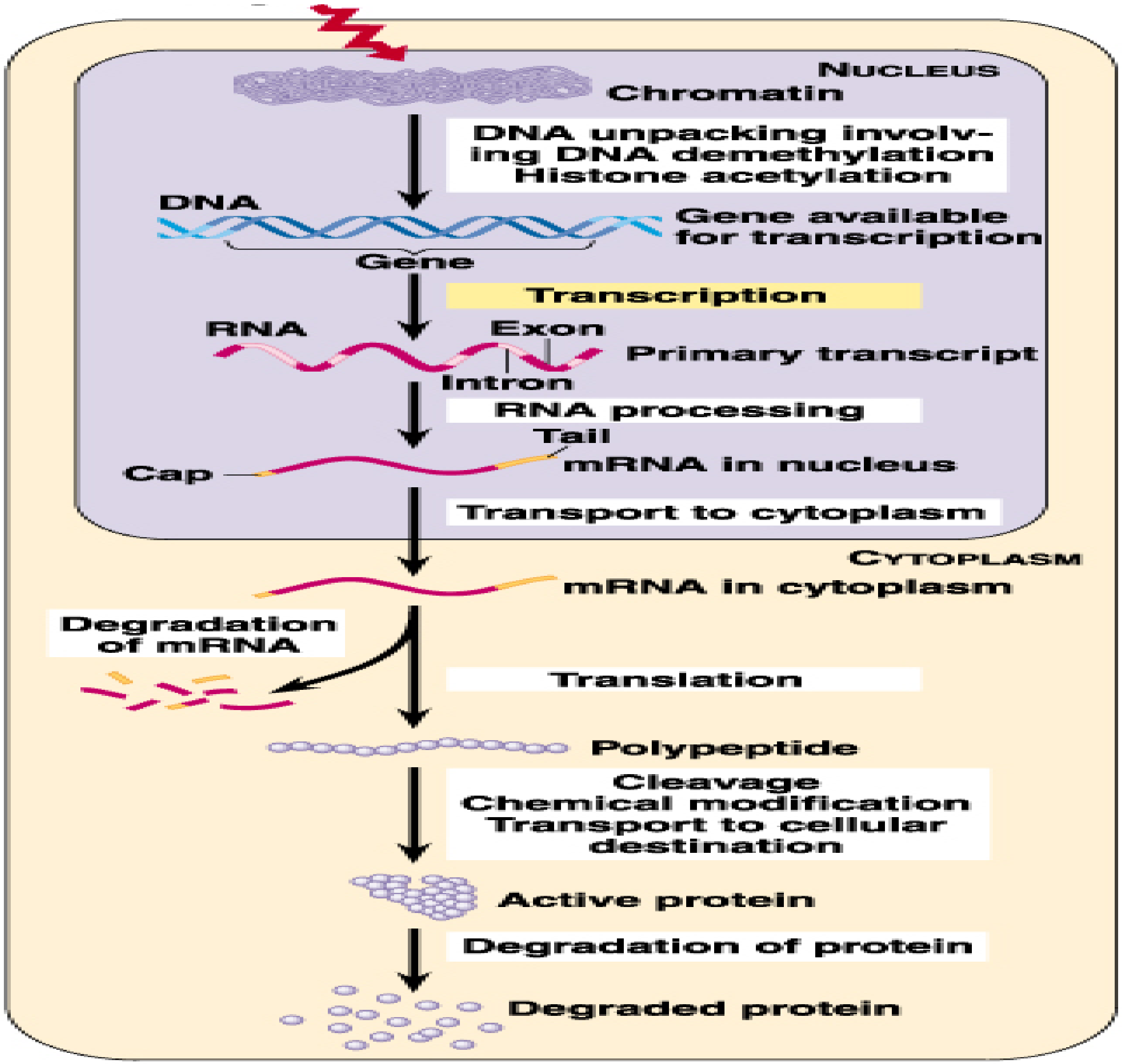
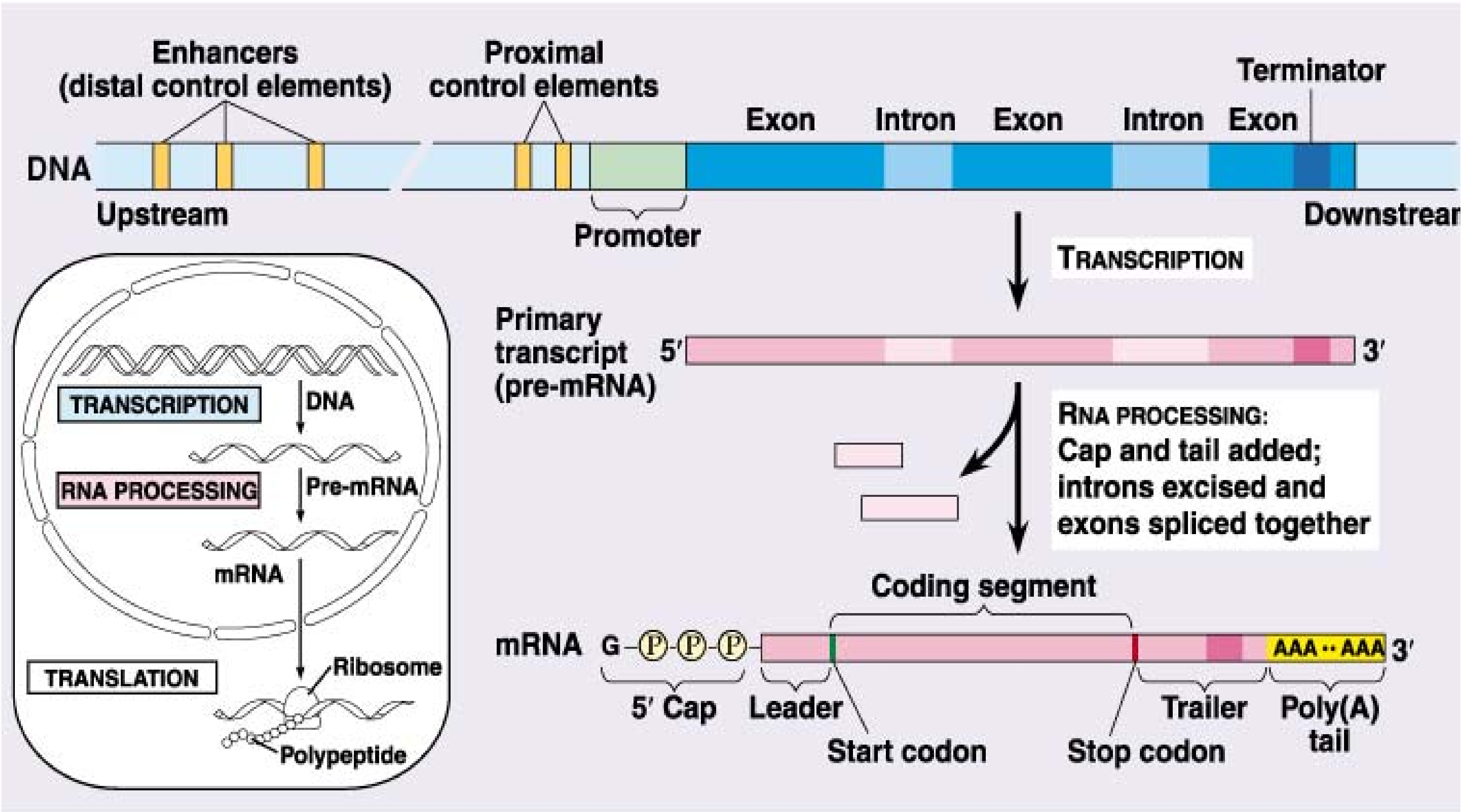
Regulation of Gene Expression in Eukaryotes



NUCLEUS
Chromatin
DNA unpacking involving DNA demethylation
Histone acetylation
DNA
Gene available for transcription
Gene
Transcription
RNA
Exon
Primary transcript
Intron
RNA processing
Tail
Cap
mRNA in nucleus
Transport to cytoplasm
CYTOPLASM
mRNA in cytoplasm
Degradation of mRNA
Translation
Polypeptide
Cleavage
Chemical modification
Transport to cellular destination
Active protein
Degradation of protein
Degraded protein

Opportunities for the control of gene expression in the eukaryotic cell



Gene Expression

- Spatial – not every gene product needed in every cell type
- Temporal – Different genes expressed at different times
  - Environmental stimuli
  - Hormones
  - Especially seen in development- formation of tissues and organs

Regulation

- –RNA processing
  - 5’ cap
  - Poly A tail
  - Intron removal
- In eukaryotes, more level of regulation than prokaryotes due to complex organelles

Cytoplasmic control

- mRNA stability:
  - Long vs. short lived mRNAs
  - Long- many rounds protein synthesis from one mRNA
  - Short – rapidly degraded, needs more transcription to replenish (half-life)
- Rapid mRNA degradation may be desirable
- Half-life problem with making a drug, too

mRNA stability

- Poly A tails – can add stability
- Longer tails stabilize message more
- E.g., histone mRNAs no poly A tails; message very short lived

Induction of transcription

- Not found as often in eukaryotes as in prokaryotes
- Induction can work by:
  - Temperature
  - Light
  - hormones

Induction of transcription

- Temperature
  - Synthesize heat shock proteins (HSPs)
  - Transcriptional regulation – stress of high heat signals HSPs to be transcribed
  - Studied in *Drosophila*- but occurs in humans also

Induction of transcription

- Light
  - RBC (ribulose 1,5 bisphosphate carboxylase)
  - Plants must absorb light energy
  - RBC produced when plants are exposed to light (see Northern blot in figure)- remember what is a Northern blot?)

Induction of transcription

- Hormones
  - Secreted, circulate through body, make contact with target cell and regulate transcription
  - Called signal molecules
  - 2 classes of hormones that activate transcription
    - Steroid hormones
    - Peptide hormones

Steroid hormones

- Small, lipid molecules derived from cholesterol
- Easily pass through cell membranes
- Examples
  - Estrogen
  - Progesterone
  - Testosterone
  - Glucocorticoids

Steroid hormones

- HRE’s- hormone response elements – mediate hormone induced gene expression
- Number of HRE’s dictate strength of response (work cooperatively)

Peptide hormones

- Linear chain of amino acids
- Examples
  - Insulin
  - Growth hormone
  - prolactin

Peptide hormones

- Cannot pass through cell membrane easily, so convey signals through membrane bound receptors
- Signal transduction – hormone binds receptor on cell surface, signal gets internalized, then cascade of events begin

Molecular control

- Transcription factors – accessory proteins for eukaryotic gene expression
- Basal transcription factors
  - Each binds to a sequence near promotor
  - Facilitates alignment of RNA polymerase

Special transcription factors

- Bind to enhancers
- Promotor specific (HRE's for e.g.)
- Properties of enhancers:
  - Can act over several thousand bp
  - Function independent of orientation
  - Function independent of position – upstream, downstream, etc. (different than promotors- close to gene and only one orientation)

- Yellow gene in *Drosophila*
- Tissue specific enhancers for pigmentation for each body part
- Mosaic patterns- alterations in yellow gene transcription in some body parts but not others
- Also see SV40 enhancer (simian virus 40) –

How do enhancers work?

- Influence activity of proteins that bind promoters
- RNA pol and basal transcription factors
- Physical contact with other proteins?
  - Enhancer and promotor regions brought together by DNA folding

Transcription factors

- 2 chemical domains
  - DNA binding
  - Transcriptional activation
- Can be separate or overlapping
- Physical interaction also quite possible

Transcription factor motifs

- Zinc finger – DNA binding
- Helix-turn-helix - DNA binding (COOH required)
- Leucine zipper - binding
- Helix-loop-helix – helical regions allow for dimerization
  - Homo and hetero dimers

Gene expression and chromosomes

- DNA needs to be accessible to RNA pol for transcription initiation
- Place on chromosome may affect this
- So, gene exp influenced by chromosomal structure
- E.g., lampbrush chromosomes

Is transcribed DNA more “open”?

- Used DNAse I treatments
- Groudine and Weintraub – showed transcriptionally active DNA more easily degraded by DNAse I than untranscribed DNA (more “open” to nuclease digestion)
- Have DNAse I hypersensitivity sites – near promotors and enhancers

Whole chromosomes: activation and inactivation

- Equalizing activity of X chromosomes in XX versus XY organisms
- Recall mechanisms:
  - Humans, inactivate one X chromosomes in females
  - In *Drosophila*, male X makes double the gene product

X compensation

- Inactivation, hyperactivation, hypoactivation
- What is molecular mechanism of dosage compensation?
  - Specific factor(s) bind to X- regulate its gene expression above all other regulatory elements

Dosage Compensation – example of X in humans

- XIC- X inactivation center – makes XIST (X inactive specific transcript) - 17kb mRNA with no ORF- so likely does not encode a protein
- RNA is the functional product of the gene
- Found only in nucleus and not associated with active

Opportunities for the control of gene expression in the eukaryotic cell