Transcription

Objectives

Difference between DNA and RNA synthesis
Chromatin structure and gene expression
Direction of transcription
Promoter
Transcription unit
RNA polymerase
Steps of RNA synthesis
Processing of RNA (mRNA, tRNA, rRNA)
Splicing and clinical implication
Inhibitors

Case

- A 24 year old man who is being evaluated as a follow up to to a preplacement medical evaluatio prior to starting his new job.
- He has no significant medical issues. His family history is unremarkable, but he knows little of the health status of those family members.
- The physical examination was normal. Routine analysis of his blood included the following results

- RBC 4.8x1066/mm3 (4.3-5.9)
- Hb: 9.6g/dl
- MCV 70microm3 (80-100)
- Serum iron 150 microgram /dl (50-170)

- Based on the data, Hb electrophoresis was performed. The results were as follows
- HbA 90% (96-98)
- HbA2 6% <3
- HbF 4% <2

- What is the possible diagnosis?
- What is the pathophysiology?

Classes of Eukaryotic RNA

RNA	Types	Abundance	Stability
Protein Coding RNAs			
Messenger (mRNA)	$\geq 10^5$ Different species	2%-5% of total	Unstable to very stable
Nonprotein Coding RNAs (ncRNAs)			
Large ncRNAs			
Ribosomal (rRNA)	28S, 18S, 5.8S, 5S	80% of total	Very stable
lncRNAs	~1000s	~1%-2%	Unstable to very stable
Small ncRNAs			
Transfer RNAs	~60 Different species	~15% of total	Very stable
Small nuclear (snRNA)	~30 Different species	≤1% of total	Very stable
Micro/Silencing (mi/SiRNAs)	100s-1000	<1% of total	Stable

Comparison in RNA and DNA synthesis

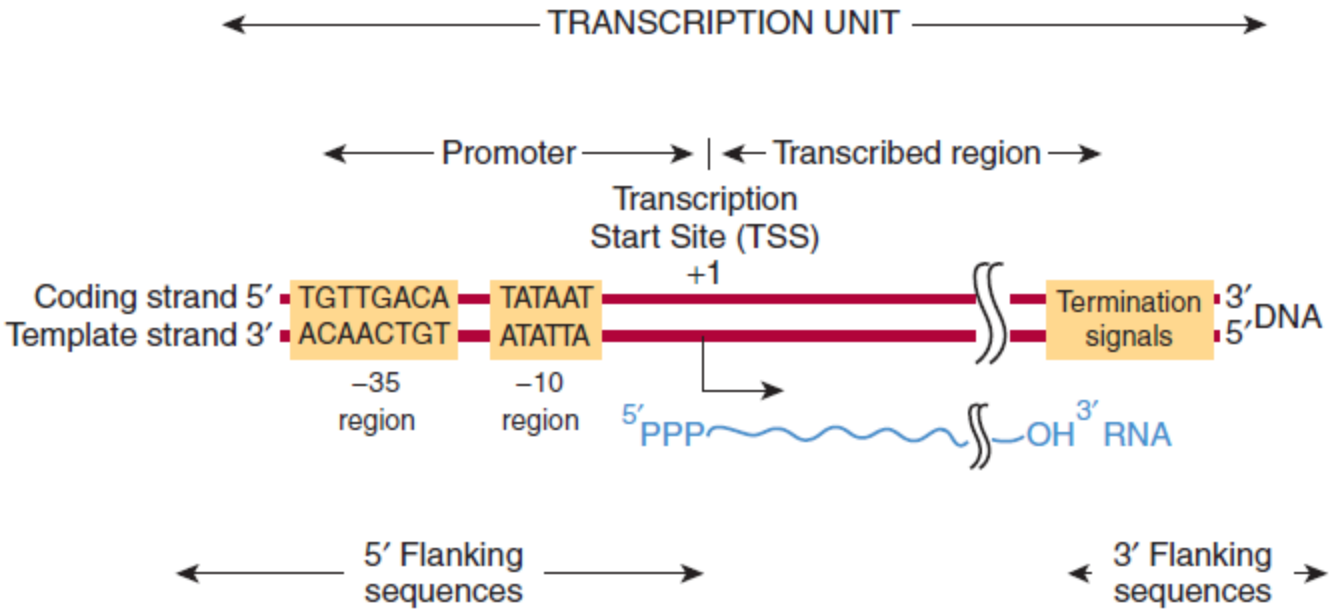
	DNA synthesis	RNA synthesis
Nucleotide	dNTP	NTP
Primer	Yes	No
Length of the genome to be copied	Entire genome	Portion of the genome
Proof reading function	Highly effective	Not highly effective
Polarity	Yes 5’ to 3’	Yes 5’to 3’
Base pairing rule	Adherence	Adherence

Transcription Unit

- A **transcription unit is defined as that region of DNA that includes the signals**

  for transcription initiation, elongation, and termination.

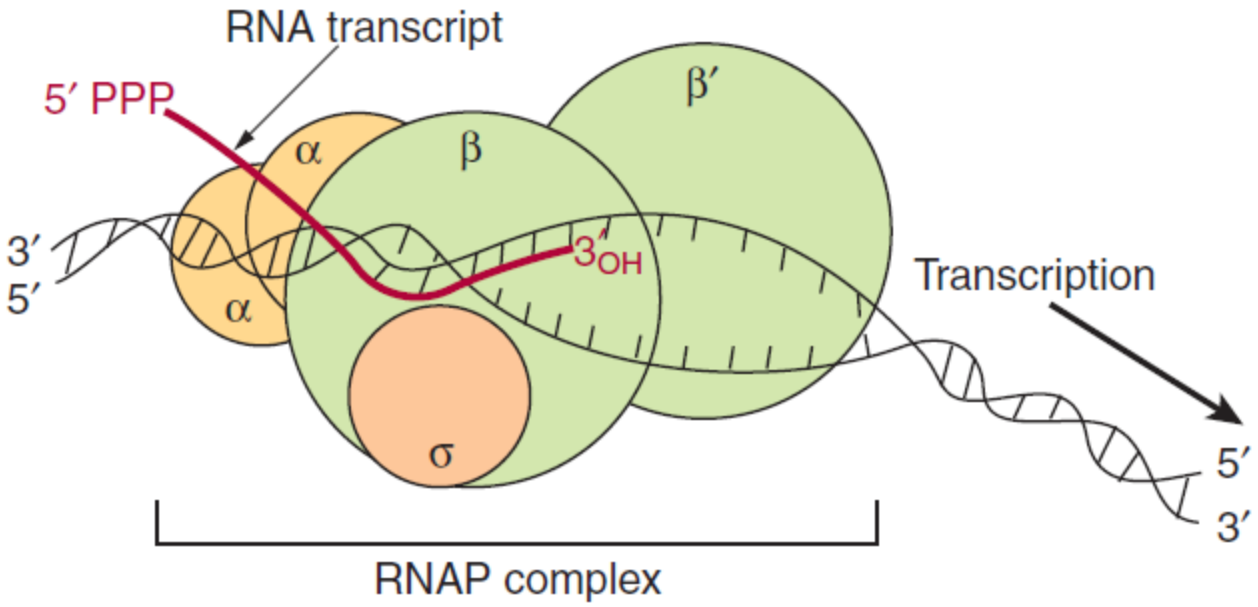
Prokaryotic promoters share two regions of highly conserved nucleotide sequence



Promoter: A regulatory region o DNA that serves to bind RNA polymerase II that in turn binds other substances that will lead to initiation of transcription

Adapted from Harper's Biochemistry

RNA polymerase catalyzes the polymerization of ribonucleotides

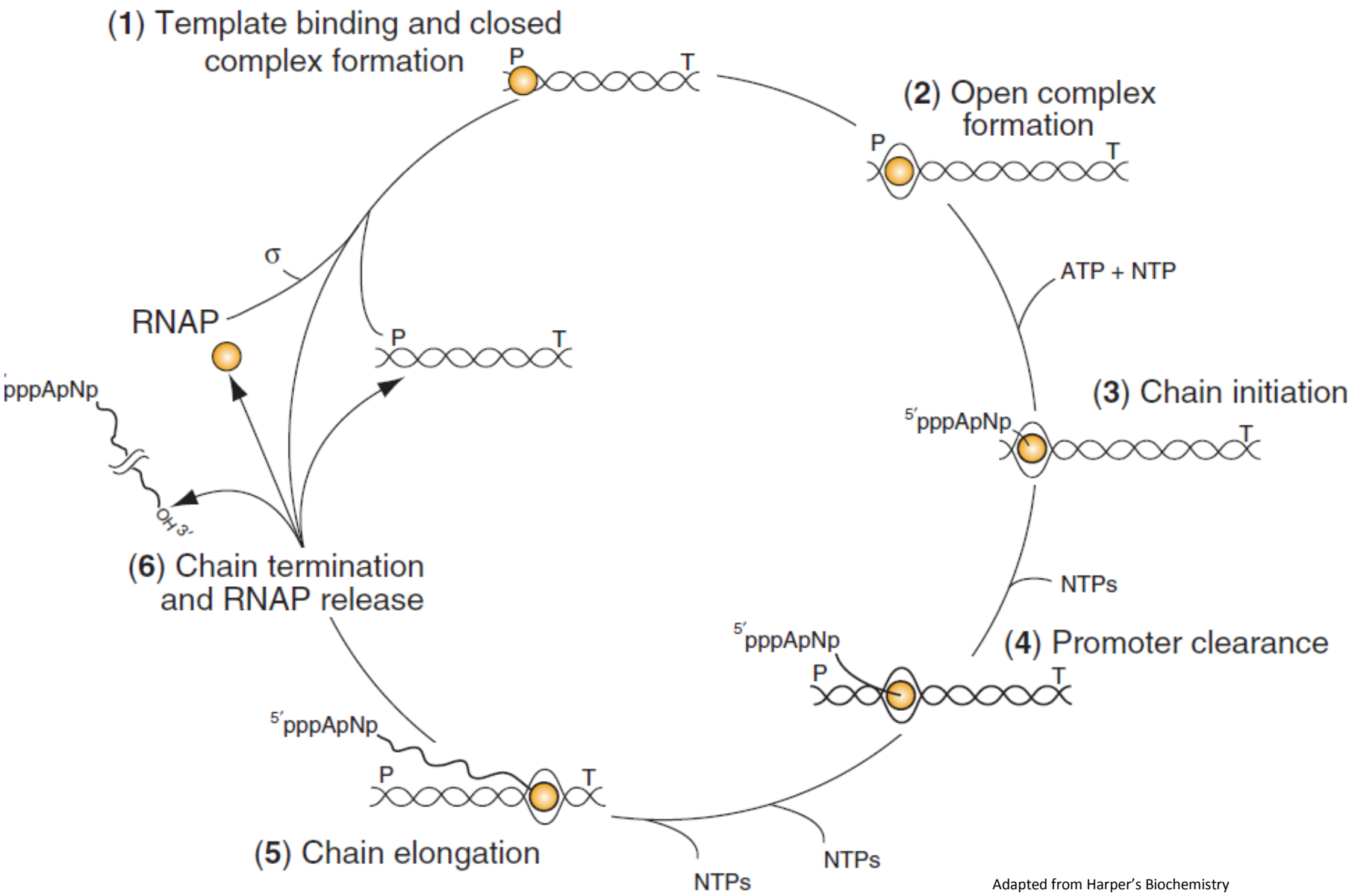


Adapted from Harper's Biochemistry

Mammalian Nuclear DNA-Dependent RNA Polymerases

Form of RNA Polymerase	Sensitivity to α-Amanitin	Major Products
I	Insensitive	rRNA
II	High sensitivity	mRNA, lncRNA, miRNA, SnRNA
III	Intermediate sensitivity	tRNA, 5s rRNA

The transcription cycle



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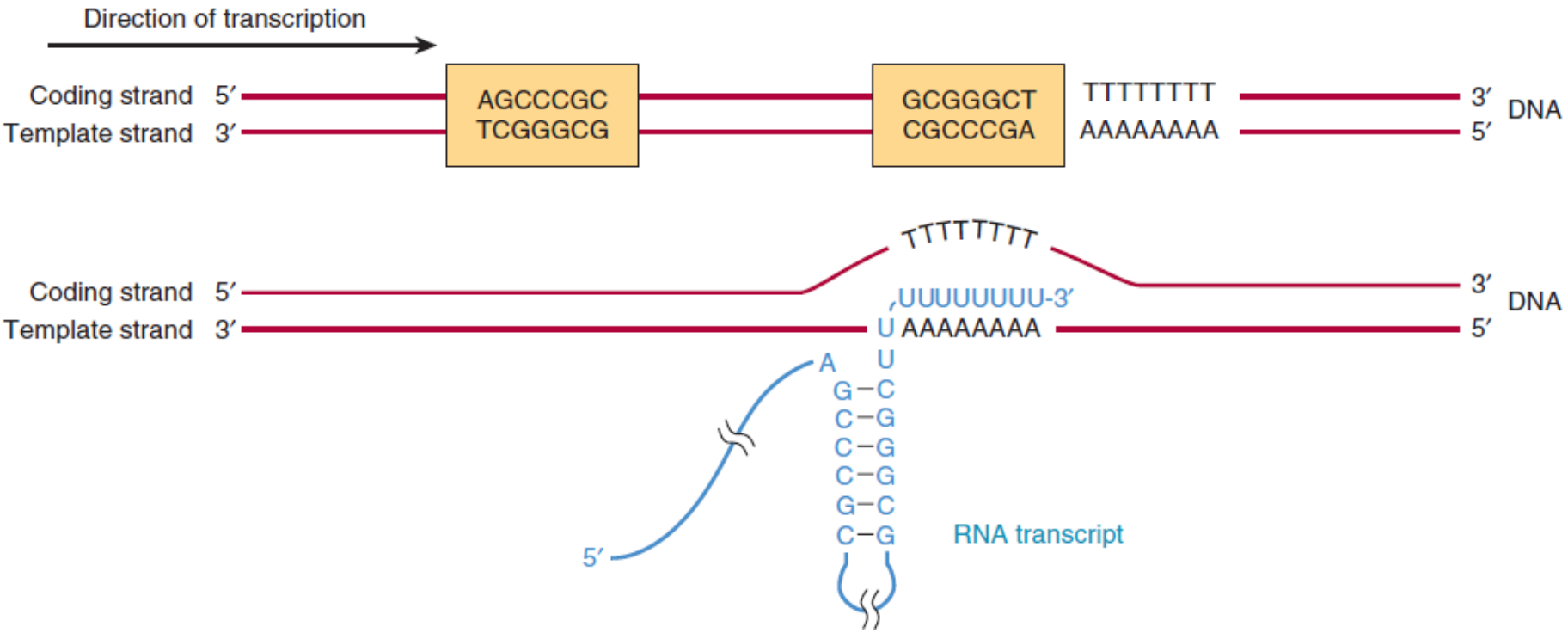
bacterial transcription termination signal



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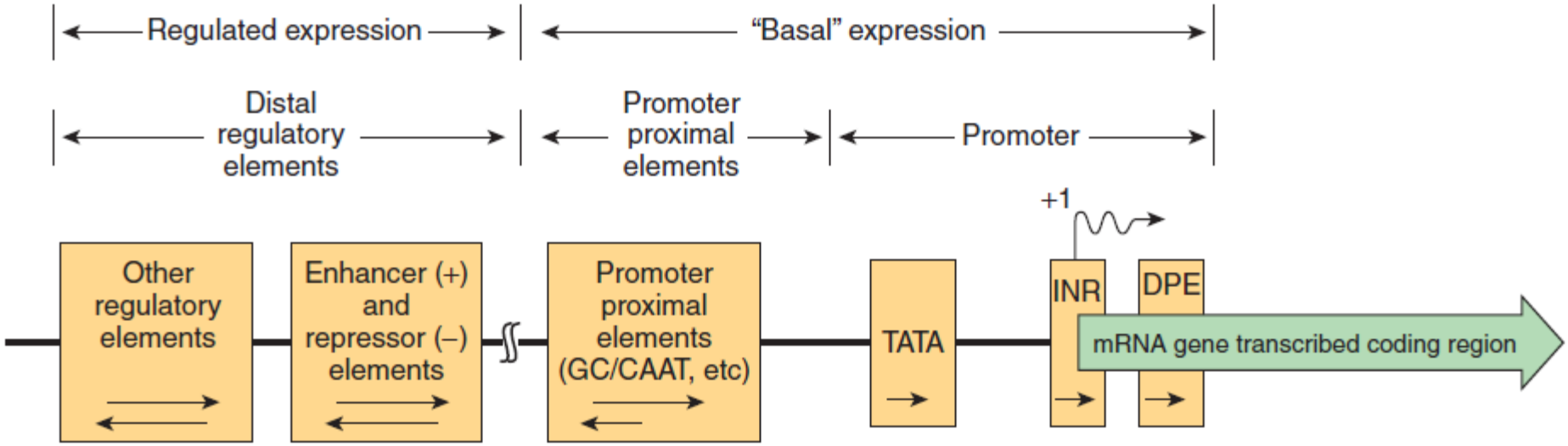
Transcription control regions in an mRNA-producing eukaryotic gene



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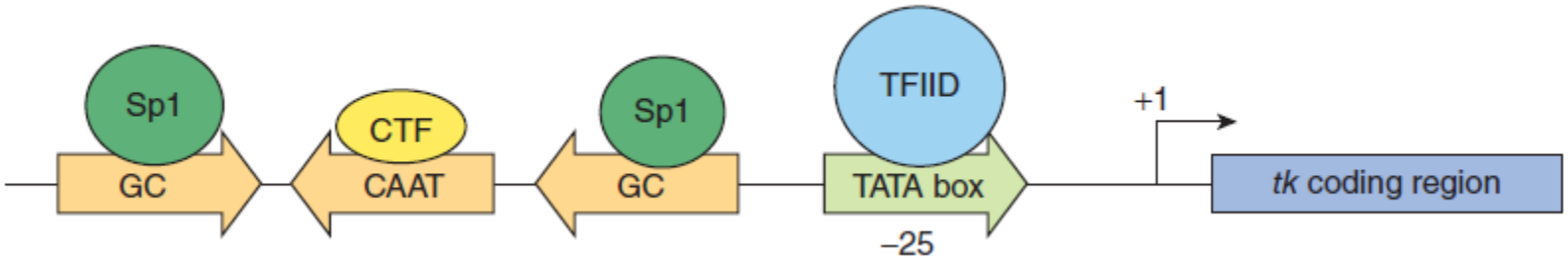
Transcription elements and binding factors



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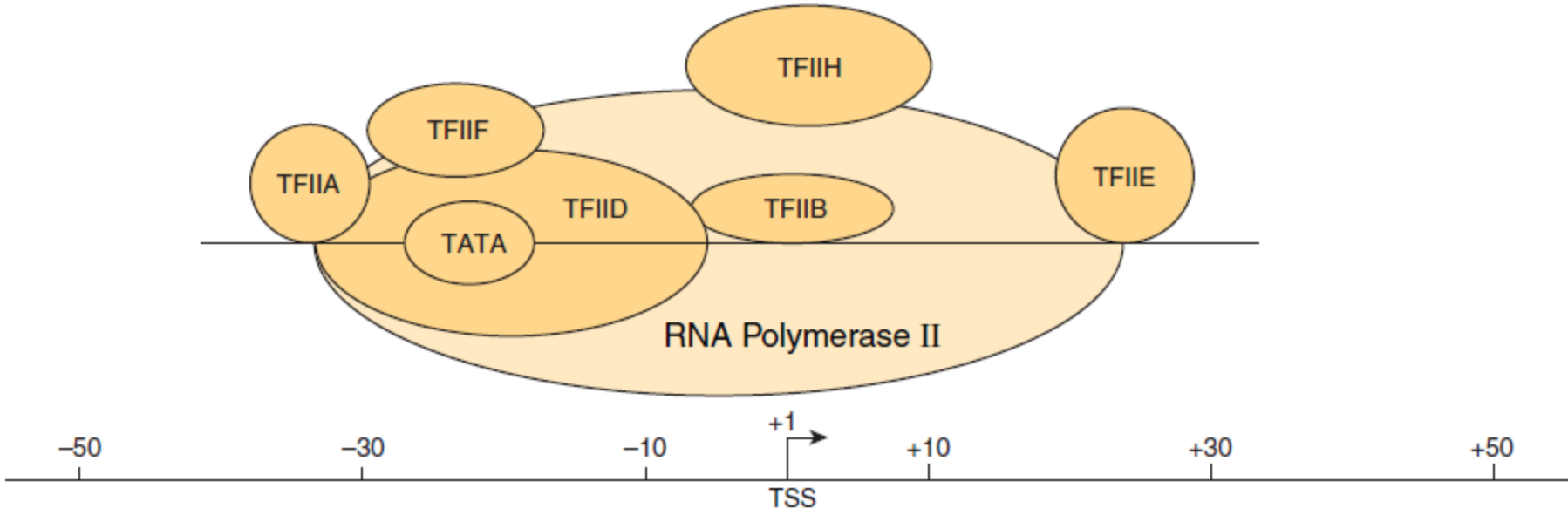
The eukaryotic basal transcription complex



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Video on transcription

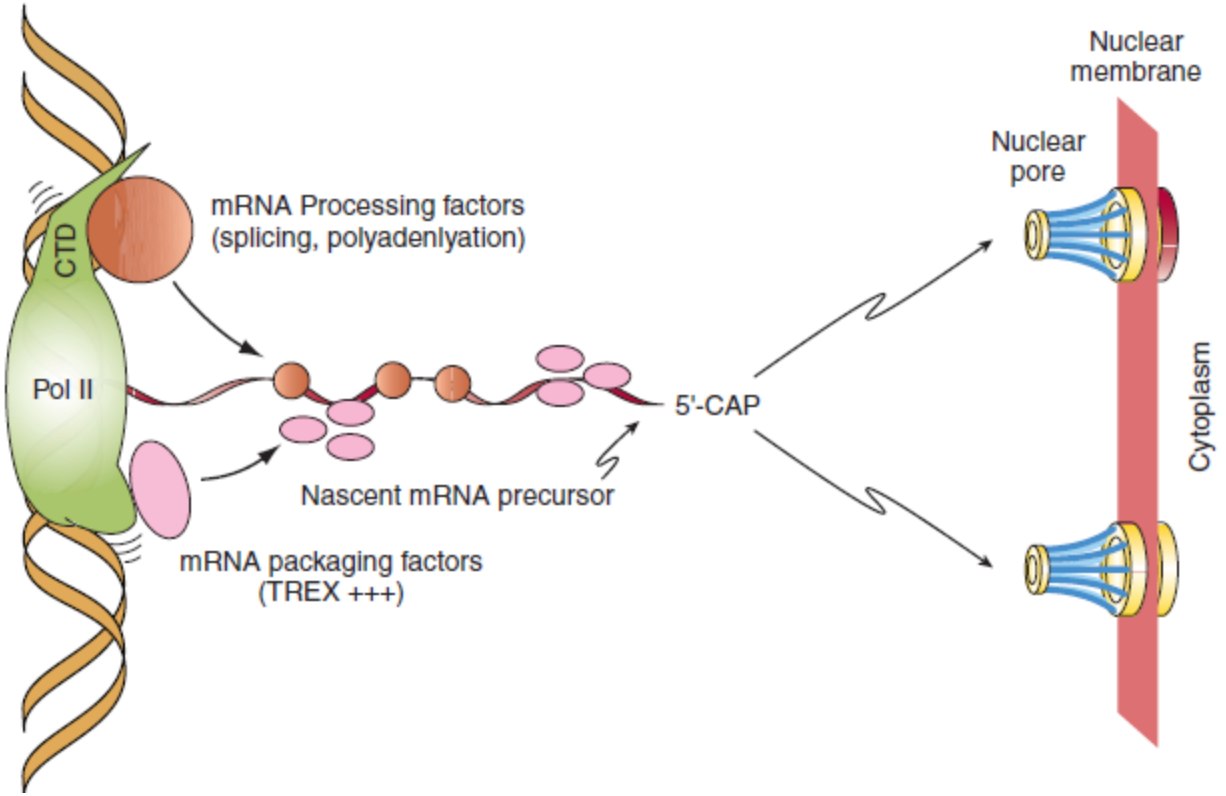
Some of the Mammalian RNA Polymerase II Transcription Control Elements, Their Consensus Sequences, and the Factors That Bind to Them

Element	Consensus Sequence	Factor
TATA box	TATAAA	TBP/TFIID
Inr	T/CT/CANT/AT/CT/C	TFIID
DPE	A/GGA/TCGTG	TFIID
CAAT box	CCAATC	C/EBP*, NF-Y*
GC box	GGGCGG	Sp1*
	CAACTGAC	Myo D
	T/CGGA/CN$_5$GCCAA	NF1*
Ig octamer	ATGCAAAT	Oct1, 2, 4, 6*
AP1	TGAG/CTC/AA	Jun, Fos, ATF*
Serum response	GATGCCCATA	SRF
Heat shock	(NGAAN)$_3$	HSF

Three Classes of Transcription Factors Involved in mRNA Gene Transcription

General Mechanisms	Specific Components
Basal components	RNA Polymerase II, TBP, TFIIA, B, D, E, F, and H
Coregulators	TAFs (TBP + TAFs) = TFIID; certain genes
	Mediator, Meds
	Chromatin modifiers
	Chromatin remodelers
Activators	SP1, ATF, CTF, AP1, etc

RNA polymerase II-mediated mRNA gene transcription is cotranscriptionally coupled to RNA processing and transport.



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Modification/processing of mRNA

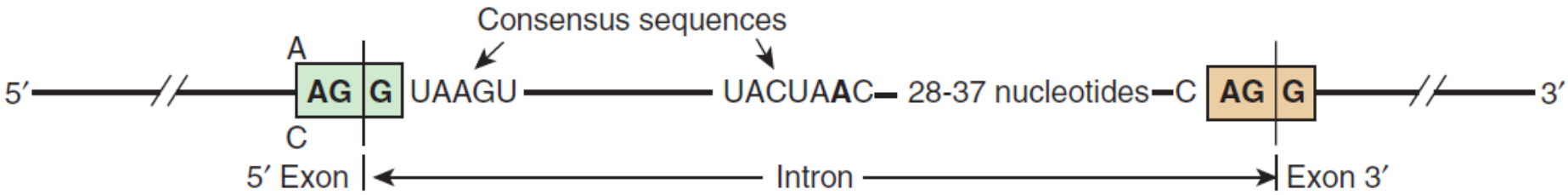
- Addition of 5’ cap
  - In nucleus
  - Efficient translation initiation
  - Protection
- Addition of polyA tail (200)
  - Cleaved about 20 nt downstream **from AAUAA** recognition sequence
  - **Poly A polymerase**
  - Facilitates translation
  - Protection
- Splicing

SPLICING

- **Spliceosome:**

- Involved in converting the primary transcript into mRNA
- Consists of
  - primary transcript
  - five snRNA
  - >60 proteins containing conserved RRM (RNA recognition) and SR (serine arginine) protein motif

Consensus sequences at splice junctions



PyNPyPyPuAPy

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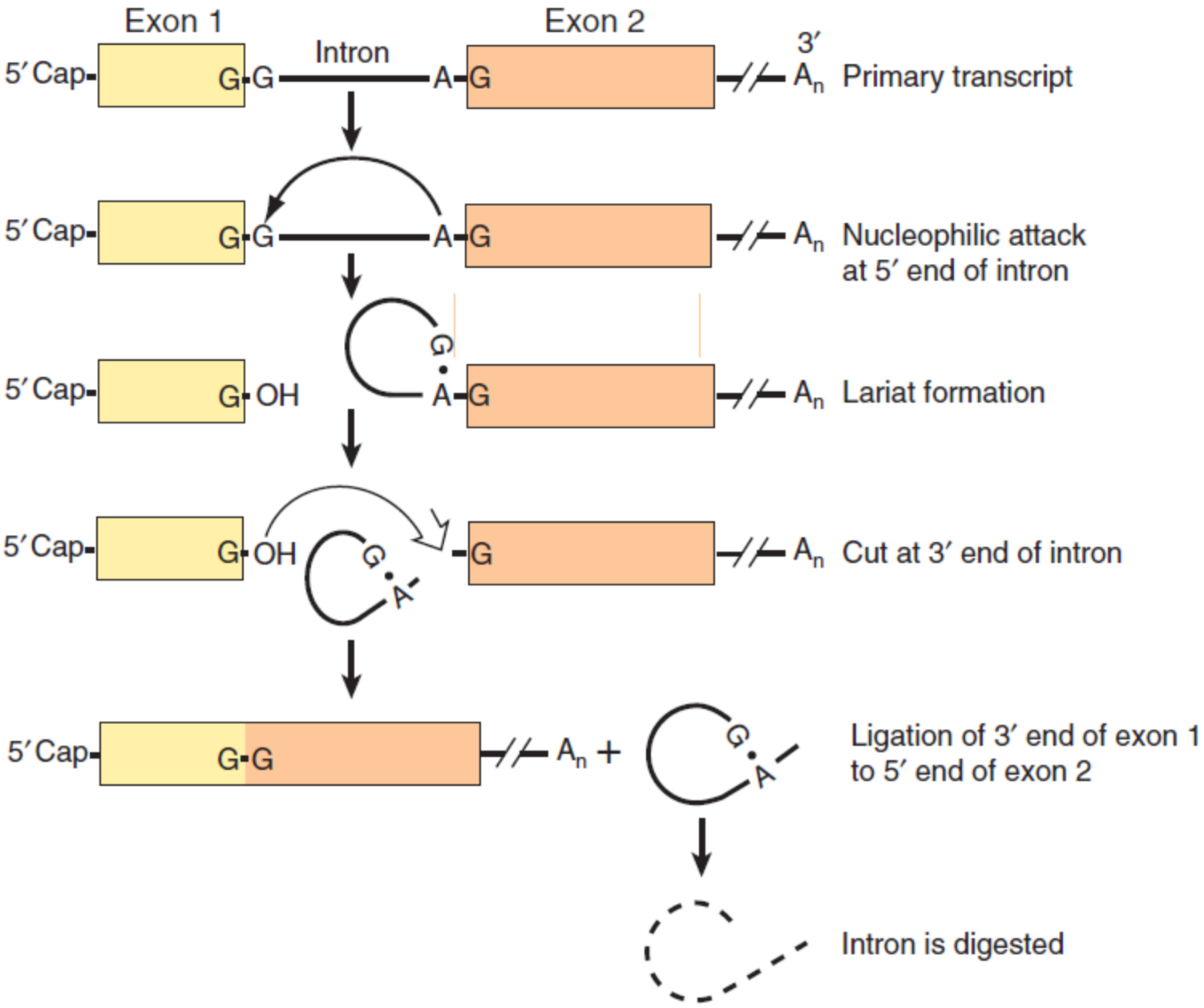
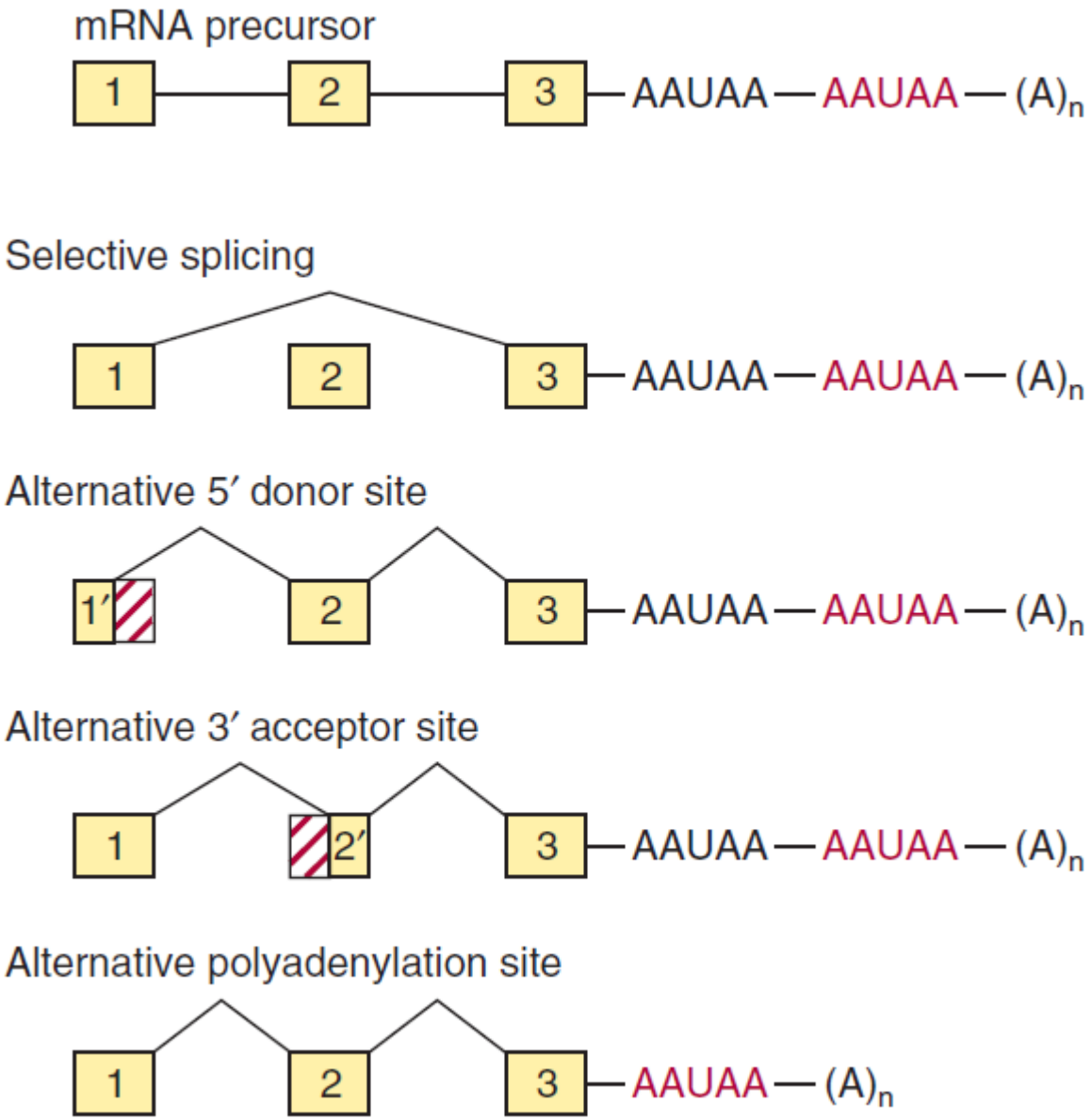


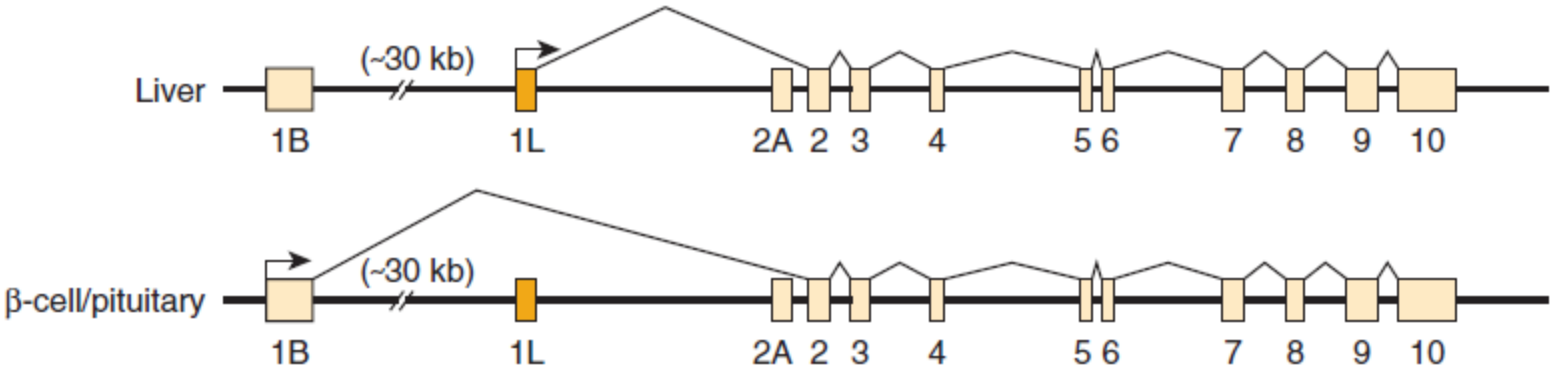
Adapted from Harper's Biochemistry

- Video on splicing

Mechanisms of alternative processing of mRNA precursors



Alternative promoter use in the liver and pancreatic a-cell glucokinase (*GK) genes*



Adapted from Harper's Biochemistry

Clinical implications of splicing

- Mutation at splice site lead to improper splicing
- At least 20% of all genetic diseases result of mutation affecting splicing
- Incorrect splicing of β-globin mRNA responsible for βthalassemia
- Splice site mutation: exons removed and introns retained
- Activate cryptic splice site
- In SLE: Antibodies against nuclear protein such as Sn RNP

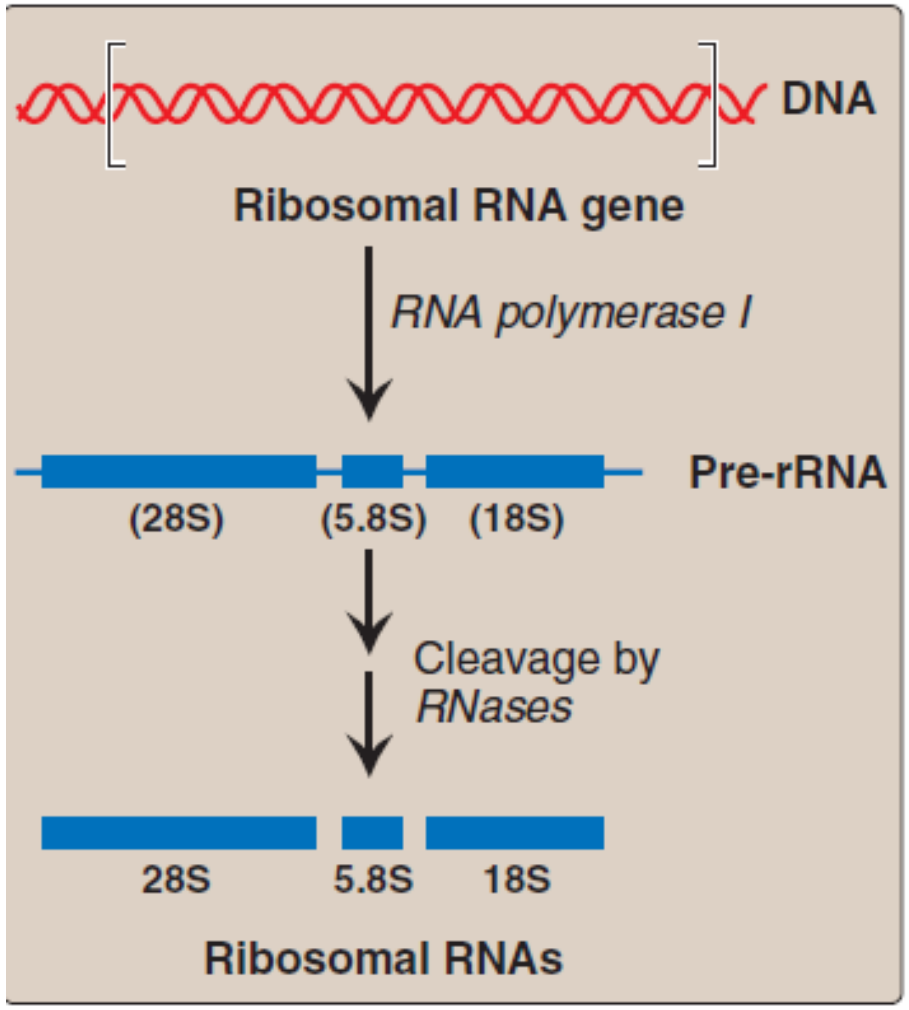
mRNA Editing

- Change of Coding information at the mRNA level
- the coding sequence of the mRNA differs from that in the cognate DNA
- Example: apolipoprotein B *(apoB) gene and mRNA*
  - In liver, synthesis of a 100-kDa apoB100.
  - In the intestine synthesis of a apoB48
    - Cytidine deaminase converts a CAA codon (Glutamine)in the mRNA to UAA
- Example: glutamine to arginine change in the glutamate receptor in trypanosome mitochondrial mRNAs

Processing of rRNA

- generated from 45S long precursor molecules called **pre-rRNAs.**
- 23S, 16S, and 5S rRNA of prokaryotes are produced from a single pre-rRNA molecule,
- Eukaryotic 5S rRNA is synthesized by RNA polymerase III and modified separately
- pre-rRNAs are cleaved by **ribonucleases** to yield intermediate-sized pieces of rRNA
- In eukaryotes, rRNA genes are found in long, tandem arrays
- rRNA synthesis and processing occur in the **nucleolus**, with base and sugar modifications facilitated by **small nucleolar RNAs** (snoRNA)

Posttranscriptional processing of eukaryotic ribosomal RNA by *ribonucleases (RNases).*

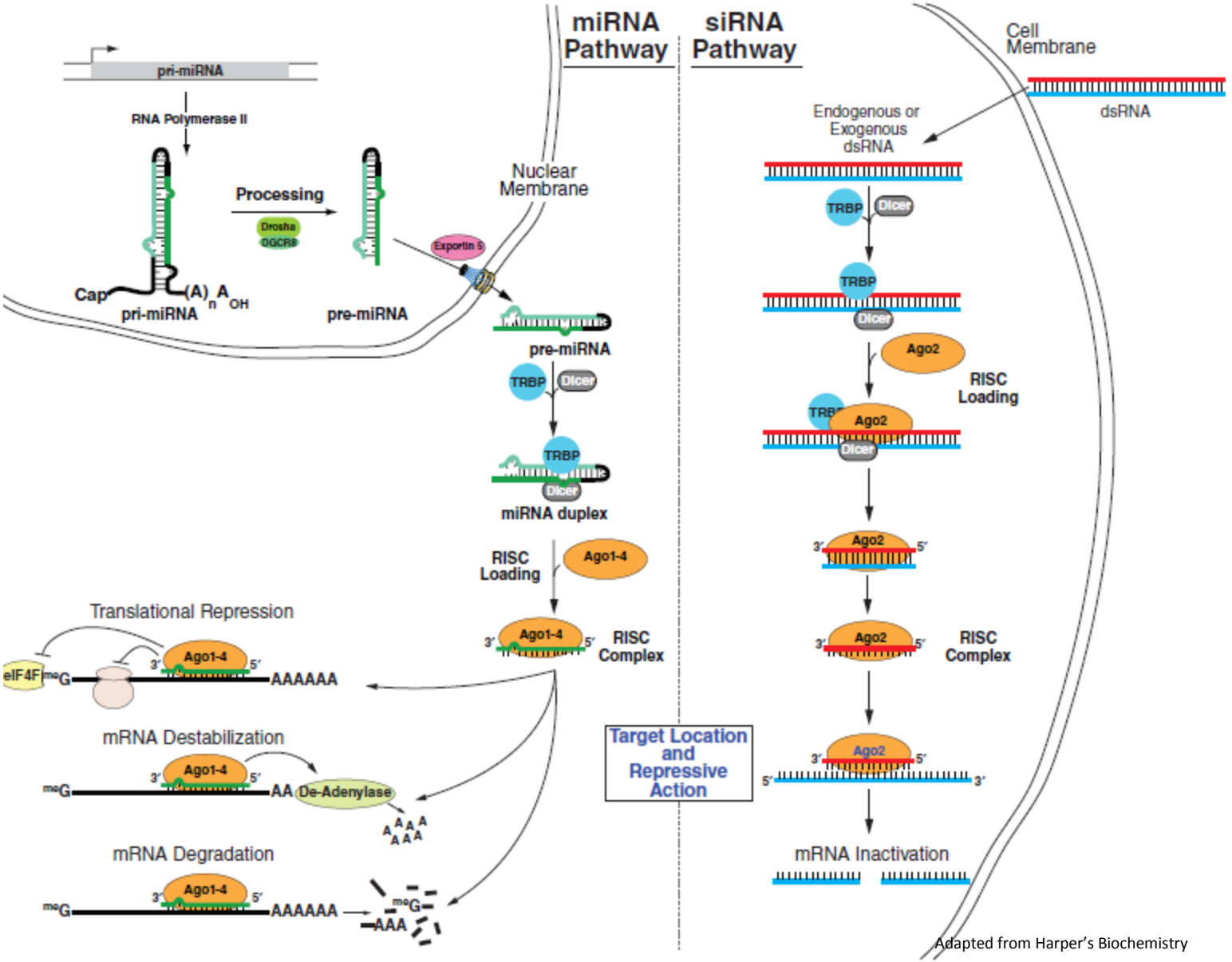


Adapted from Lippincotts Biochemistry

Processing of tRNA

- made from longer precursor molecules
- Sequences at both ends of the molecule are removed and intron is removed from the anticodon loop by nucleases.
- attachment of CpCpA terminal at the 3′ end of the molecule by the enzyme **nucleotidyl transferase**.
- modification of bases at specific positions to produce the “unusual bases” characteristic of tRNA; methylation, reduction, deamination, and rearranged glycosidic bonds, nucleotide alkylations
- Methylation in the nucleus, whereas the attachment of CpCpAOH are cytoplasmic functions

Biogenesis of micro (mi) and silencing (si)RNAs



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Inhibitors of RNA synthesis

Inhibitor	Source	Mode of action
Rifampicin	Synthetic derivative of Rifamycin	Binds to **beta subunit** of RNA polymerase which is inactivated
Alpha amanitin	Toxin from mushroom	Prevents translocation of RNA pol II During phospho diester bond formation
3' –deoxy adenosine	Synthetic analog	Incorrect entry into chain causing chain termination

Summary

- RNA is synthesized from a DNA template by the enzyme DNA dependent RNA polymerase
- While bacteria contain a single RNA polymerase (ββα2σω) there are three distinct nuclear DNA-dependent RNA polymerases in mammals
- RNA polymerases interact with unique *cis-active regions* of genes, termed promoters, in order to form preinitiation complexes (PICs) capable of initiation. In eukaryotes, the process of pol II PIC formation requires, in addition to polymerase, multiple general transcription factors (GTFs), TFIIA, B, D, E, F, and H.
- Transcription exhibits three phases: initiation, elongation, and termination

Summary

- The presence of nucleosomes can occlude the binding of both transfactors and the transcription machinery to their cognate DNA *cis-elements, thereby inhibiting* transcription
- Most eukaryotic RNAs are synthesized as precursors that contain excess sequences which are removed-additional potential steps for regulation of gene expression.
- All steps—from changes in DNA template, sequence, and accessibility in chromatin to RNA stability and translatability—are subject to modulation and hence are potential control sites for eukaryotic gene regulation.

MCQ1

- A 1-year-old male with chronic anemia is found to have β-thalassemia. Genetic analysis shows that one of his β-globin genes has a mutation that creates a new splice acceptor site 19 nucleotides upstream of the normal splice acceptor site of the first intron. Which of the following best describes the new mRNA molecule that can be produced from this mutant gene?
-

  A. Exon 1 will be too short.
  B. Exon 1 will be too long.
  C. Exon 2 will be too short.
  D. Exon 2 will be too long.
  E. Exon 2 will be missing

MCQ2

- The base sequence of the strand of DNA used as the template for transcription is GATCTAC. What is the base sequence of the RNA product? (All sequences are written 5'→ 3' according to standard convention.)
-

  A. CTAGATG.
  B. GTAGATC.
  C. GAUCUAC.
  D. CUAGAUG.
  E. GUAGAUC

MCQ3

- A 4-year-old child who becomes easily tired and has trouble walking is diagnosed with Duchenne muscular dystrophy, an X-linked recessive disorder. Genetic analysis shows that the patient's gene for the muscle protein dystrophin contains a mutation in its promoter region. Of the choices listed, which would be the most likely effect of this mutation?
-

  A. Initiation of dystrophin transcription will be defective.
  B. Termination of dystrophin transcription will be defective.
  C. Capping of dystrophin mRNA will be defective.
  D. Splicing of dystrophin mRNA will be defective.
  E. Tailing of dystrophin mRNA will be defective.

MCQ4

- A mutation to this sequence in eukaryotic mRNA will affect the process by which the 3'-end poly-A tail is added to the mRNA.

A. CAAT
B. CCA
C. GGGGCG
D. AAUAAA
E. TATAAA

	Polycistronic mRNA	Monocistronic mRNA
Messenger	Polycistronic mRNA is that messenger RNA which encodes for two or more proteins.	Monocistronic mRNA is that messenger RNA which encodes for only one or specific protein or polypeptide.
Codons	Polycistronic mRNA contains many codons of cistrons.	Monocistronic mRNA contains single codon of cistron.
ORF	Polycistronic mRNA have multiple ORFs (open reading frames).	Monocistronic mRNA have single ORF (open reading frame).
Available in	Polycistronic mRNA is present mostly in prokaryotes like bacteria etc.	Monocistronic mRNA is present in eukaryotes like human cells.
Post-Transcriptional Changes	Polycistronic mRNA do not require post-transcriptional changes.	Monocistronic mRNA requires post-transcriptional changes.