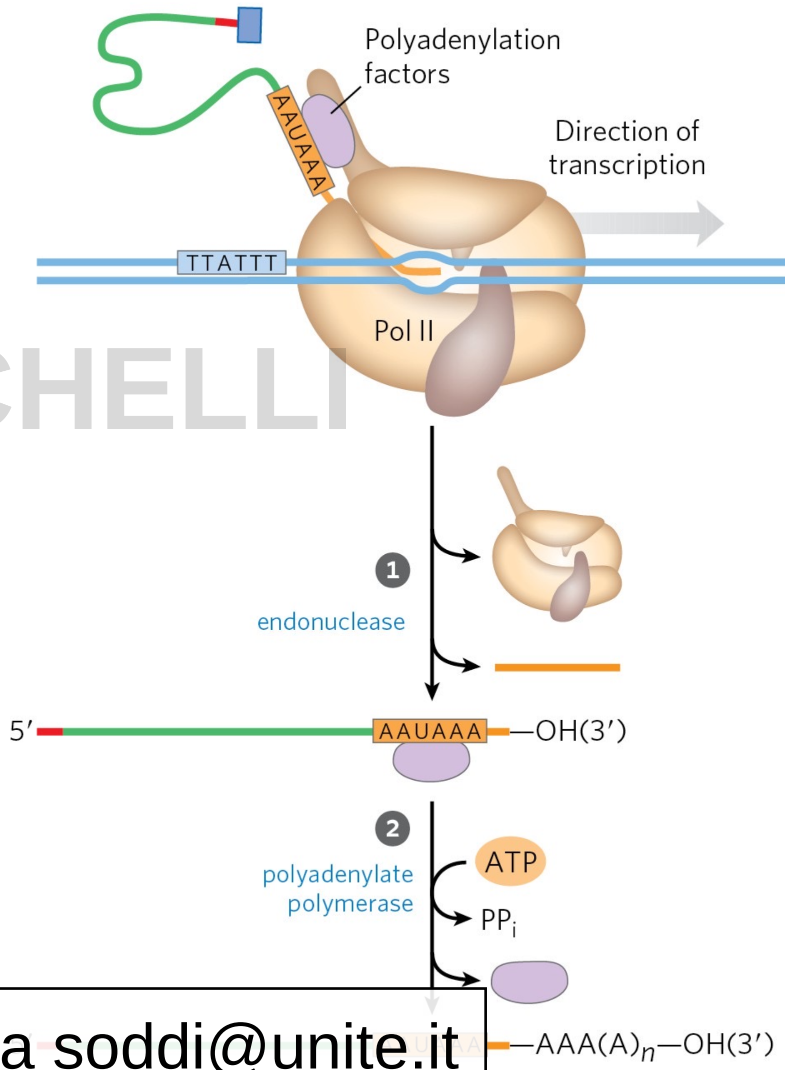
- found in certain tRNAs and a few mRNAs
- endonucleases cleave the phosphodiester bonds at both ends of the intron
- ligases join the two exons

Eukaryotic mRNAs Have a Distinctive 3′ End Structure

- **poly(A) tail** = a string of A residues added to the 3′ end of most eukaryotic mRNAs that undergo translation
  - ~30 residues in yeast and 50-100 in animals
  - serves as a binding site for specific proteins
  - may help protect mRNA from enzymatic destruction

Addition of the Poly(A) Tail

- endonuclease cleaves at the poly(A) addition site
- the addition site is:
  - 10-30 nucleotides downstream from the highly conserved sequence (5′)AAUAAA(3′)
  - 20-40 nucleotides upstream from a sequence rich in G



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A Gene Can Give Rise to Multiple Products by Differential RNA Processing

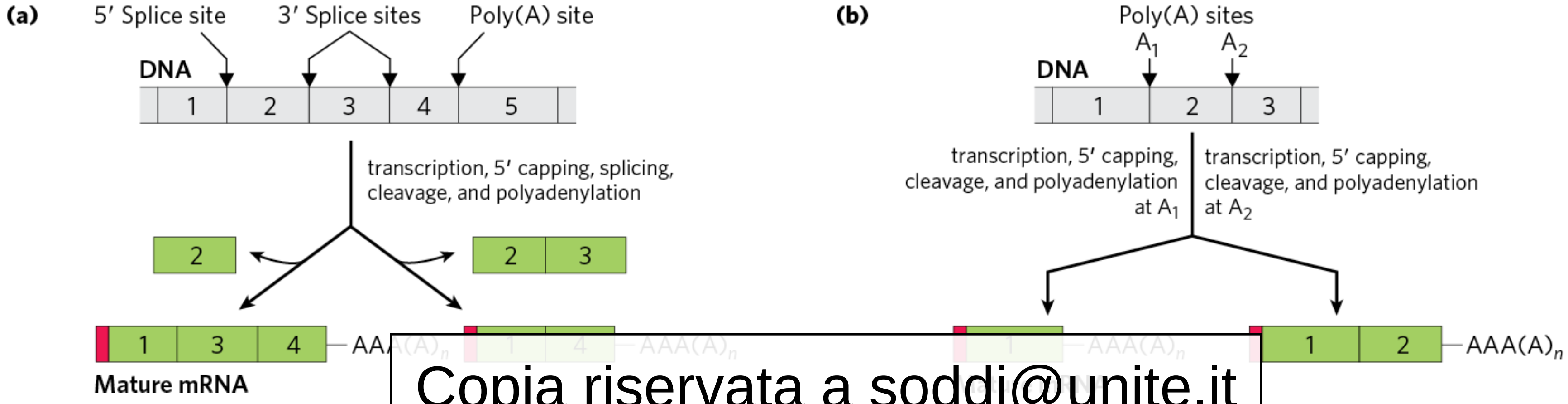
- some eukaryotic mRNA transcripts can be processed in more than one way to produce different mRNAs and thus different polypeptides

- **alternative splicing** = process in which a particular exon may or may not be incorporated into the mature mRNA transcript
  - occurs in >95% of human genes

Alternative Transcript Production in Eukaryotes

- pre-mRNA contains molecular signals for all the alternative processing pathways
  - RNA-binding proteins promote one particular path

- complex transcripts can have more than one site where poly(A) tails can form (**poly(A) site**)



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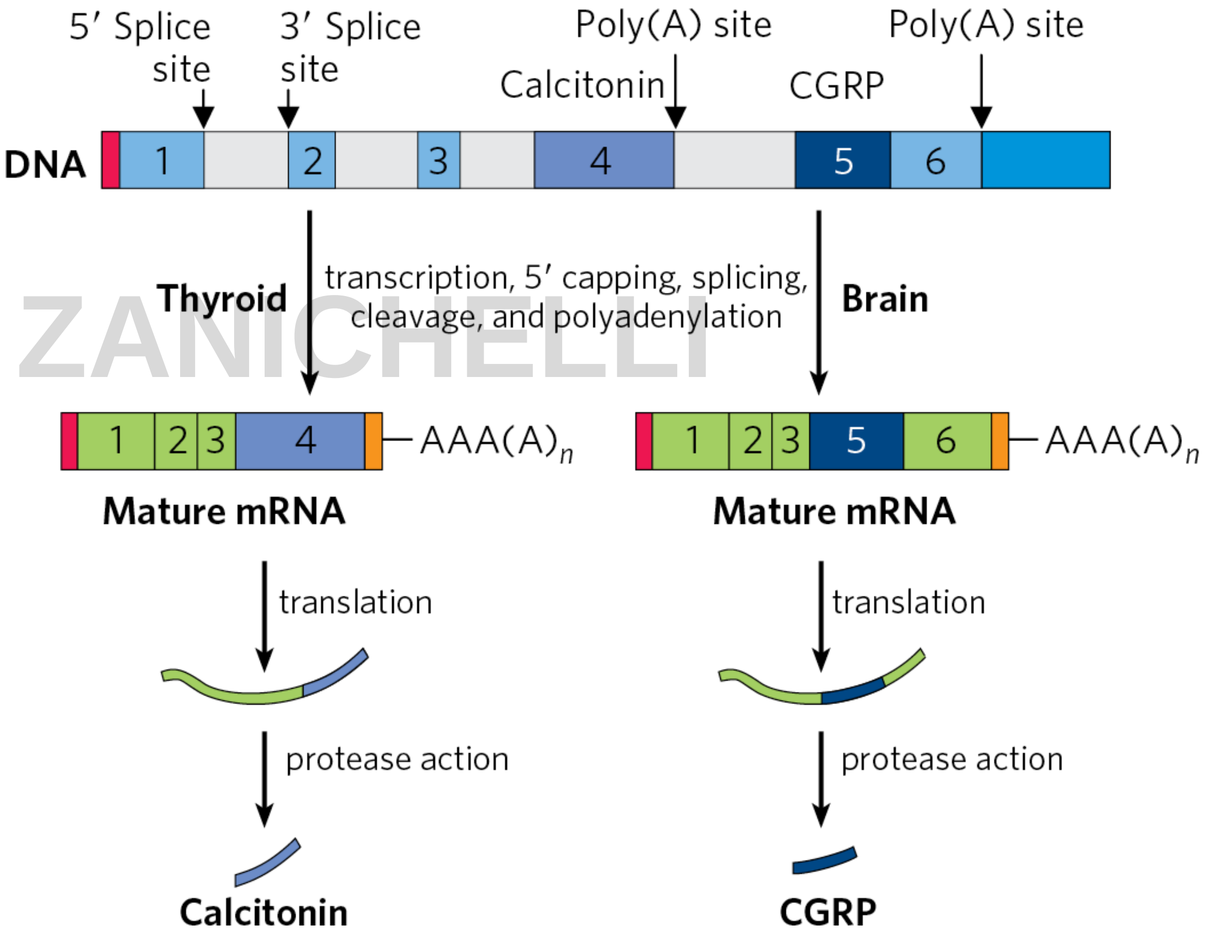
Alternative Processing of the Calcitonin Gene Transcript in Rats

- alternative splicing and poly(A) site choice increase the variety of proteins generated from the genomes of higher eukaryotes



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Eukaryotic Ribosomal RNAs

- processing is initiated in the nucleolus, in large complexes that assemble on the rRNA precursor as it is synthesized
  - tight coupling occurs between rRNA transcription, rRNA maturation, and ribosome assembly in the nucleolus

- each complex includes:
  - ribonucleases that cleave the rRNA precursor
  - enzymes that modify particular bases
  - **small nucleolar RNAs (snoRNAs)** = ncRNAs that guide nucleoside modification and some cleavage reactions
  - ribosomal proteins

Homing

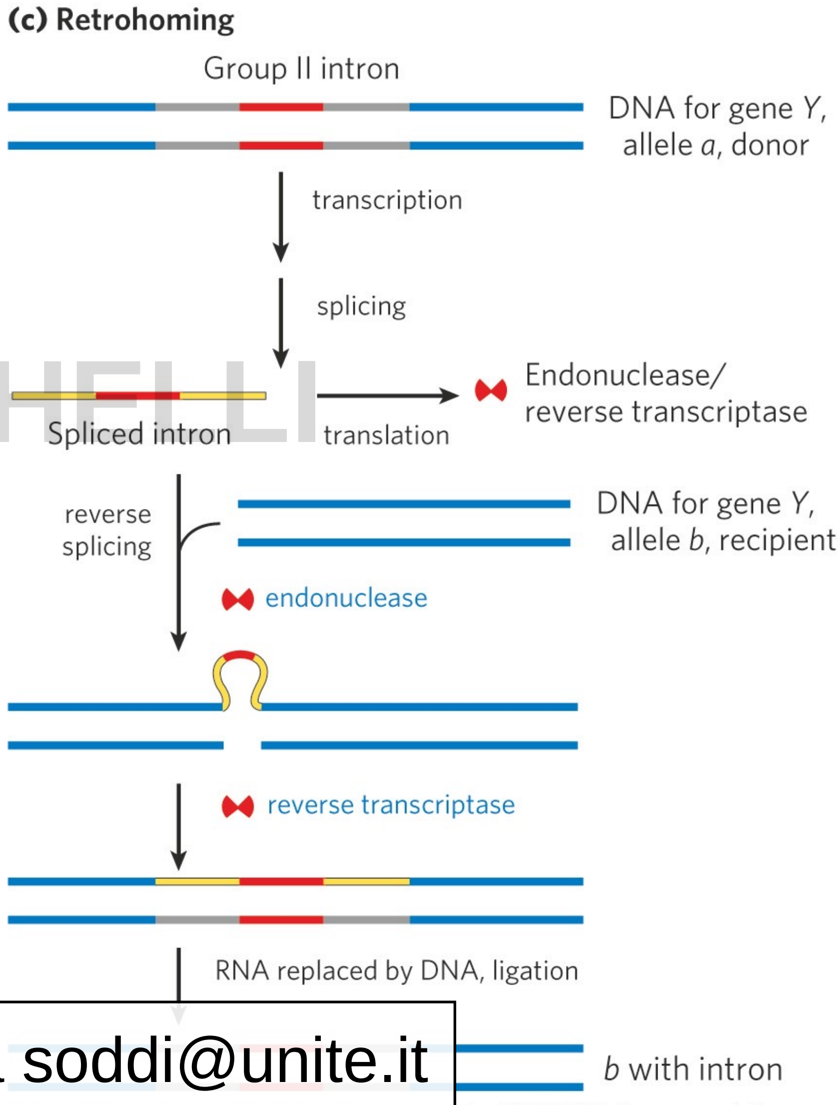
- many group I and group II introns are also mobile genetic elements
  - encode DNA endonucleases that promote their movement

- **homing** = process by which DNA endonucleases promote insertion of the intron into an identical site in a homologous gene that does not contain the intron
  - group I intron homing is DNA-based
  - group II intron homing occurs through an RNA intermediate

Retrohoming

- group II intron endonucleases have associated reverse transcriptase activity

- retrohoming = process by which in an RNA intron is inserted into a DNA gene followed by production of a DNA copy by a reverse transcriptase



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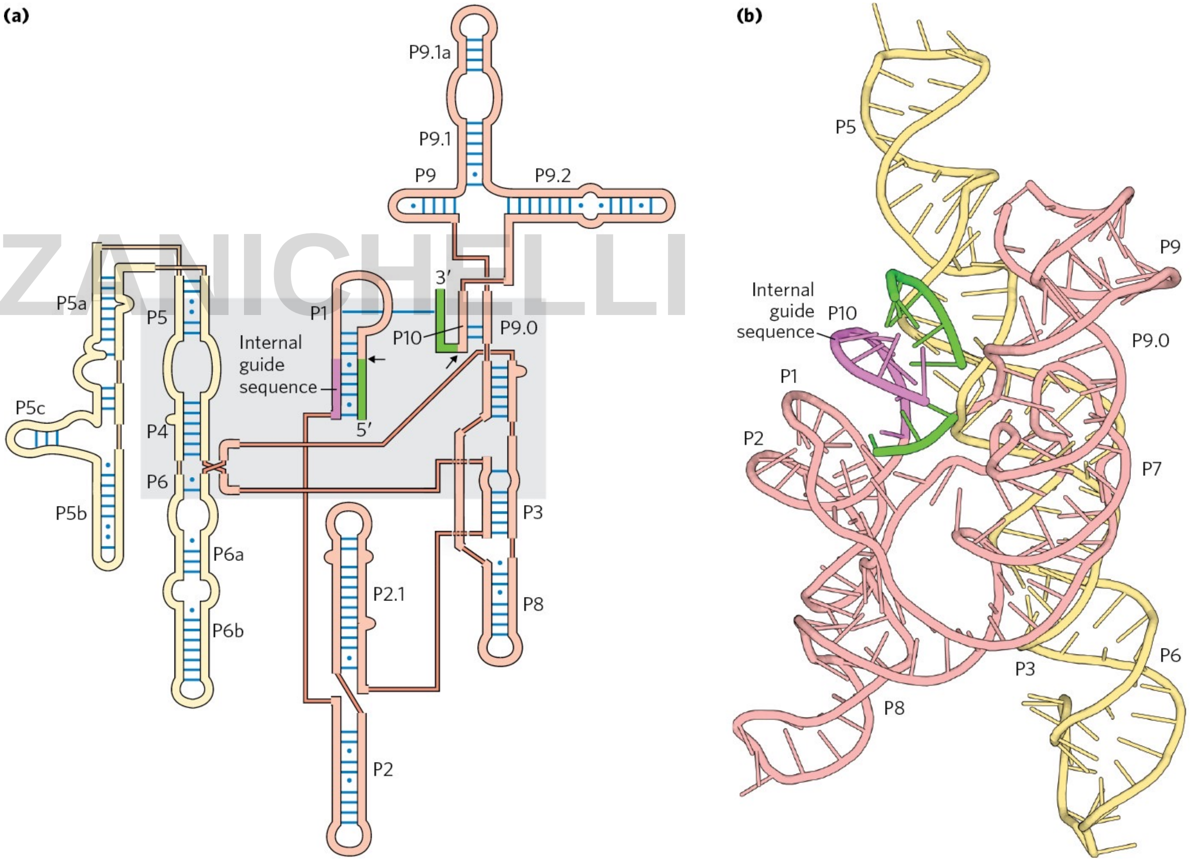
Some RNAs Are Replicated by RNA-Dependent RNA Polymerase

- Other RNA viruses include *E. coli* bacteriophages, influenza virus, and coronaviruses (SARS, COVID-19)

- **RNA-dependent RNA polymerase (RNA replicase)** = replicate RNA in the host cell
  - template-specific for viral RNA
  - lack a separate proofreading endonuclease activity
  - found in viruses (except retroviruses), plants, protists, fungi, and some simpler animals

Secondary Structure of the Self-Splicing rRNA Intron of *Tetrahymena*

- **internal guide sequence** = base-pairs with exon sequences near the 5′ splice site to promote the alignment of specific bonds to be cleaved and rejoined



Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company