RNA Processing
Posttranscriptional processing

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5’ cap of mRNA: unusual 5’,5’-triphosphate linkage..

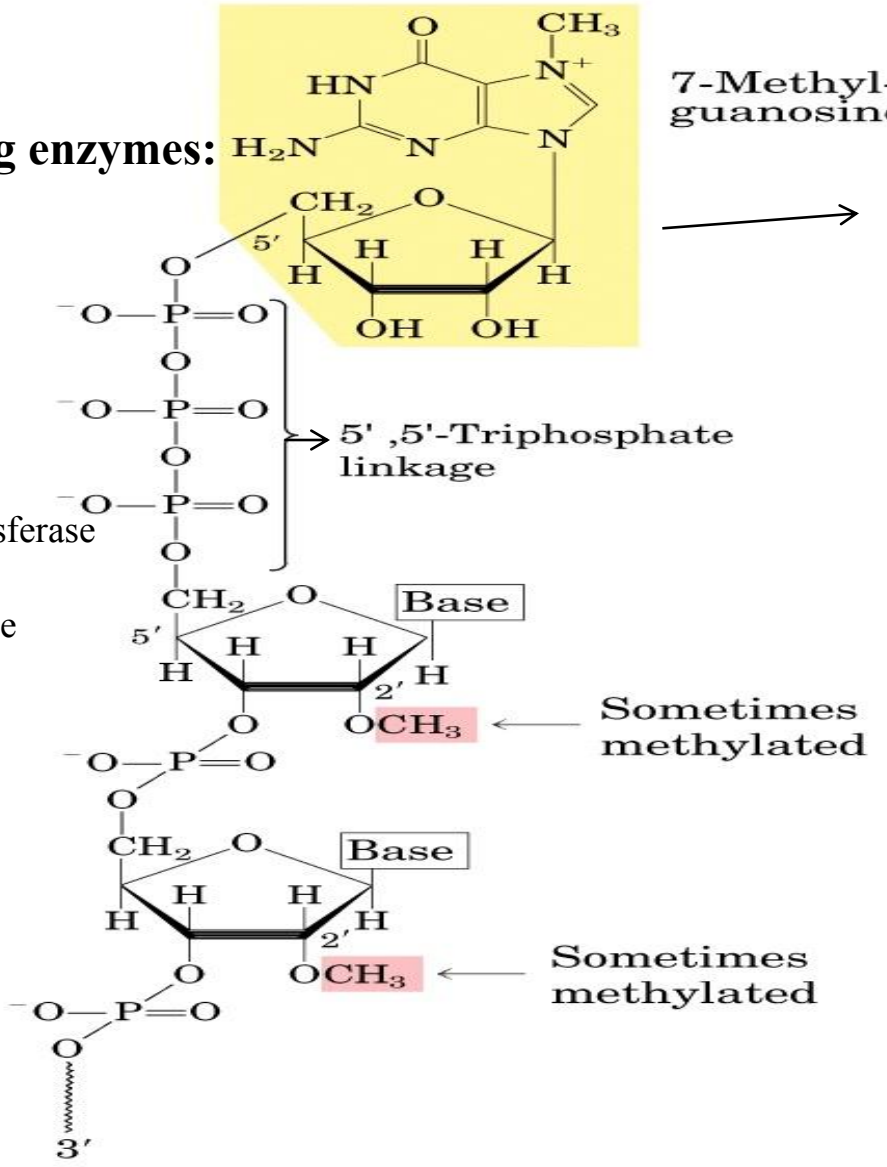
Cap synthesizing enzymes:

Phosphohydrolase

guanylyltransferase

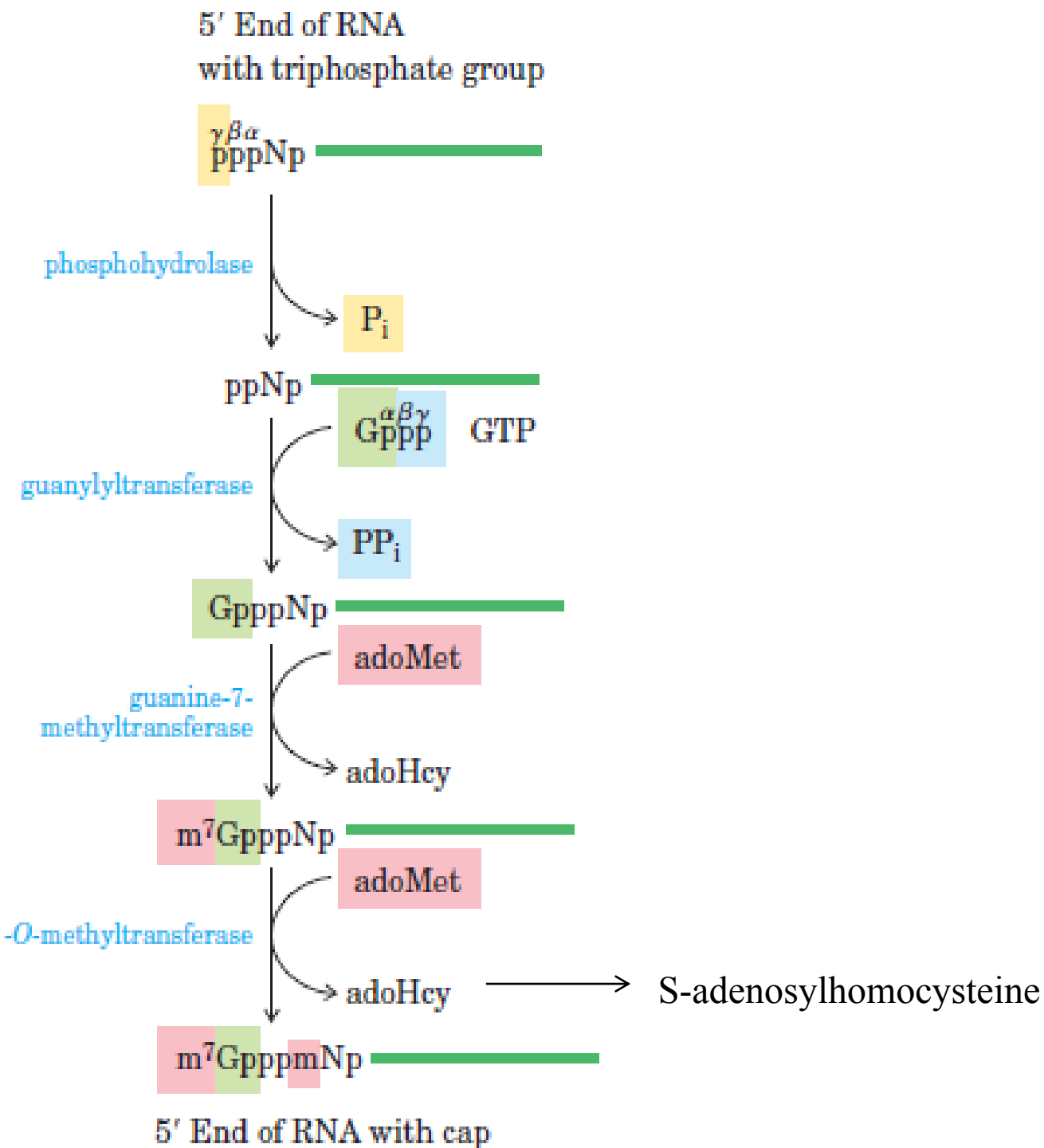
guanine-7-methyltransferase

2’-O-methyltransferase



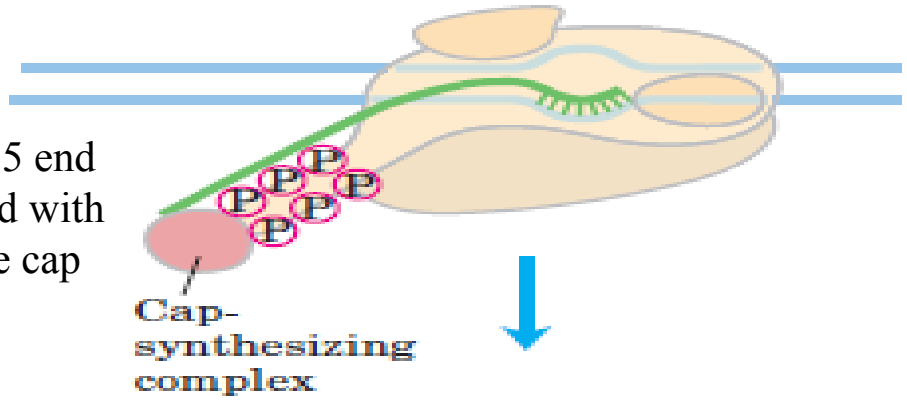
(a)

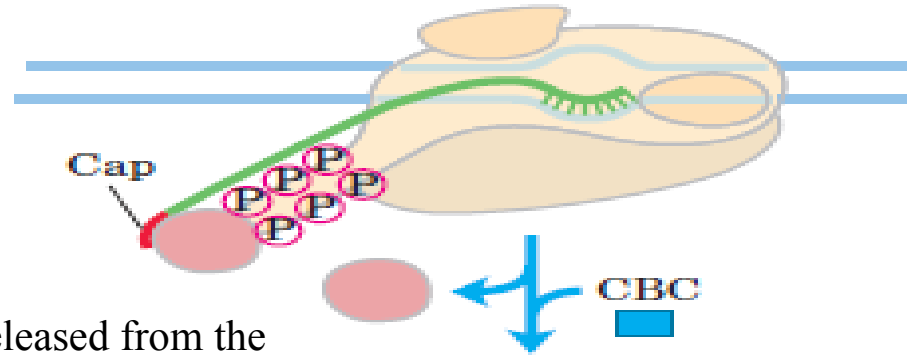
-Protects mRNA from ribonucleases.
-participates in binding of the mRNA to the ribosome to initiate translation


5′ End of RNA
with triphosphate group
pppNp
phosphohydrolase
Pi
ppNp
Gppp
GTP
guanylyltransferase
PPi
GpppNp
adoMet
guanine-7-
methyltransferase
adoHcy
m7GpppNp
adoMet
-O-methyltransferase
adoHcy
S-adenosylhomocysteine
m7GpppmNp
5′ End of RNA with cap

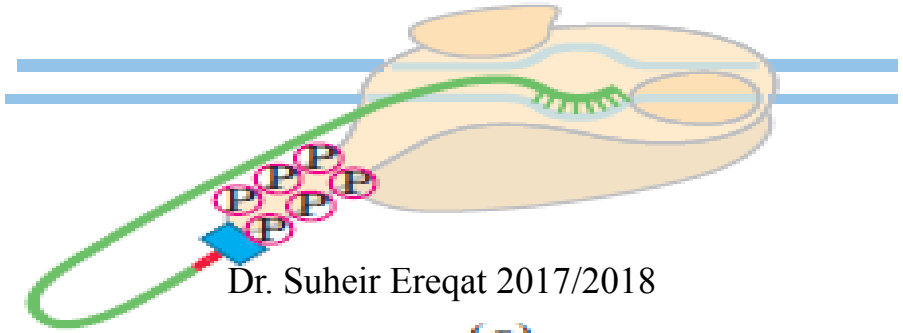
Eukaryotic mRNAs Are Capped at the 5' End

the capping enzymes and the 5 end of the transcript are associated with the RNA pol. II CTD until the cap is synthesized.





The capped 5 end is then released from the capping enzymes and bound by the cap-binding complex



(c)

RNA splicing

Group I + II : self splicing. Group I : guanosine cofactor

Group III : splicesome ,The spliceosome is made up of specialized RNA-protein complexes, small nuclear ribonucleoproteins (snRNPs,). Each snRNP contains one of a class of eukaryotic RNAs, known as **small nuclear RNAs (snRNAs) (**U1, U2, U4, U5, and U6)

Class four : (tRNA) the only class spliced by enzymes. the splicing reaction requires ATP and an endonuclease. The splicing endonuclease cleaves the phosphodiester bonds at both ends of the intron, and the two exons are joined by a mechanism similar to the DNA ligase reaction

Group I: nuclear, mitochondrial, genes that code for rRNAs, mRNAs, and tRNAs.

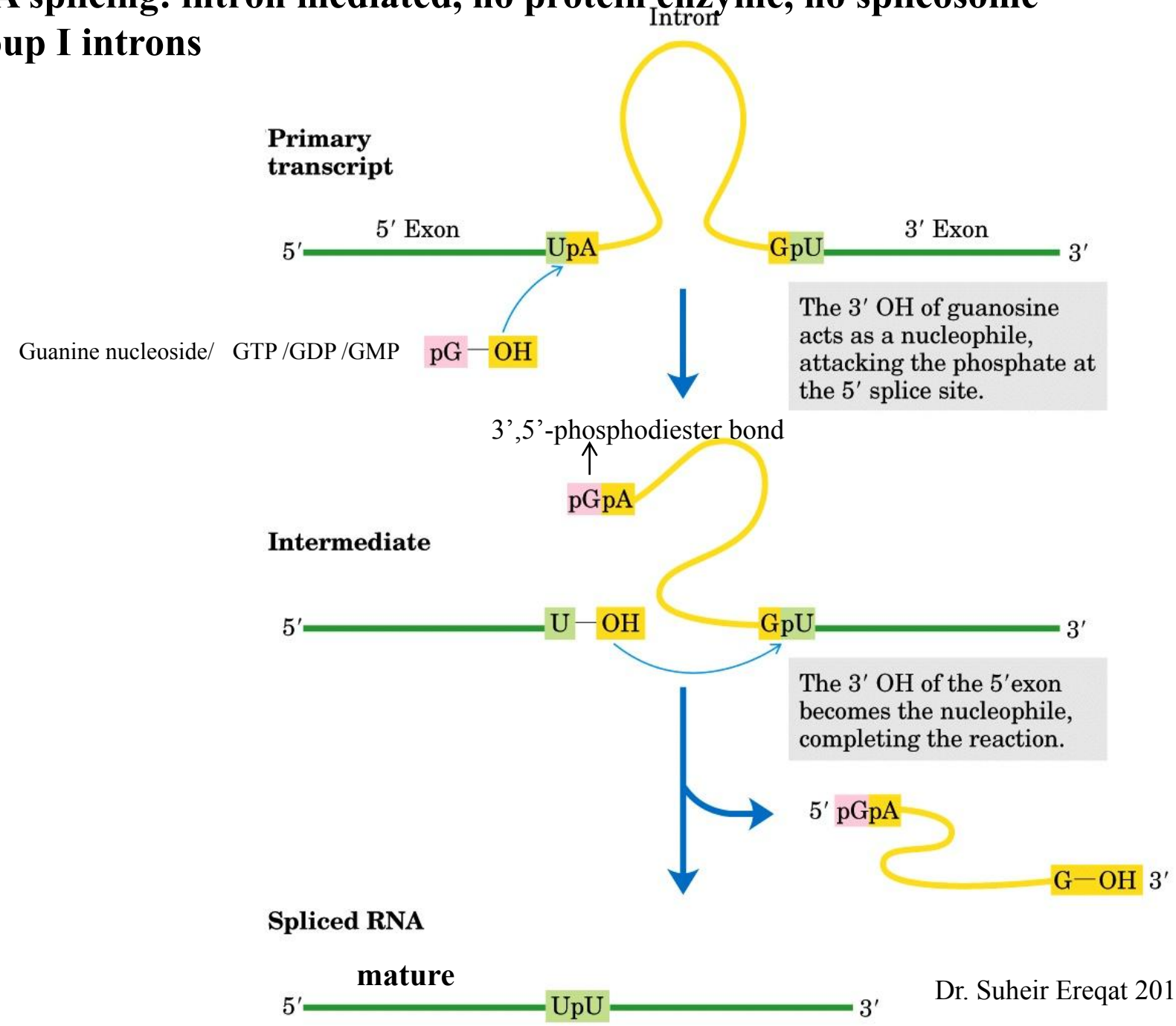
Group II introns are generally found in the primary transcripts of mitochondrial mRNAs

Group I and group II introns do not require cofactor (such as ATP) for splicing. The splicing mechanisms in both groups involve two transesterification reaction steps

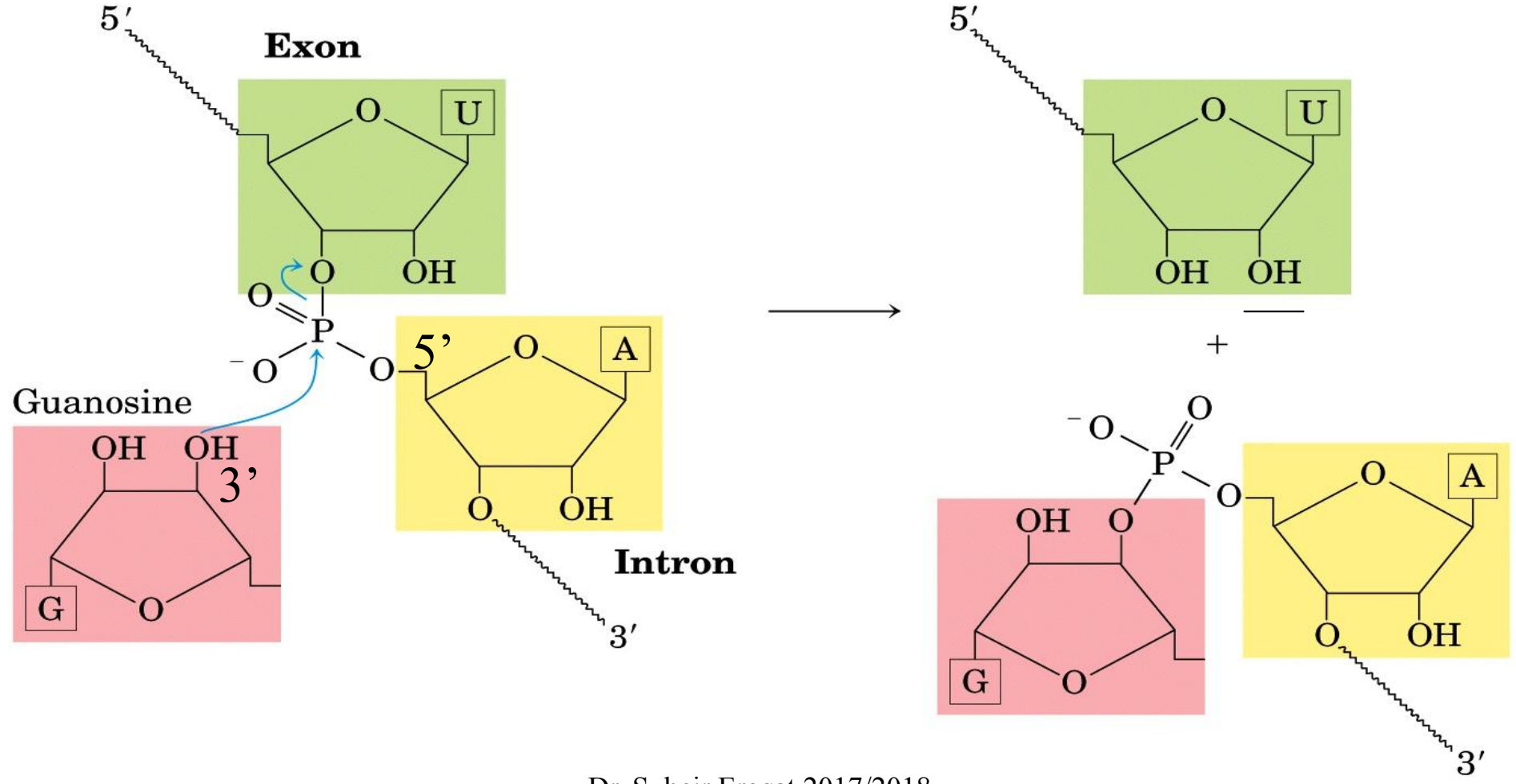
The third and largest class of introns includes those found in nuclear mRNA primary transcripts. These are called **spliceosomal introns,** catalyzed by a large protein complex called a **spliceosome**

RNA splicing: intron mediated, no protein enzyme, no splicosome

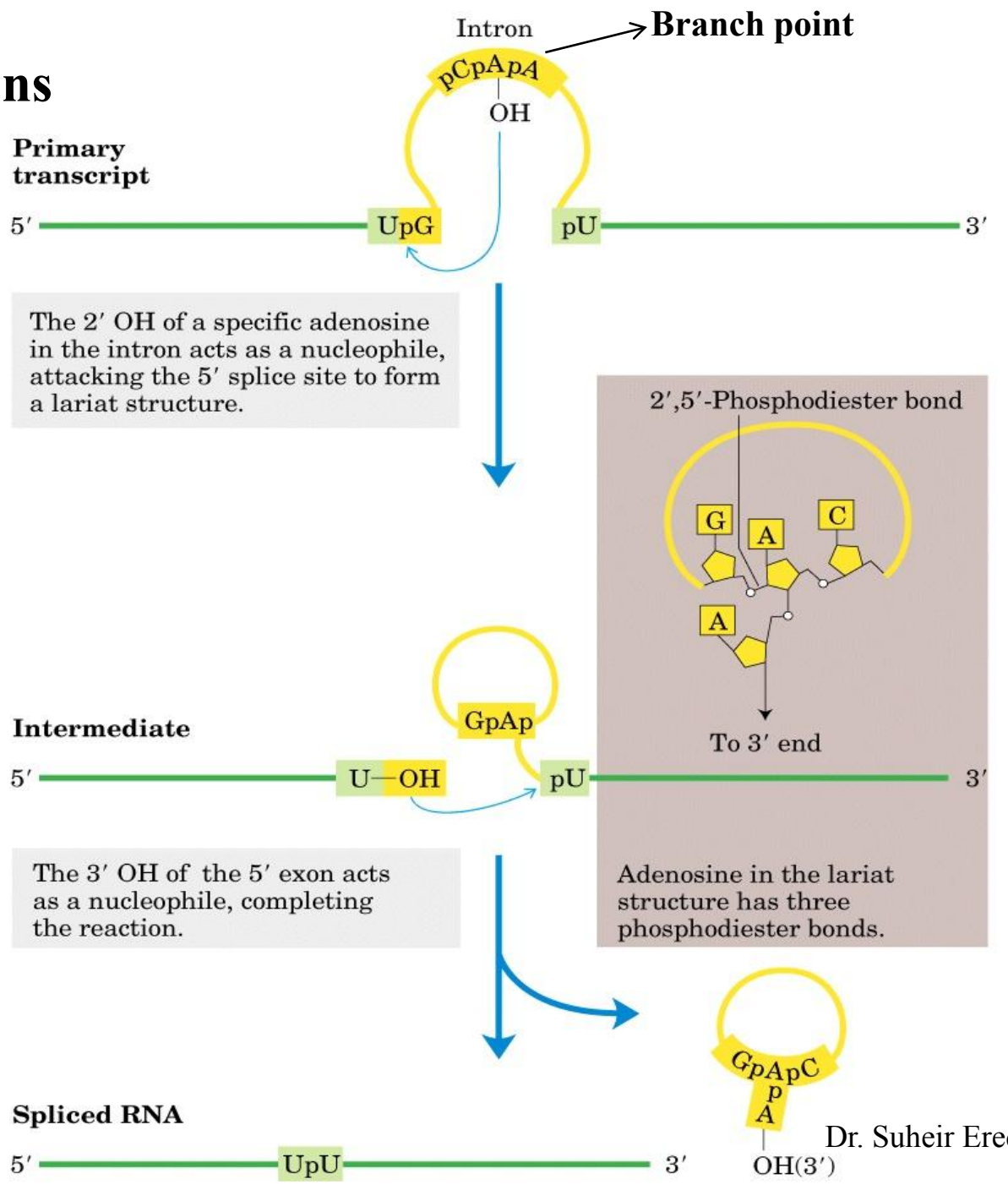
Group I introns



Transesterification : a ribose 2' or 3'-hydroxyl group makes a nucleophilic attack on a phosphorus and a new phosphodiester bond is formed at the expense of the old=maintaining the balance of energy



RNA splicing: Group II introns

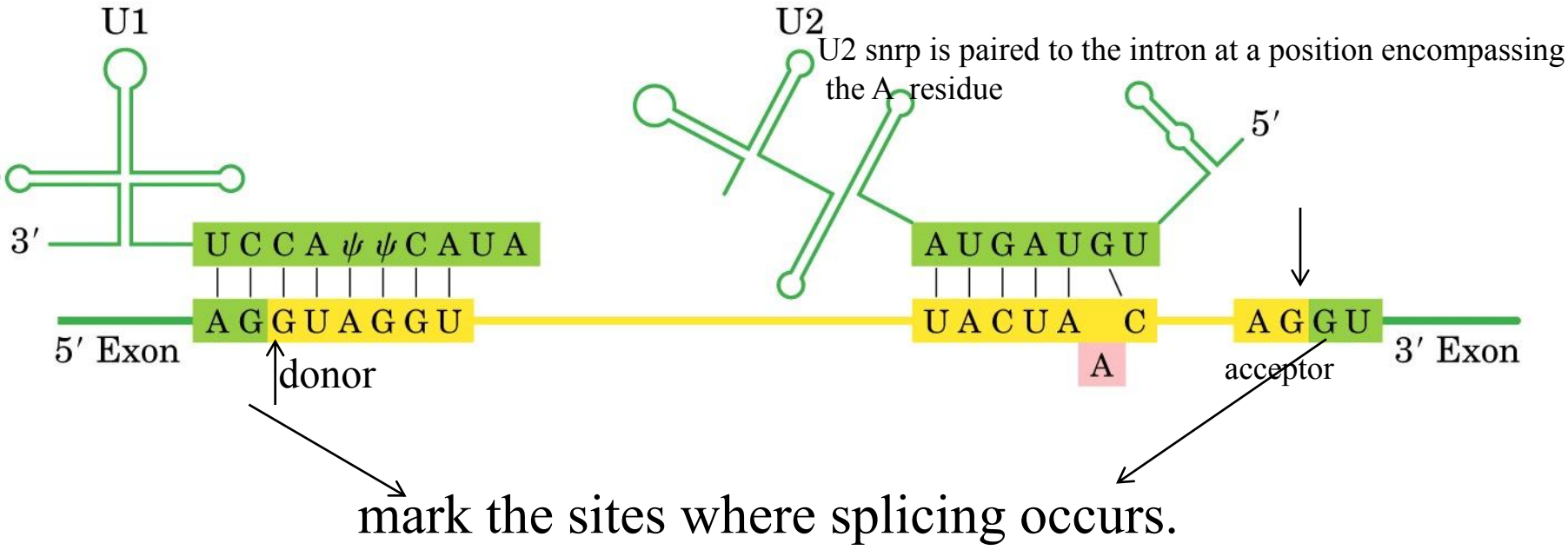


mature

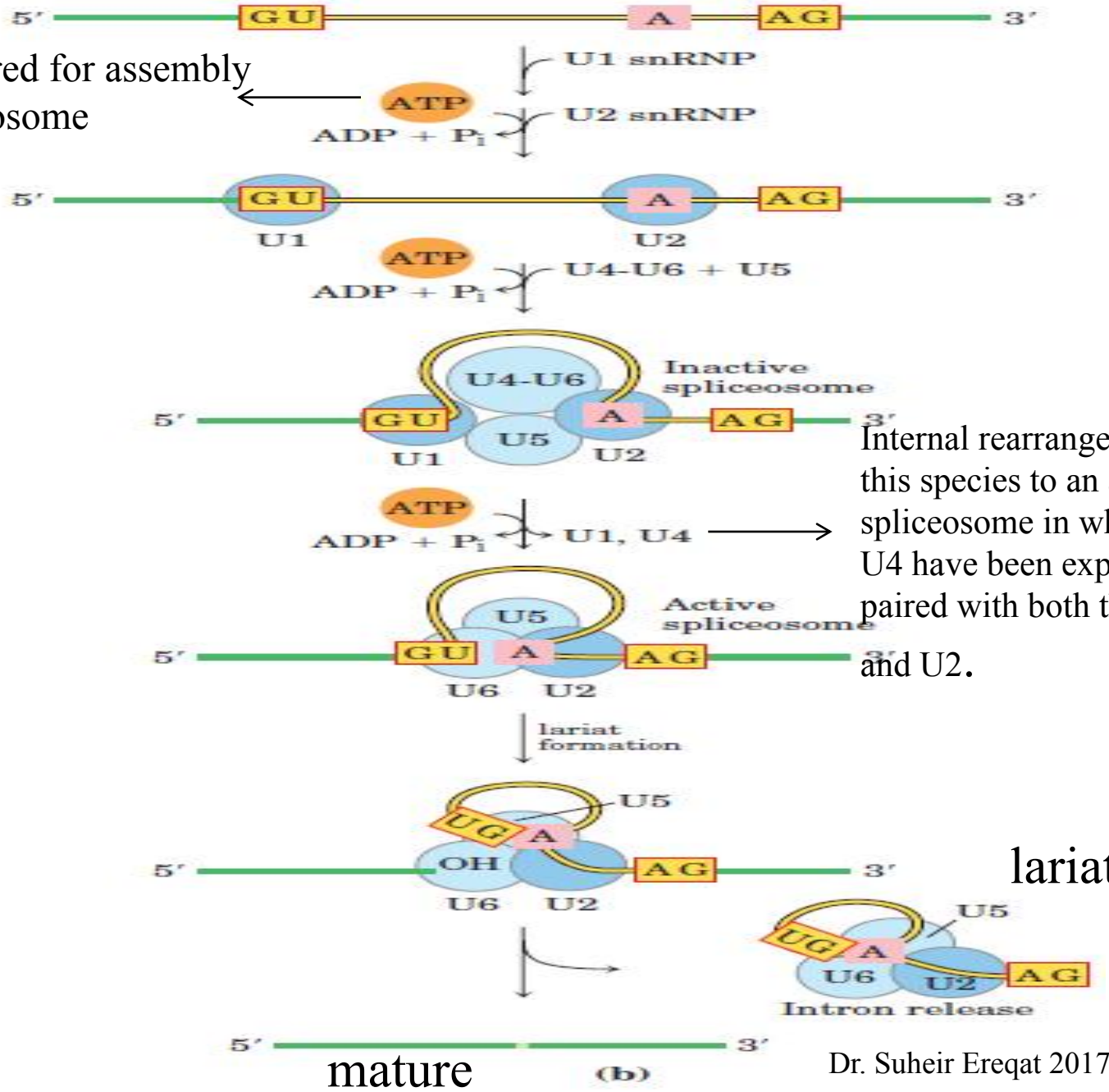
RNA splicing:
Group III introns

The U1 snrp has a sequence near its 5 end that is complementary

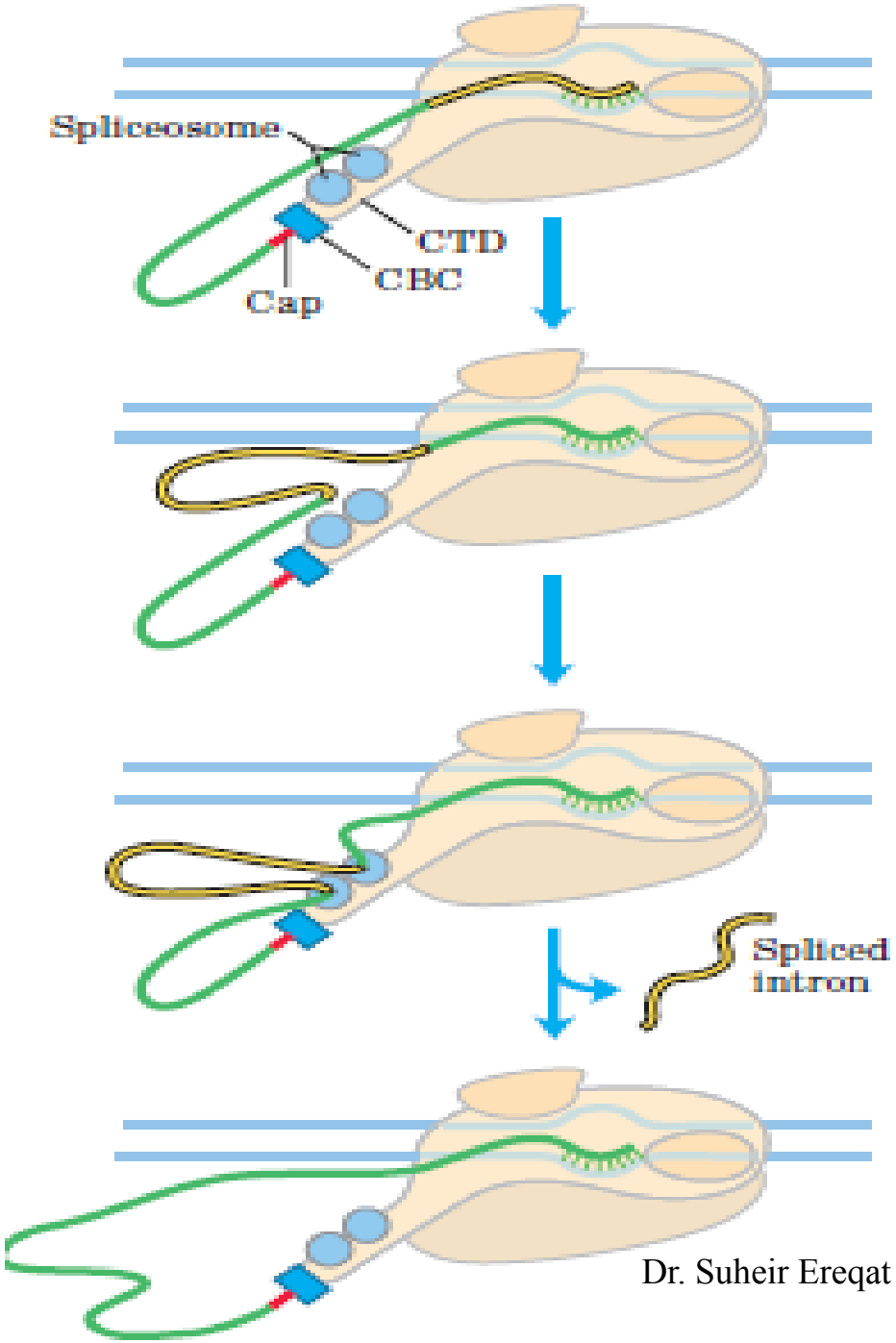
to the splice site at the 5 end of the intron.



ATP is required for assembly of the spliceosome

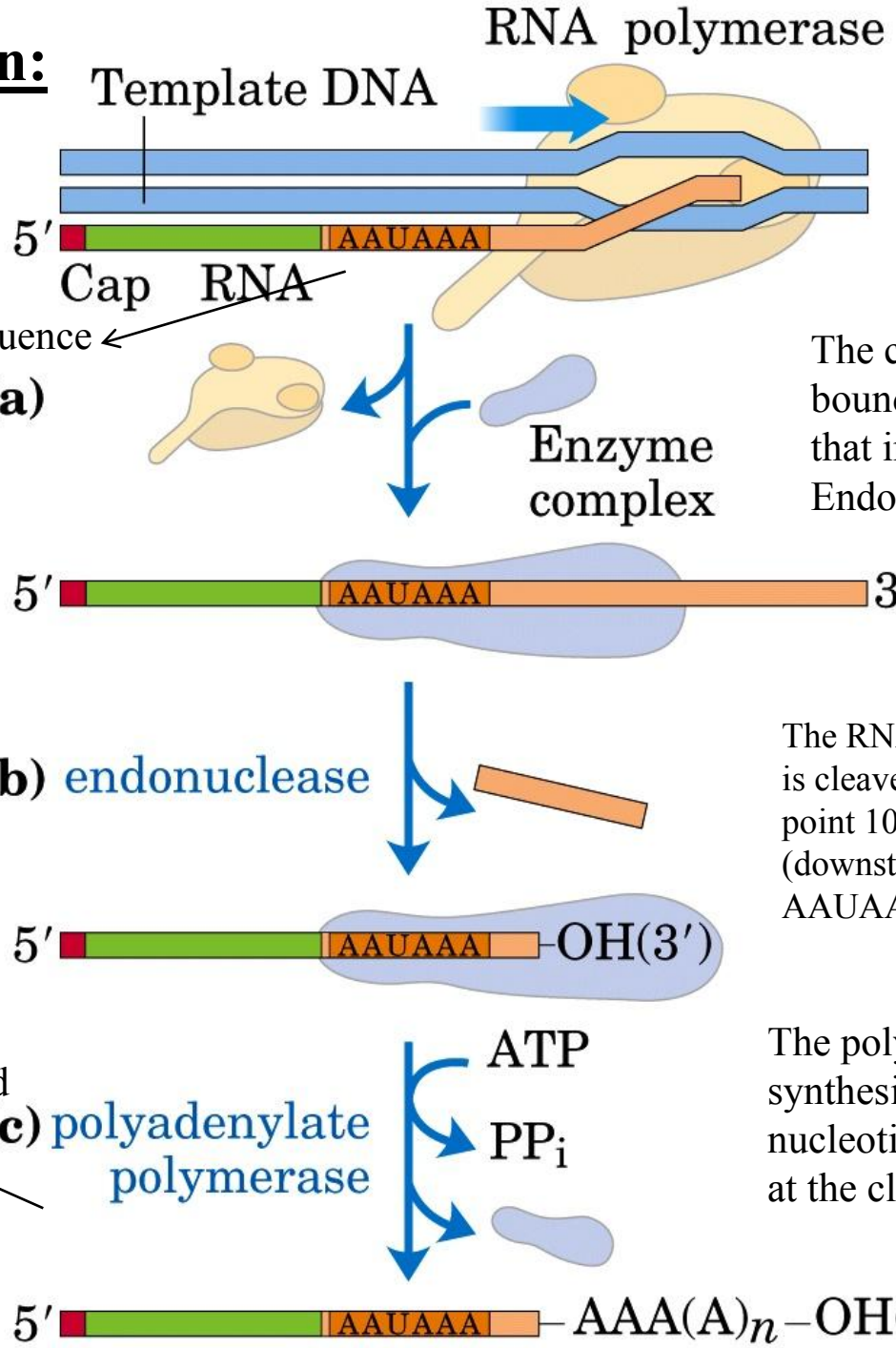


Internal rearrangements convert this species to an active spliceosome in which U1 and U4 have been expelled and U6 is paired with both the 5 splice site and U2.



Coordination of splicing and transcription provides an attractive mechanism for bringing the two splice sites together

Polyadenylation:



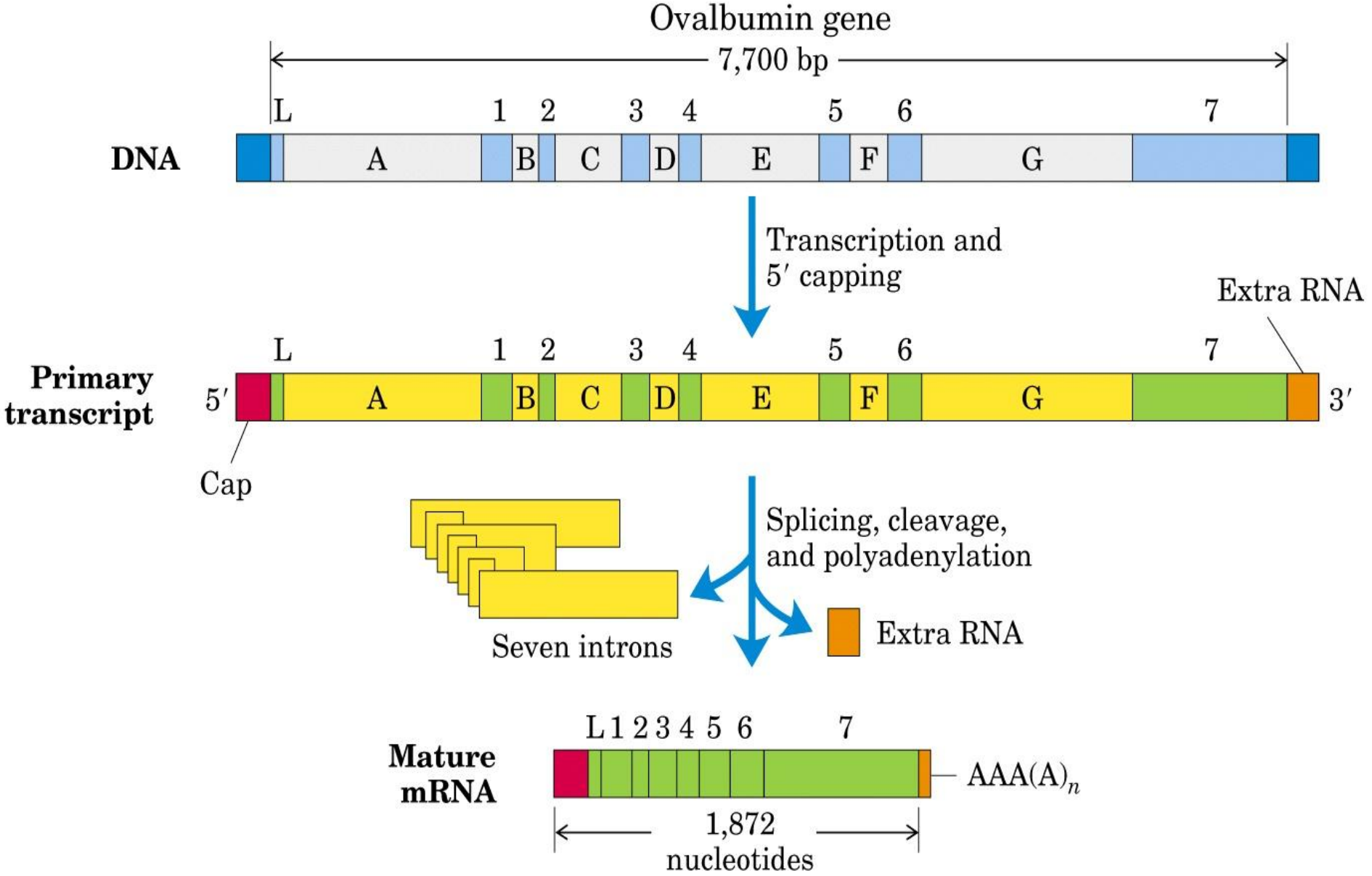
The cleavage signal sequence is bound by an enzyme complex that includes an Endonuclease and apolyadenylate

The RNA is cleaved by the endonuclease at a point 10 to 30 nucleotides to (downstream of) the sequence AAUAAA.

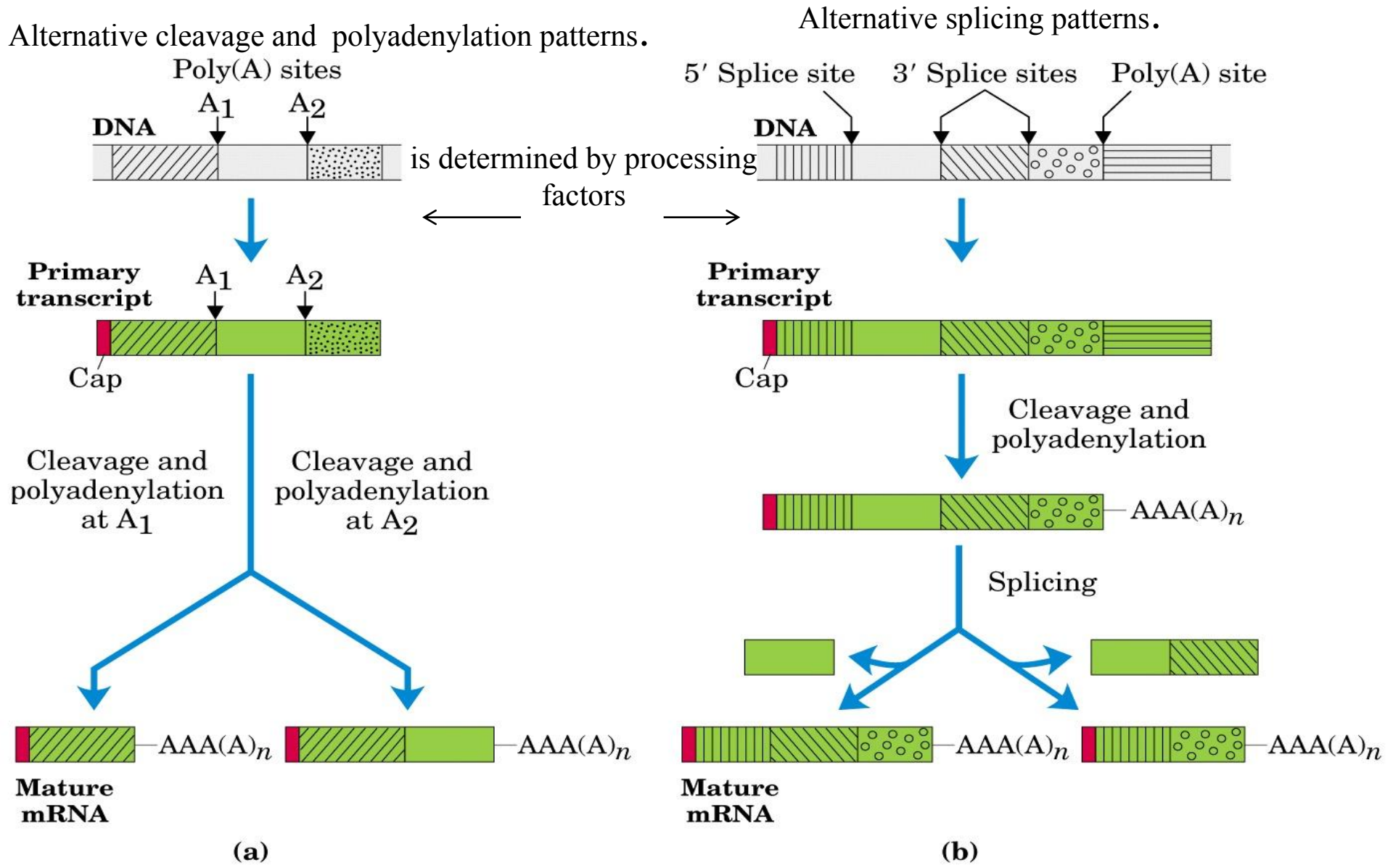
This enzyme does not require a template but does require the cleaved mRNA as a primer

The polyadenylate polymerase synthesizes a poly(A) tail 80 to 250 nucleotides long, beginning at the cleavage site.

Overview of the processing of a eukaryotic mRNA.



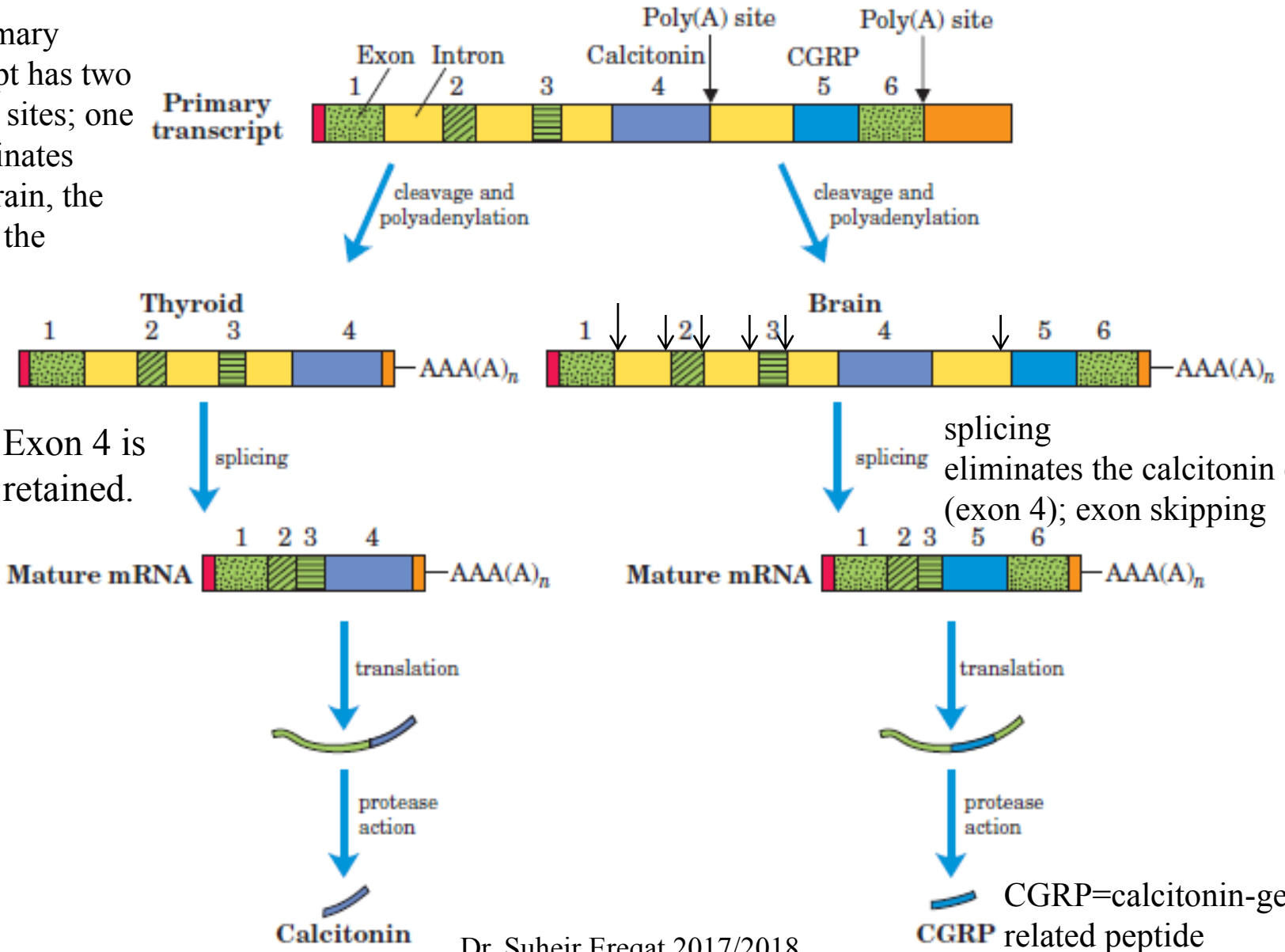
Alternative processing of complex transcripts in eukaryotes.



a single gene coding for multiple proteins.

Alternative processing of the calcitonin gene transcript in rats.

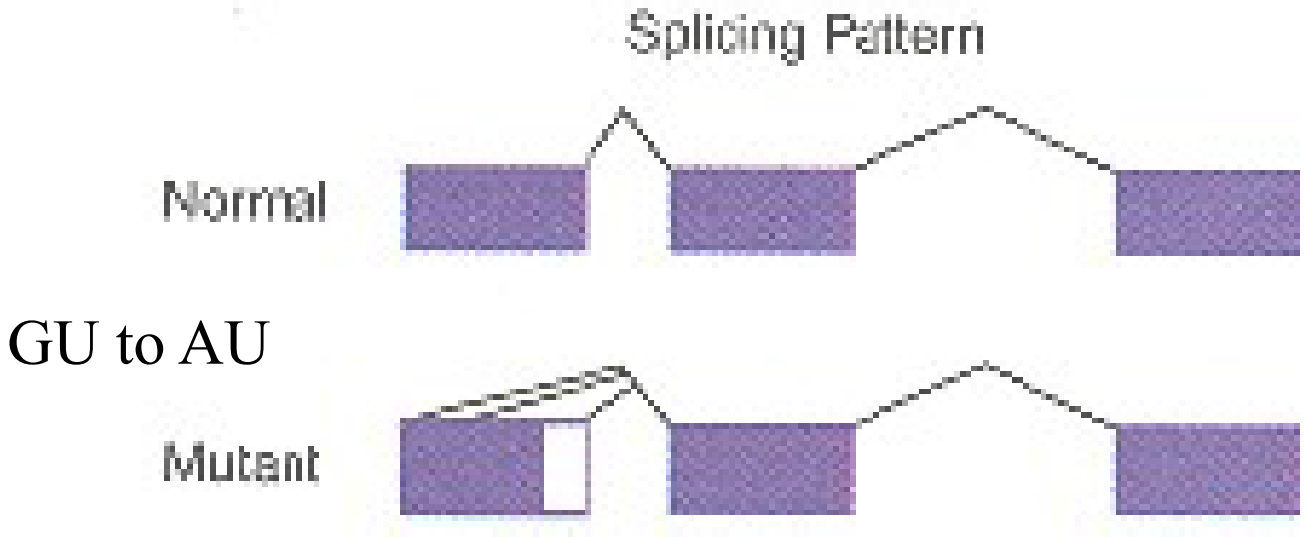
The primary transcript has two poly(A) sites; one predominates in the brain, the other in the thyroid.



Exon 4 is retained.

splicing eliminates the calcitonin exon (exon 4); exon skipping

CGRP=calcitonin-gene-related peptide



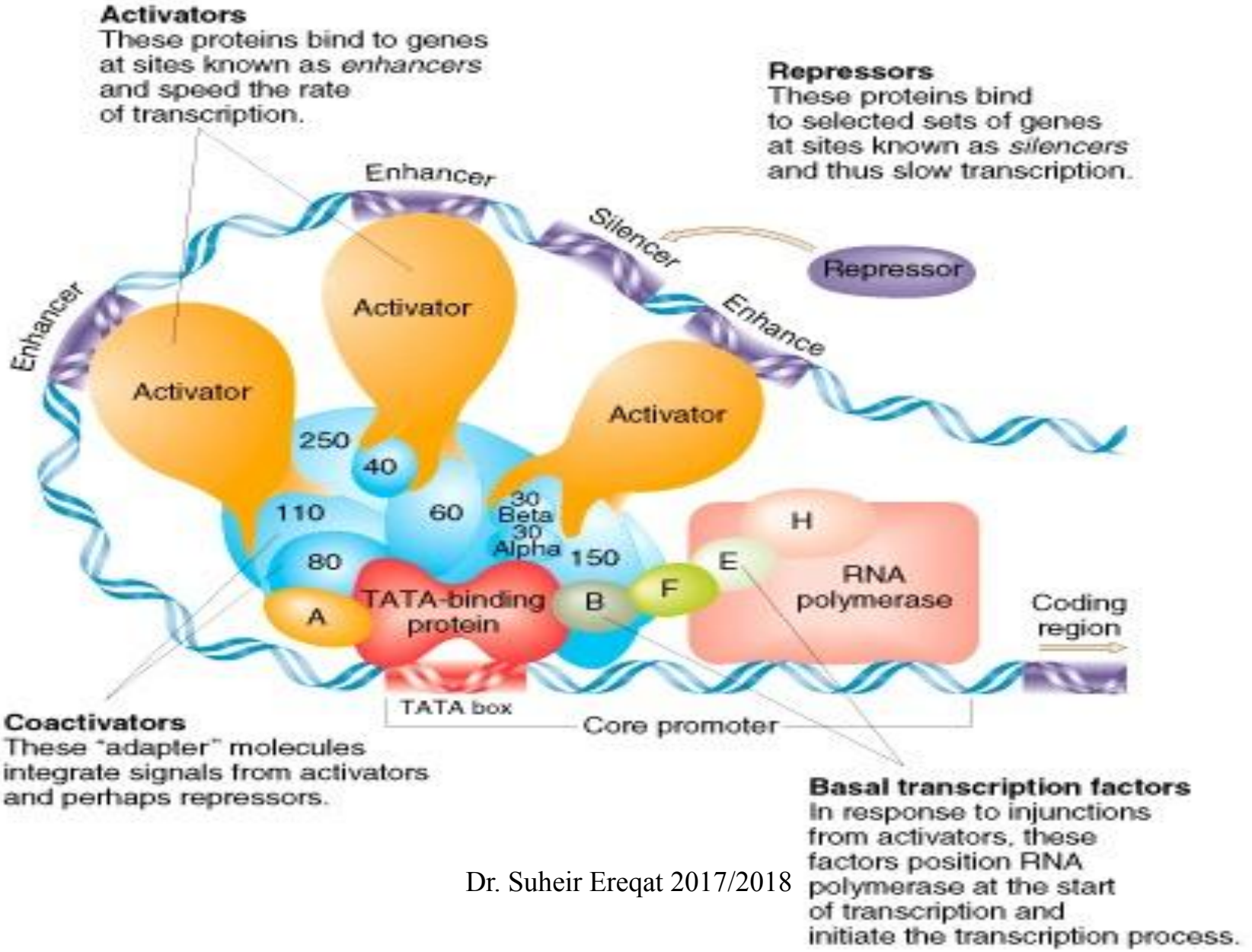
Nucleotide change at an intron–exon junction of the human b globin gene, which leads to aberrant splicing and b thalassemia

Each cell expresses, or turns on, only a fraction of its genes. The rest of the genes are repressed, or turned off. The process of turning genes on and off is known as **gene regulation**

Housekeeping genes: expressed constitutively, essential for basic processes involving in cell replication and growth.

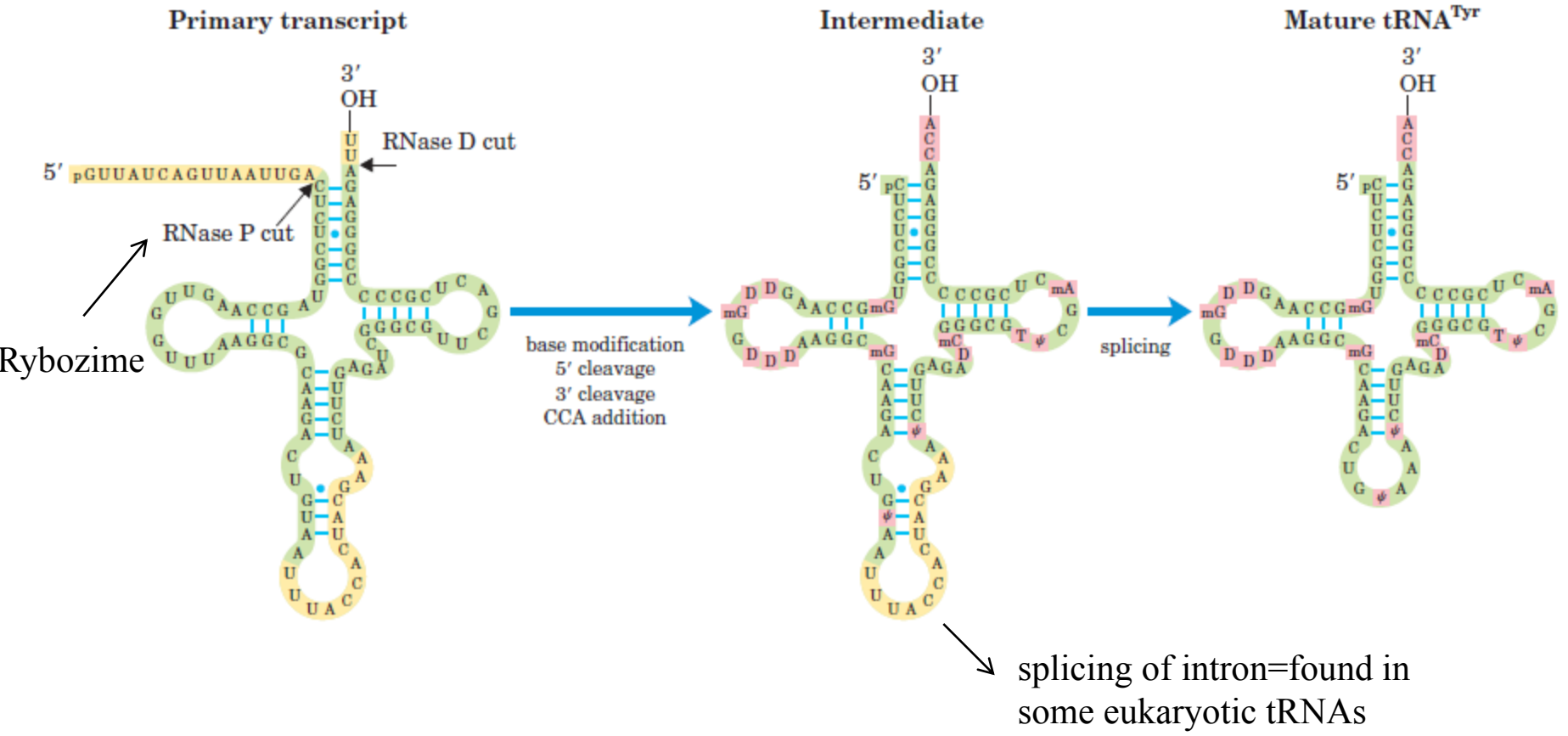
Inducible genes: expressed only when they are activated by inducers or cellular factors.

Promoters, enhancers, silencers etc.

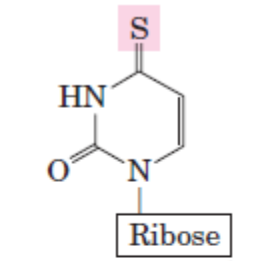


Ribosomal RNAs and tRNAs Also Undergo Processing

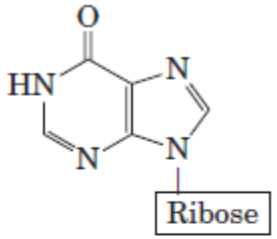
Processing of tRNAs in bacteria and eukaryotes.



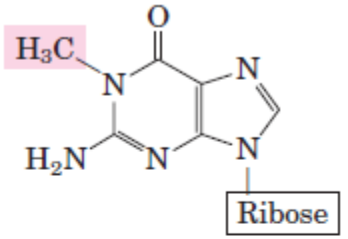
Some modified bases of rRNAs and tRNAs, produced in posttranscriptional reactions



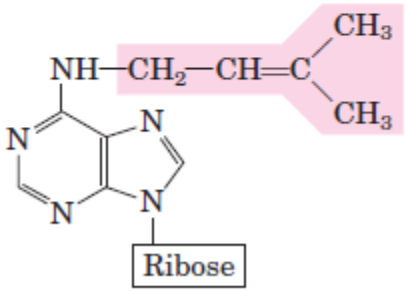
4-Thiouridine (S^4U)



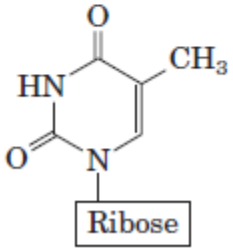
Inosine (I)



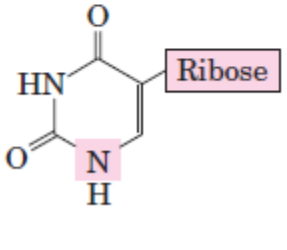
1-Methylguanosine (m^1G)



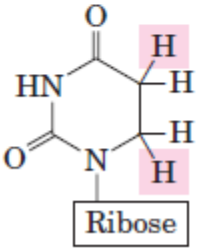
N^6-Isopentenyladenosine (i^6A)



Ribothymidine (T)



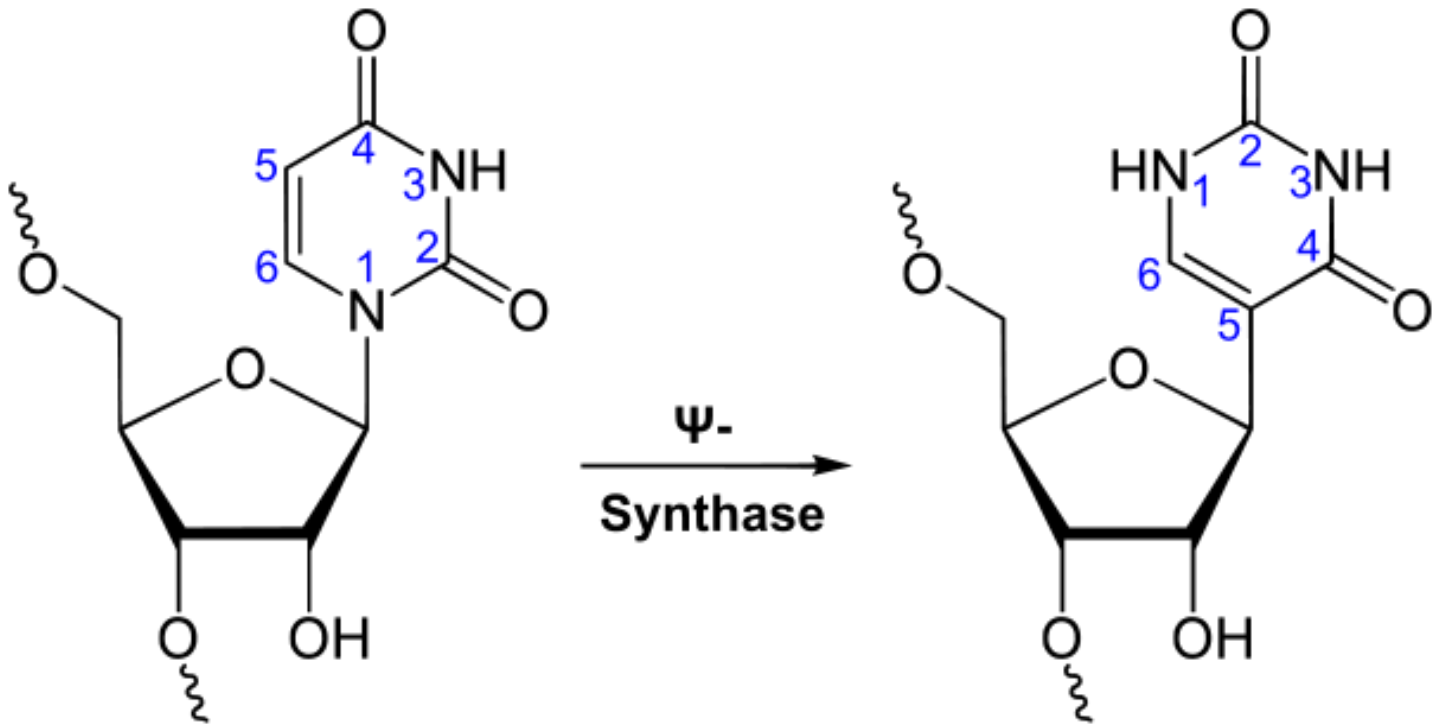
Pseudouridine (ψ)



Dihydrouridine (D)

most common nucleoside modifications

It is commonly found in tRNA, associated with thymidine and cytosine in the TΨC arm and is one of the invariant regions of tRNA. The function of it is not very clear



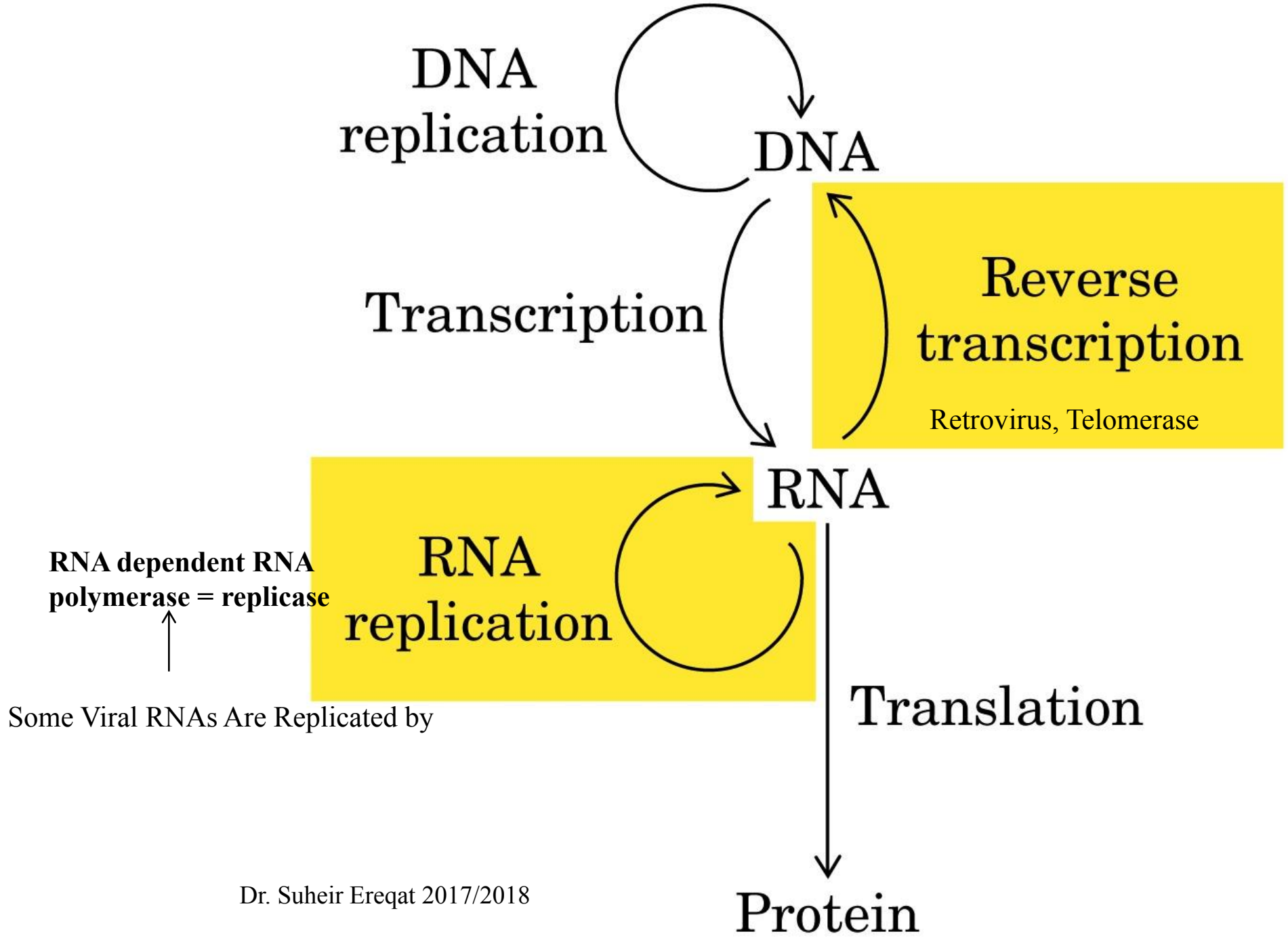
Concentration of RNA depends on:

1) Rate of synthesis.
2) Rate of degradation. (ensures mRNA not build up in the cell)

Prokaryotes:
endoribonucleases and 3'→5' exoribonucleases.

Eukaryotes: shortening the poly A tail and decapping 5' end and 3'→5' exoribonuclease (10 types) = exosome

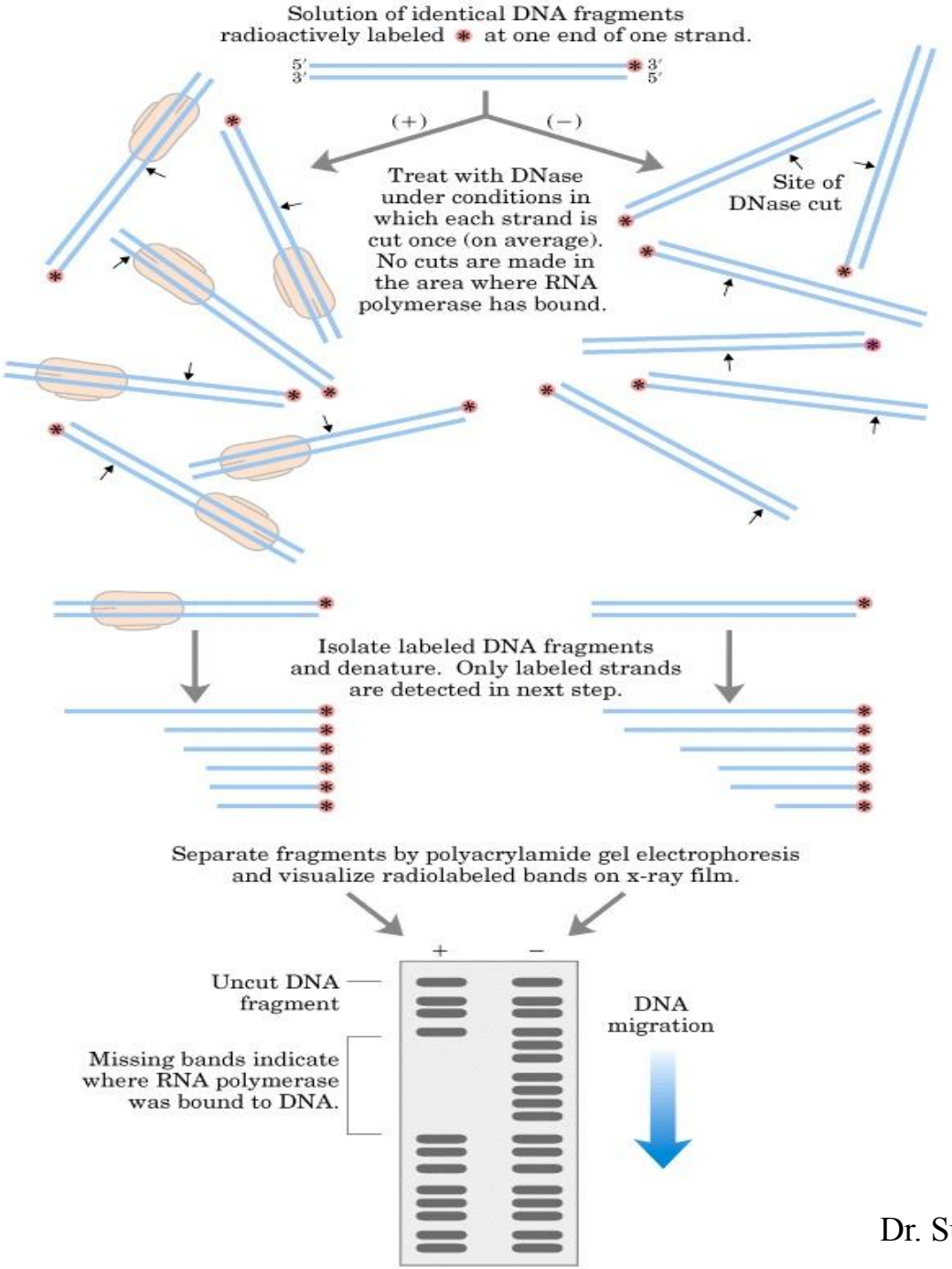
In lower eukaryotes 5'→3' exoribonuclease



Some Viral RNAs Are Replicated by

DNA Footprinting:

identifies the DNA sequences bound by a particular protein.



RNA polymerase leaves its footprint on a promotoer

DNA footprinting: identifies the DNA sequences bound by a particular protein