SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 201: Fundamentals of Molecular Biology (Unit III)

Post-transcriptional Modifications

Post-transcriptional Modifications & Processing

Almost all major types of RNA synthesized by cellular DNA dependent RNA polymerases undergo changes before they can carry out their functions

Post-transcriptional Modification

Involves addition to or alterations of existing bases or sugars

Post-transcriptional Processing

Involves phosphodiester bond cleavage and loss of certain nucleotides from the transcript.

- **The nascent RNA, also known as primary transcript, needs to be modified to become functional tRNAs, rRNAs, and mRNAs.**
- **The modification is critical to eukaryotic systems.**

Changes Associated

with

Primary Transcript

Changes Associated with Primary Transcript

RNA	Organism	Precursor	Modification	Processing	Product
mRNA	Prokaryotes	-	-? Polyadenylation ?	In some cases specific cleavage by endonucleases	mRNAs
	Eukaryotes	hnRNA (2-14 kb long in mammals) Average Size = 8-10 kb 4-5 times longer than mRNA	5' Capping, methylation and 3' Polyadenylation	In most cases, splicing of introns	mRNA
rRNA	Prokaryotes	Pre-rRNA	Methylation	Specific cleavage	16S, 23S and 5S rRNA and Spacer tRNA
	Eukaryotes	Pre-rRNA	Methylation	Specific cleavage and splicing of introns	18S, 28S and 5.8S rRNA

Changes Associated with Primary Transcript

RNA	Organism	Precursor	Modification	Processing	Product
tRNA	Prokaryotes	Pre-tRNA	Many modified bases	Specific cleavage by endonucleases, trimming by exonucleases, CCA end addition	Mature tRNA
	Eukaryotes	Pre-tRNA	Many modified bases	Specific cleavage by endonucleases, trimming by exonucleases, CCA end addition splicing of introns	Mature tRNA

Modifications of Eukaryotic mRNA

Modification of hnRNA

- **Primary transcripts of mRNA are called as heterogeneous nuclear RNA (hnRNA).**
- **hnRNA are larger than matured mRNA by many (~4-5) folds.**
- **Modification includes**
  - **Capping at the 5′- end**
  - **Tailing at the 3′- end**
  - **mRNA splicing**
  - **RNA editing**

5’ End Modification

5’ Capping & Methylation

Types of Methylated caps

- **Two types of methylated caps are found**
  - **a) Mono-methylated caps –**

    commonaly found in most eukaryotic mRNAs
  - **b) Tri-methylated caps –**

    less frequently observed (e.g., only on some non-coding RNAs) but highly conserved throughout the eukaryotes {e.g., small nuclear (sn) RNAs, small nucleolar (sno) RNAs, and telomerase RNA TLC1}

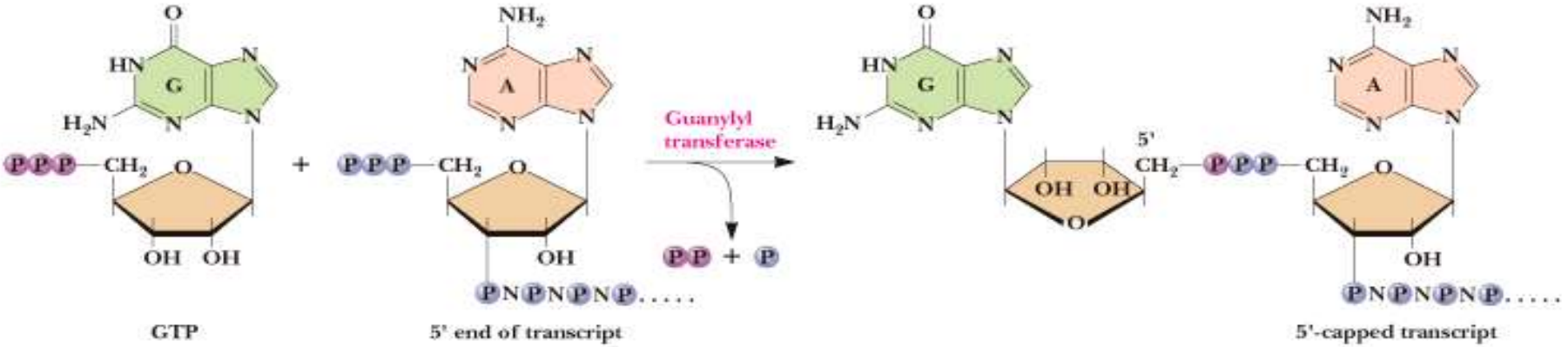
Mono-methylated cap

5’ Capping and Mono-methylation

1. The presence of an unusual methylated nucleotide at the 5’ terminus most viral & cellular mRNAs was discovered by A. Shatkin & co-workers in 1975.
2. The entire methylated terminal oligonucleotide is called as ‘cap structure’.
3. Site of biogenesis = Nucleus by a series of enzymatic reaction.
4. Capping process is completed before the completion of nascent transcript.
5. 5’cap of most mRNA is monomethylated, but some small noncoding RNAs are trimethylated.

Monomethylated cap

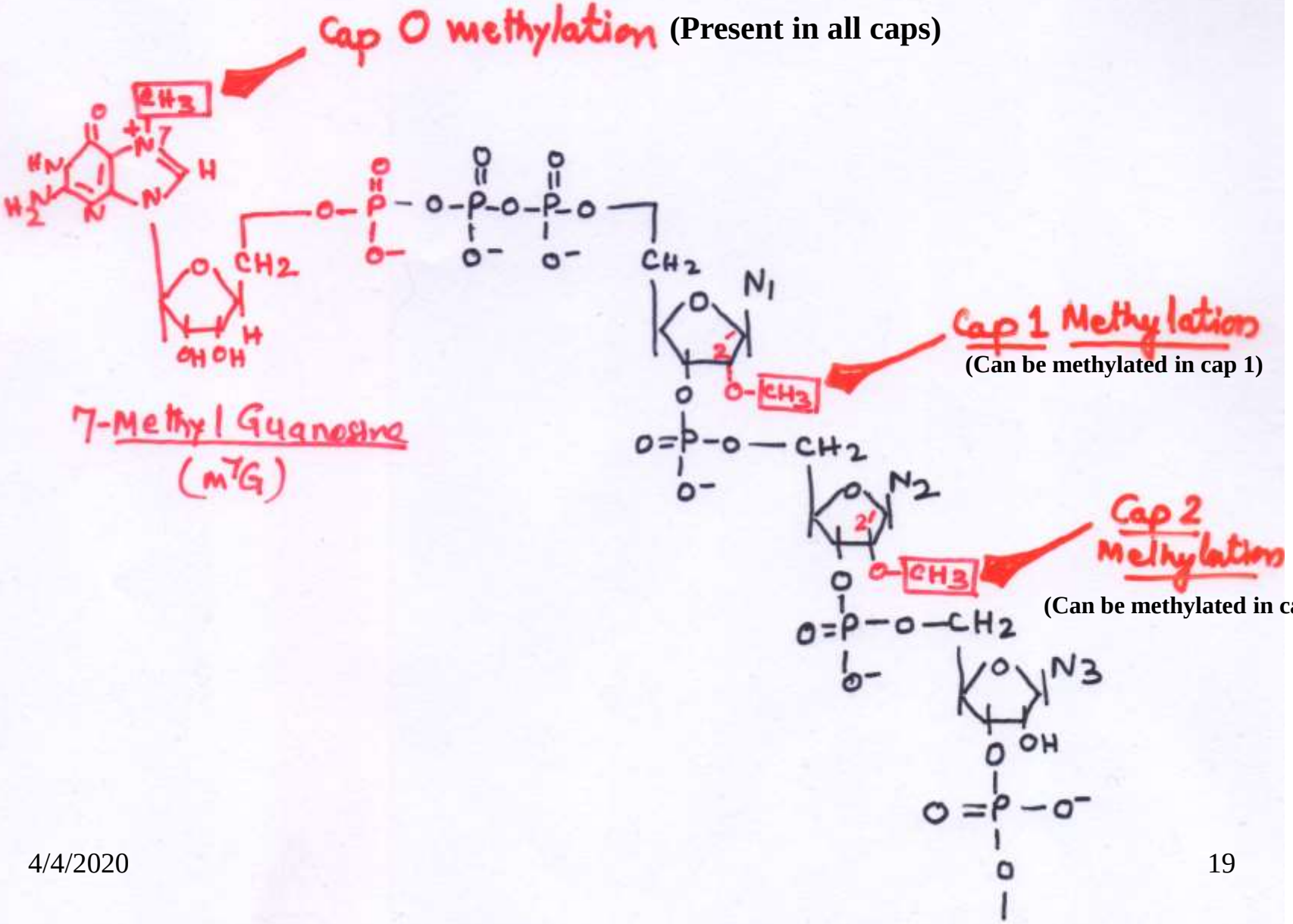
1. Primary transcripts (pre-mRNAs or heterogeneous nuclear RNA) are usually first "capped" by a guanylyl group
2. The reaction is catalyzed by guanylyl transferase
3. Capping G residue is methylated at 7-position
4. Additional methylations occur at 2'-O positions of next two residues and at 6-amino of the first adenine


Guanylyl transferase
GTP
5' end of transcript
5'-capped transcript

Full Structure
of
5’ monomethylated cap

Structure of 5′ methylated cap of eukaryotic mRNA

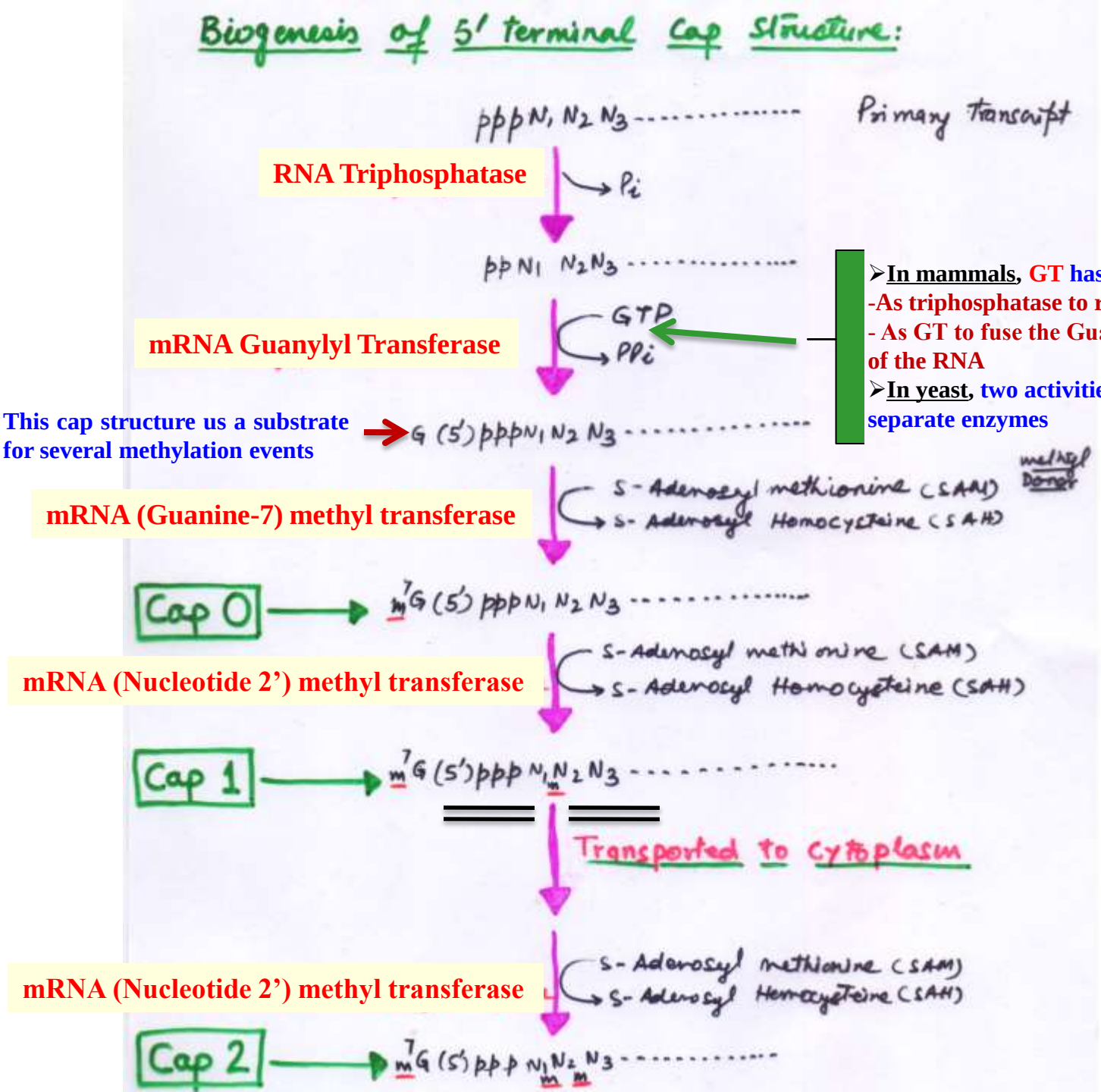
Full structure of cap



Biogenesis
of
5’ terminal cap structure

REMEMBER……

- **$TF_{II}H$ kinase phosphorylate S-5 at CTD tail (-Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7-)n and recruits enzymes required for 5' capping of mRNA (in yeast n= 26; in mouse n= 52).**
- **What are the enzymes/enzyme complexes?**
- **5' capping enzymes include:**
  1) **RNA triphosphatase**
  2) **mRNA Guanylyl Transferase (GT)** ←
  3) **mRNA (Guanine-7) methyl transferase**
  4) **mRNA (Nucleotide 2') methyl transferase**



➢**In mammals**, GT has two enzymatic activities:
-As triphosphatase to remove two phosphate from GTP
- As GT to fuse the Guanine to the original 5' terminus of the RNA

➢**In yeast**, two activities are carried out by two separate enzymes

- **The 5′- cap structure is found on hnRNA too. ⇒ The capping process occurs in nuclei.**
- **The cap structure of mRNA will be recognized by the cap-binding protein required for translation.**
- **The capping occurs prior to the splicing.**

5’ Cap Structure & Evolutionary Complexity

Relative abundance of the different cap structures changes with evolutionary complexity

1. **Yeast mRNA have Cap 0**
2. **Slime moulds are mainly Cap 0 but 20% have Cap 1 structure**
3. **Messages of Brine shrimpe & Sea urchin terminate with Cap 1**
4. **Mammals have high percent of Cap 1 and Cap 2**

Points to be remembered……..

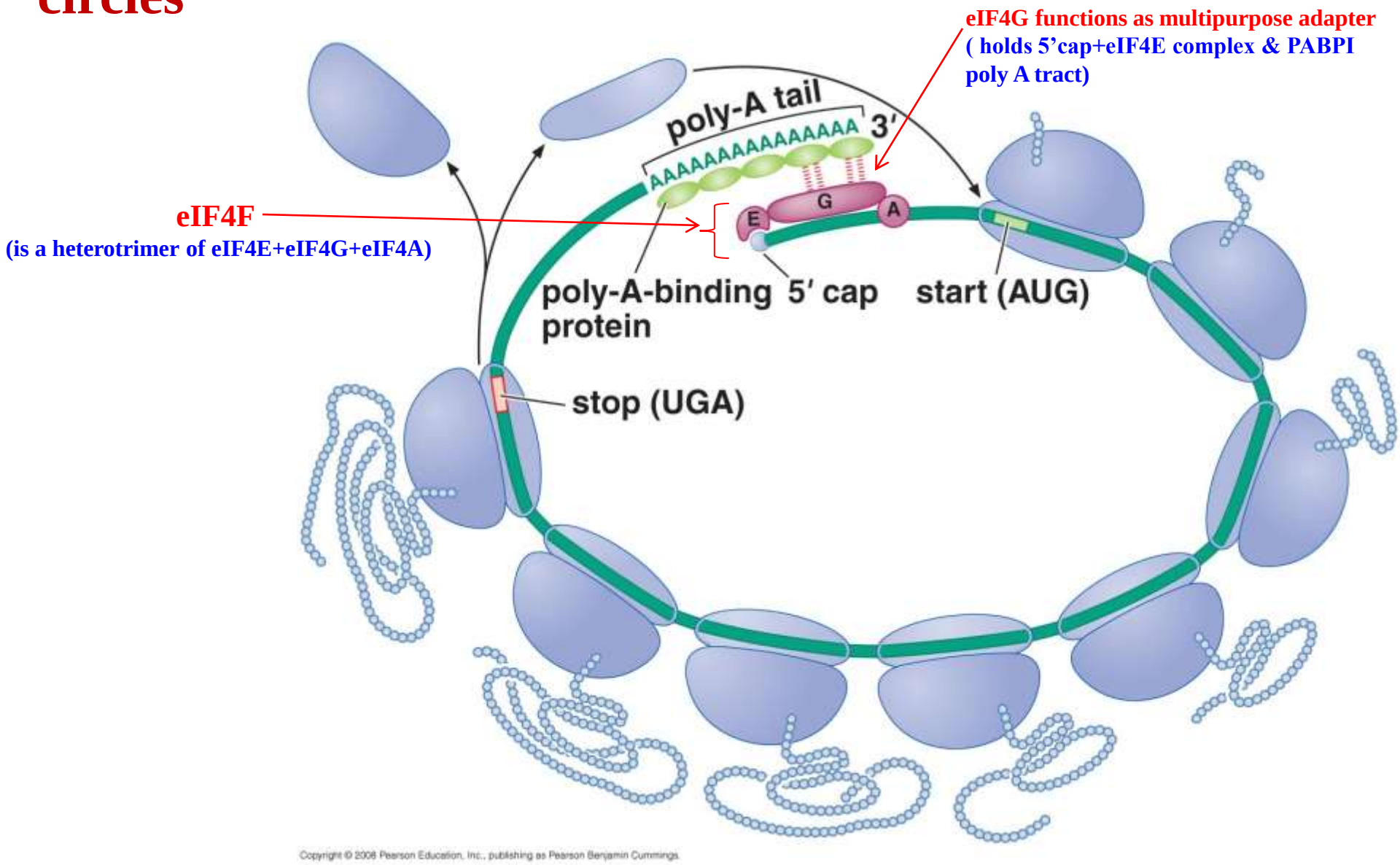
1. **Between the Cap and the translational initiation codon, AUG, there is a length of non-translated RNA known as the leader sequence.**
2. **The length of leader sequence varies from mRNA to mRNA e.g.,**

a) **Ig kChain - 3 nucleotiedes**

b) **α-amylase - 256 nucleotides**

3. **In nucleus, cap is recognized by the cap binding (CBP 20/80) heterodimer which stimulate splicing of first intron and also interact with TREX complex to facilitate mRNA export out of the nucleus.**
4. **In cytoplasm, cap interact with eIF4F to initiate translation process.**
5. **rRNA and tRNA are not capped.**
6. **Most small nuclear RNA (snRNA) contain a trimethylguanosine as a cap.**

Translation initiation factors hold eukaryotic mRNAs in circles



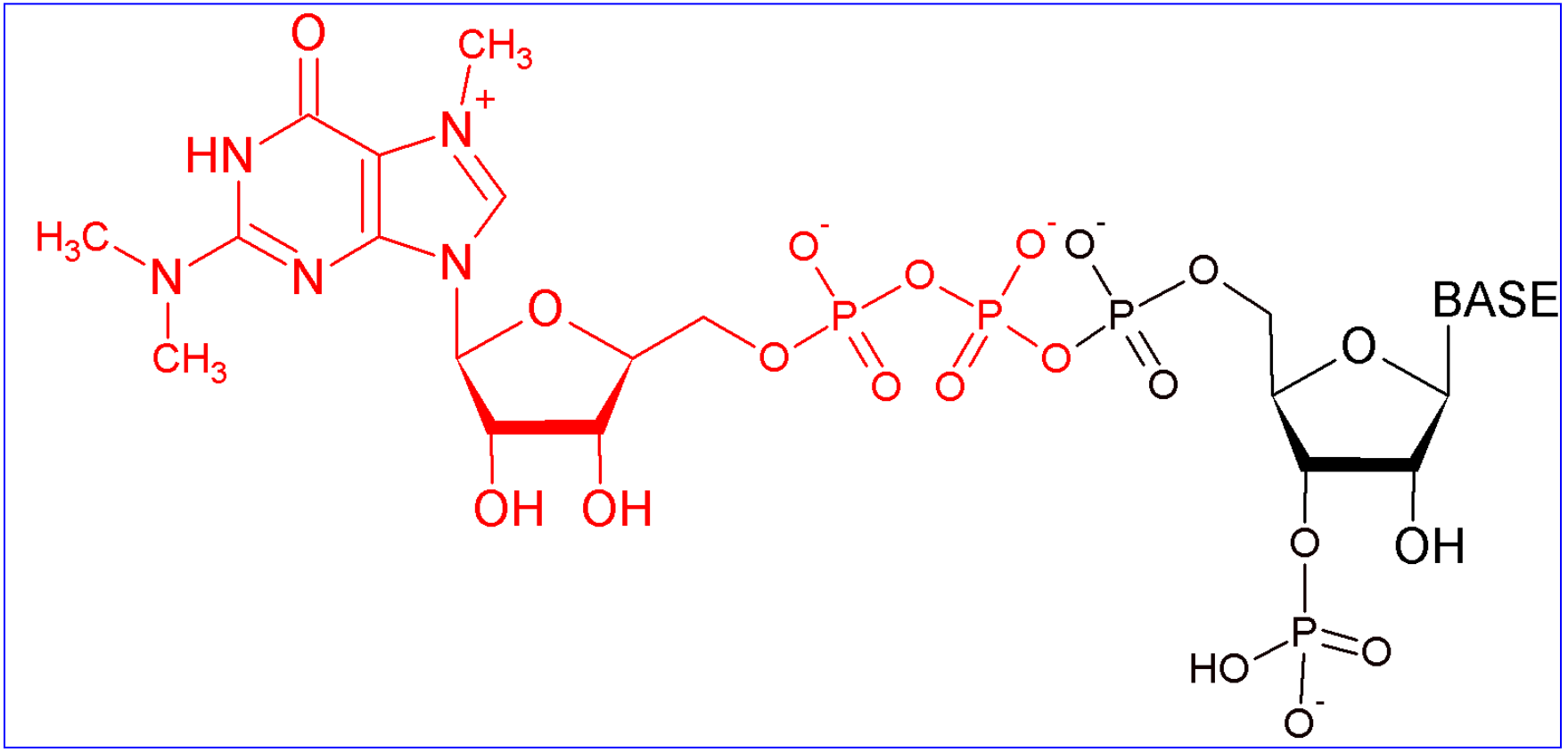
Functions of 5’ Cap Structure

Functions of 5’ Cap Structure

1. **Cap may function in the transport of mRNA from nucleus to cytoplasm** (CBP 20/80 interact with TREX component & facilitate transport of mature mRNA).
2. **It protect mRNA from phosphatases & nucleases attack and degradation** (enzymatic decapping is one of the major mechanisms in eukaryotic cells to regulate mRNA turnover)
3. **Required for productive mode of elongation of transcription by RNA Pol II** (It represents a checkpoint for transcription re-initiation from the initial pausing)
4. **Helps in ribosomal attachment**
5. **May play role in splicing**
6. **The leader sequence & Cap binding proteins (CBP) may play a role in enhancing & stabilizing the interaction of the mRNA with the ribosome & translational initiation process**
7. **Required for efficient translation**

TRI-METHYLATED CAP

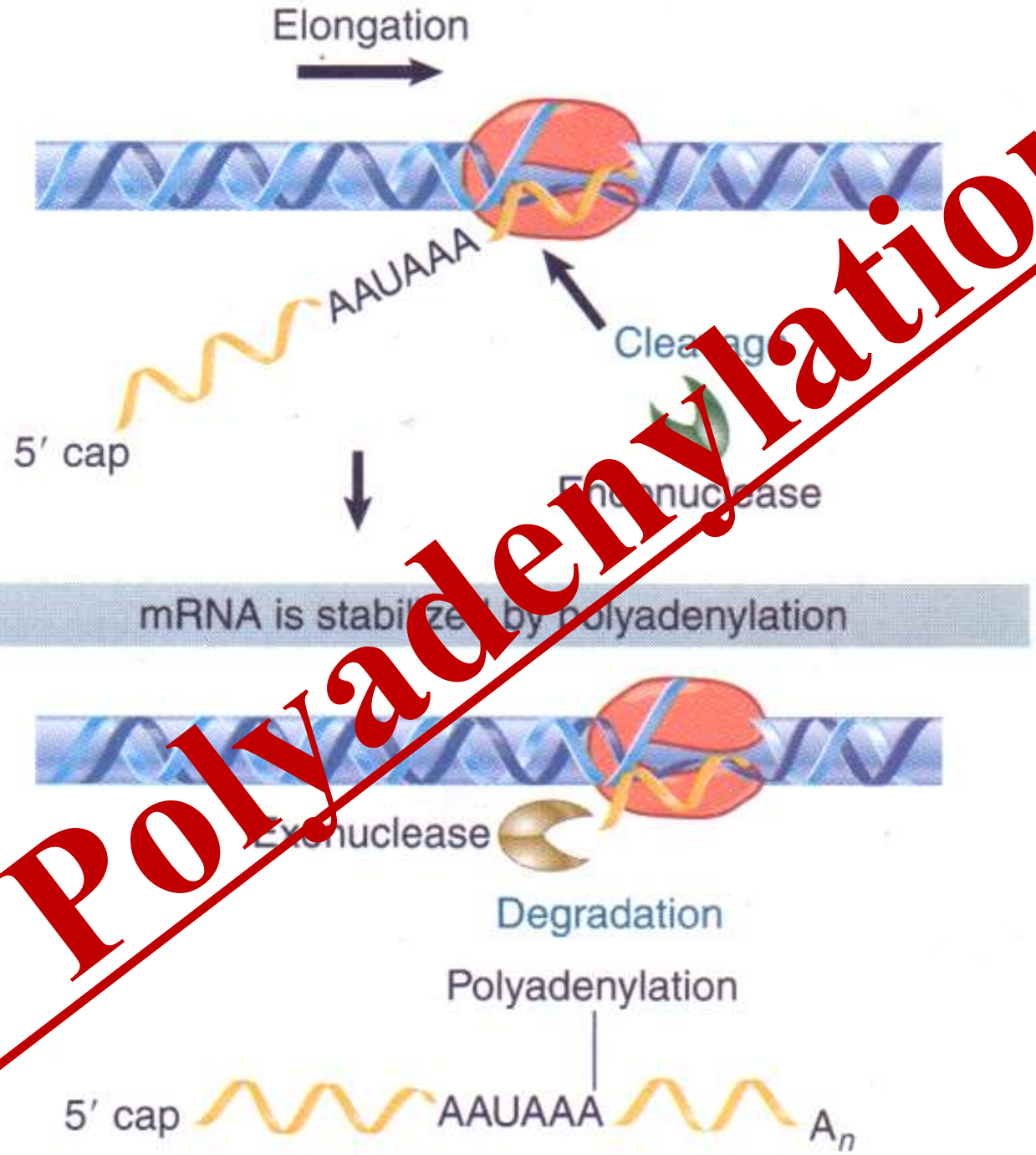
TRIMETHYL GUANOSINE CAP



The trimethylguanosine (TMG) cap modification is highly conserved throughout the eukaryotes.

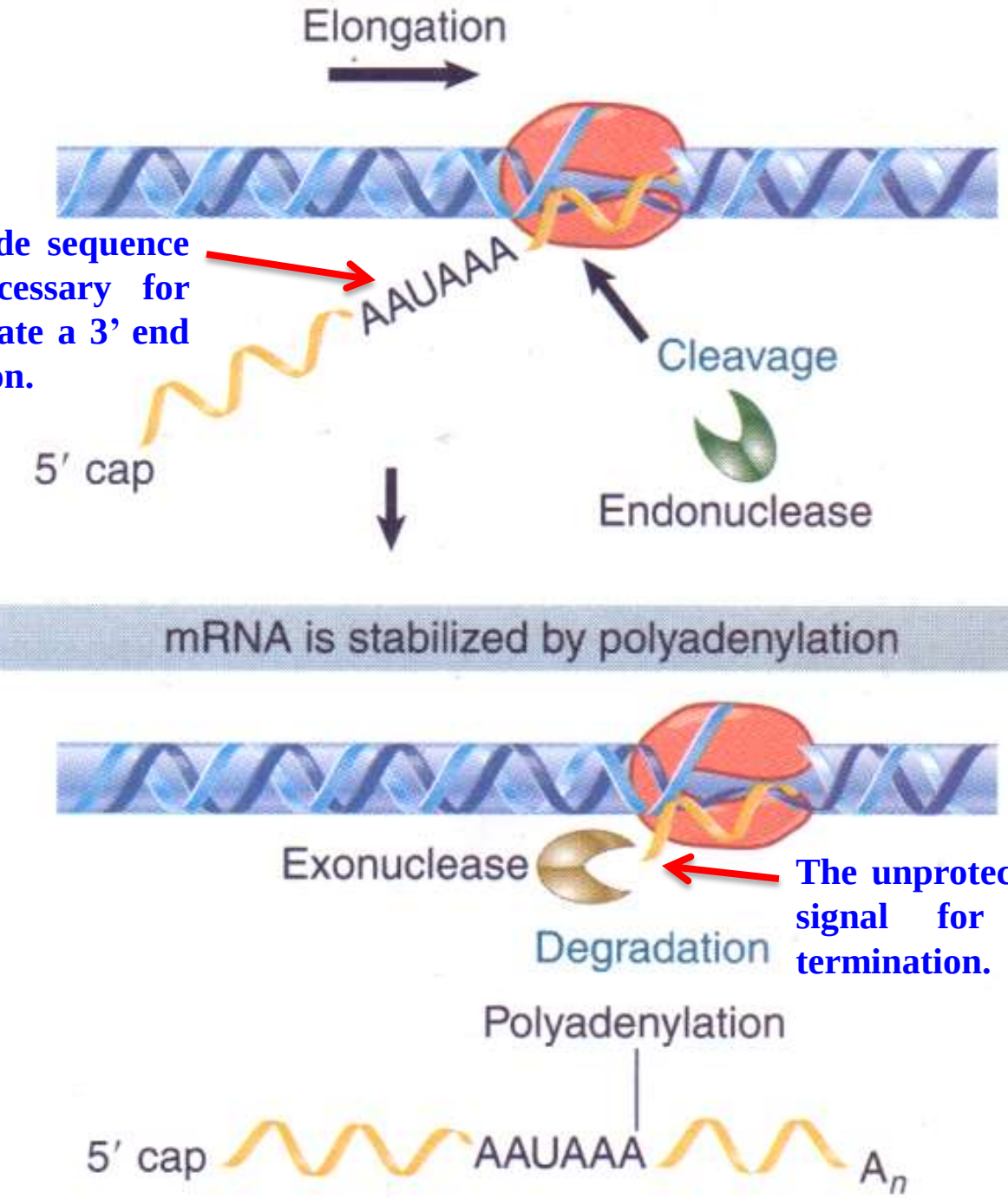
TRIMETHYL CAP

#	Component(s)	Description(s)
1	Example	*S. Cerevisiae* & Other organisms too
2	Substrate	Small nuclear (sn) RNAs, Small nucleolar (sno) RNAs, and telomerase RNA TLC1
3	Structure	$m_3^{(2,2,7)}G$
4	Enzyme	Trimethyl guanosine synthase 1 (Tgs1) - in yeast Receptor-interacting protein with methyltransferase domain (PIMT) – in human
5	Biogenesis site	➢ m^7G in nucleus ➢ Hypermethylation occurs (addition of other two methyl gps on 2'C) in cytosol
6	Functions	a) Efficient pre-mRNA splicing & pre-rRNA processing b) Small ribosomal subunit synthesis c) Maintenance of the structural organization of nucleolus



3' Polyadenylation

The 3’ ends of Pol II transcribed mRNAs are generated by cleavage followed by polyadenylation.



The hexanucleotide sequence AAUAAA is necessary for cleavage to generate a 3' end for polyadenylation.

The unprotected 5' end gives signal for transcriptional termination.

Poly-A tailing at 3′ - end

- **There is no poly(dT) sequence on the DNA template.**
- **The tailing process dose not depend on the template.**
- **The tailing process occurs prior to the splicing.**
- **Site of Polyadenylation → nucleus.**

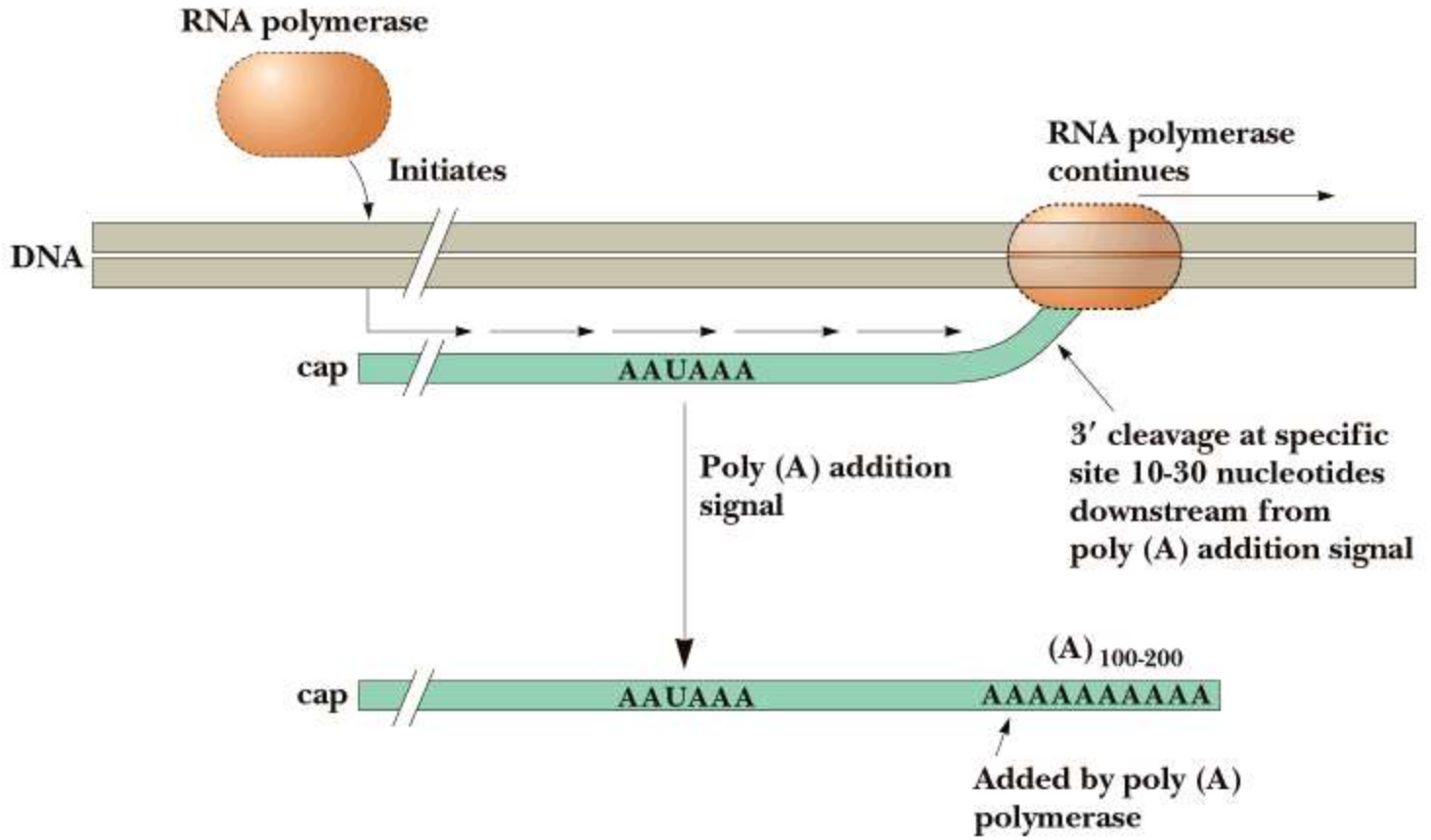
REMEMBER……

pTEFb kinase phosphorylate S-2 at CTD tail (-Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7-)$_n$ and recruits the machinery required for 3' polyadenylation & splicing of hnRNA (in yeast n= 26; in mouse n=52).

3'-Polyadenylylation

- **Termination of transcription occurs only after RNA polymerase has transcribed past a consensus AAUAAA sequence - the poly(A)$^+$ addition site**
- **10-30 nucleotides past this site, a string of 100 to 200 adenine residues are added to the mRNA transcript - the poly(A)$^+$ tail**
- **poly(A) polymerase adds these A residues**
- **Functions not known for sure**

Garrett & Grisham: Biochemistry, 2/e
Figure 31.49



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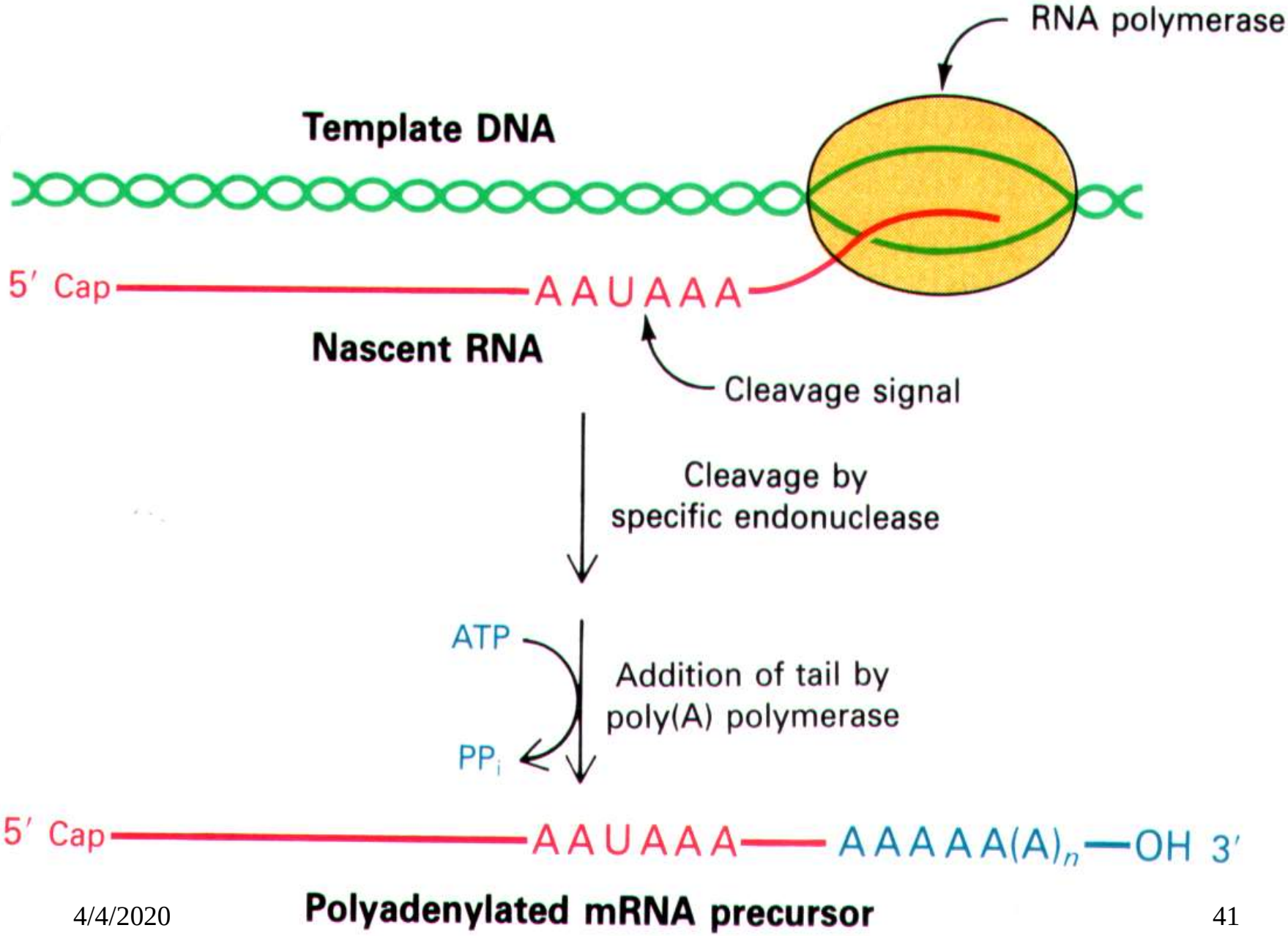

RNA polymerase
Template DNA
5′ Cap
A A U A A A
Nascent RNA
Cleavage signal
Cleavage by
specific endonuclease
ATP
PPi
Addition of tail by
poly(A) polymerase
5′ Cap
A A U A A A
A A A A A(A)n
OH 3′
Polyadenylated mRNA precursor

Components of 3’ end Formation Reaction

- **Two components:**

  1. **Cleavage component**
  2. **Polyadenylation component**

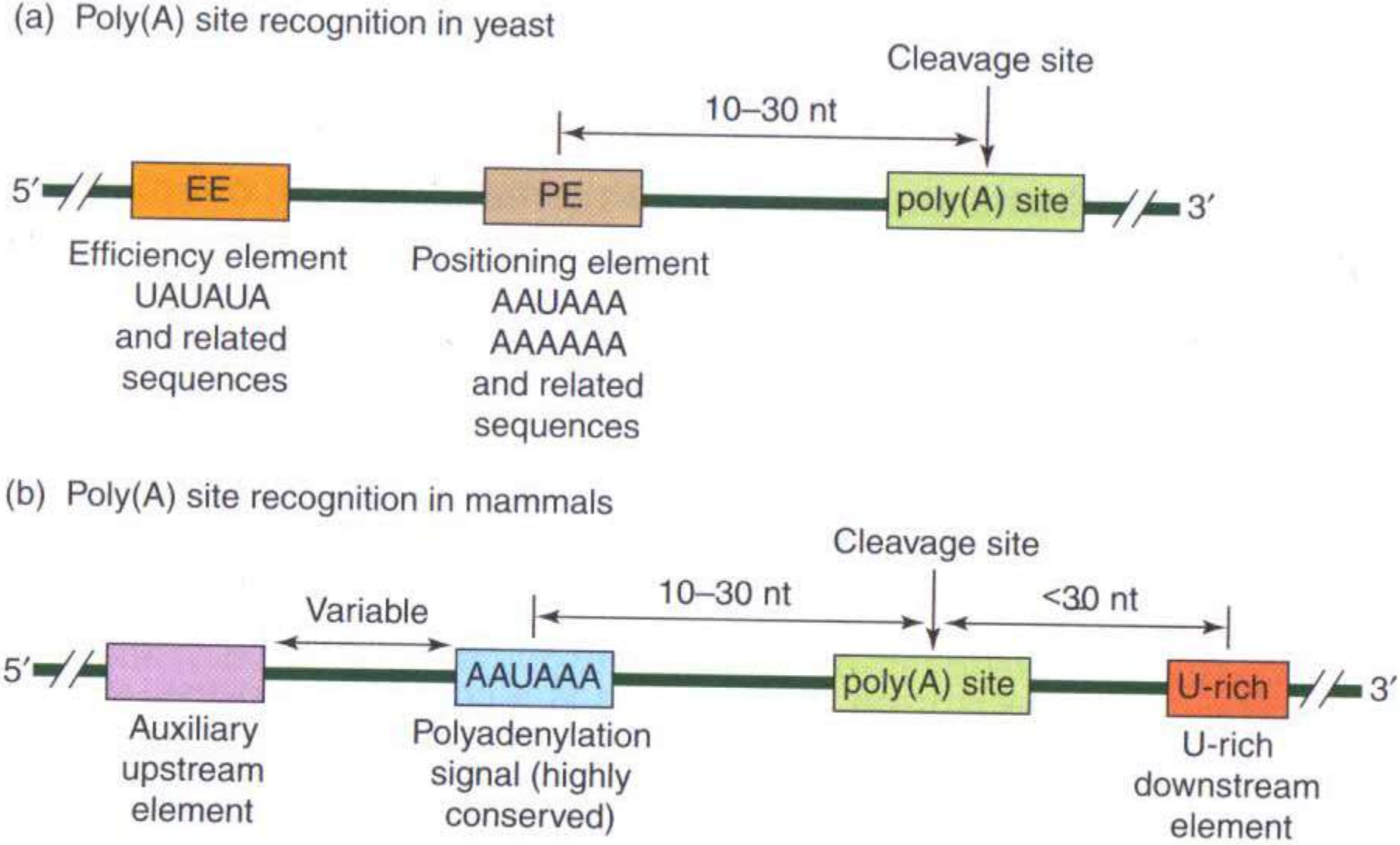
- **Cleavage component is the primary event that determines the 3’ end & polyadenylation component is the secondary event.**
- **But the reactions may be coordinated in vivo perhaps by forming a complex containing both activities.**

Molecular Components of 3' end Formation Reaction

The 3' polyadenylation complex has been analyzed at the molecular level.

Some of the identified proteins of the complex with their known functions are listed below:

#	Factor(s)	Description(s)
1	CPSF	Cleavage/Polyadenylation specificity factor
2	CstF	Cleavage stimulatory factor
3	CFI	Cleavage factor I
4	CFII	Cleavage factor II
5	PAP	Poly A Polymerase
6	PABII	Poly A binding Protein II
7	CTD	Carboxyl terminal domain of large subunit of RNAP II
8	Symplekin	Symplekin



Efficiency & Auxillary Elements: Enhance the efficiency of cleavage & polyadenylation

TABLE 19.3 Components of the Mammalian Cleavage/Polyadenylation Machinery

Factor	Processing Step	Function
CPSF cleavage/polyadenylation specificity factor	Cleavage and poly(A) addition	Contains five subunits. CPSF-73 cleaves pre-mRNA at the poly(A) site. CPSF-160 binds to AAUAAA. The functions of the other three subunits CPSF-30, CPSF-100, and Fip1 remain to be determined.
CstF cleavage stimulation factor	Cleavage	Contains four subunits (CstF-77, CstF-64, CstF-60, and SCP1). CstF-64 binds to the U-rich sequence. The functions of the other subunits remain to be determined.
CFI cleavage factor I	Cleavage	Recognizes sequence elements in poly(A) site.
CFII cleavage factor II	Cleavage	Unknown.
PAP poly(A) polymerase	Cleavage and poly(A) addition	Catalyzes poly(A) formation.
PAB II poly(A) binding protein	Poly(A) elongation	Binds poly(A) and CPSF-30. Responsible for processive poly(A) elongation and for the tail length.
CTD Carboxyl terminal domain of large subunit in RNA polymerase II	Cleavage	Binds CPSF and CstF.
Symplekin	Cleavage and poly(A) addition	Symplekin helps to assemble or stabilize the CstF complex and thereby helps to hold the complete cleavage/polyadenylation machinery together.

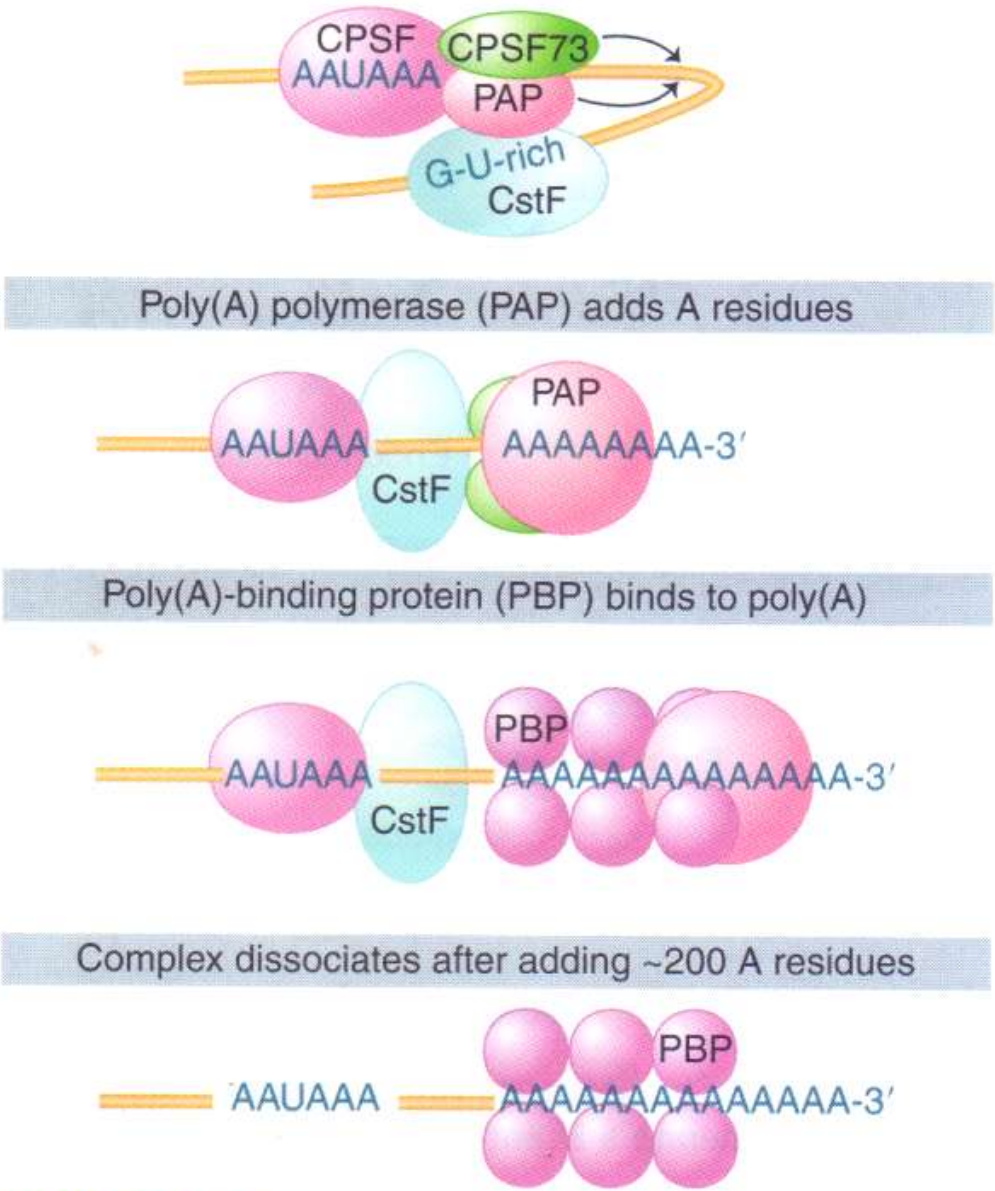
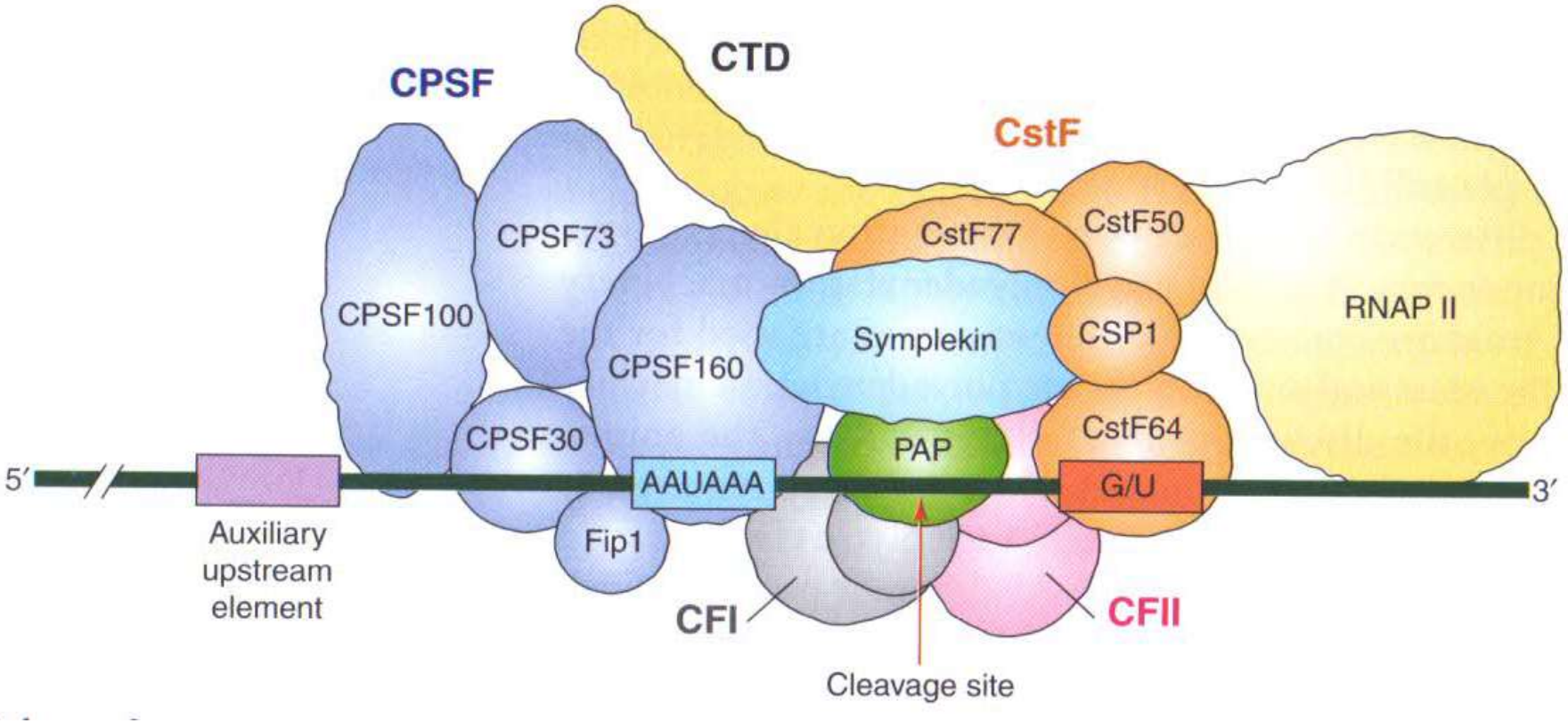
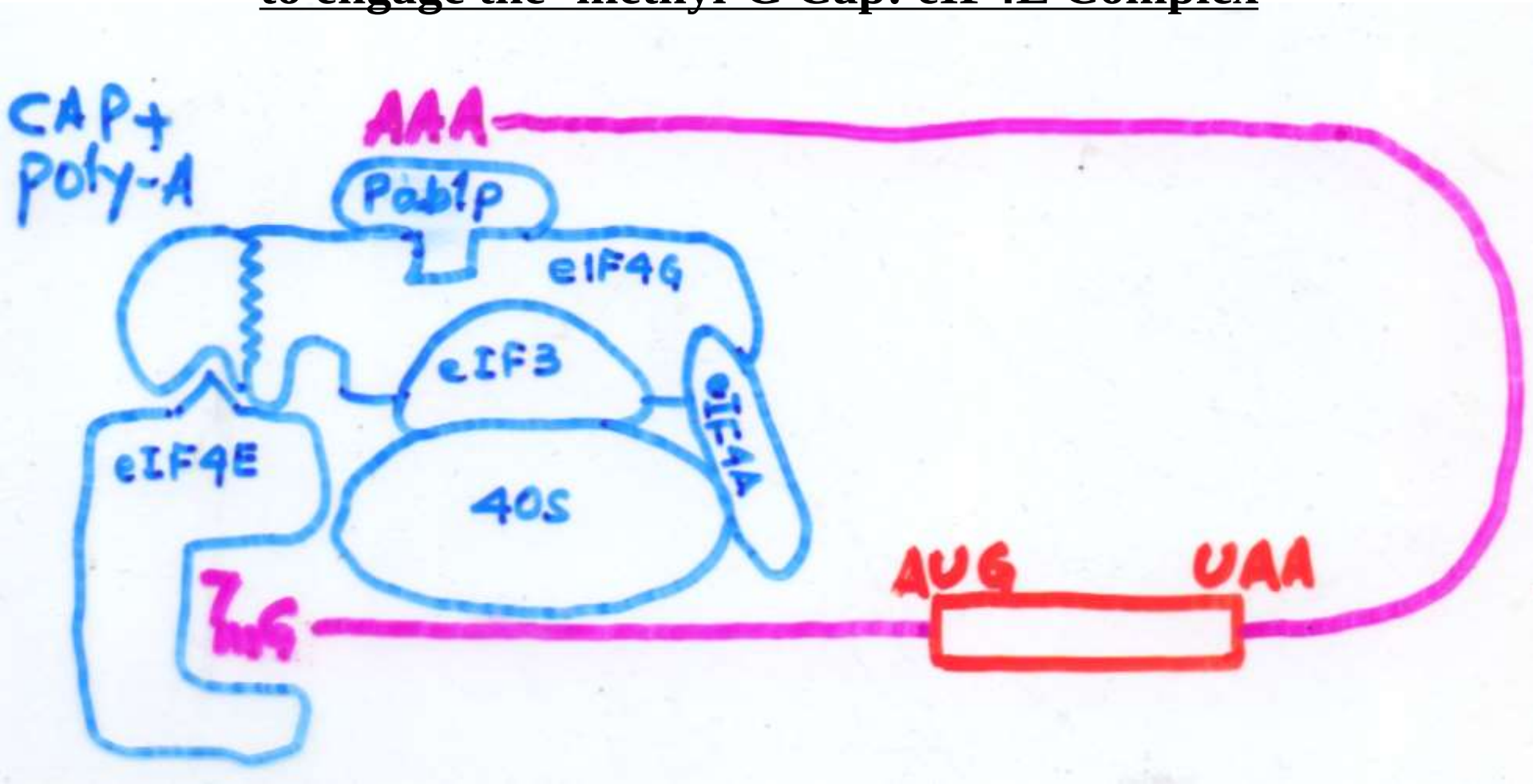


FIGURE 21.30 The 3′ processing complex consists of several activities. CPSF and CstF each consist of several subunits; the other components are monomeric. The total mass is >900 kD.

Schematic representation of the mammalian polyadenylation machinery



Initiation factor eIF4G serves as a multipurpose adapter to engage the ^{7}methyl-G Cap: eIF4E Complex



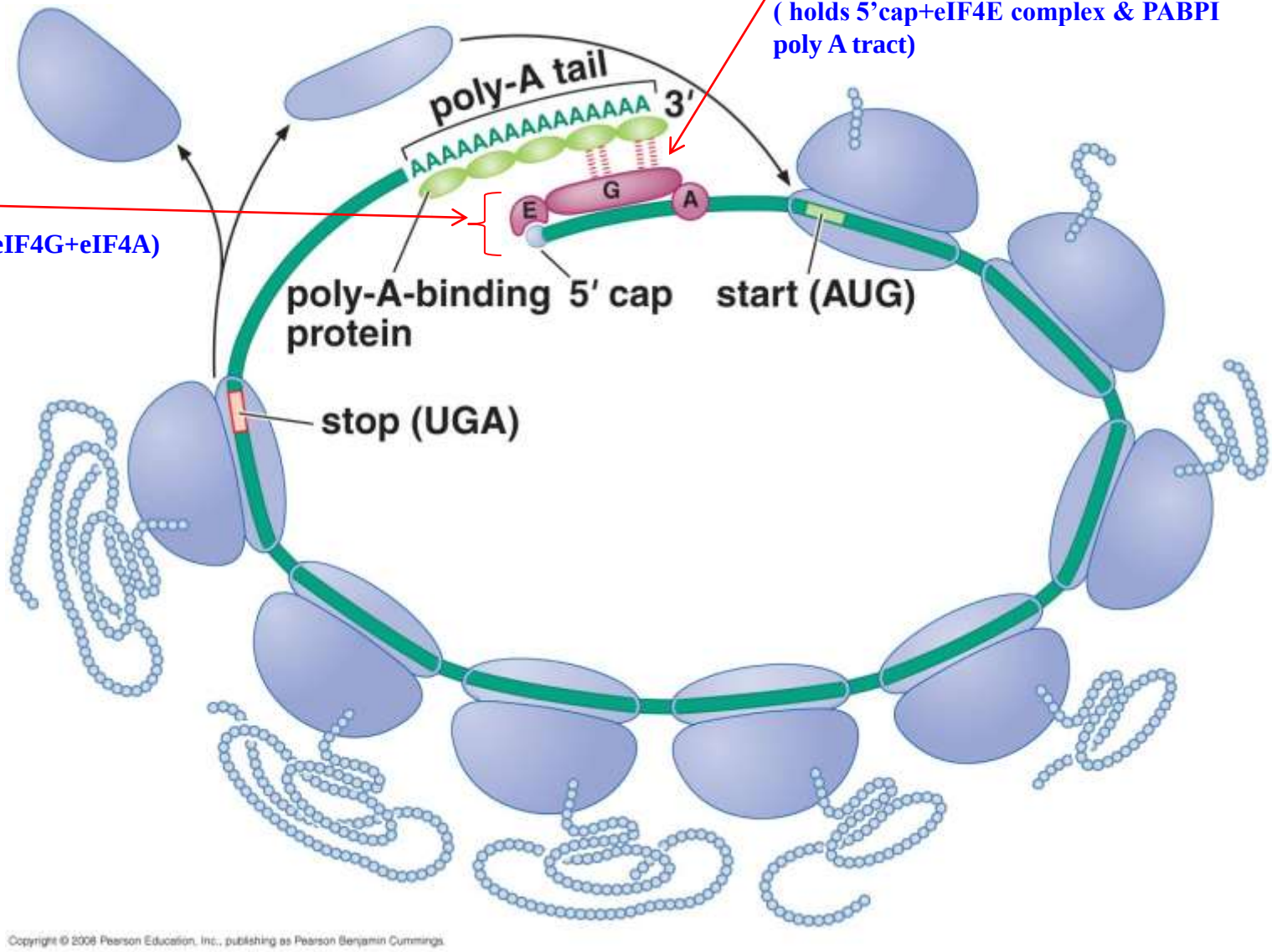
eIF4F is a hetero-trimer consisting of:

- **eIF4G – A scaffold protein**
- **eIF4E – Binds to 5' methyl cap**
- **eIF4A – is a helicase that unwinds 5' structure**

Translation initiation factors hold eukaryotic mRNAs in circles

eIF4G functions as multipurpose adapter
(holds 5'cap+eIF4E complex & PABPI poly A tract)

eIF4F
(is a heterotrimer of eIF4E+eIF4G+eIF4A)



Functions of Polyadenylation

1. Maturation of mRNA from nuclear RNA
2. Stability
   a) Protect mRNA from 3’ to 5’ end
   b) Protect stability of cap structure
3. Facilitate export of mRNA from nucleus to cytoplasm
4. Influences splicing events
5. Influences translation reaction