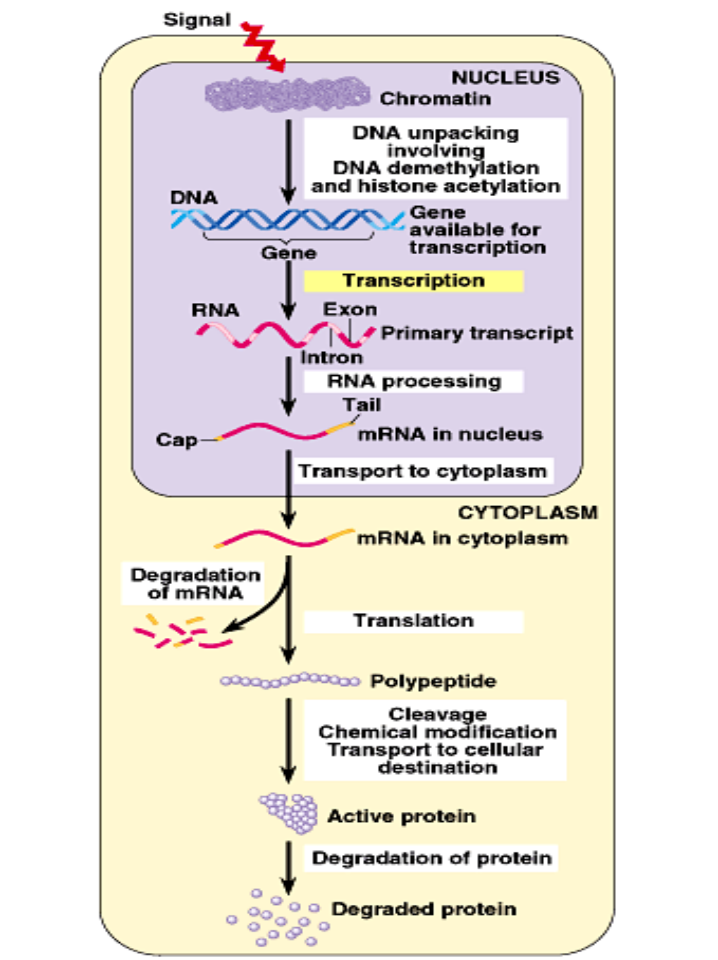
How is gene expression in eukaryotes accomplished ?

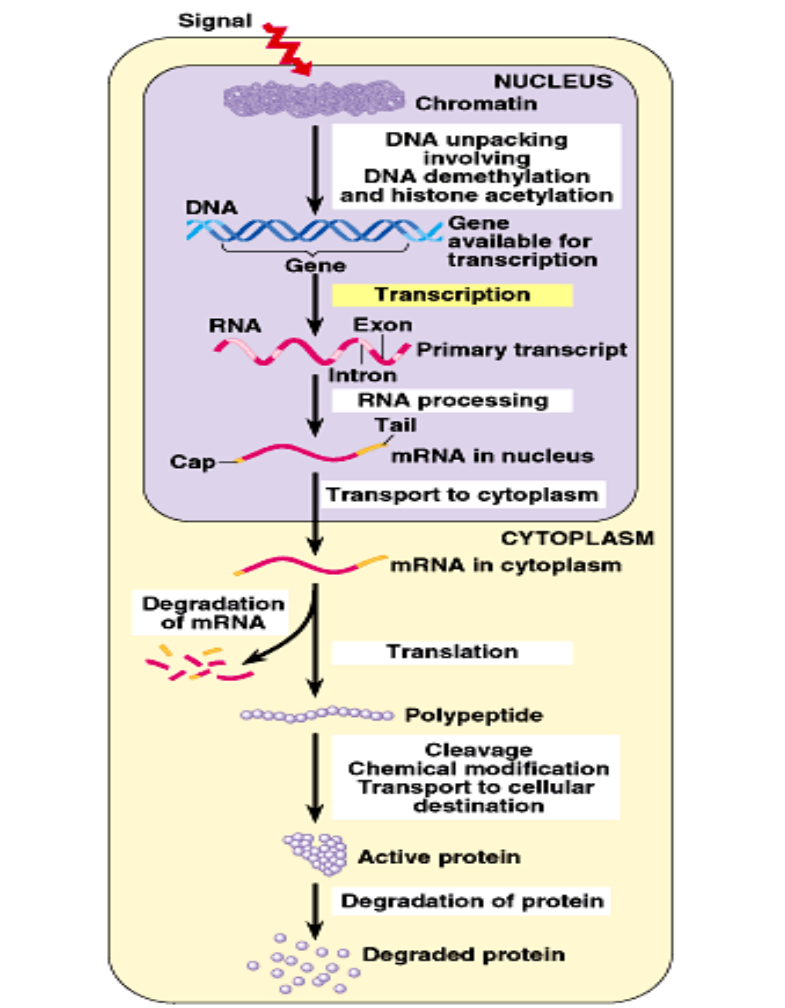
The control of eukaryotic gene expression: an overview

- **In the nucleus:**
- **1) DNA unpacking involving**
  - **a) DNA demethylation**
  - **b) histone acetylation**
- **2) Transcription control**
- **3) primary mRNA transcript processing**
- **4) preparing mRNA for transport**

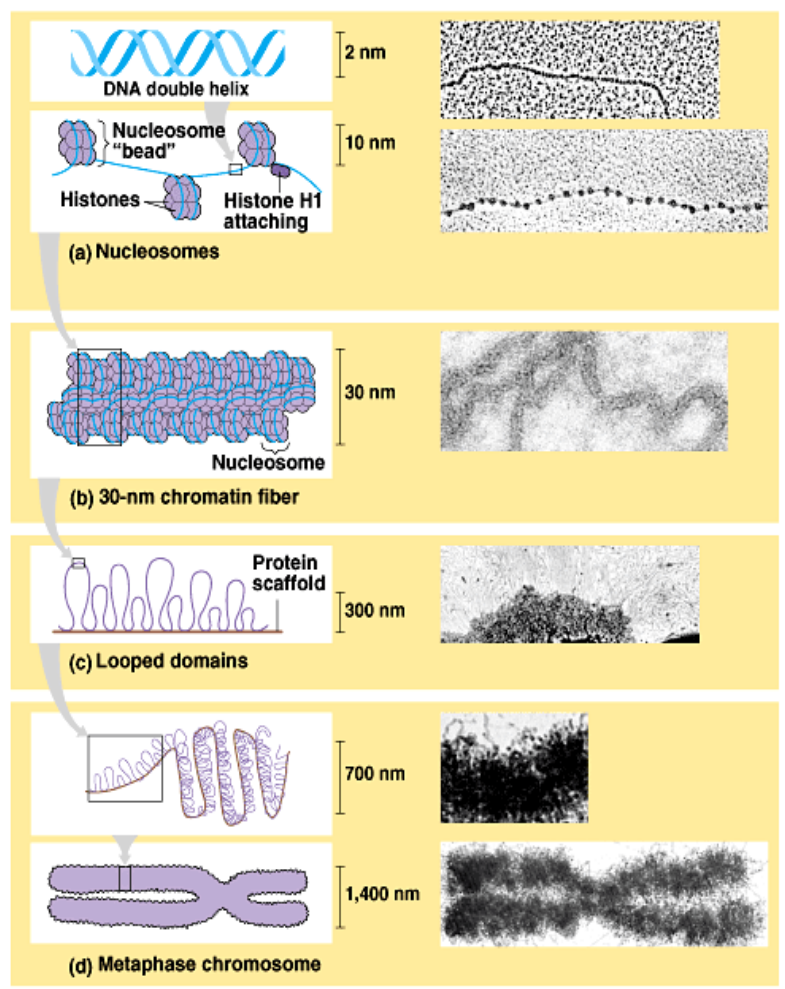


The control of eukaryotic gene expression: an overview

- **In the cytoplasm:**
- **1) Translation controls**
- **2) Degradation of mRNA**
- **3) Post-translational Modification**
  - **a) cleavage of long polypeptides**
  - **b) tagging and chemical modification**
  - **c) transport to cellular destinations**
  - **d) activating proteins**
- **4) Degradation of proteins**



Chromatin organization



- **Condensed heterochromatin is not expressed.**
- **A gene's location relative to nucleosomes and nuclear membrane (scaffold) influences its expression**

DNA Demethylation

DNA methylation = addition of methyl groups (CH_3) after DNA synthesis

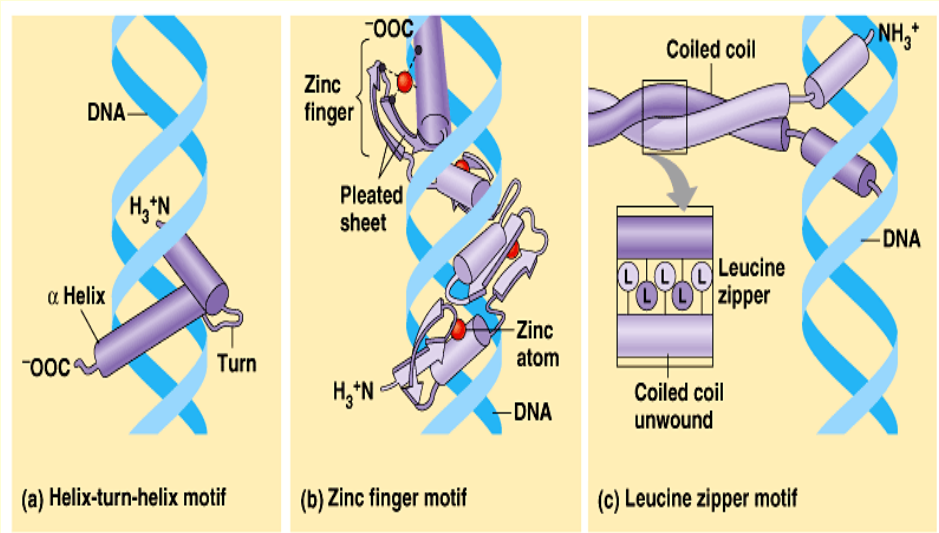
- **Usually cytosine can become methylated**
- **Genes that are not expressed are more heavily methylated**
- **Once methylated, genes usually stay that way through later divisions.**
- **Demethylating certain inactive genes turns them on.**

Histone Acetylation

- Acetylation enzymes attach acetyl groups ($COCH_3$) to certain amino acids of histone proteins.
- Acetylated histones bind to DNA less tightly; transcription factors have easier access to genes

Transcription Control

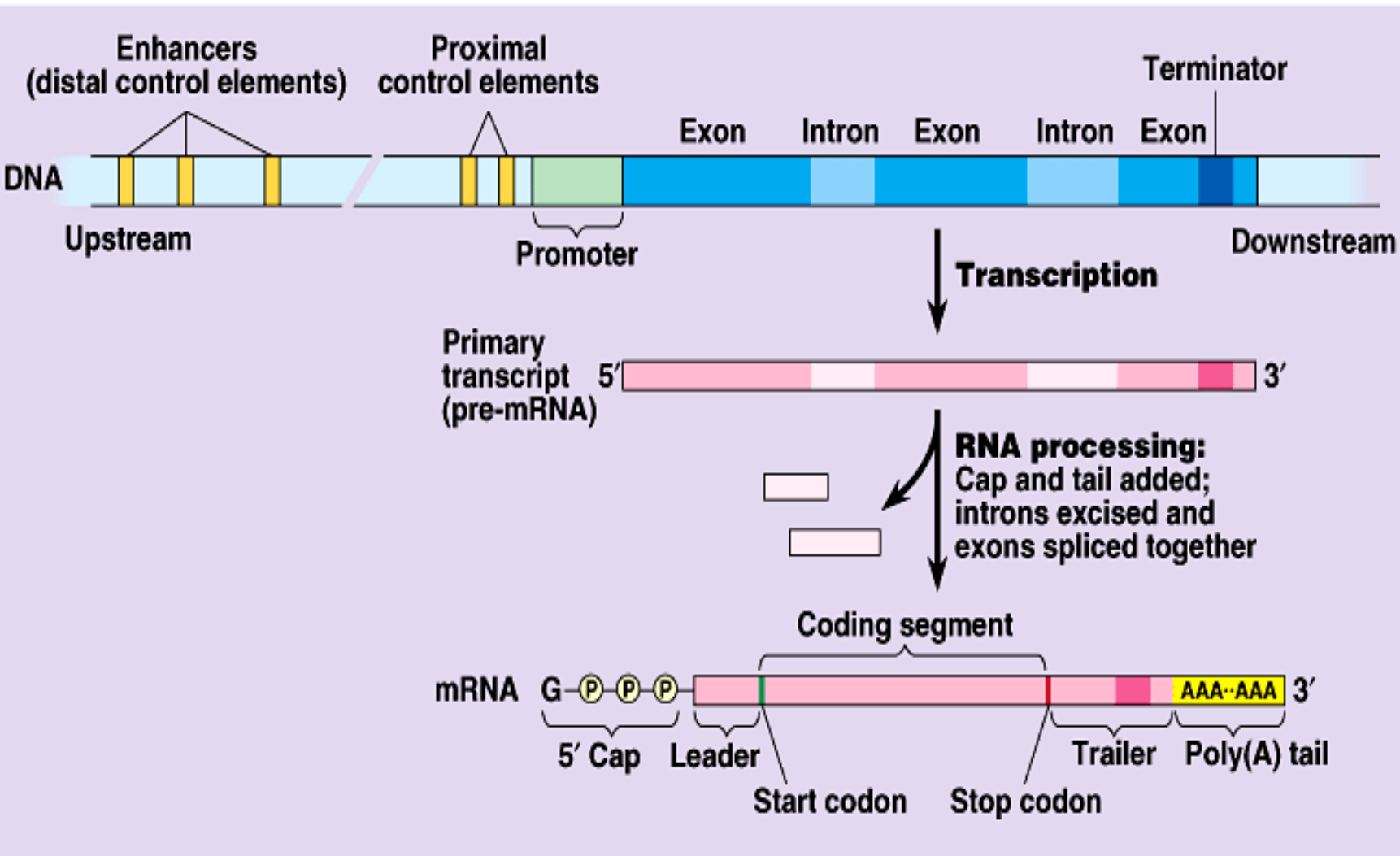
- **Transcription factors are necessary for RNA polymerase to bind to DNA during transcription.**
- **Transcription factors must be able to bind to DNA (DNA-binding domain) and to proteins (protein binding domain)**
- **Similar transcription factors activate or repress groups of genes in synchrony.**



Transcription Control

Eukaryotic genes have the following structural organization:

- **Promoter – RNA polymerase binding**
- **Proximal control elements – transcription factor binding sites near promoter**
- **Enhancers (distal control elements) – DNA that bind proteins called activators at sites very remote from the promoter**
- **Silencers (distal control elements) – DNA that binds proteins called repressors at sites not to far from enhancers**


Enhancers
(distal control elements)
Proximal
control elements
Terminator
Exon
Intron
Exon
Intron
Exon
DNA
Upstream
Promoter
Downstream
Transcription
Primary
transcript
(pre-mRNA)
5′
3′
RNA processing:
Cap and tail added;
introns excised and
exons spliced together
Coding segment
mRNA
G
P
P
P
AAA···AAA
3′
5′ Cap
Leader
Start codon
Stop codon
Trailer
Poly(A) tail

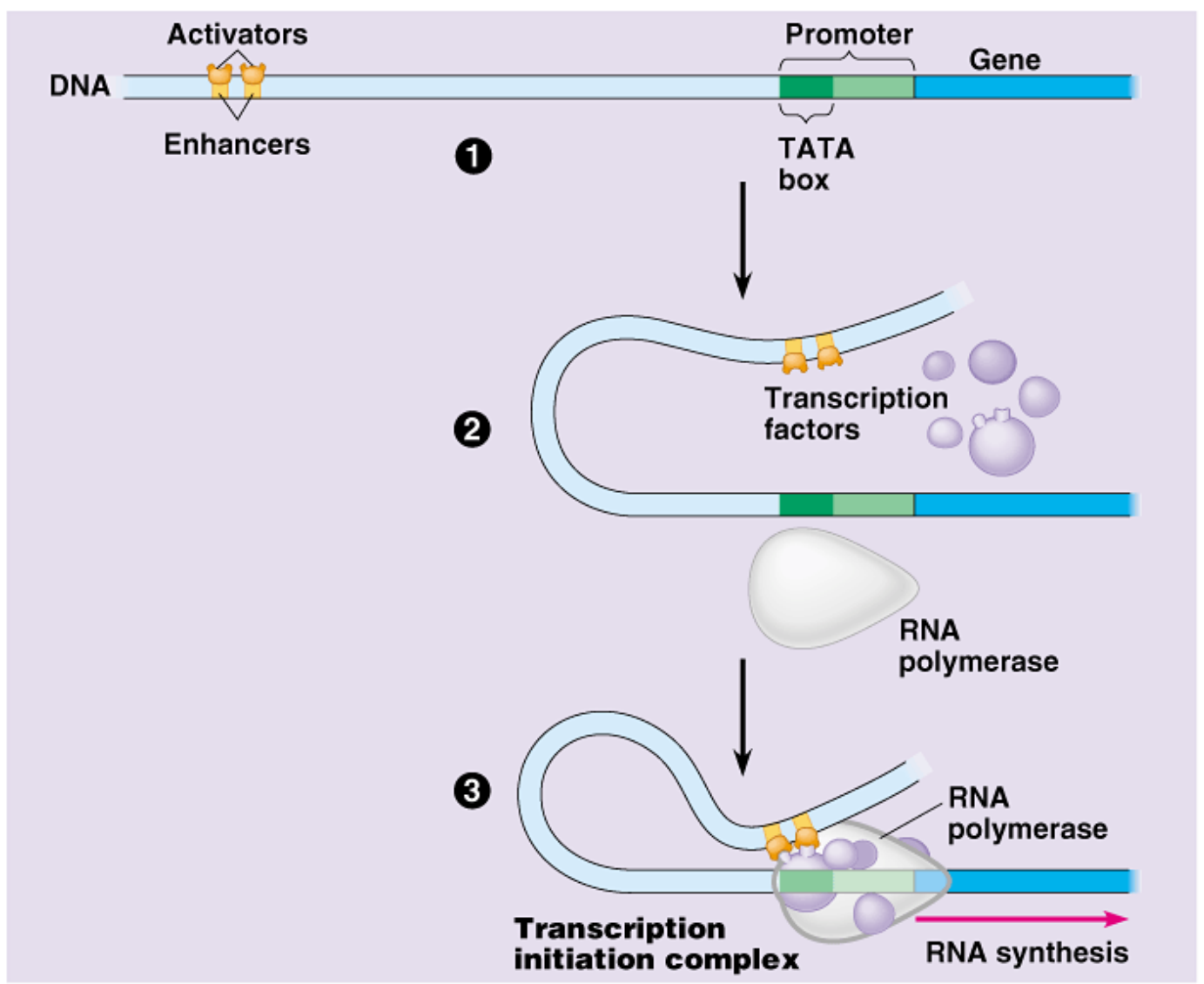
Transcription Control (Hormone Signaling)

- **Steroid (fat-soluble) hormone diffuses through the cell membrane and nucleus.**
- **Steroid binds to inactive receptor protein and activates it.**
- **Active receptor molecule attaches to specific sites within the enhancer.**
- **Enhancer, now active, can bind to activator protein.**

Transcription Control

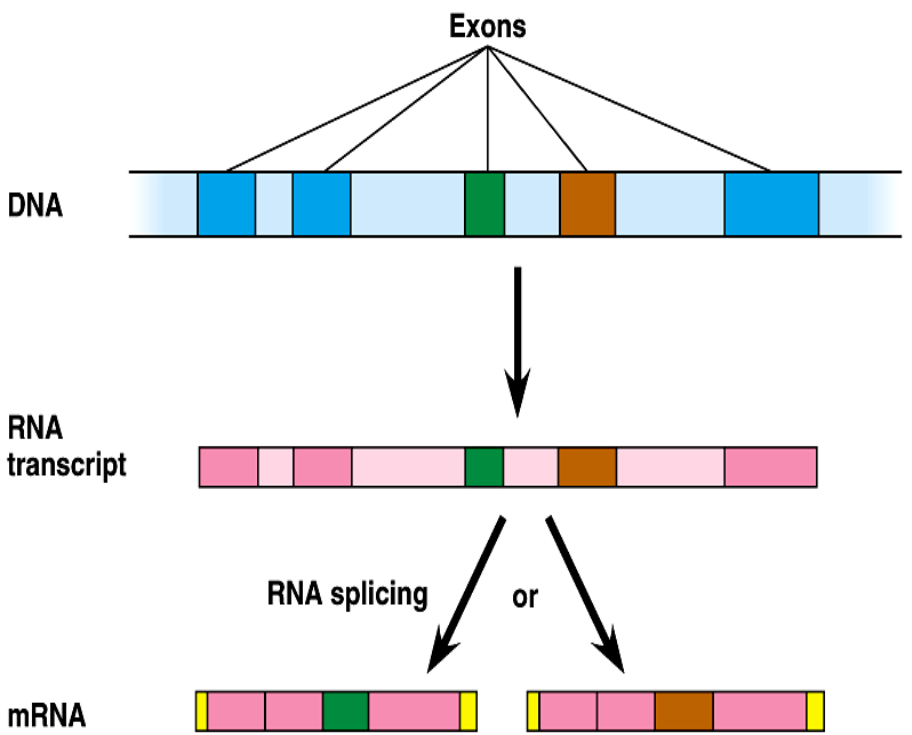
How eukaryotic genes are transcribed:

1) **Activator proteins bind to enhancer sites on DNA (or repressor proteins bind to silencer sites near enhancer sites and inhibit transcription).**
2) **DNA bending brings bound activators closer to other transcription factors.**
3) **Protein-binding domains on the activators attach to transcription factors and help form an active transcription complex**
4) **RNA polymerase is now free to bind and move along the DNA.**


Activators
Promoter
Gene
DNA
Enhancers
TATA box
1
Transcription factors
2
RNA polymerase
3
RNA polymerase
Transcription initiation complex
RNA synthesis

Primary mRNA Transcript Processing and Preparation for RNA Transport

- Introns must be removed and exons must be spliced.
- Alternative RNA splicing can occur as exons are arranged in various ways.
- A 5’ cap and a poly-A tail are added.



mRNA Degradation

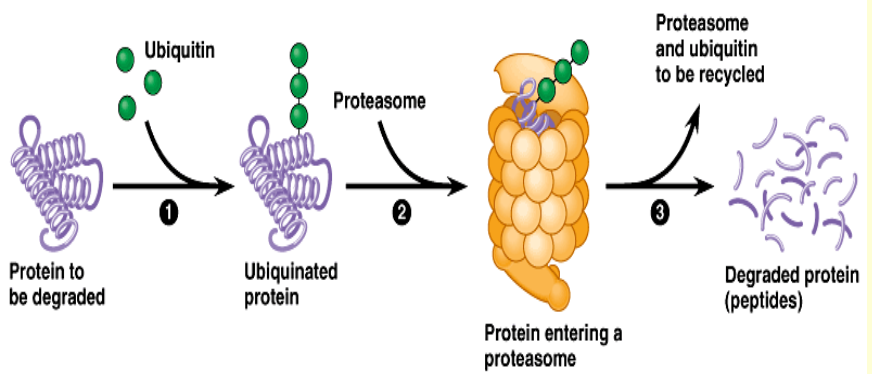
- Eukaryotic mRNA can exist for long periods of times (hours to weeks) example – mRNA for hemoglobin
- mRNA is degraded when:
  - Poly-A tail is hydrolyzed.
  - 5’ cap is removed. (mRNA codes for this)
  - Nucleases hydrolyze the remaining mRNA molecule from 5’ end.

Control of Translation

- **mRNA is stopped from initiating translation by:**
  - **Binding translation repressor protein to 5’ end of a mRNA to prevent ribosome attachment**
  - **Inactivating certain initiation factors**
    - **Occurs in early embryonic development**
    - **Egg has stored inactive mRNA prior to fertilization**
    - **New cells respond with a burst of protein synthesis after fertilization**

Protein Processing and Degradation

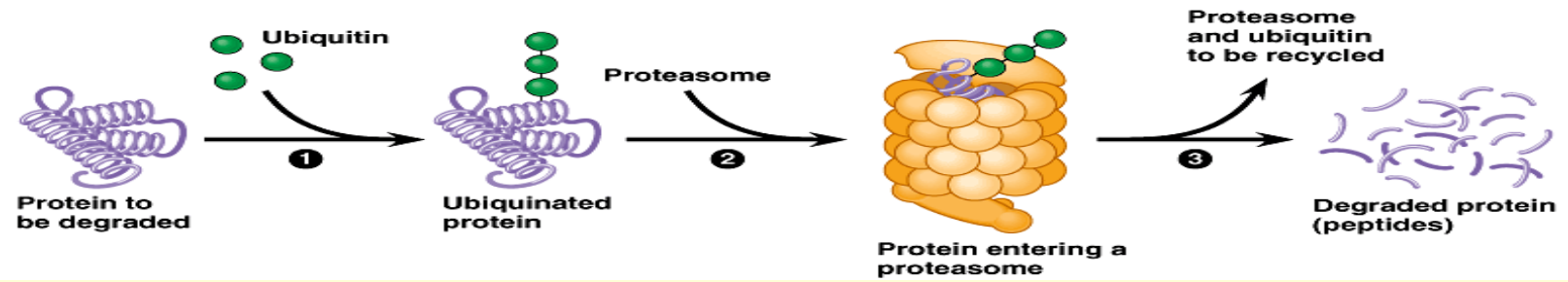
- Many eukaryotic polypeptides must be modified or transported before becoming active.
- Modifications include adding phosphates, cleaving large polypeptides, tagging with sugar, marking for export

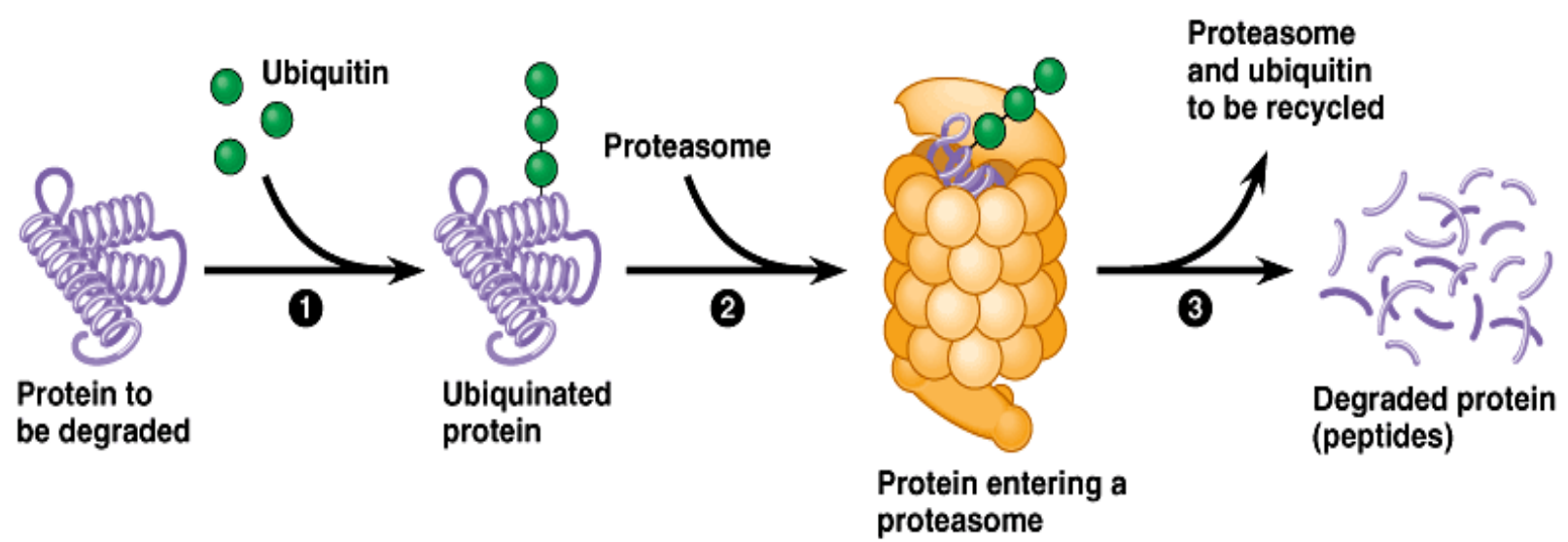


Protein Processing and Degradation

Selective degradation occurs when;

- **Ubiquitin is added to mark for destruction.**
- **Proteosomes, huge protein hydrolyzing complexes, recognize ubiquitin and degrade the tagged protein**
- **Dangerous exception to the above are mutated cell cycle proteins that proteosomes can not recognize. Cancer may result.**




Ubiquitin
Proteasome
Proteasome and ubiquitin to be recycled
1
2
3
Protein to be degraded
Ubiquinated protein
Protein entering a proteasome
Degraded protein (peptides)