RNA synthesis and processing

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RNAs of protein synthesis

- Messenger RNA (mRNA)
- Ribosomal RNA (rRNA)
- Transfer RNA (tRNA)

Transcription

- **Ribonucleotides used**
- **Uracil instead of thymine used**
- **Primer not required by RNA polymerase**
- **Only a portion of genome is transcribed.**
- **The template strand is transcribed**
- **The other strand is coding strand as RNA resembles except U instead of T**

Direction

- Reading is 3’ to 5’ direction
- Synthesis is from 5’ to 3’ direction

RNA synthesis

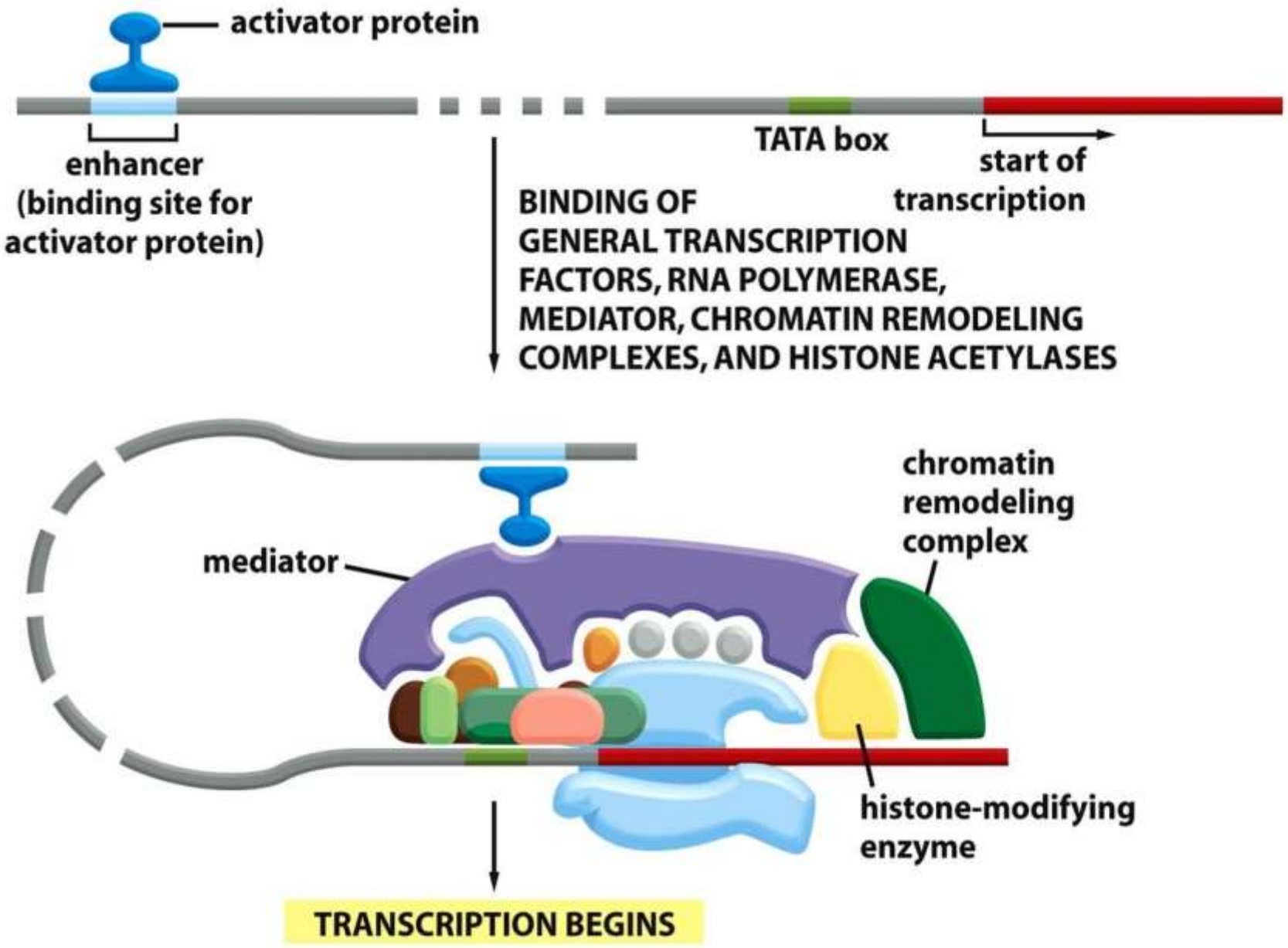
- Initiation
- Elongation
- termination

Initiation

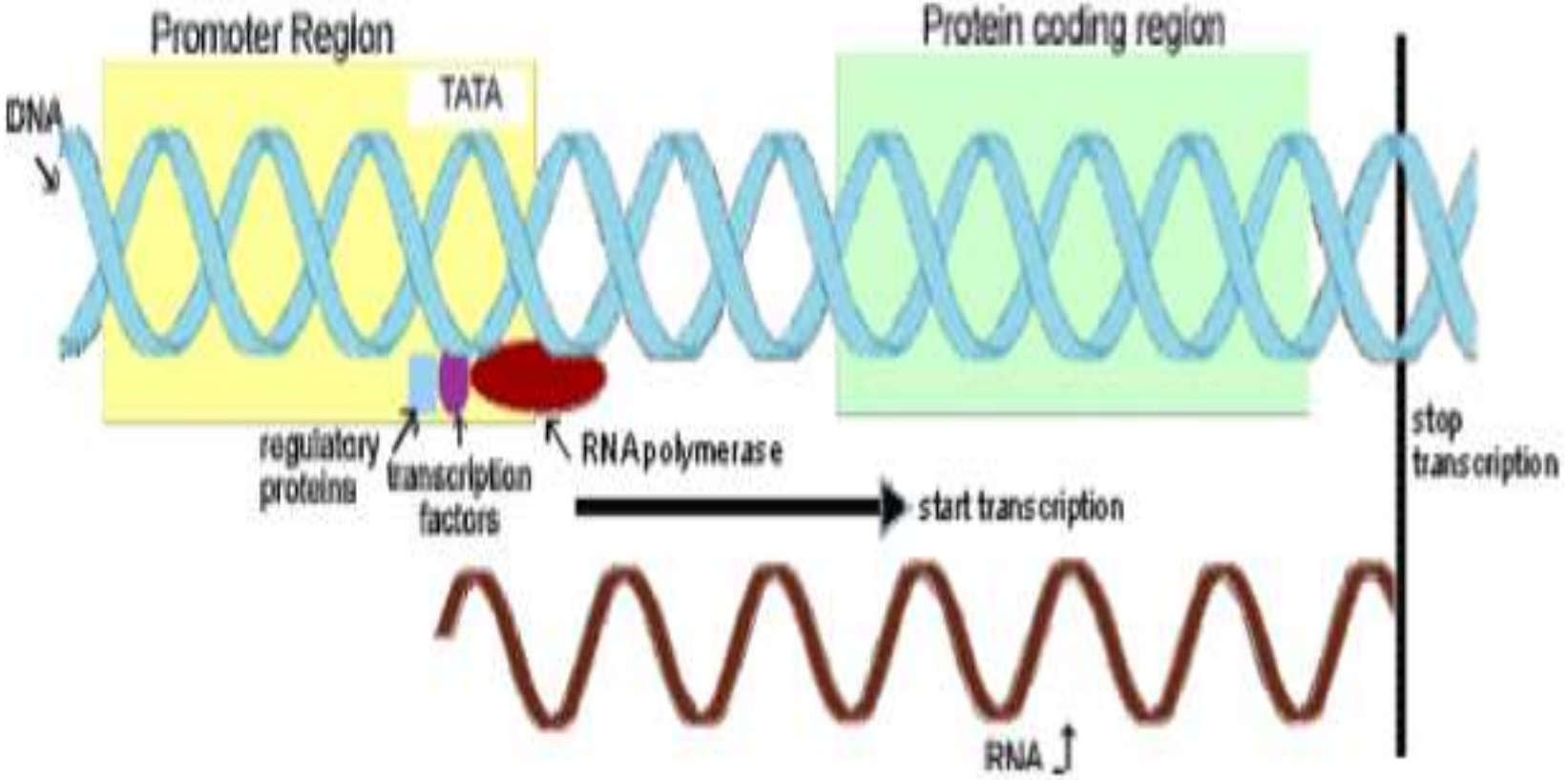
- Promoter site
- 10^5 transcription sites on the entire DNA
- 5’ end of RNA transcript is +1 nt
- The bases upstream are -1,-2,-3 …….n

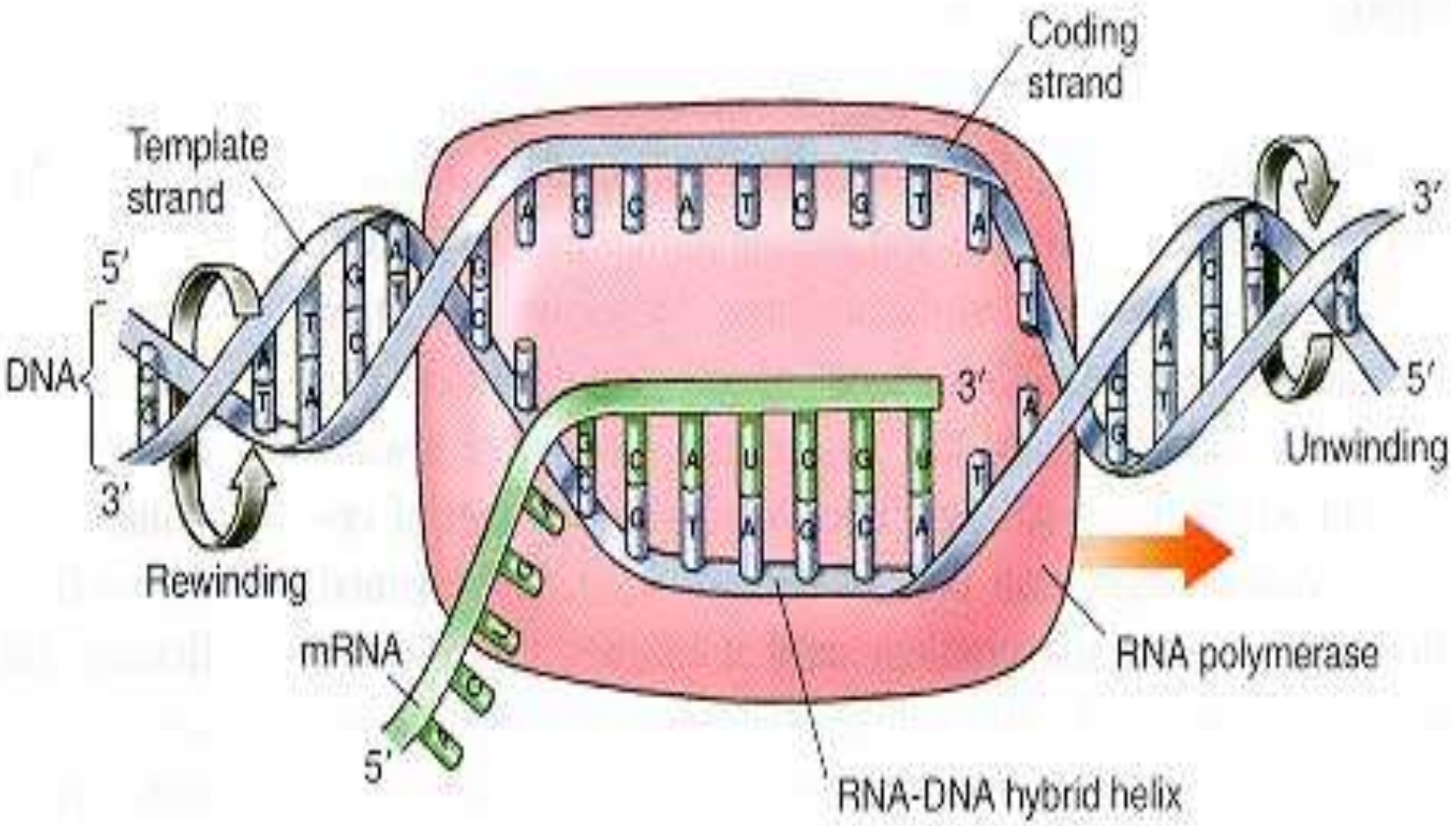
TATA box

- In bacteria - 10 bp (upstream), sequence of 5’- TATAAT-3’. TATA box – In the coding region- TATA-binding protein (TBP) binds to this region.
- Transcription factors and RNA polymerase combine the TATA box to form Pre-initiation complex (PIC).
- Activators and repressors also bind.


activator protein
enhancer
(binding site for
activator protein)
TATA box
start of
transcription
BINDING OF
GENERAL TRANSCRIPTION
FACTORS, RNA POLYMERASE,
MEDIATOR, CHROMATIN REMODELING
COMPLEXES, AND HISTONE ACETYLASES
chromatin
remodeling
complex
mediator
histone-modifying
enzyme
TRANSCRIPTION BEGINS

Typical gene organization





Transcription

One of the strands of DNA functions as a template on which nucleotide building blocks are assembled into mRNA by RNA polymerase as it moves down the DNA strand.

In Eukaryotes

- `TATAAA' sequence known as Golberg-Hogness box - -25 to -30 position- start site. – in the coding strand
- upstream -70 to -80, there is another sequence GGCCAATCT, known CAAT box.- cis-acting signal

In bacteria

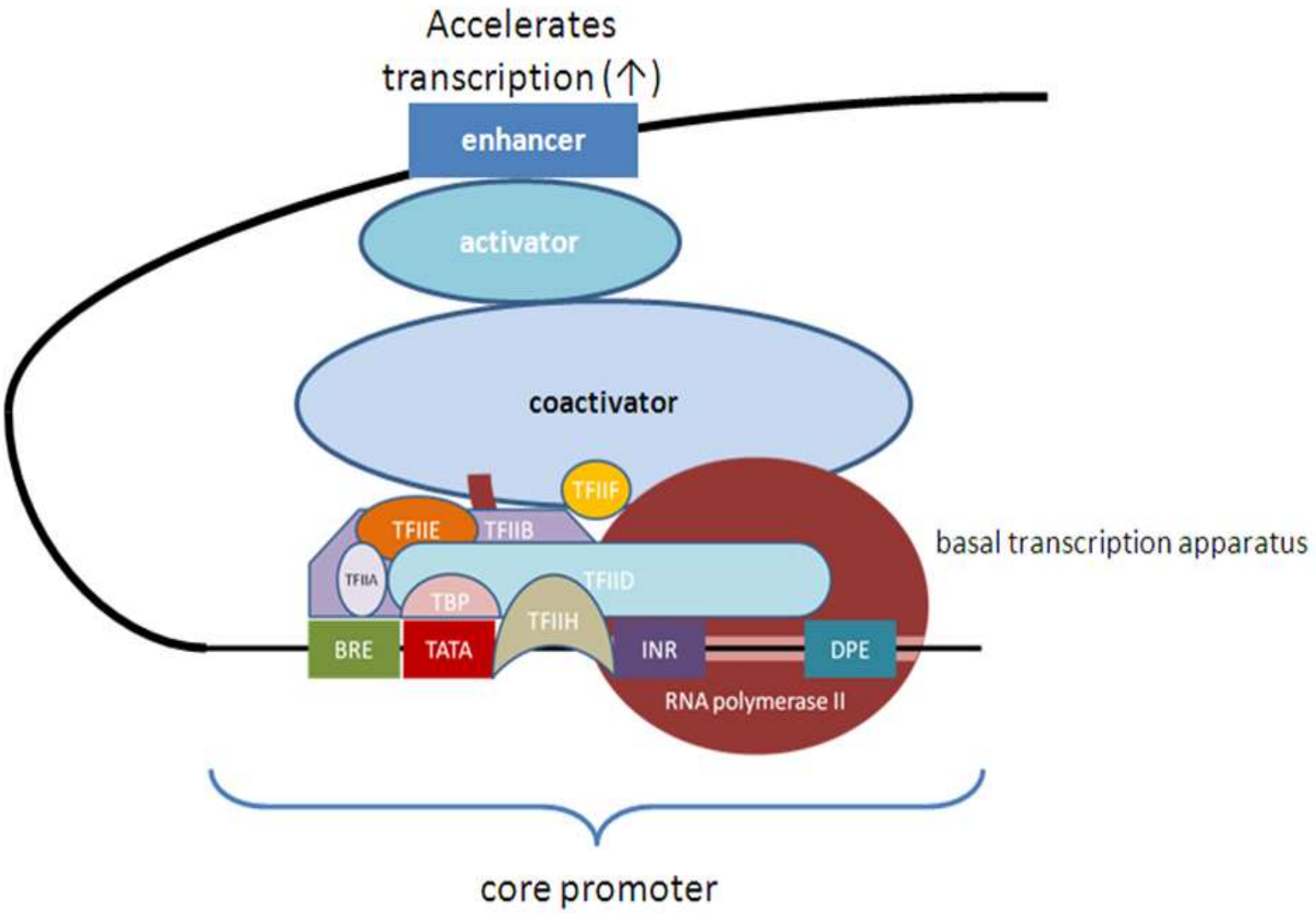
- RNAP binds to promoter site -35 bp upstream and forms a **closed complex.**
- TATA box eases the dissociation of DNA strands so that RNAP bound to promoter.
- It can have access to downstream - **open complex.**

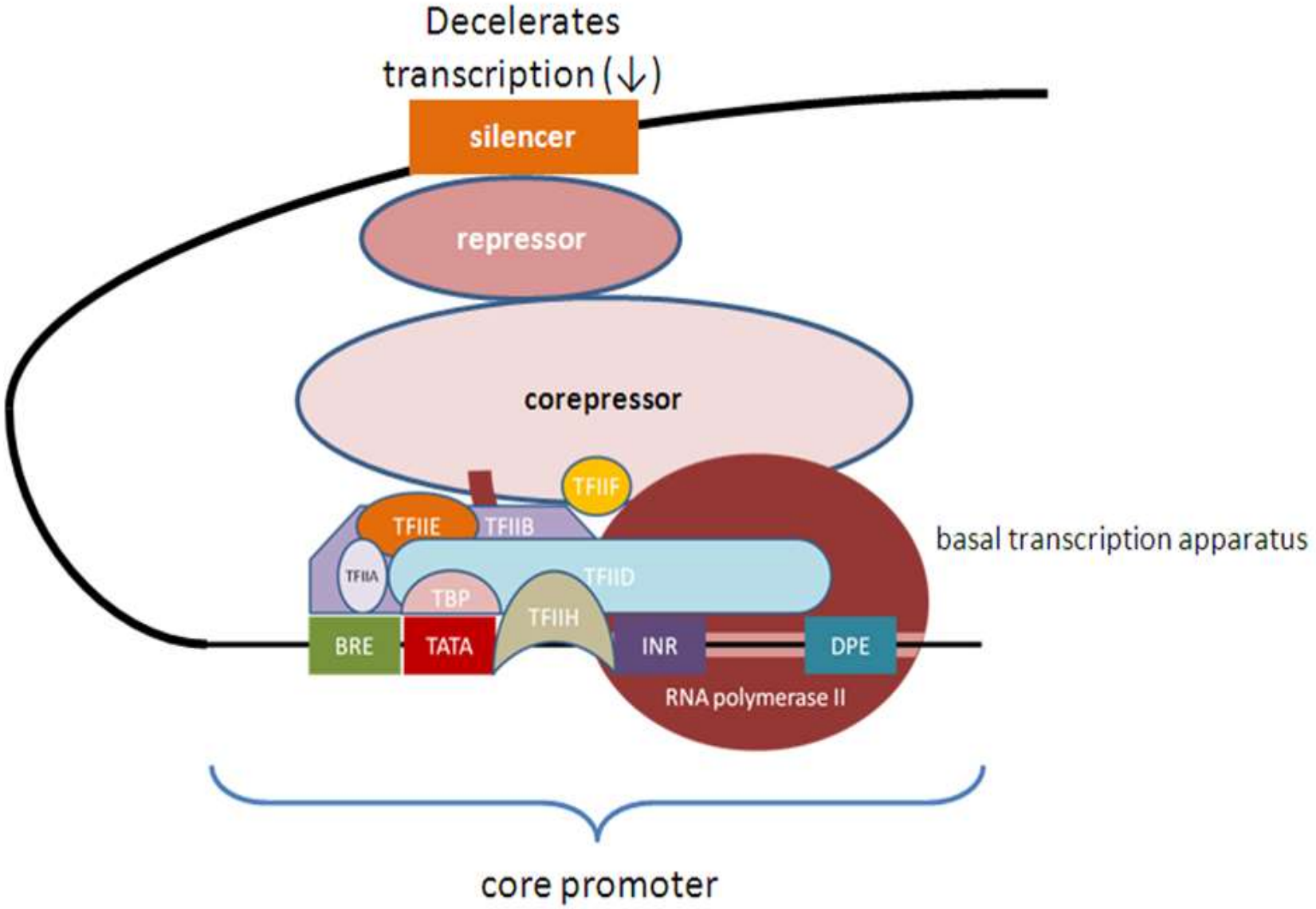
Human TATA box

- The human TATA box is bound by TATA binding protein (TBP) having many subunits.
- Non-TBP subunits are called TBP associated factors (TAFs).
- This complex of TBP and TAFs is TF IID.

No TATA box

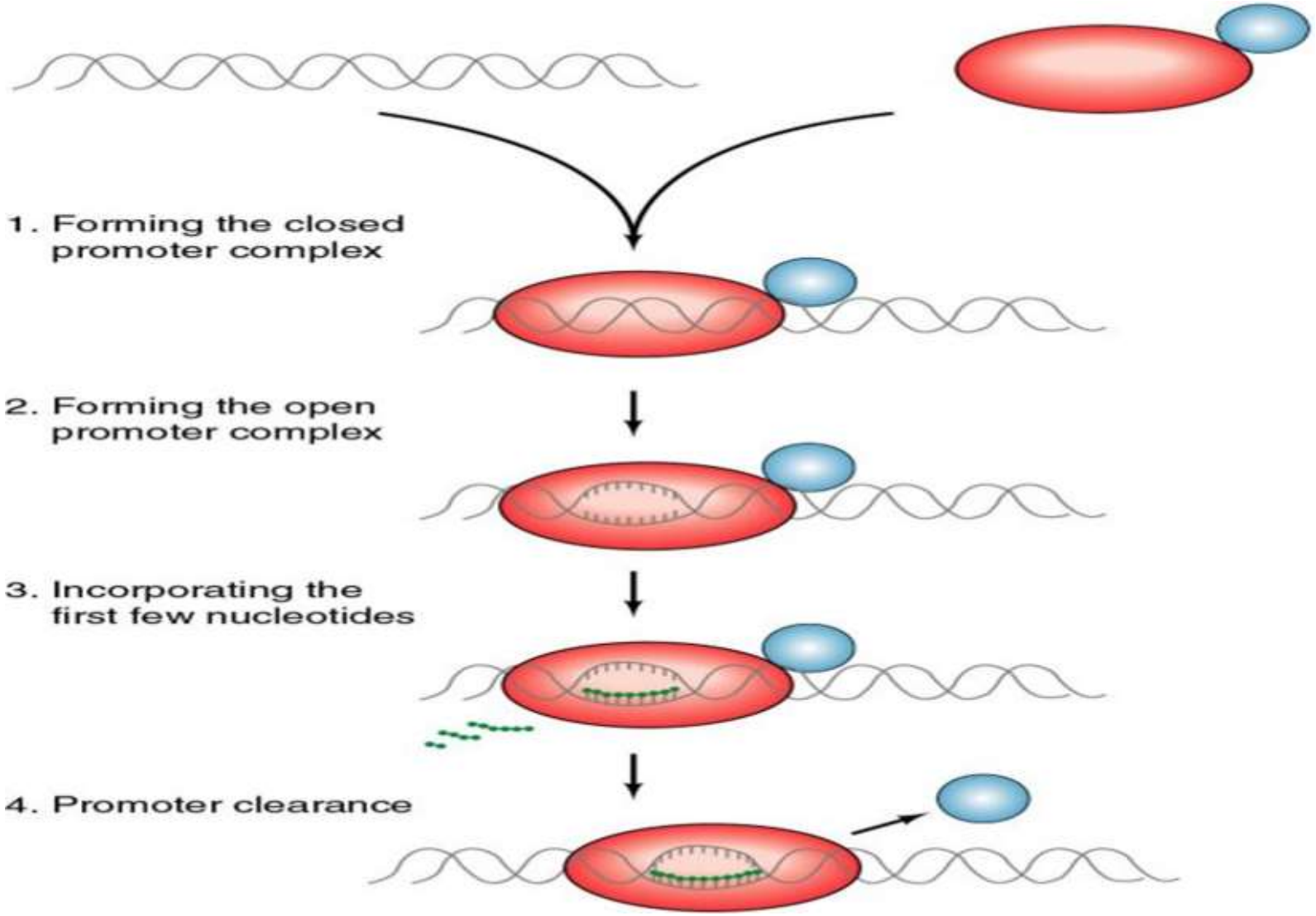
- Additional CIS elements such as initiator sequence (Inr) and or
- the downstream promoter element (DPE) which direct RNAP.


Accelerates
transcription (↑)
enhancer
activator
coactivator
TFIIF
TFIIE
TFIIB
TFIIA
TFIID
TBP
TFIIH
BRE
TATA
INR
DPE
RNA polymerase II
basal transcription apparatus
core promoter


Decelerates
transcription (↓)
silencer
repressor
corepressor
TFIIF
TFIIE
TFIIB
TFIIA
TFIID
TBP
TFIIH
BRE
TATA
INR
DPE
RNA polymerase II
basal transcription apparatus
core promoter

RNAPs

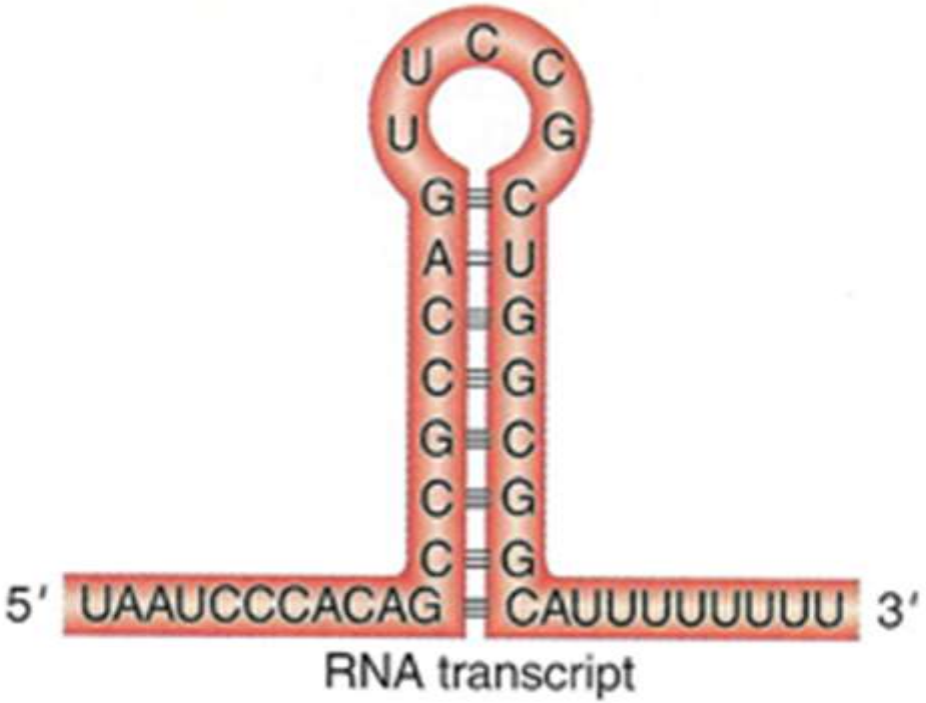
- RNAP I is insensitive to α- amanitin - rRNA synthesis.
- RNAP II is highly sensitive to α- amanitin-s mRNA, miRNA, snRNA synthesis.
- RNAP III is intermediate sensitive to α-amanitin - tRNA, 5S rRNA synthesis.


1. Forming the closed promoter complex
2. Forming the open promoter complex
3. Incorporating the first few nucleotides
4. Promoter clearance

Termination

- In Rho-independent transcription termination, intrinsic termination, RNA transcription stops when the newly synthesized RNA molecule forms a G-C-rich hairpin loop followed by a run of Us.
- When the hairpin forms, the mechanical stress breaks the weak rU-dA bonds,
- This pulls the poly-U transcript out of the active site of the RNA polymerase, terminating transcription.

Bacterial transcription terminator



Rho dependent termination

- A sequence in the template strand of DNA - recognized by a termination protein, the rho(p factor).
- Rho factor is an ATP dependent RNA stimulated helicase that disrupts RNAP, nascent RNA and DNA.
- Transcription termination in eukaryotes is less understood

Elongation phase

- Pyrophosphate is released following each cycle of polymerization
- this is rapidly degraded to phosphate by inorganic pyrophosphatase enzyme.
- DNA unwinding occurs for RNAP to have access to the template.
- The unwinding results in transcription bubble which is constant throughout transcription.

Unwinding

- Unwinding of DNA is dictated by RNAP which has intrinsic unwindase activity.
- Topoisomerase both precedes and follows RNAP and prevents supercoil tensions.
- RNAP – no nuclease activity . No proof reading

PTM of RNA

- **mRNA-** 7-methyl guanosine tri -P cap structure to 5’ end
- Poly A tail to 3’ end mRNA precursor.
- The cap protects mRNA from attack by 5’to 3’ exonuclease.
- Poly(a) tail protects 3’ end of mRNA from attack by 3’to 5’ exonuclease.

Cytoplasmic modification

- Cytoplasmic enzymes can both add and remove adenylyl residues from the poly A tails.
- It alters mRNA stability and translatability.
- In cytoplasmic organelles called P- bodies (Processing bodies or P-bodies are involved in mRNA turnover).

UTR

- Extra nucleotides found in untranslated regions (UTR) on both ends of 5’ and 3’ of coding region.
- The function of UTR is not known. The micro RNAs target sequences within the 3’ UTR.

RNA capping and polyadenylation

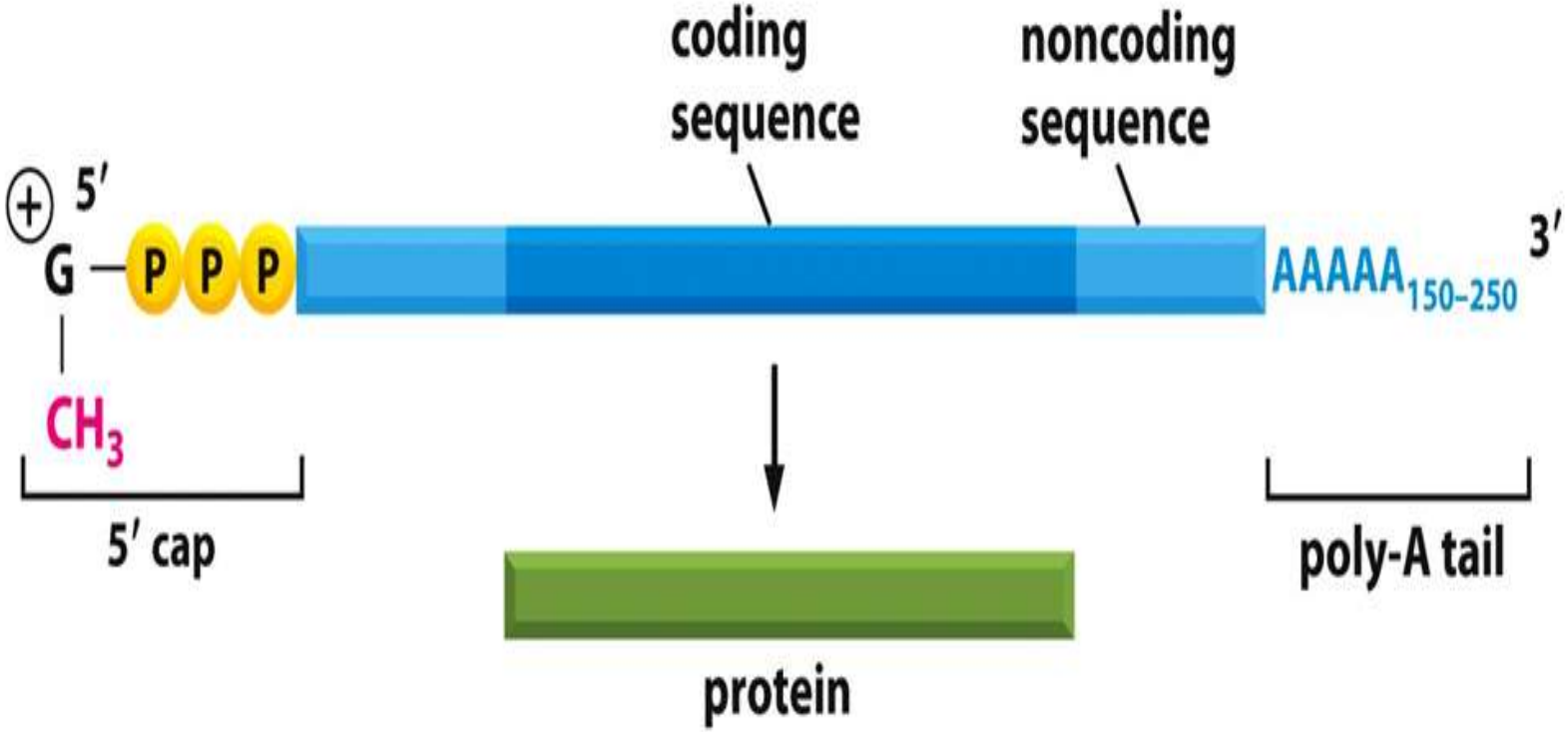


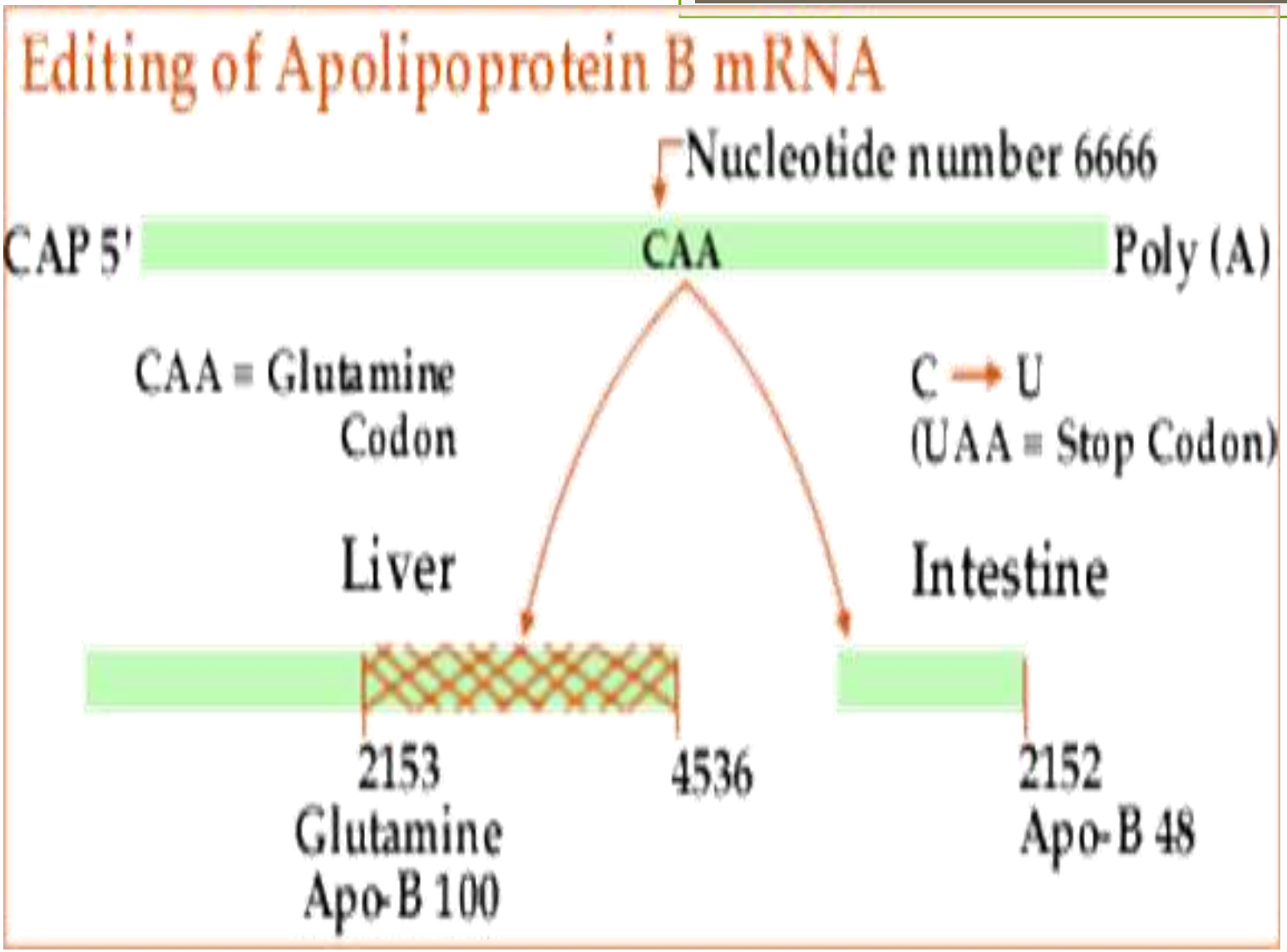
Figure 7-16a Essential Cell Biology 3/e (© Garland Science 2010)

UTR- clinical applications

- Mutations in the untranslated regions of mRNA (UTR) can also lead to diseases.
- Eg: breast cancer, Fragile X syndrome, bipolar disorder and Alzheimer's disease.

RNA editing

- RNA editing changes mRNA- coding information - changed at the level of mRNA editing.
- Apo B gene in liver - B100 (100 kDa) protein.
- In the intestine -the same gene- cytidine deaminase enzyme converts CAA codon in the mRNA to UAA at a single specific site.
- Instead of glutamine, it becomes termination codon. So Apo B48 (48 kDa) protein is formed


Editing of Apolipoprotein B mRNA
Nucleotide number 6666
CAP 5'
CAA
Poly (A)
CAA = Glutamine Codon
C → U
(UAA = Stop Codon)
Liver
Intestine
2153
4536
2152
Glutamine Apo-B 100
Apo-B 48

tRNA

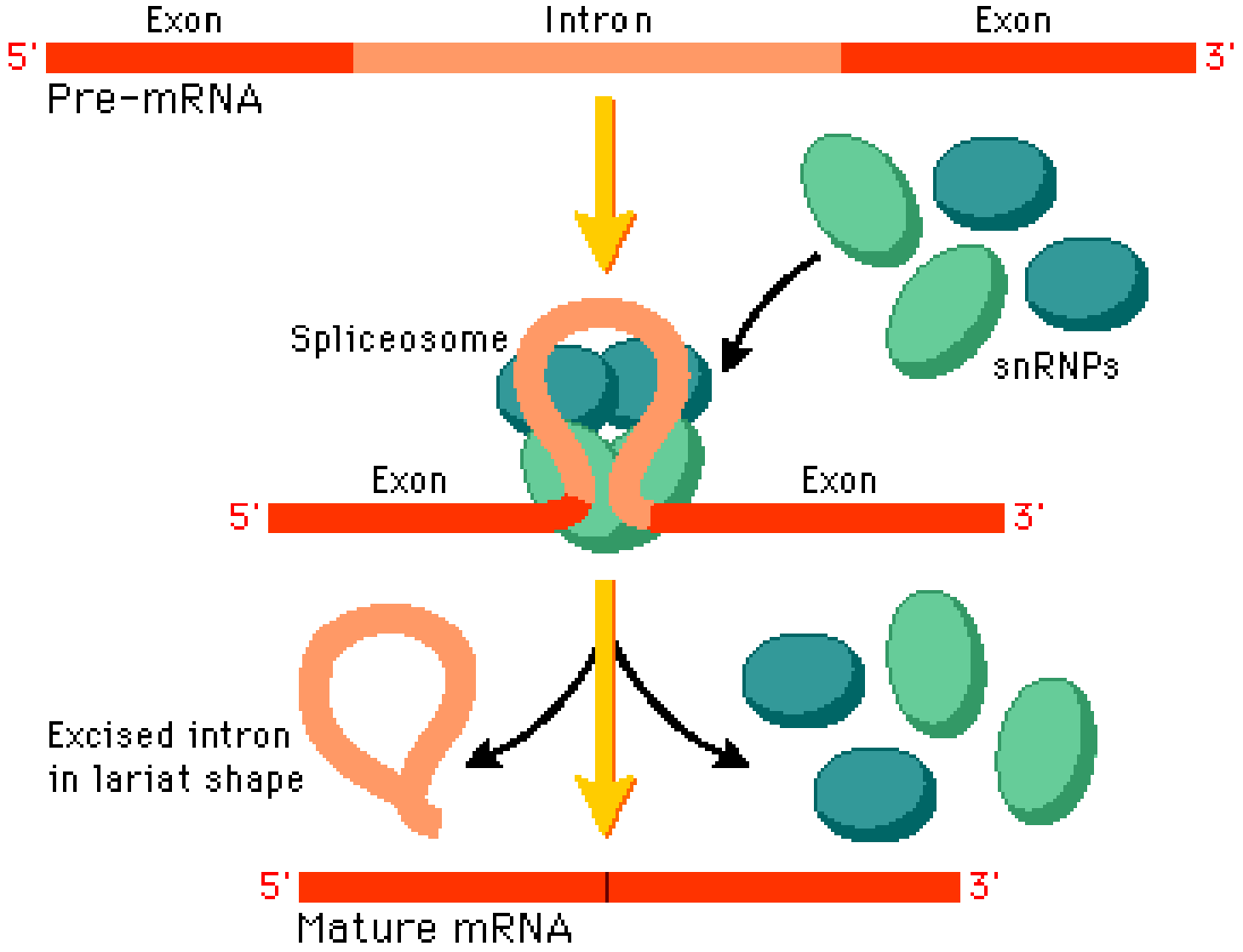
- Modification of bases A,U,G and C - methylation, reduction, deamination and rearranged glycosidic bonds.
- CCA sequence is attached to 3’ end of tRNA by nucleotidyl transferase in cytoplasm.
- The 3-OH group of A- ribose is the point of attachment of amino acid.

Introns removal and splicing of exons

- Splicing is done by spliceosomes.
- It consists of the primary mRNA transcript , five snRNAs (U1, U2, U4, U5, U6 and many proteins.
- This complex -Small nuclear ribonucleoprotein complex (snurps).

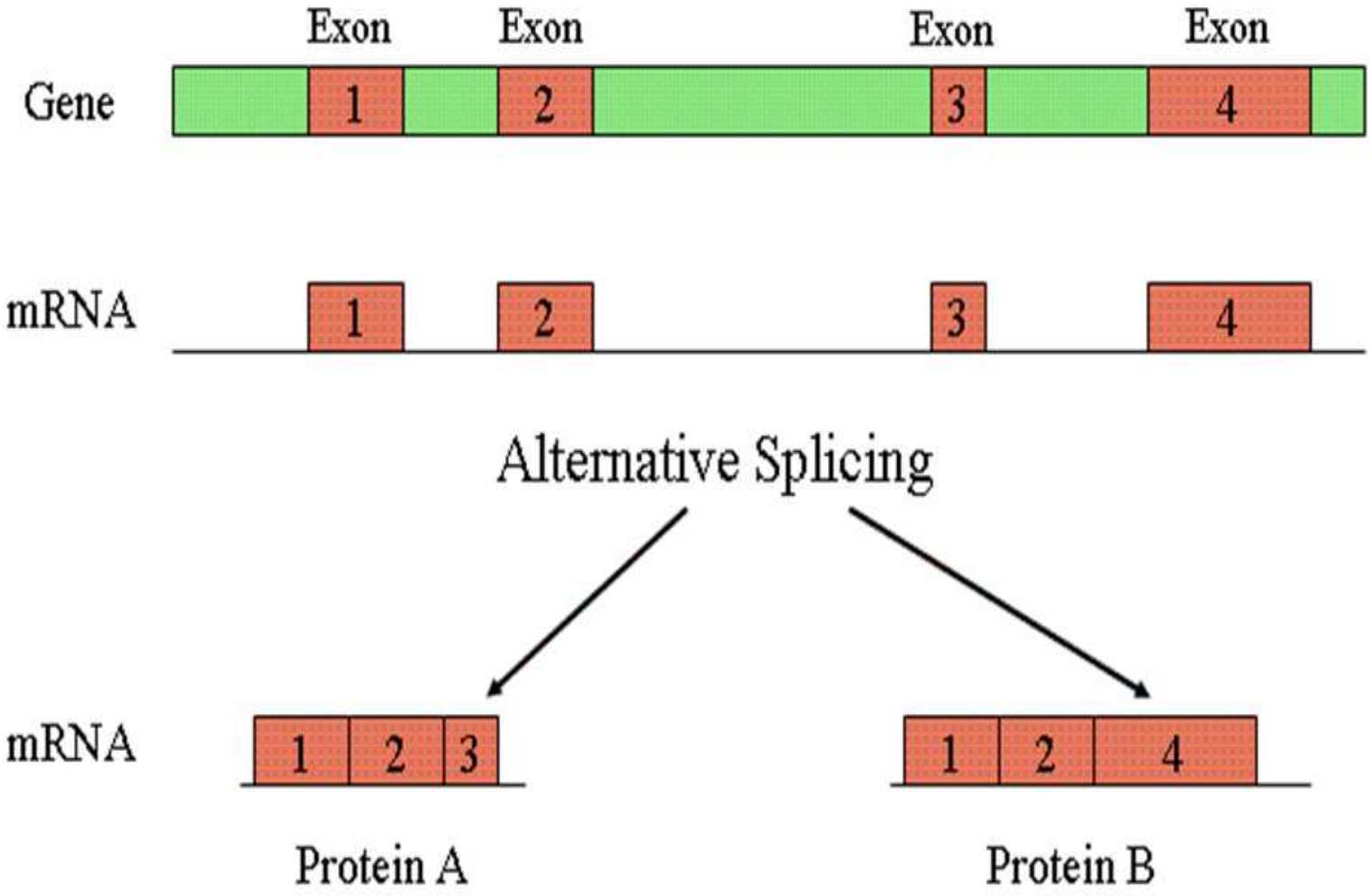
Splicing

- The splicing starts from 5’- end of exon-intron junction.
- 5’ end of intron undergoes nucleophilic attack.
- Intron forms a loop or lariat. Second cut is made at 3’ and of intron.
- Ligation of 3’ end of exon-1 with 5’ end of exon-2 is done.
- Intron is digested.


Exon
Intron
Exon
5'
3'
Pre-mRNA
Spliceosome
snRNPs
Exon
Exon
5'
3'
Excised intron
in lariat shape
5'
3'
Mature mRNA

Alternative splicing

- The processing of mRNA is also a site for regulation of gene expression.
- By selective splicing and altering donor site, alternative splicing is done.
- Different mRNAs from the same primary transcript formed.


Exon
Exon
Exon
Exon
Gene
1
2
3
4
mRNA
1
2
3
4
Alternative Splicing
mRNA
1
2
3
1
2
4
Protein A
Protein B

Faulty splicing

- e.g: In β- thalassemia, globin gene of hemoglobin- under expressed due to nucleotide change in exon- intron junction.
- Splicing modulation – helps- Duchenne muscular dystrophy, HIV.

Alternative promoter utilization

- Tissue specific gene expression by alternative splicing or by the use of alternative promoters.
- e.g:. Glucokinase gene has 10 exons and 9 introns. 2-10 exons is identical in liver and β-cells of pancreas.
- Two different promoters - β- cells, the liver promoter and exon IL - removed by splicing.

Ribosomal RNA

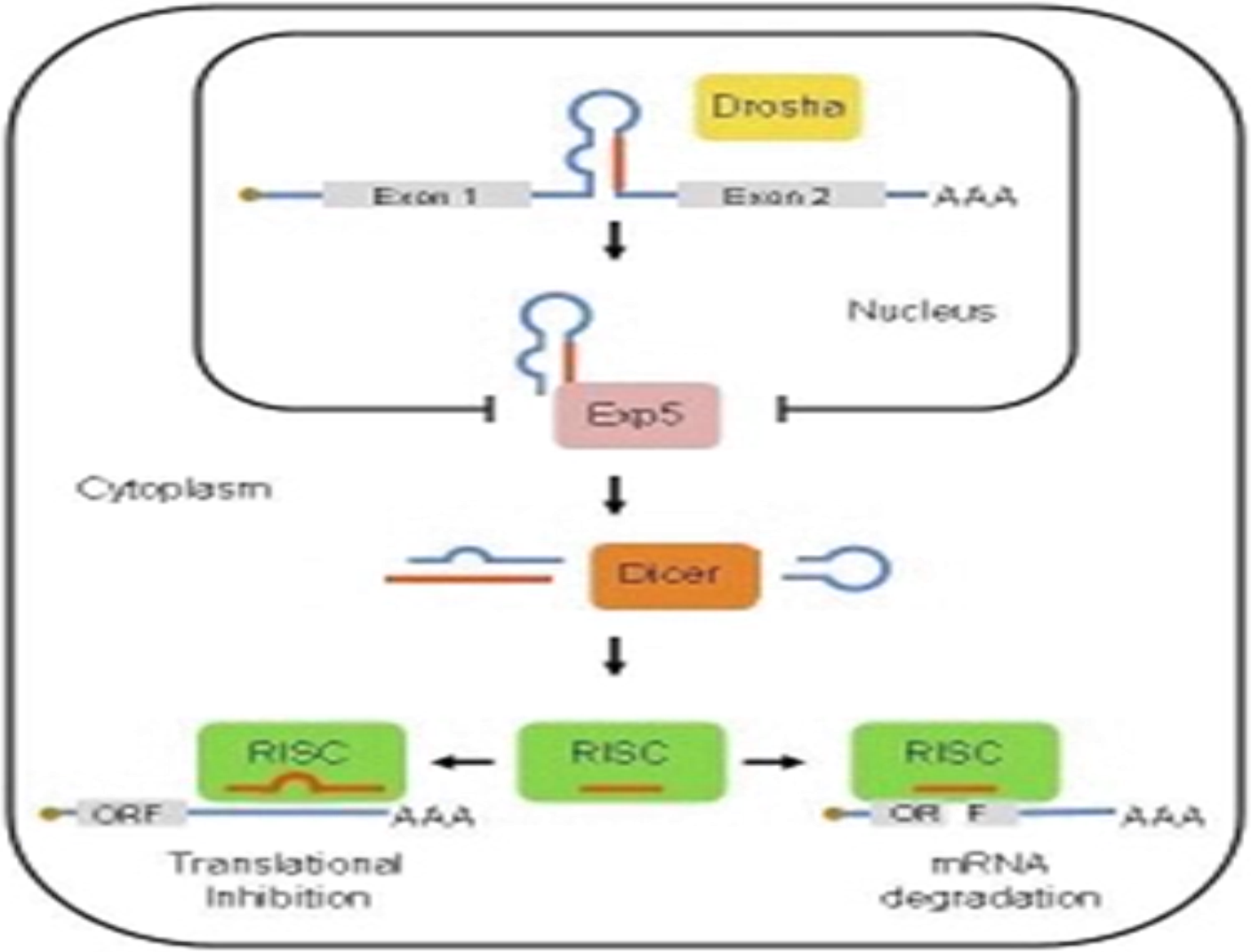
- The three rRNA molecules (28S,18S,5.8S) are from a single 45S precursor rRNA.
- The precursor is processed in the nucleolus to its components.
- The rRNA genes are present in the nucleolus of mammalian cells.

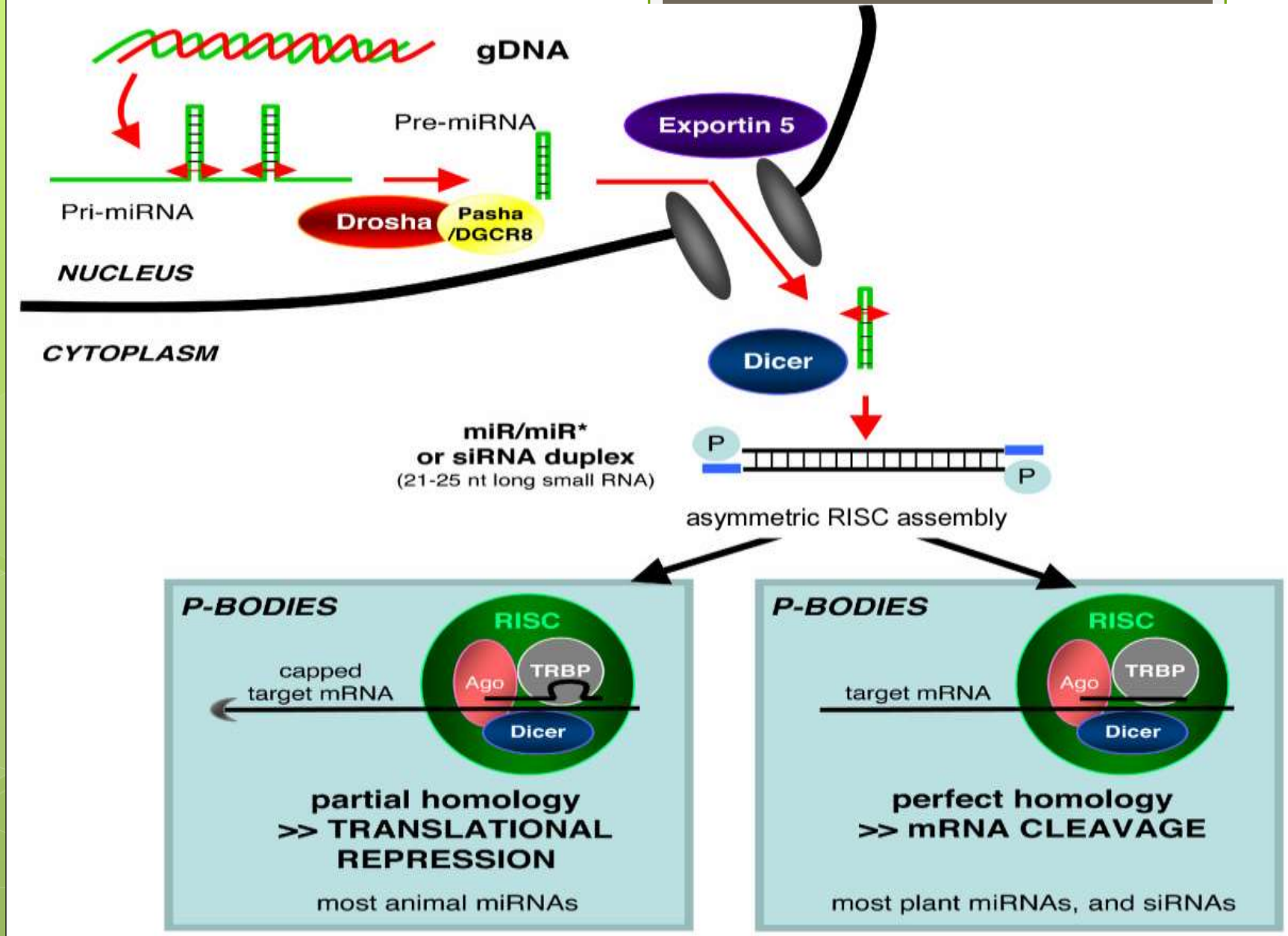
Clinical applications

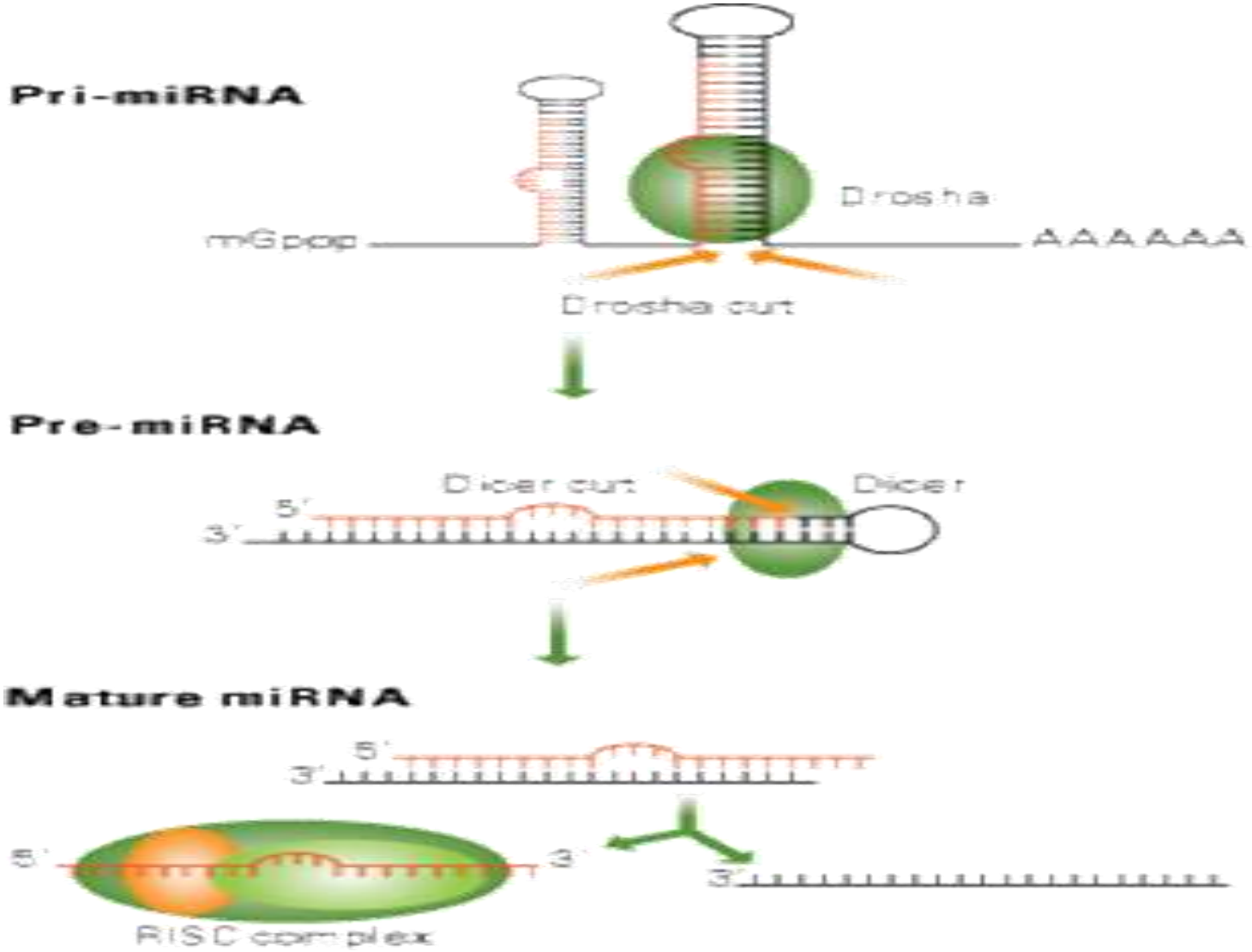
- rRNA is the target of several antibiotics: chloramphenicol, erythromycin, paromomycin, spectinomycin, streptomycin, and thiostrepton.
- Now many thousands of rRNA sequences are known
- Data stored in specialized databases such as RDP-II.

Micro RNAs

- RNAP II as primary transcripts or Pri-miRNAs are 5’ capped and 3’-polyadenylated.
- First Drosha-DGCR8 nuclease processes it but preserves its hairpin.
- In cytoplasm processed to 21-22 nucleotides lengths miRNA by Dicer nuclease.
- One of the two strands is used in the **RNA-induced silencing complex (RISC)**.
- Mature miRNA - Small interfering RNAs or short interfering RNAs or silencing RNAs (**SiRNAs**) are produced similarly.


Drosha
Exon 1
Exon 2
AAA
Nucleus
Exp5
Cytoplasm
Dicer
RISC
RISC
RISC
ORF
AAA
Translational
Inhibition
ORF
AAA
mRNA
degradation


gDNA
Pre-miRNA
Exportin 5
Pri-miRNA
Drosha
Pasha
/DGCR8
NUCLEUS
CYTOPLASM
Dicer
miR/miR*
or siRNA duplex
(21-25 nt long small RNA)
P
P
asymmetric RISC assembly
P-BODIES
RISC
capped
target mRNA
Ago
TRBP
Dicer
partial homology
>> TRANSLATIONAL
REPRESSION
most animal miRNAs
P-BODIES
RISC
target mRNA
Ago
TRBP
Dicer
perfect homology
>> mRNA CLEAVAGE
most plant miRNAs, and siRNAs


Pri-miRNA
Drosha
mGpppA
AAAAAA
Drosha cut
Pre-miRNA
Dicer cut
Dicer
5'
3'
Mature miRNA
5'
3'
5'
3'
3'
RISC complex

Clinical applications

- A mutation in the miRNA - polar cataract, hearing loss.
- miRNA deregulation -chronic lymphocytic leukemia.
- Altered expression of miRNAs causing DNA repair deficiencies leads to cancer.
- miRNAs -altered expression - schizophrenia.
- miRNAs that regulate insulin resistance, obesity, and diabetes- the let-7 family.
- Overexpression of let-7 mimics accelerated aging.

Ribozymes

- Ribozymes are RNA molecules with catalytic activity.
- e.g. RNA involved in splicing, endoribonucleases-RNase P, RNA with peptidyl transferase activity.

Reverse transcriptase

- Retrovirus is a group of RNA viruses. e.g AIDS virus. . RNA dependent DNA polymerase (reverse transcriptase) synthesize a new DNA strand.
- RNA is degraded by RNAase H.
- Another strand of DNA- using the DNA strand -to form dsDNA
- Reverse transcriptase inhibitors as drugs in the treatment of AIDS. Such as zidovudine , lamivudine and tenofovir.

Inhibitors of RNA synthesis

- Actinomycin D and Mitomycin intercalate with two GpC bp of DNA and inhibits RNA synthesis.
- Ripampicin – TB drug binds to β-subunit of RNA polymerase which is inactivated.
- α-amanitin is a toxin from mushroom which inactivates RNAP II.
- 3-deoxy adenosine is a synthetic analog that causes chain termination.
- Thiolutin, a sulfur based microbial antibiotic is an RNA polymerase inhibitor.


Thank
you